

# ACADEMIC JOURNAL OF HEALTH SCIENCES

## MEDICINA BALEAR



Diagnostic Technology Assessment in Iran: A Grounded Theory Study

Efecto del estado inflamatorio perioperatorio en el salvamento de extremidad de los pacientes con isquemia crítica sometidos a revascularización infrainguinal

Effect of sesame oil extraction by traditional and cold press methods on total aflatoxin content

Values of different index related to cardiovascular risk according the Findrisc test scores in caucasian

Influence of sociodemographic variables and tobacco consumption on the risk of nonalcoholic fatty liver disease and liver fibrosis

Resultados de la encuesta COVID-19 en alumnos, profesores y usuarios de la escuela universitaria ADEMA

*Clostridium histolyticum* collagenase (Xiapex®) in patients with peyronie disease. Expectations vs reality

The effect of Familact probiotic supplement in patients with diabetes  
(Evaluation of Blood Glucose Parameters, Lipid Profile)

La intervención de los poderes públicos frente a las pseudoterapias: posibilidades y límites

Cyto-histological correlation in cervical cytology

Genotypic and phenotypic assessment of antibiotic resistance of MRSA bacteria isolated from food stuffs

Antibiotic resistance pattern of methicillin-resistant *Staphylococcus aureus* isolates from powdered packaged medicinal plants and bottle herbal distillates

Effect of estrogen and insulin sensitivity due to exercise training with dual intensities in female rats with estradiol valerate-induced PCOS

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*Helicobacter pylori* distribution in dental plaque specimens collected from individuals referred to dental clinics

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Direct instruction and inquiry-based method in teaching endodontics among preclinical dentistry students

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Evaluation of arch bar fixation without surgical aid in jaw fracture treatment

The efficacy of Cognitive-behavior therapy based parenting for anxious children with cancer

Seckel syndrome associated dental anomaly; case report

Inestabilidad cefálica y derrame pericárdico

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## Una asignatura pendiente en cáncer ocupacional: la historia clínico-laboral

*A pending issue in occupational cancer: clinical-occupational history*

**M<sup>a</sup> Teófila Vicente Herrero**

*Médico Especialista en Medicina del Trabajo*



La notificación del cáncer laboral sigue siendo en el momento actual la más baja dentro de las enfermedades profesionales, tanto en España<sup>1</sup>, como en los países de nuestro entorno<sup>2</sup>. Esta tendencia no se ha visto modificada con el paso de los años a pesar de los esfuerzos realizados por las Organizaciones Internacionales más representativas, como la Organización Mundial de la Salud<sup>3</sup>, y por los representantes de Salud Pública y Salud Laboral en nuestro país<sup>4</sup>.

Para prevenir esta enfermedad en el entorno laboral, desde 2016 y hasta 2020, la Comisión Europea ha ido revisando la principal herramienta legal para abordar el cáncer laboral en Europa, la Directiva 2004/37/CE de agentes cancerígenos y mutágenos en el trabajo<sup>5</sup>.

Sin duda, uno de los principales motivos de la infradeclaración es la complejidad a la hora de establecer una relación de causalidad entre la exposición a los factores de riesgo laboral y el desarrollo de la neoplasia en una enfermedad multifactorial como el cáncer, donde intervienen tanto aspectos personales como medioambientales, hábitos de vida y exposiciones laborales.

El concepto de cáncer ocupacional es más amplio que el de cáncer como enfermedad profesional. No

todos los cánceres relacionados con el trabajo y con la presencia de agentes causales en el ambiente laboral son considerados enfermedad profesional. Existe asociación entre determinadas ocupaciones y la presencia de tipos de cáncer concretos, pero no todos los cánceres con evidencia de exposición laboral a agentes cancerígenos se encuentran incluidos dentro del listado de enfermedades profesionales<sup>6</sup> y pueden ser considerados como Enfermedad Profesional.

La aparición del cáncer en la población trabajadora debe llevar a averiguar sus posibles causas laborales para actuar en prevención, pero, al mismo tiempo, la detección de cualquier tipo de cáncer fuera del ámbito laboral debe inducir a incluir en la investigación un estudio exhaustivo de exposiciones laborales de riesgo, partiendo de la base de que una misma persona puede haber estado expuesta en su vida laboral a distintos compuestos y en sectores laborales diversos, lo que dificulta todavía más poder establecer una relación causa-efecto y, con ello, actuar en prevención.

La historia clínico laboral se convierte en un instrumento clave para aunar la información clínica con la ocupación u ocupaciones desarrolladas por el paciente-trabajador a lo largo de su vida laboral y encontrar las posibles relaciones con exposiciones en el lugar de trabajo que bien de



forma independiente o bien, como es más frecuente, asociadas a otros factores son causa determinante, desencadenante o agravante del proceso neoplásico.

Se trata de recabar información precisa, sistematizada y rigurosa que relacione tipos de cáncer con actividad laboral, riesgos laborales y factores personales y sociodemográficos, además de los aspectos propiamente clínicos, diagnósticos, histológicos y terapéuticos.

De este modo, todos los implicados en el cuidado del paciente con cáncer podremos empezar a tener datos que se aproximen a la realidad, que permitan trabajar

juntos a los sanitarios especialistas del ámbito laboral y extralaboral, mejorar las actuaciones preventivas y facilitar la reintegración social y laboral de la persona que ha sufrido un cáncer y vuelve al trabajo tras periodos de incapacidad, generalmente prolongada o muy prolongada.

Trabajemos juntos en los aspectos formativos que relacionan cáncer y trabajo, en los procedimientos de notificación de las neoplasias relacionadas con el trabajo y en esa historia clínico-laboral tan necesaria que será la puerta de entrada a una colaboración entre profesionales de distintas especialidades y que redundará en beneficio de todos.

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## ORIGINAL

# Diagnostic technology assessment in Iran: A grounded theory study

*Evaluación de la tecnología diagnóstica en Irán: Un estudio de teoría fundamentada*

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## Abstract

**Introduction:** Evaluation and selection of efficient medical equipment have always been among the concerns of health system managers.

**Methods:** The purpose of the present study was to present a model of diagnostic technology assessment in Iran based on a Grounded theory approach. The present qualitative Grounded Theory-based study was conducted in 2019. The participants in this study were medical equipment managers and health technology assessment experts working in university hospitals.

**Results:** The results showed that the Committee model is the most commonly used model of diagnostic technology assessment in Iran's university hospitals; it was also indicated that the organizational, economic, technological, clinical, and legal properties of diagnostic technologies are considered important in the assessment process.

**Conclusion:** Medicines, Treatment, and Medical Equipment Committees in Iran's hospitals can create an appropriate platform for diagnostic technology assessment, while the support and cooperation of managers play a decisive role in this process.

**Keywords:** Equipment and Supplies, Health, Ancillary Services Hospital.

## Resumen

**Introducción:** La evaluación y selección de equipos médicos eficientes siempre han estado entre las preocupaciones de los gestores del sistema de salud.

**Metodología:** El propósito del presente estudio fue presentar un modelo de evaluación de la tecnología de diagnóstico en Irán basado en un enfoque de la teoría fundamentada. El presente estudio cualitativo basado en la teoría fundamentada se llevó a cabo en 2019. Los participantes en este estudio fueron gerentes de equipos médicos y expertos en evaluación de tecnología de la salud que trabajan en hospitales universitarios.

**Resultados:** Los resultados mostraron que el modelo de Comité es el modelo más comúnmente utilizado de evaluación de tecnología de diagnóstico en los hospitales universitarios de Irán; también se indicó que las propiedades organizativas, económicas, tecnológicas, clínicas y legales de las tecnologías de diagnóstico se consideran importantes en el proceso de evaluación.

**Conclusiones:** Los comités de medicamentos, tratamientos y equipos médicos de los hospitales iraníes pueden crear una plataforma adecuada para la evaluación de las tecnologías de diagnóstico, mientras que el apoyo y la cooperación de los gestores desempeñan un papel decisivo en este proceso.

**Palabras clave:** Equipos y suministros, salud, Servicios técnicos en salud.

## Introduction

The diversity of the equipment market and the increase of demands and expectations of physicians and patients for the selection and application of new technologies have led to an unbridled increase in the use of new health technologies in Iran<sup>1</sup>. Over the last few years, the availability of a new generation of diagnostic and therapeutic medical devices has caused a significant increase in the use of such devices in Iranian hospitals, while hospitals' authorities are still looking forward to having more of these new devices in their hospitals<sup>2</sup>.

The results of studies in other countries show that hospitals and medical research centers spend about \$500,000,000 to \$1, 000,000,000 on medical equipment per year (plus 15-20% annual increase). It can be said that medical equipment plays the most important role in the increase in health care spending in different parts of the world<sup>3,4</sup>. In Iran, more than one-third of the total spending of various educational, medical, and health care centers is allocated to medical equipment<sup>5</sup>.

Therefore, managers are always faced with decision-making situations concerning the selection of the most appropriate technologies<sup>6</sup>. Due to the diversity of products and organizations' authority to select products based on their needs, identifying factors affecting the decision-making processes in a competitive environment and using scientific methods to select appropriate products and services are of particular importance in medical settings<sup>7</sup>.

Health Technology Assessment (HTA) has emerged mainly in response to the increasing health care costs and to ensure the efficiency of resources used in health systems<sup>8</sup>. Most studies on HTA have been conducted in high-income countries, and the HTA statuses in middle-income and low-income countries remain unattended<sup>9</sup>. Iran's current Gross Domestic Product (GDP) has been estimated at \$454.01 billion, making it a middle-income country based on the World Development Indicators (WDI)<sup>10</sup>.

In Iran, HTAs are performed at national level and there is no active HTA program at hospital level. Therefore, the present study was executed to identify the parameters considered in Iranian University Hospitals (UHs) when assessing diagnostic medical equipment. This study also presents a model of HTA in Iranian UHs based on the Grounded Theory. Considering the limited resources of the health system, the high cost of diagnostic medical equipment, and the effect of medical equipment on proper and timely treatment, the proposed model in this study can play an effective role in the optimal and nondiscriminatory selection of scarce medical resources and lead to improved service quality.

## Methodology

This qualitative study was conducted based on a Grounded Theory approach. The data were collected through unstructured interviews; the statistical population of this study consisted of medical equipment managers and HTA experts working in Iranian UHs; the data collection began in June 2019 and ended in August 2019. The demographic characteristics of the participants are presented in **table I**. Data saturation was reached after 15 interviews.

The participants were asked to describe the process of assessing diagnostic devices at hospitals where they were working. They were also asked to specify what they usually consider when assessing diagnostic medical devices? And what questions do they pose concerning those devices?

Data analysis was a regular and continuous process of data comparison, done in three stages of open coding, axial coding, and selective coding. In *open coding*, the transcribed version of the interviews was read several times, the main themes were extracted and recorded as codes; then, categories were developed by grouping similar codes. In *axial coding*, categories were linked to their subcategories to provide more accurate explanations of the phenomenon. Moreover, the primary categories, which were formed in the open coding stage, were compared and similar categories were placed on a common axis. Selective coding is the process of integrating and refining theme categorization; therefore, the present study's researcher harmonized and arranged different categories to present and form a theory. In the *selective coding* stage, while integrating and purifying the categories, it was decided to have a main theme; then, using the model paradigm, all themes were linked to the main theme. Throughout the study, methods were used to ensure the validity and reliability of the data. Four criteria have been proposed to ensure the trustworthiness of qualitative studies: credibility, dependability, confirmability, and transferability. Making long contacts with the participants, providing them with information about the project objectives to gain their trust throughout the interviews, continuously reviewing the data, audio recording each interview and transcribing it immediately after it finished, and using its feedbacks in subsequent interviews, the researchers tried to improve the study's credibility. Data dependability was tested by asking the participants and observers to review the transcribed interviews and express their corrective opinions. To test the transferability of the study, the study findings were sent to some medical equipment experts, who did not participate in the current study, to compare them with their own experiences.

**Table I:** Demographic Characteristics of the Participants.

Variables		Frequency	Percentage
Educational Level (Academic Degree)	Bachelor's Degree	3	20
	Masters' Degree	9	60
	Ph.D./Ph.D. Candidate	3	20
Organizational Status	Medical Equipment Manager	13	86.7
	Master of HTA	2	13.3
Gender	Female	9	60
	Male	6	40

**Table II:** Main categories and themes of diagnostic technology assessment in Iranian UHs.

Number	Main categories	Themes
1	Clinical Properties	Disease burden Alternative methods Attending to clinical standards Attending to the needs of specific groups of patients
2	Economic Properties	Primary cost Secondary cost Utilization of available resources Refund
3	Organizational Properties	Accreditation requirements Meeting the needs of special groups of patients in UHs (Education & Research Affairs) Accountability requirements
4	Technological Properties	Production feature Technical feature Setup requirement
5	Legal Properties	Medical Equipment Office Department of Laboratory Affairs Atomic Energy Organization Etc.
6	Assessment Model	Committee Model Mini-HTA Model no Model

## Results

The results showed that, in line with the Ministry of Health and Medical Education instructions, the Medicines, Treatment, and Medical Equipment Committees have been formed in all Iranian UHs. In other words, the technology assessment of medical devices in Iranian UHs is performed via the internal committee method. The results indicated that five main properties are considered when assessing health technologies in Iranian UHs (**Table II**).

### Step 1. Open Coding

#### Clinical properties

Clinical properties are determined based on the results of scientific studies and evidence for the effectiveness and safety of medical technologies. These properties also involve the impacts of technologies, such as their side effects and advantages in terms of safety and treatment. Moreover, meeting the needs of special patients, including those with viral infections such as AIDS, is considered here.

#### Economic properties

The economic value of the clinical effects of certain technology and its associated costs is determined

through various types of economic analyses, such as cost-minimization analysis, cost-benefit analysis, etc.

#### Organizational properties

Organizational properties are related to health institutions' operational processes and infrastructures, such as institutional expertise, educational features, types of relationships between work teams, workflow, potential barriers, and meeting accountability and accreditation requirements.

#### Technological Properties

Assessing technological properties includes examining a medical device in terms of mechanical and electrical safety, proper application, required accessories, equipment, space, maintenance, and storage.

#### Legal Properties

Legal properties include regulations (laws, resolutions & enactments) and all legal requirements before and after the application of a specific technology.

#### Assessment Model

The method each hospital uses to obtain information and assess a specific diagnostic technology.

## Step 2: Axial Coding

In the present study, five main categories of clinical properties, organizational properties, technological properties, economic properties, and legal properties were identified. Focusing on interviews, categories, themes, and reminders, “HTA characteristics in the domain of diagnostic medical equipment” was identified as the main variable in the selective coding step; this variable related all categories and themes to each other. A brief explanation of each of those categories and themes will be presented in the following sections

## Step 3: Description of the Theorizing Stage (Selective Coding)

As mentioned, the goal of fundamental theorizing is to produce a theory, not merely describing a phenomenon. To turn analyses into a theory, categories must be adequately related to each other.

### The Paradigmatic Model of Diagnostic Technology Assessment in Iran’s Public Hospitals

This theory presents mechanisms through which the target population (decision-makers in the domain of diagnostic medical equipment) identifies its needs and better assesses the diagnostic medical equipment; on the other hand, hospitals allocate their resources efficiently and nondiscriminatory. A general model is presented to develop and extend the theory of diagnostic technology assessment (within hospitals affiliated with medical sciences universities).

#### Description of the Paradigmatic Model

Hospitals are always faced with different kinds of requests from doctors, nurses, and other medical staff for applying diagnostic medical equipment and providing patients with better care. One of the most critical decision-making situations that managers always face is selecting the most appropriate technologies. Limited resources, organizational limitations, and the country’s policies in the health domain are among the factors involved in these decision-making situations. Thus, it would be appropriate to use methods that accurately and properly take into account the ambiguity of human judgment when deciding over such issues.

In the present study, the researchers sought to identify the most important factors in the assessment of diagnostic technologies in Iranian UHs. The results of this study led to the formulation of useful and practical ideas for the current condition of the country. Considering the findings of the present study, the following theory was developed:

When selecting a diagnostic technology, Medicines, Treatment, and Medical Equipment Committee is formed based on the requirements of hospitals’ accreditation rules, and the committee examines a technology from different dimensions and finally makes a decision.

One of the most important issues in such decisions is the hospital’s ability to provide “primary” and “secondary” costs of diagnostic technology. In many cases, when a hospital is not “economically” capable to pay the costs of a certain technology, other dimensions of the technology will not be assessed. “special needs patients”, “alternative diagnostic methods” and “clinical standards” are also considered in decision-making situations; in fact, clinical issues are of great importance in the decision making process. Each hospital’s grade is determined based on the Ministry of Health and Medical Education’s accreditation assessments, which has a direct impact on hospitals’ revenues. Therefore, medical equipment experts and hospital managers usually consider this when selecting diagnostic technologies. In addition to accreditation requirements, due to the accountability requirements, if regulatory institutions (e.g. the State Inspectorate Organization, municipalities, provincial governments, Ministry of Health and Medical Education, Medical Council of the Islamic Republic of Iran, Department of Environment, etc.) have expectations from the hospitals, they usually try to purchase the desired technologies. In other words, considering the “organizational properties” of a hospital is evident in every decision.

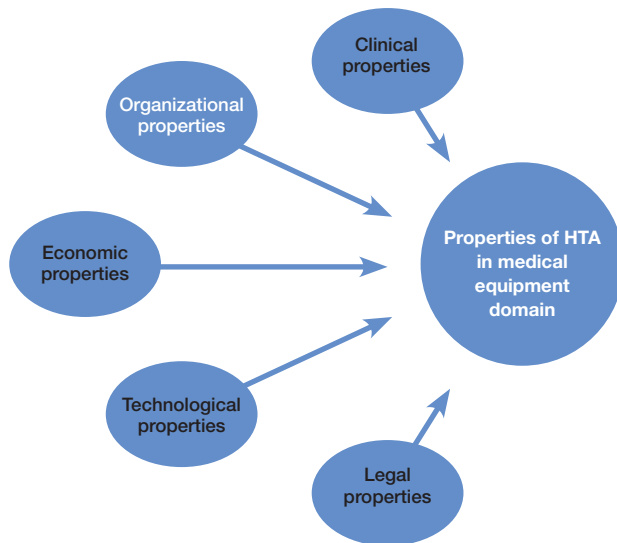
When deciding to purchase a medical device, its “technological properties” play vital roles in its “setting up”. The “quality”, organizations’ capacity to provide “after-sales-services” ... are of great importance in “brand selection”.

Furthermore, compliance with the current “regulations” in the domain of medical equipment was among the main features that were considered in the assessment of diagnostic technologies in Iranian UHs.

It can be said that the issues considered in HTA practices in Iranian UHs depend on various factors, the most important of which are “clinical”, “organizational”, “technological”, “economic” and “legal” properties. Taking into account all of these properties in decision-making situations will optimize hospitals’ budget allocations; it also helps provide doctors with the facilities they need to meet the needs of the community and minimize possible human misjudgments.

The following figure (**Figure 1**) is the basic conceptual model of HTA in Iranian UHs:



**Figure 1:** Basic conceptual model of HTA in Iranian UHs.

## Discussion

Hospital-based HTA consists of specific hospital needs<sup>11</sup>. This approach considers the local organizational conditions in which health technologies are used<sup>12</sup>. Studies have shown that many initiatives have emerged

in the area of hospital-based HTA in the world<sup>13,14,15</sup>. In 2008, the Health Technology Assessment International (HTAI) proposed four models of HTA at hospital level: 1) Ambassador Model; 2) Mini-HTA Model; 3) Internal Committee Model, and 4) HTA Unit Model<sup>16,17</sup>. The results of the present study showed that Iranian UHs use Internal Committee and Mini HTA models of HTA; moreover, it was indicated that clinical, organizational, economic, technological, and legal properties of medical devices are considered important factors in the assessment of diagnostic medical technologies in Iranian UHs.

Research shows that clinical and economic aspects of medical devices are considered very important in hospital-based HTA<sup>18</sup>, which is in line with the presets study's findings.

Besides, previous studies have reported that legal properties had been considered in only 23% of their examined hospital-based HTA documents<sup>19</sup>, while those properties were quite widespread in the context of Iranian UHs.

In line with the present study's findings, the technological properties of medical technologies have received considerable attention in previous studies, including the one conducted in France<sup>20</sup>.

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## ORIGINAL

# Efecto del estado inflamatorio perioperatorio en el salvamento de extremidad de los pacientes con isquemia crítica sometidos a revascularización infrainguinal

*Effect of perioperative inflammatory status on limb salvage in critically ischemic patients undergoing infrainguinal revascularization*

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**Resumen**

**Introducción:** Múltiples estudios han demostrado que la alteración de diversos marcadores inflamatorios se relaciona con el éxito tras la revascularización en pacientes con isquemia crónica.

El **objetivo** de este trabajo es evaluar la utilidad del índice plaqueta/linfocito (IPL) preoperatorio, índice neutrófilo/linfocito (INL) y de la Proteína C Reactiva-no ultrasensible (PCR) postoperatorios, como marcadores pronósticos de curación de lesión trófica y salvamento de extremidad en pacientes con isquemia crítica.

**Material y métodos:** Estudio retrospectivo, seguimiento de 24 meses. Se incluyen 261 pacientes con diagnóstico de isquemia crítica tratados mediante revascularización. Los índices analizados fueron obtenidos a partir de analítica sanguínea.

**Resultados:** La media de edad fue de 68 años. El 61% de los casos fueron tratados de forma endovascular. La tasa de supervivencia fue del 93,5% y la tasa de salvamento de extremidad fue del 85%. De los marcadores analizados un valor de PCR postoperatorio > 3,03 mg/dl se asoció con un incremento en el riesgo de amputación ( $p=0,024$ ). El INL > 3,2 ( $p=0,51$ ) y el IPL > 160 ( $p=0,6$ ) mostraron relación con la tasa de cicatrización y el salvamento de extremidad. La diabetes mellitus (OR 2,22 IC95%1,13-4,36;  $p=0,019$ ) con la amputación mayor.

**Conclusiones:** El estado inflamatorio perioperatorio se relaciona con el éxito de la revascularización. La PCR no ultrasensible se asocia con un incremento de la tasa de amputación. EL INL y el IPL son marcadores ampliamente disponibles que podrían estar relacionados como marcadores pronósticos, siendo necesario el desarrollo de estudios adicionales.

**Palabras clave:** Isquemia crítica, inflamación, salvamento de extremidad, revascularización, índice neutrófilo/linfocito, índice plaqueta/linfocito, proteína C reactiva.

**Abstract**

**Introduction:** multiple studies have shown that the alteration of several inflammatory markers is related to success after revascularization in patients with chronic ischemia.

**The aim** of this study is to evaluate the usefulness of preoperative platelet/lymphocyte index (PLI), neutrophil/lymphocyte index (NL) and postoperative non-ultrasensitive C-reactive protein (CRP) as prognostic markers of trophic lesion healing and limb salvage in patients with critical ischemia.

**Material and methods:** retrospective study, 24-month follow-up. We included 261 patients with a diagnosis of critical ischemia treated by revascularization. The indexes analyzed were obtained from blood tests.

**Results:** the mean age was 68 years. Sixty-one percent of the cases were treated endovascularly. The survival rate was 93.5% and the limb salvage rate was 85%. Of the markers analyzed a postoperative CRP value > 3.03 mg/dl was associated with an increased risk of amputation ( $p=0.024$ ). INL > 3.2 ( $p=0.51$ ) and IPL > 160 ( $p=0.6$ ) showed relationship with healing rate and limb salvage. Diabetes mellitus (OR 2.22 CI95%1.13-4.36;  $p=0.019$ ) with major amputation.

**Conclusion:** Perioperative inflammatory status is related to revascularization success. Non-ultrasensitive CRP is associated with increased amputation rate. INL and IPL are widely available markers that could be related as prognostic markers, being necessary the development of further studies.

**Keywords:** Critical ischemia, inflammation, limb salvage, revascularization, neutrophil/lymphocyte index, platelet/lymphocyte index, C-reactive protein.

## Introducción

La enfermedad arterial periférica (EAP) es una patología frecuente y generalmente diagnosticada de forma tardía<sup>1</sup>. Si el abordaje de la EAP no se realiza de manera oportuna la enfermedad progresa y una de las principales complicaciones asociadas es la isquemia crítica de la extremidad (ICE)<sup>2</sup>. La ICE es una entidad asociada con una elevada mortalidad y alto riesgo de amputación, que a pesar de las diversas técnicas desarrolladas en las últimas décadas se mantiene en tasas elevadas<sup>3</sup>.

La EAP se define como una enfermedad inflamatoria, en la que diversos mediadores celulares (monocitos/macrófagos) y humorales (Proteína C Reactiva (PCR), Interleucinas(IL)) intervienen en el proceso de formación y evolución de la placa ateromatosa<sup>4</sup>. Se han relacionado marcadores inflamatorios como la PCR ultrasensible o los niveles de IL-6 con el desarrollo de EAP y coronariopatía, determinando su uso como mecanismo pronóstico de evolución de la enfermedad arterial<sup>5-7</sup>.

Los leucocitos y en especial los neutrófilos juegan un rol importante en la aterogénesis y aterotrombosis. Estudios previos han sugerido que la PCR<sup>1,8-10</sup>, el INL<sup>4,11-16</sup>, el IPL<sup>5,8,17,18</sup>, y el índice linfocito monocito (ILM)<sup>19</sup> perioperatorios, se asocian con incremento en el riesgo de desarrollo de isquemia crítica, mortalidad cardiovascular, y pérdida de extremidad, en paciente con EAP o coronariopatía.

## Objetivos

Determinar si la PCR, el INL, el IPL y el ILM en la admisión o en las primeras horas del postoperatorio pueden ser considerados como marcadores pronósticos en pacientes con isquemia crítica sometidos a revascularización del sector infrainguinal.

## Materiales y Métodos

Se desarrolló un estudio retrospectivo en el que se incluyeron 261 pacientes sometidos a revascularización realizada tanto de manera endovascular como mediante cirugía abierta, a nivel de sector femoro-poplíteo o de troncos distales por isquemia crítica de miembros inferiores, admitidos entre enero del 2014 y diciembre del 2015.

El objetivo primario analizado fue la amputación (supervivencia de extremidad), como objetivos secundarios se analizaron la curación de lesiones tróficas y el control del dolor.

Los datos clínicos y demográficos así como los correspondientes al seguimiento se obtuvieron del registro médico de cada paciente. Comorbilidades como la hipertensión arterial y diabetes mellitus se documentaron, independientemente del tratamiento administrado. La insuficiencia renal crónica se definió como una filtración glomerular menor de 60 ml/min calculada en base a la fórmula CKD-EPI. Se documentó de forma independiente el uso de aspirina, clopidogrel o estatinas.

Para determinar los diferentes parámetros objeto del estudio se usaron los valores absolutos obtenidos de la analítica sanguínea realizada a cada paciente en las 24 horas previas y las 12 horas siguientes al procedimiento. El cálculo se efectuó mediante la división del valor absoluto de los neutrófilos entre el valor absoluto de los linfocitos para calcular el INL, el valor absoluto de las plaquetas entre el valor absoluto de los linfocitos para el IPL y el valor absoluto de los monocitos para el ILM.

Se determinó la tasa de salvamento de extremidad y de supervivencia mediante el uso de curvas de Kaplan Meier.

## Análisis estadístico

Las características clínicas de los sujetos de estudio fueron analizadas usando técnicas de estadística descriptiva. Los grupos fueron comparados usando test de chi-cuadrado para variables categóricas, test t de Student para variables continuas con distribución normal y test de U de Man Whitney para variables continuas con distribución no normal

El punto de corte óptimo de los valores de INL, IPL, ILM, PCR se determinó mediante la aplicación de curvas de características receptor-operador (curvas ROC) y la detección de interacción automática por Chi-cuadrado (CHAID), para determinar los posibles puntos de corte que podían discriminar entre amputación o no amputación.

Tras obtener los puntos de corte para cada una de las variables analizadas, se desarrollo un modelo de regresión logística binario mediante el cual se calcularon los odds ratio de cada factor, con sus correspondientes intervalos de confianza (IC) al 95% para los diferentes factores de riesgo. En todos los test se determinó un valor de  $p < 0,05$  como estadísticamente significativo. Todos los análisis estadísticos fueron realizados en el software R Studio 1.3.

## Resultados

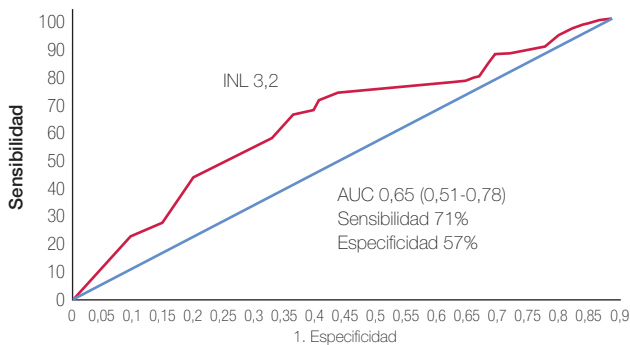
Un total de 261 pacientes fueron incluidos en el análisis. Las características de los pacientes se pueden observar en la **tabla I**.

**Tabla 1:** Datos demográficos de la población a estudio.

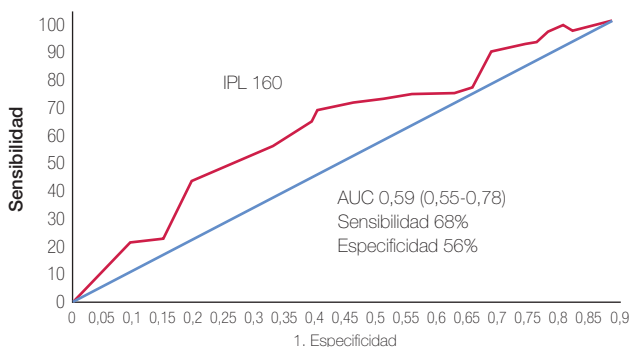
Edad media: años +/- desviación estándar	68,72 +/- 10,9
Sexo (masculino): n (%)	207 (79,3%)
Tabaquismo: n (%)	197 (75,5%)
Hipertensión arterial: n (%)	228 (87,4%)
Diabetes mellitus: n (%)	178 (68,2%)
Enfermedad renal crónica: n (%)	87 (33,3%)
Enfermedad coronaria: n (%)	102 (39,1%)
Enfermedad cerebro vascular: n (%)	32 (12,3%)
Dislipemia: n (%)	189 (72,4%)
Tipo de intervención quirúrgica	
Endovascular: n (%)	158 (60,5%)
Convencional: n (%)	103 (39,5%)

Los puntos de corte significativos valorados mediante la aplicación de la curva receptor-operador y el análisis CHAID de las diferentes razones se presentaron solo cuando la variable de resultado era la amputación, y fueron: 3,2 para el INL postoperatorio (área bajo la curva 0,65 IC 95% 0,54-0,78) (**Imagen 1**), de 160 para el IPL preoperatorio (área bajo la curva 0,59 IC 95% 0,55-0,78) (**Imagen 2**), y de 3,03 para la PCR postoperatoria (área bajo la curva 0,65 IC 95% 0,61-0,75) (**Imagen 3**).

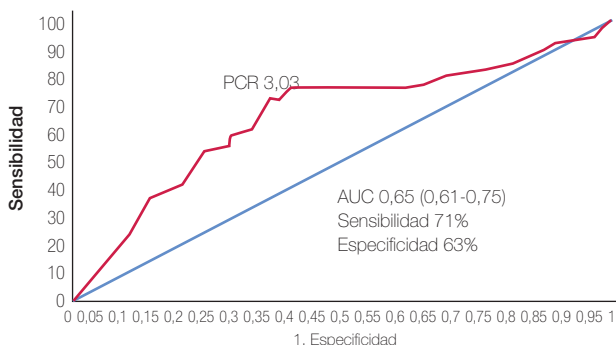
**Imagen 1:** Curva ROC INL.



**Imagen 2**



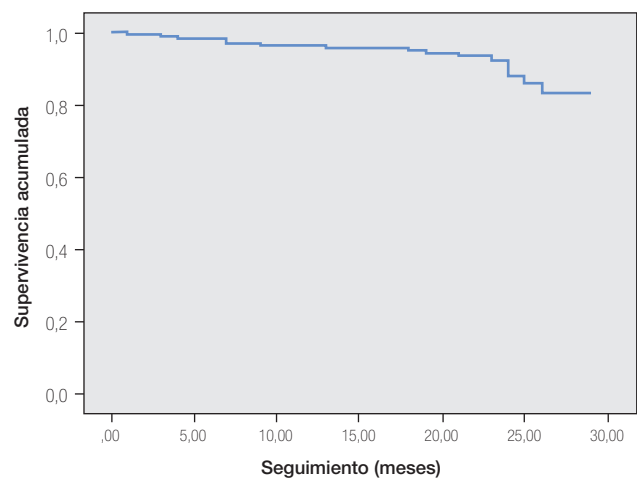
**Imagen 3**



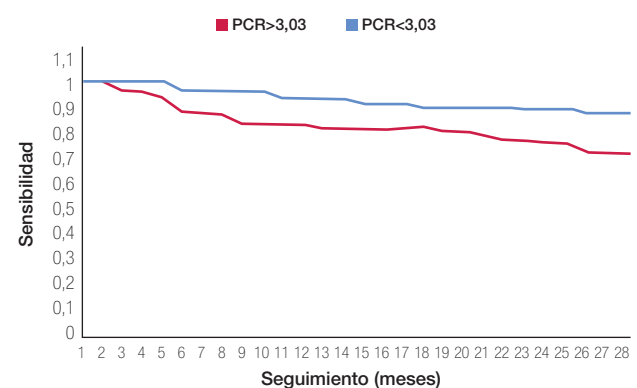
Con estos puntos de corte se realizó una regresión logística bivalente para evaluar los diversos marcadores como factores de riesgo independientes para amputación. Se incluyeron en este análisis las siguientes variables: el sexo, la diabetes, la edad > 75 años, la historia de ictus, la insuficiencia renal crónica, el tabaquismo, el tipo de intervención quirúrgica fueron incluidos. El valor de PCR postoperatoria > 3,03 mg/dl se asoció significativamente con la necesidad de amputación, OR 2,56 (IC95% 1,26-3,57 p 0,024). El INL con un OR 1.14 (IC95% 0,7-1,7 p 0,058) y el IPL con un OR 1,6 (IC95% 0,9-2,89 p 0,06) no se asociaron de manera significativa con la necesidad de amputación durante el seguimiento, aunque demostraron tendencia a actuar como factores pronósticos. De las covariables incluidas en el estudio la diabetes mellitus con un OR 2,22 (IC95% 1,13-4,36 p 0,019) se asocio con un menos salvamento de la extremidad.

Al desarrollar las curvas de supervivencias, la tasa de supervivencia global de la serie fue del 93,5% (**Imágenes 4 y 5**). Cuando se valoró la tasa de salvamento de la extremidad en base a los niveles de PCR, se encontraron diferencias significativas, teniendo aquellos pacientes con PCR mas elevada mayor riesgo de pérdida de extremidad.

**Imagen 4:** Curva de supervivencia global.



**Imagen 5:** Salvamento de Extremidad según valor de PCR.





## Discusión

En este estudio se concluye que un valor de PCR mayor de 3,03 mg/dl en la analítica postoperatoria se relaciona con un riesgo incrementado de amputación, esto ya que incluso tras ajustar para otros factores de riesgo conocidos como diabetes o consumo de tabaco, un valor de PCR mayor de 3,03 mg/dl se asocia con un incremento de 2,56 veces en el riesgo de amputación mayor. La diabetes mellitus también se presenta como un factor pronóstico de amputación tras revascularización. Otros marcadores analizados como el INL o el IPL también se mostraron una tendencia a actuar como factores asociados con el salvamento de la extremidad, aunque las relaciones no presentaron significación estadística.

La inflamación es un proceso fundamental en el desarrollo y evolución de la patología aterosclerótica, y los múltiples mediadores que intervienen en la misma pueden servir como marcadores pronósticos de la evolución postoperatoria de aquellos pacientes con ICE. De todos ellos en nuestro estudio hemos analizado la influencia de la PCR y diversos mediadores de la inmunidad celular valorados mediante los índices INL e IPL.

La PCR es un mediador inflamatorio de producción hepática y relacionado con el desarrollo de ICE<sup>20</sup>. Se ha demostrado que actúa como mediador en la acumulación de lípidos a nivel de placa por parte de los macrófagos mediante la activación de receptores *scavenger*<sup>21</sup>. Si bien la evidencia existente hasta el momento ha relacionado niveles elevados de PCR con la progresión de la EAP hacia ICE, no existen hasta donde conocemos estudios que la relacionen como factor pronóstico del salvamento de la extremidad tras revascularización a nivel de sector femoro-poplíteo y de troncos distales, tomando en cuenta la necesidad de intervenciones endovasculares o de cirugía abierta.

Los leucocitos en general juegan un rol importante en el desarrollo de lesiones ateroscleróticas, especialmente en la deposición lipídica en la placa, y las plaquetas se hallan en relación directa con el proceso inestabilidad y rotura de la placa<sup>3</sup>. El INL y el IPL se obtienen a partir de las concentraciones absolutas de neutrófilos, linfocitos y plaquetas todos estos valores fácilmente obtenidos mediante una analítica sanguínea.

Desde el punto de vista fisiopatológico los neutrófilos median la respuesta inflamatoria por mecanismos como la liberación de metabolitos del ácido araquidónico, mientras que la linfopenia reactiva refleja la producción de cortisol inducida como respuesta al estrés, y también por la activación de mecanismos de apoptosis a nivel de placa ateromatosa<sup>12</sup>. Los linfocitos también se han asociado con el desarrollo de circulación colateral por

medio de la IL-16 en modelos murinos<sup>16</sup>. Es por ello que la relación entre neutrófilos y linfocitos en una muestra de sangre periférica puede darnos una idea del estado proinflamatorio en los pacientes afectados de ICE, lo que sirve como un marcador indirecto de la severidad de la enfermedad aterosclerótica.

Además las plaquetas también se relacionan con el grado de respuesta inflamatoria sistémica y la presencia de trombocitosis se ha reportado como un marcador pronóstico adverso en pacientes con enfermedad arterial periférica<sup>17</sup>. La fase proinflamatoria asociada con la liberación de IL-1, IL-3 e IL 6 incrementa la cantidad de plaquetas circulantes<sup>16</sup>. Por lo tanto si tenemos en cuenta la linfopenia reactiva de los pacientes con aterosclerosis, la relación entre plaquetas y linfocitos puede ser de utilidad para la valoración del estado inflamatorio a nivel endoluminal y, como tal de la evolución de la enfermedad tras la aplicación de una medida terapéutica.

## Conclusión

Los resultados de este estudio señalan que el estado inflamatorio en el perioperatorio de los pacientes con isquemia crítica que son sometidos a revascularización puede ser un factor pronóstico de salvamento de extremidad. En nuestro estudio la PCR postoperatoria elevada > 3,03 mg/dl se asoció con una mayor frecuencia de amputación.

Otros marcadores del estado inflamatorio del paciente pueden ser de utilidad debido a que son fácilmente obtenidos a partir de la analítica sanguínea, y aunque no han presentado resultados estadísticamente significativos, se requieren estudios encaminados a analizar su rol como marcadores con mayor tamaño muestral o estratificados en base al tipo de intervención para dilucidar su posible utilidad.

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# Effect of sesame oil extraction by traditional and cold press methods on total aflatoxin content

*Efecto de la extracción de aceite de sésamo por métodos tradicionales y de prensado en frío sobre el contenido total de aflatoxinas*

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## Abstract

**Background:** Aflatoxin contamination is an undesirable issue in the oil industry. Methods of extraction have efficient effects on the aflatoxin contents of achieved oil.

**Aim:** The present survey was performed to assess the effects of traditional and cold press extraction methods on the aflatoxin contents of sesame oil.

**Methods:** A total of 17 crude sesame, 17 unfiltered oil, and 17 filtered oil samples were collected from different steps of oil production by cold press. Additionally, a total of 9 crude sesame, 9 unfiltered oil, and 9 filtered oil samples were collected from different steps of oil production by the traditional method. All samples were tested for aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>. Aflatoxin content was determined by high-performance liquid chromatography.

**Results:** Dehulling reduced total aflatoxin as 79.79%. Traditional and cold press procedures caused 8.2% and 70.22% transferring of total aflatoxin from crude sesame to achieved oil, respectively.

**Conclusion:** Traditional method was more effective than cold press in decreasing aflatoxin contents of achieved sesame oil.

**Keywords:** Sesame, oil extraction, traditional method, cold press, aflatoxin content.

## Resumen

**Antecedentes:** La contaminación por aflatoxinas es un problema indeseable en la industria del aceite. Los métodos de extracción tienen efectos eficaces sobre el contenido de aflatoxinas del aceite obtenido.

**Objetivo:** El presente estudio se realizó para evaluar los efectos de los métodos de extracción tradicionales y de prensado en frío sobre el contenido de aflatoxinas del aceite de sésamo.

**Metodología:** Se recogieron un total de 17 muestras de aceite crudo de sésamo, 17 de aceite sin filtrar y 17 de aceite filtrado, procedentes de diferentes etapas de la producción de aceite por prensado en frío. Además, se recogieron un total de 9 muestras de sésamo crudo, 9 de aceite sin filtrar y 9 de aceite filtrado de diferentes etapas de la producción de aceite por el método tradicional. Todas las muestras fueron analizadas para detectar aflatoxinas B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> y G<sub>2</sub>. El contenido de aflatoxinas se determinó mediante cromatografía líquida de alto rendimiento.

**Resultados:** El descascarillado redujo el total de aflatoxinas en un 79,79%. Los procedimientos tradicional y de prensado en frío provocaron una transferencia del 8,2% y del 70,22% del total de aflatoxinas del sésamo crudo al aceite obtenido, respectivamente.

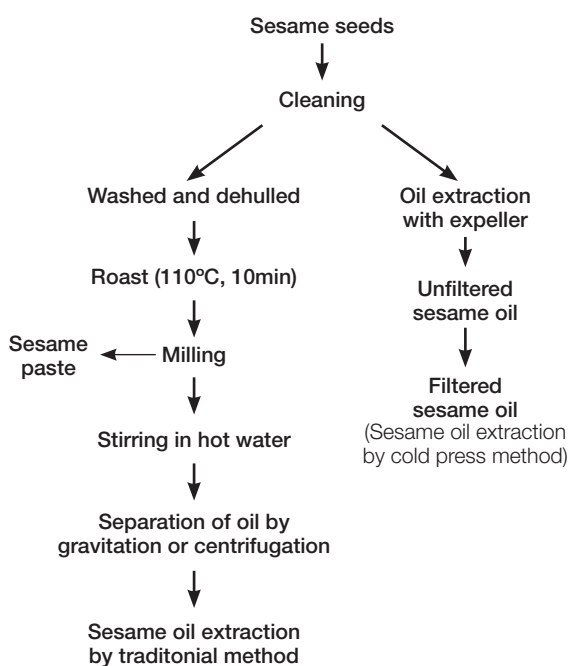
**Conclusiones:** El método tradicional fue más eficaz que el prensado en frío para reducir el contenido de aflatoxinas en el aceite de sésamo obtenido.

**Palabras clave:** Sésamo, extracción de aceite, método tradicional, prensado en frío, contenido de aflatoxinas.

## Introduction

Food security is one of the most critical challenges in the present century<sup>1-5</sup>. Edible oil is among the most commonly used food products playing a crucial factor in human health. Vegetable oils have an essential role in health status. Impurities and contamination of vegetable oils may vary based on the materials, weather, soil, harvesting, storage and processing. Also different oil extraction methods including organic solvent, water (traditional) and press effect on the transferring contamination from materials to the final product. Given consumers' concerns about chemicals used in food products, oil extraction by water and press has been much-paid attention. Sesame (*Sesamum indicum* L.) is an oilseed cultivated in tropical and temperate regions. Sesame oil is known as the queen of oilseeds due to its high-quality oil<sup>6</sup>. Sesame oil is one of the most widely used products as 4756000 t sesame oil was produced in 2013 worldwide<sup>7</sup>. This kind of oil is extracted by traditional, cold press, and solvent methods. Diagram of sesame oil exaction by traditional and cold press methods are shown in **figure 1**.

**Figure 1:** Diagram of sesame oil exaction by traditional and cold press methods.



Water flotation is a traditional method in Iran and Sudan for the extraction of sesame oil. Sesame seed is heated to 110°C for 10 min and milled. Enough boiling water is then added to suspend the ground seed on stirring. The mixture is boiled with stirring for 15 min. After cooling, the upper oil layer is separated off and dried by heating. The oil recovery from 0.5 kg seed is 108 ml, equivalent to an oil extraction efficiency of 41%<sup>8</sup>. Cold

press is a popular method of sesame oil extraction in Iran where no chemicals, solvent or additives are used, and it is produced in the presence of customers in some shops<sup>9</sup>. However, the product's safety is not assessed in Iran and aflatoxin is produced under improper storage conditions. Sesame oil contamination with aflatoxin has been reported in China, Sudan, and Senegal<sup>10</sup>.

Materials for edible oil production are often stored under such conditions for weeks that promote the growth of moulds producing mycotoxin aflatoxins such as *Aspergillus flavus*, *A. parasiticus* and *A. nomius* naturally occurring in foods. Among 18 known aflatoxins, B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> are classified in group A of carcinogenic factors by International Agency for Research on Cancer (IARC)<sup>11,12</sup>. Given the harmful effects of aflatoxins on public health, most studies on mycotoxins are presently concentrated on aflatoxin<sup>13</sup>. Furthermore, aflatoxin is continuously monitored in most products by health monitoring systems. Thus, the various national and international standard for the permitted limit of aflatoxin in food products has been developed<sup>14-17</sup>. Aflatoxin produced in oilseeds may be transferred to the final oil product. However, the concentration of these contaminants can be reduced through the processing method (extraction and purification). Traditional and cold press extraction are the two most widely used extraction techniques in Iran. This study aimed to examine the effect of different processes in sesame oil extraction using traditional and cold press methods on total aflatoxin content.

## Materials and methods

### Sampling

Sufficient amount of sesame seeds were collected from sesame oil extraction shops in Isfahan, Iran. Sesame oil was extracted by traditional method including washing, dehulling, roasting (110°C, 10 min) and oil extraction steps. The collected sesame seeds were processed by the traditional oil extraction method. In addition to 9 samples of raw sesame seeds, 9 samples were collected from each of the stages of washing, peeling, roasting and oil production. Moreover, 17 raw sesame seeds samples were collected from 17 cold press extraction shops in Isfahan, Iran. First, aflatoxin content in the raw seeds was determined, then the aflatoxin levels of the extracted oil samples were measured, and finally aflatoxin content of the filtered oil was determined. For a complementary assessment, 19 filtered oil samples obtained by the cold press extraction were collected, the aflatoxin content was determined, and the results were reported.

The amounts of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in the samples were measured according to Iranian national standard No. 6872 (ISIRI, 2004). This analysis included three steps of purification, detection and determination. The toxins were purified by using immunoaffinity columns.

They were detected by Kobra cell and determined by use of fluorescence detector at 365 nm excitation wavelength and 435nm emission wavelength. The procedure was validated via spiking with different levels of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> and then efficacy parameters such as linearity, accuracy, precision and limit of detection (LOD) were measured. The validity of method was confirmed and it was used for our purpose.

### Calibration and standard curve drawing

To draw the calibration curve, 1 mg of each aflatoxin pure powder was dissolved in 5 ml toluene: acetonitrile (90:10) and a standard solution (200 µg/ml) was prepared. After dilution, four standard solutions were obtained from the secondary standard solution and injected into High-Performance Liquid Chromatography (HPLC). To prepare mobile phase solvent, water: acetonitrile: methanol (6:2:3) were mixed, and 300 µL 4M nitric acid and 120 mg potassium bromide were added per L mobile phase. The samples were extracted with 200 mL 80% methanol and 5 g salt. The mixture was filtered and added to 130 mL water to obtain 150 mL diluted extract.

### Aflatoxin measurement

The samples were purified using immunoaffinity columns (IAC) with antibodies against aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> and a vacuum Manifold system. The first 70 mL of the solution passed through the column. Afterwards, the column was washed with double distilled water. The column was dried, and aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> were removed with 1500 µL distilled water as 100 ML were injected into HPLC. To examine the accuracy and precision of the applied method, a control sample was spiked with aflatoxin B<sub>1</sub> and was analyzed with the test samples. A calibration curve was drawn every day using 4 standards, and then the samples were injected, and aflatoxin content was determined. Principles of the previous survey were applied<sup>19</sup>.

### Recovery

To calculate the recovery, a certain amount of standard solution of aflatoxin was added to control the contaminated sample then was injected into HPLC and aflatoxin content was calculated by the following relation:

$$R = (C_{\text{Sample spike}} - C_{\text{Sample}}) * 100 / C_{\text{Spike}}$$

Where R is recovery value, and C sample spike is aflatoxin concentration in the contaminated sample.

### LOD and LOQ

The detection limit (LOD) and limit of quantitation (LOQ) were used to calculate the method's sensitivity. LOD is the lowest concentration in a detectable matrix but cannot be measured precisely, and LOQ is the lowest concentration that is detectable and measurable precisely. These parameters were calculated by using the calibration curve.

To do so, calibration gradient (a) and standard deviation of independent variable regression (Sb) were put in  $LOD = 3 * Sb/a$  equation and  $LOQ = 10 * Sb/a$  and LOD and LOQ were calculated.

### Statistical analysis

Data were analyzed by SPSS software version 20 (Chicago, IL, version, SPSS Inc). Mean, and standard deviation was presented in frequency distribution tables. Total aflatoxin mean was compared to International and national standard by one-sample t-test<sup>20-22</sup>. Aflatoxin mean in different steps of oil extraction was compared by Wilcoxon signed ranks test.

## Results

### Standard curve

Aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> were analyzed by four standard solutions at 1, 3, 5 and 7 ppm. An example of chromatograms is shown in figure 2. As illustrated in the figure, the peak is in a proper form with a retention time of about 4 min.

Figure 2: Chromatogram using 1ppm by HPLC.

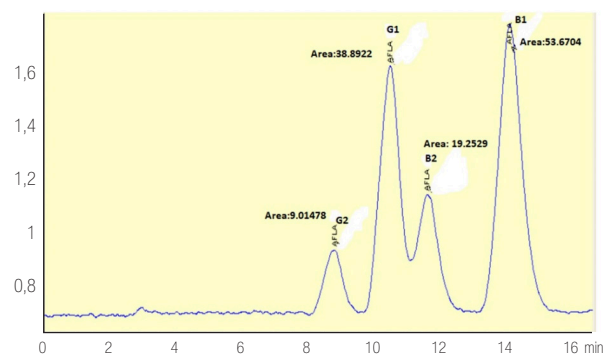
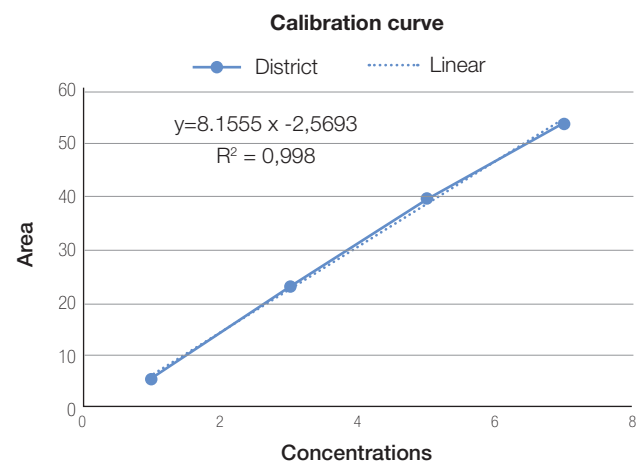


Figure 3: Calibration curve of Aflatoxin B.





### Calibration curve

The calibration curve of aflatoxin is shown in **figure 3**.

The calibration curve results revealed a linear relationship between different concentrations of aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> and area under the curve with the correlation coefficient (*r*) of all calibration curves being > 0.998.

### Results of recovery

Results of validation by spiking sesame and its oil with aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> were studied. The amounts of spiking of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in examined samples were 1, 1, 1, and 1 ppb, respectively. The amounts of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in examined samples were 133, 1.099, 1.155, and 1.127 ppb, respectively. Additionally, the recovery rates of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in examined samples were 133%, 109.9%, 155.5% and 112.7%, respectively.

### Results of LOD and LOQ

The lowest LOQ for aflatoxins B<sub>1</sub> and G<sub>1</sub> was <0.5, and aflatoxins B<sub>2</sub> and G<sub>2</sub> were <0/1. The lowest LOD for B<sub>1</sub> and G<sub>1</sub> was <0/16, and B<sub>2</sub> and G<sub>2</sub> was <0.03, being the lowest LOD.

### Results of aflatoxin measurement in oil extracted by cold press

Minimum, maximum and mean contents of four aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> are displayed in **table I**. In the present study, 25 samples showed 69.4% aflatoxin contamination with the mean value being 1.337± 0.329 ppb. Total aflatoxin content in all samples ranges < LOQ to 3.1 ppb. Comparison of the result to Iranian national standard for

total aflatoxin (15 ppb) suggest that it is lower than the specified amount. Mean aflatoxin B<sub>1</sub> contamination was 0.437±0.33. Aflatoxin B<sub>1</sub> contamination was found in 27.8% of the samples. Aflatoxin B<sub>2</sub> contamination in the samples was 0.106±0.033 ppb. There was no aflatoxin G<sub>1</sub> and G<sub>2</sub> in the samples. In the present study, total aflatoxin, B<sub>1</sub> and B<sub>2</sub> contents were 1.337±0.329 ppb, 0.473± 0.426 ppb and 0.33±0.106, respectively. G<sub>1</sub> and G<sub>2</sub> content was zero. Only one sample showed B<sub>1</sub> content higher than the amount permitted by EU (2 ppb).

### Effect of cold press method on aflatoxin content in the extracted sesame oil

Maximum, minimum, mean and standard deviation of four aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> in different steps of oil extraction by cold press are presented in **table II**. The mean of total aflatoxin in crude sesame, extracted oil and filtered oil was 1.88±1.15 ppb, 1.32±0.32 ppb and 1.21± 0.032 ppb, respectively. Mean aflatoxin B<sub>1</sub> contents in crude sesame, oil, and filtered oil were 1.31±0.865 ppb, 0.421±0.34 ppb, and 0.192±0.23 ppb, respectively. Mean aflatoxin B<sub>2</sub> in crude sesame and oil was 0.224±0.31 ppb and 0.107±0.032 ppb, respectively. Aflatoxin B<sub>2</sub> in filtered oil was < LOQ. No aflatoxins G<sub>1</sub> and G<sub>2</sub> were found in the samples. Total aflatoxin, B<sub>1</sub> and B<sub>2</sub> in oil samples extracted by cold press were 63.5, 48.68, and 12.06, respectively. These toxins showed 8.33, 54.39 and 10% significant decrease in filtered oil, respectively. As shown in the table, extraction steps and filtration reduced total aflatoxin, B<sub>1</sub> and B<sub>2</sub>, as cold press method resulted in 35.63%, 77.80% and 59.83% decrease in total aflatoxin, B<sub>1</sub> and B<sub>2</sub>.

**Table I:** Max., Min., Mean, and SD of four aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in sesame oil produced by cold press.

Aflatoxin type	Min	Max	Mean±SD	Contaminated oil		Non-contaminated oils	
B <sub>1</sub>	< LOQ*	2.3	0.473±0.426	69.4	25	30.6	11
B <sub>2</sub>	< LOQ	0.2	0.106±0.033	27.8	10	72.2	26
G <sub>1</sub>	< LOQ	< LOQ	< LOQ	0	0	0	0
G <sub>2</sub>	< LOQ	< LOQ	< LOQ	0	0	0	0
Total aflatoxin	< LOQ	3.1	1.337±0.329	69.4	25	30.6	11

\*LOQ: limit of quantitation (B<sub>1</sub>, G<sub>1</sub>= 0.5 ppb B<sub>2</sub>, G<sub>2</sub>= 0.1 ppb)

**Table II:** Max., Min., Mean, and SD of four aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in different steps of oil production by cold press.

Sample Type	Sample number	Aflatoxin type	Min	Max	Mean±SD
Crude Sesame	17	B <sub>1</sub>	< LOQ*	4.57	0.865±1.31
		B <sub>2</sub>	< LOQ	1.35	0.224±0.31
		G <sub>1</sub>	< LOQ	< LOQ	< LOQ
		G <sub>2</sub>	< LOQ	< LOQ	< LOQ
		Total	<1/2	5.67	1.88±1.15
Unfiltered Oil	17	B <sub>1</sub>	< LOQ	1.04	0.421±0.34
		B <sub>2</sub>	< LOQ	0.19	0.107±0.032
		G <sub>1</sub>	< LOQ	< LOQ	< LOQ
		G <sub>2</sub>	< LOQ	< LOQ	< LOQ
		Total	<1/2	1.75	1.32±0.15
Filtered Oil	17	B <sub>1</sub>	< LOQ	0.63	0.192±0.23
		B <sub>2</sub>	< LOQ	< LOQ	< LOQ
		G <sub>1</sub>	< LOQ	< LOQ	< LOQ
		G <sub>2</sub>	< LOQ	< LOQ	< LOQ
		Total	<1/2	1.33	1.21±0.032

\*LOQ: Limit Of Quantitation (B<sub>1</sub>, G<sub>1</sub>= 0.5 ppb B<sub>2</sub>, G<sub>2</sub>= 0.1 ppb).

### Effect of traditional water method on aflatoxin content in the extracted sesame oil

Maximum, minimum, mean and standard deviation of four aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in different steps of the oil extraction by traditional method are presented in **table III**. Mean total aflatoxin in crude sesame, dehulled sesame, roasted sesame, and oil was 17.46±13.35, 3.53±2.17, 2.86±1.33 and 1.43±0.628 ppb, respectively. Mean aflatoxin B<sub>1</sub> in crude sesame, dehulled sesame, roasted sesame and oil was 16.1±12.946, 2.71±2.04, 2.07±1.26 and 0.36±0.74 ppb, respectively, and mean aflatoxin B<sub>2</sub> was 0.77±0.605, 0.21±0.135, 0.18±0.09 and 0.11±0.48 ppb, respectively. No G<sub>1</sub> and G<sub>2</sub> were found in the samples. Dehulling resulted in 79.79%, 86.95% and 72.72% in total aflatoxin, B<sub>1</sub> and B<sub>2</sub>, respectively. However roasting at 110°C had no significant (p>0.05) effect on aflatoxin decrease. Traditional (water) method resulted in 91.80%, 97.76%, and 85.71% decrease in total aflatoxin, B<sub>1</sub> and B<sub>2</sub>, respectively. Comparative results of total aflatoxin, B<sub>1</sub> and B<sub>2</sub> measurement in different steps of oil extraction by traditional method.

### Effect of cold press and traditional method on aflatoxin content in the extracted sesame oil

The method of edible oil extraction may affect aflatoxin decrease depending on the type of oil, extraction and purification methods. There were no studies on the effect of the sesame oil extraction process, so studies on similar oilseeds were used. Filtration resulted in 8.33, 54.39 and 100% decrease in total aflatoxin, B<sub>1</sub> and B<sub>2</sub> in sesame oil produced by the cold press method. Cold press and filtration decreased total aflatoxin, B<sub>1</sub> and B<sub>2</sub>.

## Discussion

Aflatoxin contamination is an undesirable issue in the oil industry. Consumption of oil contained diverse aflatoxin

levels caused severe health-related complications, including cancer, neoplasia, mutations, hepatic toxicities, renal failure, abortion and even fetal malformation<sup>23</sup>. Thus, it is essential to control the aflatoxin content of edible oils. The oil extraction stage from seeds is one of the most significant steps in controlling aflatoxin in achieved oils<sup>10</sup>. Several reports disclosed that the oil extraction method caused significant effects on the aflatoxin contents of oils<sup>10,24</sup>.

The present survey was performed to assess sesame oil extraction by traditional and cold press methods on total aflatoxin contents of achieved oil. The present research findings disclosed that the traditional method of sesame oil extraction was more effective in reducing aflatoxin contents than the cold-press technique. One of the main reasons for the high aflatoxin contamination in sesame oil samples may be attributed to unhygienic conditions of the sesame oil extraction in producing units. Thus, good hygiene, safety and supervision in the sesame oil-producing units may be crucial to decrease the aflatoxin content of sesame oil. Additionally, using high-quality sesame is another way to decrease the risk of aflatoxin residues in achieved oil. Aflatoxin content found in the present investigation was within the range permitted by the Iranian national standard. However, regarding the daily consumption of contaminated sesame oil, this aflatoxin content may have some health threats to the consumers. In a study conducted in Sudan, aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> contents were measured, and the amount of G<sub>1</sub> and G<sub>2</sub> at present study were inconsistent with their findings, with our found amounts being lower<sup>25</sup>. A study on aflatoxin content in edible oils (sesame, peanut, and cottonseed) using HPLC was found that aflatoxin content in all samples was within the permitted limit<sup>26</sup>. In contrast to our results, no aflatoxin B<sub>2</sub> was found in samples. Elzupir et al. (2010)<sup>27</sup> determined aflatoxin content in edible oils (unpurified sesame oil, cottonseed oil and peanut oil) by HPLC. In

**Table III:** Max., Min., Mean, and SD of four aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in different steps of oil production by the traditional method.

Sample Type	Sample number	Aflatoxin type	Min (ppb)	Max (ppb)	Mean±SD (ppb)
Crude Sesame	9	B <sub>1</sub>	1.8	40.5	16.1±12.94
		B <sub>2</sub>	0.1	1.9	0.77±0.605
		G <sub>1</sub>	< LOQ*	< LOQ	< LOQ
		G <sub>2</sub>	< LOQ	< LOQ	< LOQ
		Total	2.4	41.9	17.46±13.35
Washed and dehulled sesame	9	B <sub>1</sub>	0.65	6.4	2.71±2.04
		B <sub>2</sub>	< LOQ	0.5	0.21±0.135
		G <sub>1</sub>	< LOQ	< LOQ	< LOQ
		G <sub>2</sub>	< LOQ	< LOQ	< LOQ
		Total	1.38	7.3	3.53±2.17
Roasted sesame	9	B <sub>1</sub>	0.5	4	2.07±1.26
		B <sub>2</sub>	< LOQ	0.4	0.18±0.09
		G <sub>1</sub>	< LOQ	< LOQ	< LOQ
		G <sub>2</sub>	< LOQ	< LOQ	< LOQ
		Total	1.2	4.8	2.86±1.33
Oil	9	B <sub>1</sub>	< LOQ	2.3	0.36±0.74
		B <sub>2</sub>	< LOQ	0.2	0.11±0.048
		G <sub>1</sub>	< LOQ	< LOQ	< LOQ
		G <sub>2</sub>	< LOQ	< LOQ	< LOQ
		Total	1.2	3.1	1.43±0.628

\*LOQ: Limit Of Quantitation (B<sub>1</sub>, G<sub>1</sub>= 0.5 ppb B<sub>2</sub>, G<sub>2</sub>= 0.1 ppb)

contrast to our results, total aflatoxin in all sesame oil was above the level specified by FDA (20 µg/kg). In their study, aflatoxins G<sub>1</sub> and G<sub>2</sub> were found in all samples, while in our study, no G<sub>1</sub> and G<sub>2</sub> were observed in the samples<sup>25</sup>. Asadi et al. (2011)<sup>28</sup> measured aflatoxin content in 182 sesame samples in Iran. 18/1% of the samples showed B<sub>1</sub> contamination. Also, 8 samples had aflatoxin B<sub>2</sub>, and one sample had G<sub>1</sub> and G<sub>2</sub>. Abalaka (1984)<sup>29</sup> studied aflatoxins in purified and unpurified peanut oil and found that purified oil had no aflatoxins.

A study on aflatoxin content in peanut was conducted in India, and it was found that the cold-press method resulted in 85% decrease in total aflatoxin<sup>10</sup>. Our results are not consistent with the results of the study mentioned above. In the present study, washing and dehulling decreased total aflatoxin, B<sub>1</sub> and B<sub>2</sub> significantly, while roasting at 90-100°C had no significant effect on aflatoxin. Aflatoxin is resistant to heating as it is degraded at 237-306°C<sup>30</sup>. Jalili (2016)<sup>30</sup> studied the effect of roasting at different temperatures on aflatoxin content in peanut and concluded that roasting at 150°C resulted in 60.8-79 decrease in B<sub>1</sub> and G<sub>1</sub> and roasting at 140°C resulted in 5.8-64.58 decrease in B<sub>1</sub> and G<sub>1</sub> and roasting at 100°C for 30°C did not affect aflatoxin decrease. Our finding was consistent with their results. Arzandeh and Jinap (2011)<sup>31</sup> examined the effect of roasting on aflatoxin content in peanut. The results showed that roasting at 90-150°C resulted in 78.4, 57.3, 73.9 and 25.2% decrease in B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>. In another study, Hussain et al. (2011)<sup>32</sup> studied peanut roasting at 150°C

for 120 min and observed a noticeable decrease (95%) in aflatoxin content. Our results are not in agreement with their finding. The roasting procedure also had significant effects on the aflatoxin degradation among the Nigerian peanut seeds<sup>33</sup>. One of the limitation of our study was the lack of similar national or international studies on sesame oil extraction by water (traditional) and cold press.

## Conclusion

In the present study, the traditional method was more effective than the cold press in decreasing aflatoxin. One of the main reasons for high aflatoxin contamination in sesame oil samples is inappropriate materials storage conditions in sesame oil extraction. Thus proper hygiene, safety and supervision are of great importance. In the present study, aflatoxin content was within the Iranian national standard range; however, high contamination in the samples may pose severe threats to human health.

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# Values of different index related to cardiovascular risk according the Findrisk test scores in caucasian

*Valores de diferentes índices relacionados con riesgo cardiovascular según las puntuaciones del test Findrisk en caucásicos*

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## Abstract

**Introduction:** Diabetes is a very prevalent disease and therefore it is very important to have tools to assess the risk of suffering from it.

**Methods:** A descriptive and cross-sectional study was carried out on 59,042 workers from different productive sectors. The relationship between different scales of overweight, obesity and cardiovascular risk such as waist circumference, waist to height ratio, body shape index, visceral adiposity index, conicity index, hypertriglyceridemic waist and Cholindex with the Findrisk type 2 diabetes risk scale was evaluated.

**Results:** A worsening in the mean values of the different scales analyzed is observed as the value of the Findrisk test increases in both sexes. An increase in the prevalence of altered values of these same scales is also observed as the values of the Findrisk test increase and equally in both sexes.

**Conclusion:** As expected, the Findrisk test is directly related to the different scales of overweight, obesity and cardiovascular risk analyzed.

**Keywords:** Cardiovascular diseases, diabetes, obesity, abdominal obesity, Finrisk test.

## Resumen

**Antecedentes:** La Diabetes es una enfermedad prevalente y por lo mismo es muy importante tener las herramientas para evaluar el riesgo de sufrirla.

**Material y métodos:** Se realizó un estudio descriptivo y transversal en 59,042 trabajadores de diferentes sectores productivos. Se evaluó la relación entre diferentes escalas de sobrepeso, obesidad y riesgo cardiovascular como circunferencia de la cintura, índice cintura/altura, índice de forma corporal, índice de adiposidad visceral, índice de conicidad, cintura hipertrigliceridémica y cholindex con la escala findrisk de riesgo de diabetes tipo 2.

**Resultados:** Se observó un empeoramiento de los valores medios en las diferentes escalas analizadas cuando los valores del test findrisk aumentaban en ambos sexos. Se observó también un incremento en la prevalencia de valores alterados en estas mismas escalas cuando los valores del Findrisk test se incrementaban, igualmente en ambos sexos.

**Conclusiones:** El test Finrisk se relaciona directamente con las escalas de sobrepeso, obesidad y riesgo cardiovascular analizadas.

**Palabras clave:** Enfermedades cardiovasculares, diabetes mellitus, obesidad, obesidad abdominal, test de Finrisk.



## Introduction

Cardiovascular diseases (CVD) cause great morbidity and mortality both in the developed and undeveloped countries. In recent years 80 percent of deaths from CVD have occurred in countries with medium or low income, and the number is growing<sup>1</sup>. The cardiovascular risk (CVR) is defined as the likelihood of an event in a given period, usually 10 years, for its determination generally scales are based on cohort studies are used. Determining the CVR it is based on clinical guidelines that address cardiovascular prevention.

In the occurrence of CVD is influenced by different factors such as tobacco consumption, obesity, dyslipidemia and diabetes. The risk of diabetes can be determined with different scales among which we highlight the FINDRISC (FINnish Diabetes Risk Score) questionnaire for being perhaps the most widely used. FINDRISC has been successfully implemented as a practical screening instrument to assess diabetes risk and to detect undiagnosed type 2 diabetes in European populations<sup>2-4</sup>. However it has also become evident that it is not universally applicable among all ethnic groups and populations<sup>5,6</sup>.

There are many indexes that help predict CVD from classic Body Mass Index (BMI), waist circumference and waist to height ratio to the most recent Body Adiposity Index (BAI)<sup>7,8</sup>. There are other indices that could perhaps be useful in predicting these CVD among which are the Body Shape Index (ABSI) at some authors they have linked to an increased risk of cardiovascular mortality<sup>9</sup>, the Visceral Adiposity Index (VAI) which has been linked with visceral fat levels<sup>10</sup>, type 2 diabetes<sup>11</sup> and coronary artery disease<sup>12</sup>, Cholindex which has been linked with coronary artery disease<sup>13</sup>, the Conicity Index (CI) which has been linked with high coronary risk<sup>14</sup> and Hypertriglyceridemic waist (HTGW) has been associated with type 2 diabetes<sup>15</sup>, coronary artery disease<sup>16</sup> and even acute myocardial infarction<sup>17</sup>.

An analysis of the scientific literature shows that previous indexes have not been used too much in cardiovascular prevention but perhaps can provide valuable information on the assessment of CVR.

For all these reasons, and trying to improve cardiovascular prevention, this study presents the main objective is to determine what relationship exists between FINDRISC test values and the values of these indices.

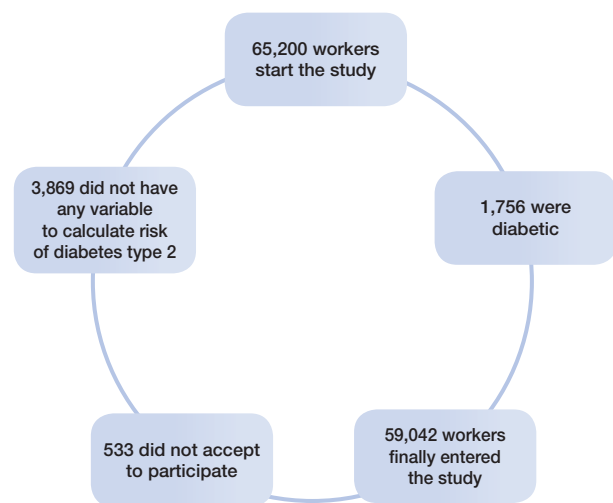
## Materials and Methods

### Subjects and Study Protocol

A cross-sectional study with Caucasians adult workers was performed. All subjects were from different productive sectors. Participants in the study were systematic selected

during their work health periodic examination between January and December 2019. Every day each worker was assigned a number and half of the examined workers were randomly selected using a random number table. Thus, from a total population of 130,487 workers, 65,200 of them were invited to participate in the study. 3,869 They do not have any parameter necessary to calculate any of the scales. 533 refused to participate and 1,756 they are excluded to be diabetic and not being able to perform the FINDRISC test, being the final number of participants 59,042 (see flowchart in **figure 1**), with 25,510 women (43.2%) and 33,532 men (56.8%). The mean of age of participants in the study was 39.7 years (SD±10.30).

Figure 1: Flowchart of participants.



The following inclusion criteria were considered: age between 18 and 70 (working age population), no diabetic, agreement to participate in the study and to be gainfully employed. Subjects who did not meet any of the inclusion criteria and those who refused to participate were excluded from the study.

### Measurements and Calculations

All anthropometric measurements were made in the morning, after an overnight fast, at the same time (9 a.m.), and according to the recommendations of the International Standards for Anthropometric Assessment (ISAK)<sup>18</sup>. Furthermore, all measurements were performed by well trained technicians or researchers to minimize coefficients of variation. Each measurement was made three times and the average value was calculated. Weight and height were determined according to recommended techniques mentioned above. Body weight was measured to the nearest 0.1 kg using an electronic scale (Seca 700 scale, Secagmbh, Hamburg). Height was measured to the nearest 0.5 cm using a stadiometer (Seca 220 (CM) Telescopic Height Rod for Column Scales, Secagmbh, Hamburg). BMI was calculated as weight (kg) divided by height (m) squared (kg/m<sup>2</sup>). Criteria

to define overweight were the ones of the World Health Organization (WHO)<sup>19</sup> which considers obesity when BMI  $\geq 30$  kg/m<sup>2</sup>. Abdominal waist was measured using a flexible steel tape (Lufkin Executive Thinline W 606). The plane of the tape was perpendicular to the long axis of the body and parallel to the floor. Waist circumference was measured at the level of the umbilicus and superior iliac crest. The measurement was made at the end of a normal expiration while the subject stood upright, with feet together and arms hanging freely at the sides. Waist circumference (WC) was measured using a tapeline at the level midway between the lateral lower rib margin and iliac crest. Waist-to-height ratio (WtHR) was calculated by dividing WC by height in cm.

Venous blood samples were taken from the antecubital vein with suitable vacutainers without anticoagulant to obtain serum. Blood samples were taken following a 12 h overnight fast. Participants were seated at rest for at least 15 minutes before blood samples were taken. Serum was obtained after centrifugation (15 min, 1,000 g, 4°C) of blood samples. Serum was stored at -20°C and analysis were performed within 3 days. Concentrations of glucose, cholesterol and triglycerides were measured in serum by standard procedures used in clinical biochemistry laboratory using a clinical system Beckman Coulter SYNCHRON CX@9 PRO (Beckman Coulter, Brea, CA, USA).

Blood pressure was determined after a resting period of 10 minutes in the supine position using an automatic and calibrated sphygmomanometer OMRON M3 (OMRON Healthcare Europe, Spain). As indicated for the anthropometrical measures, blood pressure was measured three times with a one-minute gap between each measurement and an average value was calculated.

FINDRISC questionnaire value 8 items: age, BMI, waist circumference, physical activity, dietary consumption of fruits, vegetables, and berries, Use of antihypertensive medication, previously measured high blood glucose and family history of diabetes. The maximum achievable score is 26. Less than 7 points is considered low risk, 7-11 point slightly elevated risk, 11-14 points moderate risk, 15-20 points high risk and 21-26 points very high risk.

Real Body shape index (ABSI)<sup>9</sup> was calculated using the equation:

- Waist circumference (cm)/BMI<sup>2/3</sup> weight<sup>1/2</sup> (kg)

Theoretical ABSI is set based on sex and age. The ratio between real and theoretical ABSI is called ABSI relative risk (ABSI RR). ABSI RR <1 is considered abnormal.

Conicity index(CI)<sup>14</sup> was calculated using the equation:

- Waist circumference (m)/ (0,109  $\sqrt{\text{weight (kg)}}$ /height (m))

The cut-off to consider high CI were 1.18 for women and 1.25 for men.

Visceral Adiposity Index (VAI)<sup>20</sup> was calculated using the equations:

Women (Waist circumference /((39.68+(1.89 BMI)) x (triglycerides/1.03) x (1.31/HDL-C)

Men (Waist circumference /((36.58+ (1.89 BMI)) x (triglycerides /0.81) x (1.52/HDL-C)

The cut-off to consider optimal VAI were < 30 years ( $\geq 2.52$ ) 30-42 years ( $\geq 2.23$ ) 43-51 years ( $\geq 1.92$ ) 52-65 years ( $\geq 1.93$ )  $\geq 66$  years ( $\geq 2.00$ )

Cholindex<sup>13</sup> was calculated using the equations:

- LDL-C-HDL-C (if triglycerides <400 mg/dl) or LDL-C-HDL-C+TG/5 (if triglycerides  $\geq 400$  mg/dl)

The cut-off to consider high Cholindex was 80 mg/dl

We believe that there are Hypertriglyceridemic waist (HTGW)<sup>15</sup> when:

-Waist circumference  $\geq 88$ cm in women and  $\geq 102$  cm in men and triglycerides  $\geq 150$  mg/dl.

### Statistical Analyses

All the data were tested for their normal distribution (Kolmogorov-Smirnov test). Results are expressed as means and standard deviations (SD) and, when required, in percentages. Student t test for unpaired data was used to evaluate differences in anthropometric and biochemical characteristics between genders. Chi-square test was used for the difference of proportions. The existence of significant bivariate correlations between parameters such as ABSI, CI, VAI and Cholindex and FINDRISC questionnaire was ascertained by determining Pearson or Spearman correlation coefficients.

Statistical analysis was carried out using IBM SPSS Statistics 27.0 software (SPSS/IBM, Chicago, IL, USA). Significance was accepted at  $p < 0.05$ .

### Ethical considerations and aspects

The study was approved by the Institutional Review Board of the Region. All procedures were performed in accordance with the ethical standards of the institutional research committee and with the 2013 Declaration of Helsinki. All patients signed written informed consent documents prior to participation in the study.

## Results

Age and anthropometrical and clinical characteristics of the participants in the study as a whole and categorized by gender are shown in **table I**. Significant differences between men and women were found in all parameters analyzed with higher values of age, anthropometric characteristics (height, weight, body mass index, waist circumference, and waist to height ratio), systolic and diastolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides in men.

The mean values for the different indices according FINDRISC questionnaire are shown in **table II**. The ABSI, VAI and Cholindex values in women are worsening in parallel with FINDRISC test values, the same applies to men. The Conicity index values behave differently in men and women, in men also they are getting worse with increasing the value of the FINDRISC test, however in women no clear relationship with the test was observed.

The prevalence of normal and altered values of the different indices according FINDRISC questionnaire values are shown in **table III**. In women, the prevalence

of high VAI, HTGW and high Cholindex is increasing in parallel with the increase in the value of the FINDRISC test, in men we can observe the same with high VAI, HTGW, high CI and high Cholindex. ABSI altered shows no clear relationship with the FINDRISC questionnaire values in women and men. In women this relationship was not seen with the high CI.

The correlations between parameters such as ABSI, CI, VAI and Cholindex and FINDRISC questionnaire was -0.087 ABSI, 0.242 CI, 0.398 VAI and, 0.329 Cholindex, p-value <0.01.

**Table I:** Anthropometric, clinical and analytical characteristics of participants in the study.

Characteristics <sup>1</sup>	Women (n=25,510)	Man (n=33,532)	Total (n=59,042)	value <sup>1</sup>
Age (years)	39.30 ± 10.10	40.01 ± 10.35	39.70 ± 10.25	<0.0001
Weight (kg)	161.32 ± 6.51	173.94 ± 7.04	168.49 ± 9.25	<0.0001
Height (cm)	64.87 ± 12.94	81.06 ± 13.75	74.06 ± 15.62	<0.0001
BMI (kg/m <sup>2</sup> )	24.94 ± 4.84	26.78 ± 4.16	25.98 ± 4.56	<0.0001
Waist (cm) circumference	75.24 ± 9.66	88.37 ± 9.54	82.69 ± 11.59	<0.0001
WtHR	0.47 ± 0.06	0.51 ± 0.06	0.49 ± 0.06	<0.0001
Systolic BP (mmHg)	114.36 ± 14.94	124.91 ± 15.36	120.35 ± 16.06	<0.0001
Dyastolic BP (mmHg)	70.29 ± 10.34	75.77 ± 10.74	73.40 ± 10.91	<0.0001
Cholesterol (mg/dl)	192.78 ± 36.39	196.74 ± 38.63	195.03 ± 37.73	<0.0001
HDL-C (mg/dl)	55.03 ± 9.17	50.68 ± 7.53	52.56 ± 8.56	<0.0001
LDL-C (mg/dl)	120.39 ± 36.92	121.82 ± 37.18	121.20 ± 37.07	<0.0001
Triglycerides (mg/dl)	86.98 ± 43.77	123.24 ± 85.76	107.58 ± 72.99	<0.0001

BMI, Body mass index. WtHR, waist-to-height-ratio. Systolic BP, Systolic blood pressure. Dyastolic BP, Diastolic blood pressure. HDL-C, high-density lipoprotein cholesterol. LDL-C, low-density lipoprotein cholesterol.

<sup>1</sup> data are expressed as means ± standard deviation.

Statistical significance was estimated by independent t-test

## Discussion

Despite the evident relationship between overweight and obesity with the onset of diabetes, we have not found in the literature consulted, and in the different databases, any study that establishes relationships between any type 2 diabetes risk scale, whether Findrisk or other, with scales that assess overweight and obesity such as those assessed in this study.

Although there are a large number of studies that analyze the Findrisk test, almost none relate it to this type of scales. We found two studies carried out in the Spanish population<sup>22,23</sup> that related the values of this test with cardiovascular risk scales such as atherogenic indices, metabolic syndrome, REGICOR, SCORE, heart age and vascular age.

The strengths of the study include the large sample size, almost 60,000 workers, and the fact that for the first time

**Table II:** Mean values of the different indices as FINDRISC test.

		n <sup>2</sup>	ABSI		CI		VAI		Cholindex	
			Mean (SD)	p value <sup>1</sup>	Mean (SD)	p value <sup>1</sup>	Mean (SD)	p value <sup>1</sup>	Mean (SD)	p value <sup>1</sup>
Women	Low	19057	0.090 (0.080)	<0.0001	1.08 (0.08)	<0.0001	2.14 (1.09)	<0.0001	60.75 (40.78)	<0.0001
	Slightly raised	4777	0.091 (0.012)		1.14 (0.14)		3.01 (1.94)		77.08 (41.26)	
	Moderate	1017	0.088 (0.011)		1.12 (0.13)		3.33 (2.10)		84.26 (40.31)	
	Hight	643	0.088 (0.012)		1.15 (0.14)		4.15 (2.83)		88.81 (41.27)	
	Very hight	16	0.084 (0.090)		1.11 (0.11)		6.06 (3.74)		99.66 (45.81)	
Men	Low	22465	0.094 (0.070)		1.17 (0.07)		2.53 (1.63)		66.36 (39.56)	
	Slightly raised	8117	0.094 (0.090)		1.23 (0.11)		4.48 (3.96)		84.35 (43.70)	
	Moderate	1856	0.093 (0.080)		1.24 (0.10)		5.17 (4.26)		89.74 (44.86)	
	Hight	960	0.093 (0.090)		1.25 (0.11)		6.28 (5.67)		90.37 (49.95)	
	Very hight	134	0.092 (0.080)		1.25 (0.10)		7.14 (5.56)		99.63 (46.40)	

ABSI, Body shape index. CI, Conicity index. <sup>1</sup> Statistical significance was estimated by independent t-test <sup>2</sup> Number of participants in the study.

**Table III:** Cataloging the various indices according on the value of FINDRISC test by gender. VAI, Visceral Adiposity Index. HTGW, Hypertriglyceridemic waist. ABSI, Body Shape Index.

	Women					Men					p value
	Low	Slightly raised	Moderate	High	Very high	Low	Slightly raised	Moderate	High	Very high	
High VAI	37.7	66.0	74.6	84.9	93.8	48.7	79.6	86.3	88.2	94.0	<0.0001
Normal VAI	62.3	34.0	25.4	15.1	6.3	51.3	20.4	13.7	11.8	6.0	
HTGW absence	99.9	90.1	85.1	71.2	43.8	98.3	76.6	67.6	57.2	44.0	<0.0001
HTGW presence	0.1	9.9	14.9	28.8	56.3	1.7	23.4	32.4	42.8	56.0	
ABSI Relative Riskaltered	89.9	76.7	85.3	81.0	100.0	82.8	74.2	81.6	76.3	84.3	<0.0001
Normal ABSI RelativeRisk	10.1	23.3	14.7	19.0	0.0	17.2	25.8	18.4	23.8	15.7	
High Conicity index	11.2	35.1	28.0	39.5	37.5	14.5	41.0	47.7	48.6	48.5	<0.0001
Normal Conicity index	88.8	64.9	72.0	60.5	62.5	85.5	59.0	52.3	51.4	51.5	
High Cholindex	31.0	46.8	51.4	60.2	75.0	34.9	51.9	57.5	54.4	68.7	<0.0001
Normal Cholindex	69.0	53.2	48.6	39.8	25.0	65.1	48.1	42.5	45.6	31.3	

a type 2 diabetes risk scale such as the Findrisk is related to other scales related to overweight and cardiovascular risk such as the body shape index, visceral adiposity index, conicity index and Cholindex, which makes this study a future reference for further research.

The limitations of this study are that it was carried out in a specific region and in a working population, so that the results cannot be extrapolated to the general population and to other regions.

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## Authorship contributions

PATRÓN O, HECTOR.: conception, study design, data collection and drafting of the original version of the manuscript. KE A. EDMUNDO: study conception, data collection and analysis, and critical revision of the paper. MANZANERO F. ZOE: conception and design of the study, analysis of the data, critical revision of the manuscript and writing of the final version of the manuscript. All authors approved the final version of the manuscript.



# Influence of sociodemographic variables and tobacco consumption on the risk of nonalcoholic fatty liver disease and liver fibrosis

*Influencia de las variables sociodemográficas y del consumo de tabaco en el riesgo de padecer hígado graso no alcohólico y fibrosis hepática*

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## Abstract

**Introduction:** Non-alcoholic fatty liver disease (NAFLD) is the leading cause of liver disease in the Western world. Although it is normal to have a certain amount of fat in the liver, when it exceeds 10% it becomes a health problem. It is not usually a serious disease, unless it is accompanied by steatohepatitis (inflammation of the liver caused by the presence of fat), which can develop into cirrhosis and/or liver cancer.

**Methods:** A descriptive and cross-sectional study was carried out in 9,550 users of the Scottish National Health System in which the influence of sociodemographic variables such as age, sex and educational level and tobacco consumption on the prevalence of non-alcoholic fatty liver disease and liver fibrosis determined with different scales was assessed.

**Results:** The prevalence of high risk of NAFLD and liver fibrosis determined by risk scales is influenced by sex (more prevalent in men), age (increasing with age), educational level (higher prevalence in people with less education) and tobacco use (somewhat more prevalent in smokers, although only with the hepatic steatosis index).

**Conclusions:** The high-risk values in the NAFLD and hepatic fibrosis scales are influenced by sociodemographic variables and only in some cases by tobacco consumption.

**Keywords:** Fatty liver, liver fibrosis, tobacco.

## Resumen

**Introducción:** El hígado graso no alcohólico (NAFLD) es la principal causa de enfermedad hepática en el mundo occidental. Aunque es normal tener una cantidad determinada de grasa en el hígado, cuando ésta supera el 10% se convierte en un problema de salud. No suele ser una enfermedad grave, a no ser que se acompañe de esteatohepatitis (inflamación del hígado causado por la presencia de grasa), lo que puede evolucionar en cirrosis y/o cáncer hepático.

**Material y métodos:** Se realizó un estudio descriptivo y transversal en 9,550 usuarios del Sistema Nacional de Salud de Escocia en los que se valoró la influencia de variables sociodemográficas como edad, sexo y nivel de estudios y, el consumo de tabaco en la prevalencia de Hígado graso no alcohólico y fibrosis hepática determinados con diferentes escalas.

**Resultados:** La prevalencia de alto riesgo de NAFLD y fibrosis hepática determinada mediante escalas de riesgo se ve influenciada por el sexo (más prevalente en hombres), edad (va incrementándose con la edad), nivel de estudios (mayor prevalencia en las personas con menores estudios) y consumo de tabaco (algo más prevalente en los fumadores aunque sólo con el hepatic steatosis index).

**Conclusiones:** Los valores de alto riesgo en las escalas de NAFLD y fibrosis hepática se ven influidos por las variables sociodemográficas y solo en algunos casos por el consumo de tabaco.

**Palabras clave:** Hígado graso, fibrosis hepática, tabaco.



## Introduction

The main and defining characteristic of non-alcoholic fatty liver disease (NAFLD) is the accumulation of free fatty acids and triglycerides in the hepatocytes, specifically in the cytoplasm, mainly in the form of large fat vacuoles, in individuals who do not consume alcohol excessively ( $\leq 3$  standard drinking unit/day in men and  $\leq 2$  standard drinking unit/day in women) and do not present other liver diseases<sup>1</sup>. Although in most cases it follows a benign course, a small percentage of patients may develop non-alcoholic steatohepatitis (NASH), characterized by the appearance of hydropic degeneration of the hepatocytes and lobular inflammation with or without perisinusoidal fibrosis, which can progress to cirrhosis and hepatocellular carcinoma, leading to death due to liver disease<sup>2</sup>.

NAFLD is currently considered the leading cause of liver disease in the Western world, with an estimated prevalence of 20-30% according to the criteria used in different studies<sup>3-5</sup>.

The causal factors of NAFLD can be divided into primary factors, which are the most important, and are related to the different components of the metabolic syndrome such as obesity, type 2 diabetes and dyslipidemia<sup>6-9</sup>. NAFLD could be considered the hepatic component of the metabolic syndrome. Insulin resistance would be the determining alteration of steatosis and this in turn would be responsible for inflammatory disorders (IL-6, TNF $\alpha$ ), oxidative stress, mitochondrial dysfunction, NASH and fibrosis<sup>10-14</sup>. Secondary factors are less frequent, and are related to the consumption of drugs (corticosteroids, estrogens, amiodarone, tamoxifen), bariatric surgery, parenteral nutrition, congenital metabolic diseases and other toxins<sup>6,7</sup>. In clinical practice, many patients with NAFLD present obesity, type 2 diabetes or dyslipidemia as a causal factor, and the association of various factors is frequent.

Blood tests (elevated liver enzymes ALT and AST), diagnostic imaging (ultrasound, CT and MRI) and even liver biopsy are used to diagnose NAFLD. There are also several scales that assess the risk of NAFLD as we will see below.

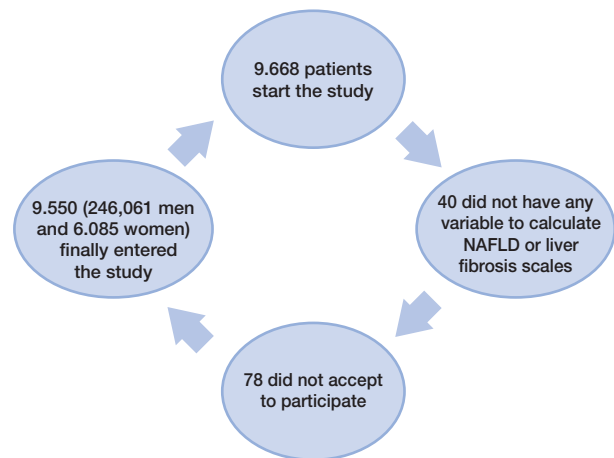
The aim of this work is to know how different sociodemographic variables such as age, sex, educational level and tobacco consumption affect the risk of NAFLD and liver fibrosis.

## Methods

Retrospective, cross-sectional study in a sample of 9668 users of the *National Health Service* between January 2018 and June 2020. 78 did not agree to participate in the study and 40 lacked some of the variables necessary to calculate the fatty liver or liver fibrosis scales. The total

number of participants who entered the study was 9550. (see Flow chart in **figure 1**).

**Figure 1:** Participant flow chart.



### Inclusion criteria

- Age between 18 and 70 years.
- Agree to participate in the study by giving up data for epidemiological purposes.

Sociodemographic variables such as age and sex, level of education (primary, secondary and university) and tobacco consumption were collected, being considered smokers when they had regularly consumed at least 1 cigarette/day (or its equivalent in other types of consumption) in the last month, or had quit less than 1 year ago.

### Fatty liver and hepatic fibrosis risk scales

- **Lipid accumulation product (LAP)**<sup>15</sup>
  - Men: (waist circumference (cm) – 65) x (triglycerides (mMol)).
  - Women: (waist circumference (cm) – 58) x (triglycerides (mMol))
- **Fatty liver index (FLI)**<sup>16</sup>

$$FLI = \left( e^{0.953 \cdot \log_e(\text{triglycerides}) + 0.139 \cdot \text{BMI} + 0.718 \cdot \log_e(\text{GGT}) + 0.053 \cdot \text{waist circumference} - 15.745} \right) / \left( 1 + e^{0.953 \cdot \log_e(\text{triglycerides}) + 0.139 \cdot \text{BMI} + 0.718 \cdot \log_e(\text{GGT}) + 0.053 \cdot \text{waist circumference} - 15.745} \right) \times 100$$

A person is considered to be at high risk when the FLI value is equal to or greater than 60

- **Hepatic steatosis index (HSI)**<sup>17</sup>  
HSI = 8 x ALT/AST + BMI (+ 2 if type 2 diabetes yes, + 2 if female)
- **Zhejiang University index (ZJU)**<sup>18</sup>  
BMI + FPG mmol L + TG mmol L + 3 ALT/AST + 2 if female

**- Fatty liver disease index (FLD)<sup>19</sup>**

BMI + TG + 3 × (ALT/AST) + 2 × Hyperglycemia  
(presence= 1; absence = 0)

Values <28.0 or >37.0 excluded the possibility of NAFLD

**- Framingham steatosis index<sup>20</sup>**

FSI = -7.981 + 0.011x age - 0.146 x sex (female = 1, male = 0) + 0.173 x BMI + 0.007 x TG + 0.593 x hypertension (yes = 1, no = 0) + 0.789 x diabetes (yes = 1, no = 0) + 1.1 x ALT/AST ratio ≥ 1.33 (yes = 1, no = 0).

**- Bard scoring system (BSS)<sup>21</sup>**

BMI ≥ 28 = 1 point, AST/ALT ≥ 0.8 = 2 points, type 2 diabetes mellitus = 1 point.

Cut off for high risk 2 points

Statistical analysis was performed with the SPSS 27.0 program, and a p value of <0.05 was considered statistically significant.

**Ethical considerations and aspects**

The study was approved by the Clinical Research Ethics Committee. The procedures were performed following the ethical standards of the institutional research committee and with the 2013 Declaration of Helsinki. All patients signed written informed consent documents before participating in the study.

**Results**

The mean age of the patients included in our study was slightly over 47 years. Most of them had primary education. Women smoked more than men. The clinical and analytical variables are more unfavorable in men, the differences being statistically significant in most cases as can be seen in **table I**.

The mean values of all fatty liver and liver fibrosis scales analyzed in this study are higher in men. An increase in the values of all scales is observed as the age of the patients increases. People with a university education are those with the most favorable values in all scales. Smokers have lower values than non-smokers. In all cases, the differences observed were statistically significant. The complete data can be found in **table II**.

**Statistical analysis**

A descriptive analysis of the categorical variables was performed and the frequency and distribution of the responses were calculated for each of them. The mean and standard deviation were calculated for quantitative variables, and the percentage for qualitative variables. A bivariate association analysis was performed using the  $\chi^2$  test (with a correction with Fisher's exact test, when conditions required it) and a Student's t test for independent samples. For the multivariate analysis, binary logistic regression was used with the Wald method, with calculation of the Odds ratio, and the Hosmer-Lemeshow goodness-of-fit test was performed.

**Table I:** Sociodemographic, anthropometric, clinical and analytical characteristics of the sample.

	Men n=6085 Mean (SD)	Women n=3465 Mean (SD)	Total n=9550 Mean (SD)	p-value
Age (years)	47.55 (8.32)	46.97 (7.92)	47.34 (8.19)	0.001
Height (cm)	174.94 (6.65)	161.90 (6.19)	170.21 (9.02)	<0.0001
Weight (kg)	83.61 (14.52)	67.47 (13.07)	77.75 (16.02)	<0.0001
Body mass index (kg/m <sup>2</sup> )	27.29 (4.31)	25.75 (4.85)	26.73 (4.58)	<0.0001
Waist circumference (cm)	84.84 (7.93)	73.25 (8.52)	80.63 (9.87)	<0.0001
Waist to height ratio	0.49 (0.04)	0.45 (0.05)	0.47 (0.05)	<0.0001
Systolic blood pressure (mmHg)	128.07 (15.93)	117.69 (16.33)	124.30 (16.83)	<0.0001
Diastolic blood pressure (mmHg)	78.83 (11.05)	73.22 (10.85)	76.79 (11.30)	<0.0001
Total cholesterol (mg/dL)	201.90 (36.33)	199.90 (34.13)	201.17 (35.55)	0.008
HDL-cholesterol (mg/dL)	52.61 (8.98)	57.77 (9.75)	54.48 (9.60)	<0.0001
LDL-cholesterol (mg/dL)	122.97 (34.38)	122.79 (32.77)	122.90 (33.80)	0.809
Triglycerides (mg/dL)	133.18 (77.00)	96.93 (48.27)	120.03 (70.19)	<0.0001
Glucose (mg/dL)	95.05 (24.19)	88.47 (17.98)	92.66 (22.36)	<0.0001
ALT (U/L)	28.79 (14.65)	19.40 (9.51)	25.39 (13.78)	<0.0001
AST (U/L)	24.12 (9.52)	18.54 (7.00)	22.10 (9.09)	<0.0001
GGT (U/L)	34.22 (26.38)	20.11 (15.00)	29.10 (23.89)	<0.0001
	Percentage	Percentage	Percentage	p-value
18-39 years	18.32	17.63	18.07	<0.0001
40-49 years	39.43	46.24	41.90	
50-69 years	42.25	36.13	40.03	
Primary school	69.09	51.95	62.87	<0.0001
Secondary school	21.74	37.23	27.36	
University	9.17	10.82	9.77	
Non-smokers	72.62	68.69	71.19	<0.0001
Smokers	27.38	31.31	28.81	

The prevalence of high-risk values of all the scales, both of nonalcoholic fatty liver disease and liver fibrosis, is much higher in men. An increase in prevalence is also observed parallel to the increase in age. As with the mean values, the most disadvantaged group in the prevalence of high-risk scales is the group with the lowest level of education. Non-smokers have a higher prevalence in all the high-risk scales. In all cases the differences show statistical significance. The complete data can be found in **table III**.

In the multivariate analysis using binary logistic regression, age 50 years or older, male sex, non-university education, and tobacco consumption were established as covariates. Age over 50 years and male sex were the variables that most increased the risk of presenting nonalcoholic fatty liver disease or liver fibrosis. Tobacco consumption increases the risk exclusively with hepatic

steatosis index. All the data from the multivariate analysis are presented in **table IV**.

## Discussion

Male sex is the variable that most increases the risk of presenting NAFLD in all the scales assessed in this study. Age is another variable that also increases the risk in all the scales. Low socioeconomic level increases the risk of NAFLD in all except the Fatty liver disease index. Tobacco consumption only influences NAFLD when assessed with the hepatic steatosis index.

The increase in the prevalence of NAFLD with age obtained in our work has also been observed in other studies consulted; thus, in 550 Japanese studies<sup>22</sup> it was seen that both NAFLD and liver fibrosis diagnosed

**Table II:** Mean values of different fatty liver and liver fibrosis scales according to sociodemographic variables and tobacco consumption.

	n	FLI Mean (SD)	HSI Mean (SD)	ZJU Mean (SD)	FLD Mean (SD)	FSI Mean (SD)	LAP Mean (SD)	BSS Mean (SD)
Men	6085	39.74 (24.93)	37.43 (6.56)	37.77 (5.56)	32.59 (5.18)	0.23 (0.19)	31.16 (25.41)	1.38 (1.06)
Women	3465	18.35 (20.68)	36.52 (6.27)	36.99 (5.62)	30.11 (5.35)	0.15 (0.16)	17.86 (16.58)	0.82 (0.89)
18-39 years	1726	29.40 (26.56)	36.80 (6.83)	36.90 (5.81)	31.26 (5.72)	0.16 (0.17)	24.77 (24.26)	0.71 (0.89)
40-49 years	4001	29.73 (25.09)	36.82 (6.26)	37.08 (5.29)	31.31 (5.22)	0.18 (0.17)	24.64 (22.69)	0.74 (0.88)
50-70 years	3823	35.47 (25.37)	37.51 (6.45)	38.17 (5.70)	32.24 (5.30)	0.24 (0.20)	28.82 (23.79)	1.84 (0.88)
Primary school	6004	34.47 (26.09)	37.37 (6.62)	37.87 (5.82)	32.15 (5.50)	0.22 (0.19)	28.03 (24.23)	1.26 (1.03)
Secondary school	2613	27.35 (24.09)	36.73 (6.26)	36.88 (5.16)	30.92 (5.09)	0.17 (0.16)	23.32 (22.07)	1.01 (1.00)
University	933	28.87 (24.72)	36.33 (5.94)	36.72 (5.06)	30.87 (5.00)	0.18 (0.17)	23.90 (21.44)	1.08 (1.06)
Non-smokers	6799	32.50 (25.69)	37.16 (6.40)	37.56 (5.57)	31.78 (5.34)	0.20 (0.19)	26.79 (23.69)	1.19 (1.04)
Smokers	2751	30.67 (25.45)	36.94 (6.64)	37.30 (5.65)	31.46 (5.46)	0.19 (0.18)	25.20 (22.99)	1.13 (1.03)

FLI: Fatty liver index, HSI: Hepatic steatosis index, ZJU: Zhejiang University index, FLD: Fatty liver disease, FSI: Framingham steatosis index, LAP: Lipid accumulation product, BSS: Bard scoring system.  
In all cases the differences are statistically significant.

**Table III:** Prevalence of high risk of different fatty liver and liver fibrosis scales according to sociodemographic variables and tobacco consumption.

	n	FLI high risk Percentage	HSI high risk Percentage	ZJU high risk Percentage	FLD high risk Percentage	LAP high risk Percentage	BSS high risk Percentage
Men	6085	22.96	54.49	42.55	65.06	36.11	41.59
Women	3465	7.04	47.22	36.25	48.66	22.42	20.81
18-39 years	1726	15.16	42.35	34.59	53.94	27.11	18.13
40-49 years	4001	15.30	49.41	38.17	58.14	27.94	18.87
50-70 years	3823	19.62	56.08	45.02	62.46	36.31	57.13
Primary school	6004	19.47	53.51	42.85	60.03	33.73	36.91
Secondary school	2613	12.94	49.67	35.82	57.90	27.02	28.93
University	933	14.36	47.37	36.01	56.59	26.05	30.01
Non-smokers	6799	17.59	52.67	40.67	59.76	31.73	34.53
Smokers	2751	16.18	49.84	39.26	57.51	29.70	32.86

FLI: Fatty liver index, HSI: Hepatic steatosis index, ZJU: Zhejiang University index, FLD: Fatty liver disease, FSI: Framingham steatosis index, LAP: Lipid accumulation product, BSS: Bard scoring system.  
In all cases the differences are statistically significant.

**Table IV:** Multivariate binary logistic regression analysis.

	50-70 years OR 95% CI	p-value	Male OR 95% CI	p-value	Non university OR 95% CI	p-value	Smokers OR 95% CI	p-value
FLI high risk	1.26 (1.13-1.40)	<0.0001	3.88 (3.36-4.48)	<0.0001	1.23 (1.01-1.49)	0.042		ns
HSI high risk	1.31 (1.20-1.43)	<0.0001	1.31 (1.20-1.43)	<0.0001	1.21 (1.06-1.39)	0.006	1.10 (1.01-1.19)	0.026
ZJU high risk	1.37 (1.26-1.49)	<0.0001	1.28 (1.17-1.39)	<0.0001	1.22 (1.06-1.40)	0.007		ns
FLD high risk	1.22 (1.12-1.33)	<0.0001	1.94 (1.79-2.12)	<0.0001		ns		ns
LAP high risk	1.45 (1.33-1.58)	<0.0001	1.91 (1.74-2.11)	<0.0001	1.30 (1.12-1.52)	0.001		ns
BSS high risk	6.01 (5.46-6.61)	<0.0001	2.86 (2.57-3.17)	<0.0001	1.29 (1.10-1.53)	0.002		ns

by biopsy were more frequent in older persons, which led to the conclusion that age is strongly associated with the development and progression of NAFLD. Something similar was observed in a study of 5<sup>22</sup> Indian diabetics<sup>23</sup>.

In our research, males have a higher risk of developing NAFLD and hepatic fibrosis, these data are similar to those found in the Chinese population<sup>24</sup> where the OR were 3.48 (similar to those found by us with FLI). In this study the diagnosis was made with ultrasound. However, other authors have found a higher prevalence in women<sup>23</sup> and others have found no differences between the sexes<sup>25</sup>.

Our data indicate that the prevalence of NAFLD is higher in people with lower socioeconomic status, and these data coincide with those found by other authors. In a study carried out in a Chinese population<sup>26</sup> it was found that people with a low socioeconomic level were 2.19 times more at risk than those with a higher standard of living. An investigation in 5272 Koreans<sup>27</sup> assessing the relationship between social status and muscle strength with the occurrence of NAFLD showed that both low socioeconomic status and decreased muscle strength were independently and synergistically associated with an increased risk of NAFLD in older persons. One study compared the prevalence of NAFLD in 21 countries<sup>28</sup> with different economic status and concluded that prevalence correlated positively with the per capita income of individuals, such that countries with higher economic status tend to have a higher prevalence of NAFLD. A study in young people under 21 years of age showed that NAFLD appeared earlier in the

more economically disadvantaged group but there was no difference in severity<sup>29</sup>.

We have only found a relationship between smoking and NAFLD when assessed with HSI, finding an increased prevalence among smokers. Two Korean studies also found a positive relationship, one in almost 200,000 people where current smoking levels, pack-years and urinary cotinine levels were positively associated with the risk of developing NAFLD, suggesting that smoking contributed to the development of NAFLD<sup>30</sup>. The other Korean study in 160 862 persons<sup>31</sup> with similar methodology concluded that cotinine-verified current smoking and self-reported current smoking were independent risk factors for NAFLD. A study in 8580 Chinese<sup>32</sup> over 40 years of age assessed the effect of passive and active smoking on NAFLD determined by ultrasound and liver enzymes and observed that passive smoking and heavy active smoking were associated with increased prevalence.

The strengths of this study include the large sample size, almost 10,000 people, and the large number of scales that evaluate fatty liver and liver fibrosis, a total of 7 scales. As limitations we would highlight the lack of objective diagnostic methods for the diagnosis of NAFLD.

## Conflicts of interest

The authors declare no conflicts of interest.

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# Resultados de la encuesta COVID-19 en alumnos, profesores y usuarios de la escuela universitaria ADEMA

*Results of the COVID-19 survey on students, teachers and users of the ADEMA University School*

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## Resumen

**Introducción:** El SARS-Cov-2 es el coronavirus responsable de la pandemia que ha asolado el mundo desde finales del año 2019, teniendo su origen en Wuhan. El cuadro clínico incluye multitud de síntomas aunque los más frecuentes son fiebre, tos seca y dificultad para respirar, y tiene como complicaciones principales la neumonía, el síndrome respiratorio agudo o la sepsis.

**Material y métodos:** Se realizó una encuesta en 1296 usuarios (profesores, alumnos y pacientes de la clínica odontológica) de la Escuela Universitaria ADEMA a los que se les preguntó sobre síntomas compatibles con la COVID en las dos semanas anteriores, sobre situaciones de riesgo de contagio y sobre el conocimiento del protocolo elaborado por el centro educativo.

**Resultados:** Más del 99% de los encuestados no han tenido síntomas compatibles con una infección por SARS-CoV-2 en las dos semanas anteriores a completar la encuesta. Un porcentaje similar han cumplido con las normas establecidas por las autoridades sanitarias en lo referente al uso de mascarillas y no han estado en contacto o conviviendo con personas sospechosas o diagnosticadas de la COVID. Menos del 1% están esperando los resultados de pruebas diagnósticas y el 40,9% de los encuestados confirman que han leído el protocolo elaborado por ADEMA para la pandemia.

**Conclusiones:** El número de usuarios de la Escuela Universitaria ADEMA que han presentado síntomas o han estado expuestos a situaciones de riesgo es muy bajo, inferior al 1%, mientras que el porcentaje de los que confirman haber leído los protocolos elaborados para esta pandemia es muy bajo, de apenas un 40%.

**Palabras clave:** COVID-19, síndrome respiratorio agudo grave, encuestas epidemiológicas.

## Summary

**Introduction:** SARS-Cov-2 is the coronavirus responsible for the pandemic that has ravaged the world since the end of 2019, having its origin in Wuhan. The clinical picture includes a multitude of symptoms although the most frequent are fever, dry cough and shortness of breath, and has as main complications pneumonia, acute respiratory syndrome or sepsis.

**Material and methods:** A survey was made of 1296 users (teachers, students and patients of the dental clinic) of the ADEMA University School who were asked about symptoms compatible with COVID in the previous two weeks, about situations of risk of contagion and about knowledge of the protocol drawn up by the educational center.

**Results:** More than 99% of respondents had not had symptoms compatible with SARS-CoV-2 infection in the two weeks prior to completing the survey. A similar percentage have complied with the rules established by the health authorities regarding the use of masks and have not been in contact or living with persons suspected or diagnosed with COVID. Less than 1% are awaiting diagnostic test results and 40.9% of respondents confirm that they have read the protocol developed by ADEMA for the pandemic.

**Conclusions:** The number of ADEMA University School users who have presented symptoms or have been exposed to risk situations is very low, less than 1%, while the percentage of those who confirm having read the protocols elaborated for this pandemic is very low, barely 40%.

**Keywords:** COVID-19, Severe acute respiratory syndrome, health surveys.

## Introducción

La COVID-19 es una pandemia ocasionada por el virus SARS-CoV-2<sup>1</sup>. El primer caso apareció en diciembre de 2019 en la ciudad de Wuhan<sup>2</sup>, al presentar un grupo de personas un tipo de neumonía desconocida. La mayoría de los individuos afectados tenían relación con trabajadores del Mercado Mayorista de Mariscos del Sur de China de Wuhan<sup>3</sup>. La Organización Mundial de la Salud (OMS) la reconoció como pandemia el 11 de marzo de 2020<sup>4</sup>.

El virus se transmite esencialmente por pequeñas gotas de saliva (microgotas de Flügge), que se emiten al hablar, estornudar, toser o espirar<sup>5-7</sup> y también está documentada la transmisión por aerosoles (< 5µm). Su período de incubación suele ser de cinco días, pero puede variar de dos días a dos semanas<sup>8,9</sup>. Los síntomas más comunes son fiebre, tos seca y dificultad para respirar<sup>8</sup> mientras que las complicaciones pueden incluir la neumonía, el síndrome respiratorio agudo o la sepsis<sup>10-12</sup>.

Las medidas de prevención incluyen el lavado de manos, la desinfección de superficies, cubrirse la boca al toser, el distanciamiento físico entre las personas y el uso de mascarillas, además del confinamiento domiciliario y el seguimiento para las personas sospechosas de estar infectadas<sup>6,13</sup>. La edad es un factor de riesgo (ser mayor de 65 años) y también lo son la presencia de enfermedades crónicas como la diabetes, cardiopatías, enfermedades respiratorias, inmunodeficiencias o hipertensión arterial.

La pandemia ha tenido un gran impacto socioeconómico<sup>14</sup>, se han cerrado centros educativos en más de 124 países lo que ha afectado a más de 2200 millones de estudiantes<sup>15</sup>. Un tercio de la población mundial está confinada existiendo importantes restricciones a la libre circulación<sup>16</sup>, lo que ha provocado una dramática reducción de la actividad económica<sup>17</sup> y un incremento paralelo del desempleo<sup>18</sup>. Uno de los pocos efectos positivos de la pandemia ha sido que la reducción de los viajes y el cierre de numerosas empresas ha provocado un importante descenso en la contaminación atmosférica<sup>19,20</sup>.

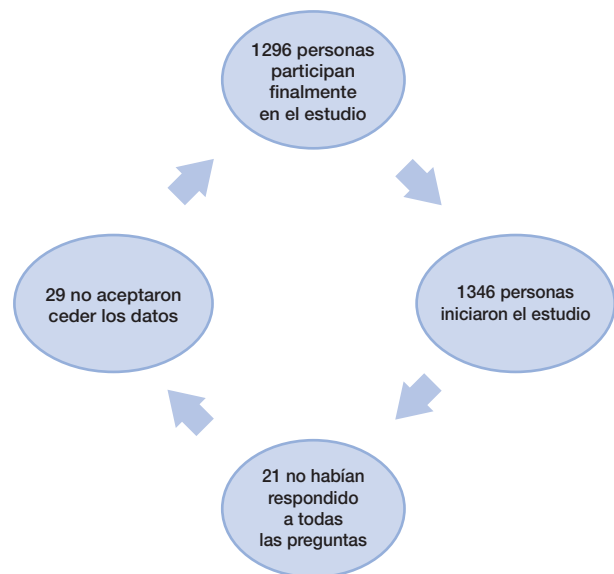
A 1 de abril de 2021 se habían contabilizado más de 130,2 millones de casos en 255 países y 2.840.729 de fallecidos. En octubre de 2020, la OMS estimaba que al menos un 10 % de la población mundial se había contagiado, debido al gran subregistro de casos a nivel mundial<sup>21,22</sup>.

El objetivo de este estudio es conocer la prevalencia de síntomas sospechosos de COVID y el conocimiento de la obligatoriedad de comunicarlos entre los usuarios de la Escuela Universitaria ADEMA.

## Metodología

Se realizó un estudio descriptivo y transversal en 1.346 usuarios (profesores, alumnos y pacientes de la clínica odontológica) de la Escuela Universitaria ADEMA en el periodo septiembre 2020 y enero 2021, de ellos 50 no se incluyeron (29 por no aceptar la cesión de los datos y 21 por no responder a todas las preguntas) por lo que finalmente entraron en el estudio 1296 personas. (ver flujograma en la **figura 1**).

**Figura 1:** Diagrama de flujo de los participantes en el estudio.



Se elaboró un cuestionario no validado de 12 preguntas, las cinco primeras valoraban la presencia de síntomas de la COVID (fiebre, tos, otros síntomas respiratorios, diarrea, cansancio o malestar general), las últimas ocho preguntas se centraban en situaciones de riesgo o en la realización de pruebas diagnósticas. La encuesta la realizó personal sanitario entrenado.

### Análisis estadístico

Se realiza un análisis descriptivo de las variables categóricas, calculando la frecuencia y distribución de respuestas de cada una de ellas. Para las variables cualitativas se calcula el porcentaje. El análisis estadístico se realiza con el programa SPSS 27.0 siendo el nivel de significación estadística aceptado de 0,05.

### Consideraciones y aspectos éticos

Todos los procedimientos se realizaron de acuerdo con las normas éticas del comité de investigación institucional y con la Declaración de Helsinki de 2013. Todos los pacientes firmaron documentos de consentimiento informados por escrito antes de participar en el estudio.

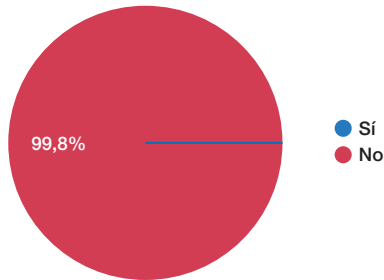
## Resultados

La inmensa mayoría de los encuestados (más del 99%) no han presentado síntomas compatibles con una infección con la COVID-19 en los días o semanas anteriores a la realización de la encuesta. También la gran mayoría de los encuestados no han estado expuestos a situaciones de riesgo de esta pandemia.

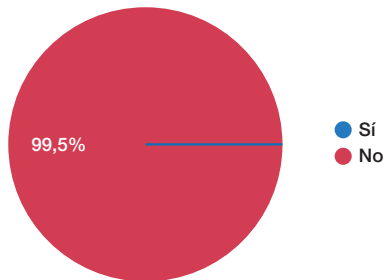
Casi ninguno de los encuestados (menos del 1%) están esperando el resultado de pruebas diagnósticas para la COVID. Prácticamente todos los encuestados conocen la obligación de comunicar cualquier cambio que pueda estar relacionado con la COVID, mientras que sólo un 40,9% conocen el protocolo de la Escuela Universitaria ADEMA frente a la pandemia. Todos los datos se presentan en las **figuras 2a, 2b y 2c**.

Figura 2a: Resultados del cuestionario.

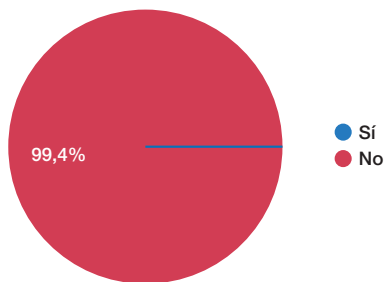
¿Presenta o ha presentado fiebre en los últimos 4 días previos?



¿Presenta o ha presentado diarrea o signo digestivo en los 14 días previos?



¿Presenta o ha presentado tos o cualquier otro signo respiratorio en los 14 días previos?



¿Presenta o ha presentado cansancio, malestar general en los 14 días previos?

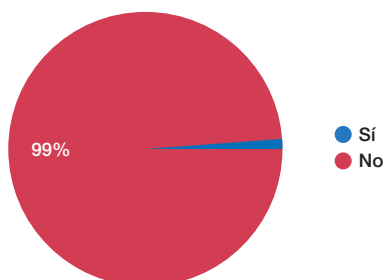
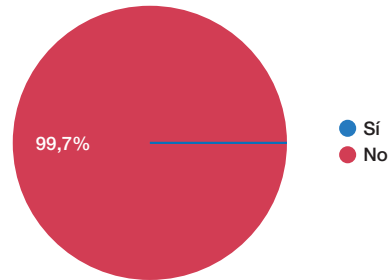
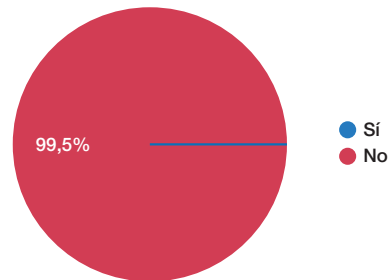


Figura 2b: Resultados del cuestionario.

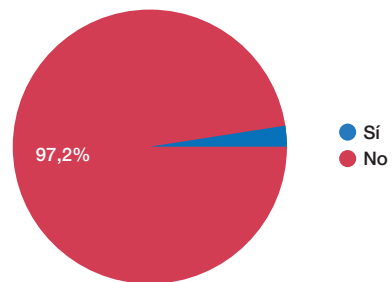
¿Presenta o ha presentado pérdida de olfato o del sentido del gusto en los 14 días previos?



Si se ha realizado algún tipo de test y este ha dado positivo



¿Ha estado en contacto o conviviendo con alguien sospechoso o confirmado de COVID-19?



Está esperando el resultado de algún test de COVID-19

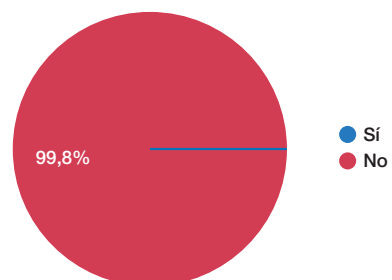
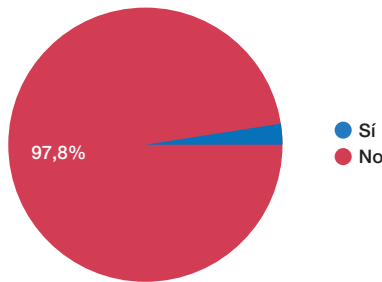
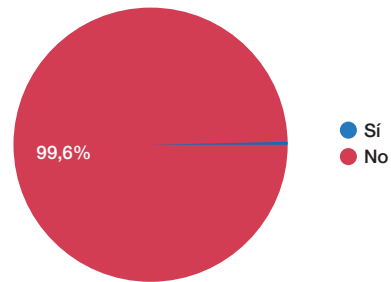


Figura 2c: Resultados del cuestionario.

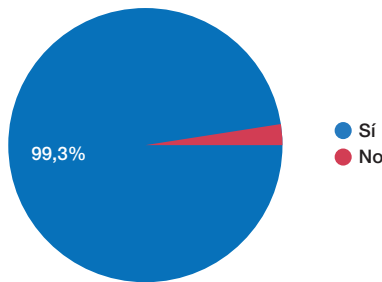
¿Se han realizado salidas o encuentros sin mantener las distancias de seguridad?



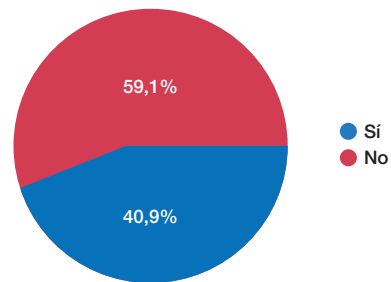
¿Ha habido encuentros con gran número de personas y sin mascarilla?



Tengo conocimiento o la obligación de informar si se produce alguna variación en los datos que he aportado en la presente encuesta



He leído el protocolo que ADEMA Escuela Universitaria ha establecido y tiene colgado en la plataforma virtual



## Discusión

Los resultados de nuestro estudio ponen de manifiesto que la práctica totalidad de las personas encuestadas no presentaban síntomas sospechosos de infección por SARS-CoV-2 en ninguna de sus modalidades clínicas, ya que más del 99% de ellos eran asintomáticos. Un porcentaje similar no recordaban haber tenido contactos con personas sospechosas de sufrir la enfermedad. Del mismo modo un porcentaje también muy elevado cumplía con las normas de seguridad en lo referente al uso de mascarillas y distancia de seguridad. Un dato a destacar es que poco más del 40% habían leído los protocolos elaborados por la Escuela Universitaria ADEMA.

No hemos encontrado en la literatura encuestas similares realizadas en centros educativos pero si dos actividades realizadas en población general. La primera de ellas la realizó Madrid Salud en 39799 personas, la mayoría de la Comunidad de Madrid. A diferencia de lo observado en nuestro estudio solo el 61% no ha tenido síntomas y piensa que no se ha contagiado y el 6% no sabe si se ha contagiado, pero ha guardado cuarentena por haber estado en contacto con personas contagiadas<sup>23</sup>. El segundo estudio se realizó en el Gobierno de Colima, en México, con el objetivo de cotejar la tasa de confirmación de personas infectadas con coronavirus identificando a los contagiados activos que no habían sido detectados

y así evitar que se expanda el número de contagios. La estrategia denominada Dapecode (Detección Acelerada de Personas Contagiadas No Detectadas) permitió localizar al 15% de personas, que en uno o más miembros de la familia, resultaron positivos al Covid-19. Estas cifras son también muy superiores a las obtenidas por nosotros.

Como fortalezas del estudio destacaremos el elevado tamaño muestral y que es la primera encuesta de estas características en el ámbito educativo. Como limitaciones que no se han podido estratificar los resultados por sexo, grupos de edad o colectivo al que pertenecían (profesores, alumnos o usuarios de la clínica).

## Conflicto de intereses

Los autores no declaran conflicto de intereses.

## Contribución de los autores

Diseño del estudio: YP, recogida de datos YP, PA, análisis estadístico HP metodología D, PA, HP, redacción del manuscrito YP revisión del manuscrito PA, HP,D.

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## ORIGINAL

# Clostridium histolyticum collagenase (Xiapex®) in patients with peyronie disease. Expectations vs reality

*Colagenasa clostridium histolyticum (xiapex®) en pacientes con enfermedad peyronie. Expectativas vs realidad*

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## Abstract

**Introduction:** Peyronie's disease conditions the deformity of the penis. Its etiology and effective treatment to prevent surgery have not been determined. The approval of Xiapex®, collagenase from *Clostridium histolyticum*, generated great expectations.

**Justification and objectives:** Drug of recent use in Europe that requires multicenter studies to support its application. The aim is to collect the clinical results obtained from the implantation of Xiapex®.

**Material and methods:** A study of 24 patients treated with Xiapex® between 2017 and 2020 was carried out. Previous data was collected in consultation and information on the results by telephone questionnaire.

**Results:** The initial deviation was 61.4 degrees with a dorsal direction in 59%. 77% of the patients completed the 4 cycles. 41% reported having improved and would recommend the use of Xiapex®. 95% improved penile pain.

**Discussion:** Numerous studies have been published on Xiapex®. Many focus on modifying the treatment scheme proposed by the manufacturer, either by reducing the number of cycles or by the use of vacuum or traction devices. It is known that it is a disease with an important psychological profile, so it is necessary to consider the assessment of subjective results.

**Conclusions:** New studies are needed to collect and assess the real ability to modify the course of the disease with Xiapex®. It is necessary to insist on clear and concise inclusion and exclusion criteria, as well as a treatment scheme that achieves reproducible results.

**Keywords:** Peyronie, colagenasa, Xiapex, Clostridium, Histlyticum, curvature.

## Resumen

**Introducción:** La Enfermedad de Peyronie condiciona la deformidad del pene. No se ha determinado su etiología ni tratamiento eficaz que evite la cirugía. La aprobación de Xiapex®, colagenasa de *Clostridium histolyticum*, generó grandes expectativas.

**Justificación y objetivos:** Fármaco de reciente uso en Europa que precisa de estudios multicéntricos que avalen su aplicación. Se busca recoger los resultados clínicos obtenidos de la implantación de Xiapex®.

**Material y métodos:** Se ha realizado un estudio de 24 pacientes tratados con Xiapex® entre 2017 y 2020. Los datos previos se recogieron en consulta y la información sobre los resultados mediante cuestionario telefónico.

**Resultados:** La desviación inicial fue de 61,4 grados con dirección dorsal en el 59%. El 77% de los pacientes completaron los 4 ciclos. El 41% refería haber mejorado y recomendaría el uso de Xiapex®. Un 95% mejoró del dolor peneano.

**DISCUSIÓN:** Han sido publicados numerosos estudios sobre Xiapex®. Muchos se centran en la modificación del esquema de tratamiento propuesto por el fabricante, bien por la disminución del número de ciclos o bien por el uso de dispositivos de vacío o tracción. Se sabe que se trata de una enfermedad con un importante perfil psicológico por lo que hay que considerar la valoración de los resultados subjetivos.

**Conclusiones:** Quedan muchos estudios por realizar que recojan y valoren la capacidad real de modificación del curso de la enfermedad con Xiapex®. Es necesario insistir en unos criterios de inclusión y exclusión claros y concisos, así como un esquema de tratamiento que consiga resultados reproducibles.

**Palabras clave:** Peyronie, colagenasa, Xiapex, Clostridium, Histlyticum, curvatura.

## Introduction

Peyronie's disease is a disorder that causes the deformity of the penis, mainly, causing its curvature. However, it can lead to other results such as shortening, narrowing... In addition, it can associate other disorders or alterations such as erectile dysfunction.

Its etiology is not clearly defined and there are several theories for its justification. The most accepted, explains that it occurs as a result of a healing process that gives rise to the appearance of fibrotic plaques in the tunica albuginea of the penis. (Figure 1).

Figure 1: Image taken from <https://www.mens-app.es/enfermedad-de-peyronie/>.



The disease occurs in two phases. A first acute phase, which associates pain and in which penile curvature appears, followed by another chronic phase, which does not usually associate pain, in which the fibrotic plaque and curvature tend to stabilize.

For many years, urology has sought medical treatments that improve the patient's symptoms, penile curvature and deformity. However, good clinical results have not been achieved.

The European Medicines Agency approved in 2014 the use of collagenase from *Clostridium histolyticum* intraplaque (Xiapex®) for the treatment of adult men with Peyronie's disease, with a palpable plaque and curvature deformity of the penis of at least 30 degrees when start treatment.

As a last resort, surgical treatment continues. It is the one that achieves the greatest penile correction. The most used involve the plication of the tunica albuginea, the excision of the fibrous plaque, the application of incisions in them and, even, the implantation of penile prostheses to solve, in turn, the usually coexisting erectile dysfunction.

## Justification and objectives

The arrival of Xiapex® was a motivation for urology, especially for andrological urologists who for many years had seen how patients who came to their consultations with this disorder could only offer not completely effective medical treatment or surgical treatment with the derived complications.

Treatment with collagenase from *Clostridium histolyticum* was already known for its use for Dupuytren's disease with palpable plaque. It is a disorder that results from a fibrotic process in the palmar fascia of unknown origin that causes progressive closure of the hand due to retraction of the superficial palmar aponeurosis.

The drug is composed of two collagenase enzymes whose coexpression and isolation is obtained from the anaerobic fermentation of a strain of the bacterium *Clostridium histolyticum* selected for its phenotypic characteristics. These collagenases, called AUX-I and AUX-II, are representative of the two main classes of collagenases produced by the germ.

Collagenases are proteinases that hydrolyze collagen, effectively cleave interstitial collagen such as that present in penile fibrotic plaques. Injection of Xiapex® into a Peyronie's plate can cause enzymatic rupture of the plate. After plaque rupture, penile curvature deformity and patient discomfort from Peyronie's disease are reduced.

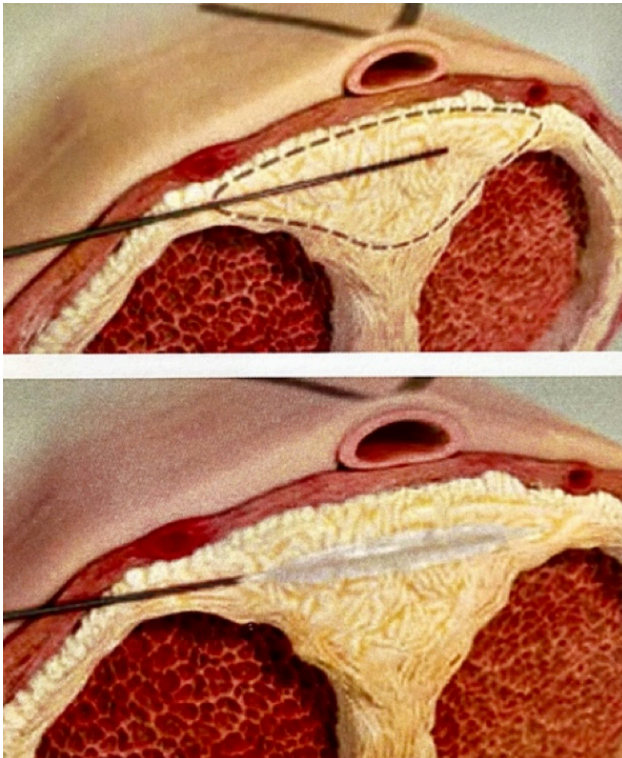
Making a review of the current bibliography, there are no works that assess the application of this novel treatment in our closest environment. For this reason, we consider it essential to carry out a study that analyzes the clinical results obtained after the implantation of treatment with Xiapex®. The elements necessary to consider if the treatment with the collagenase injection has met the expectations generated after its approval for use will be evidenced or, on the contrary, if we find another non-surgical treatment whose efficacy is not always objective.

## Materials and methods

This is a prospective study on patients who were candidates for treatment with Xiapex®. 24 patients were selected to undergo treatment with *Clostridium histolyticum* Xiapex® collagenase between January 2017 and March 2020. Of the total number of patients undergoing treatment, 2 of them refused to participate in the present study.

Initial clinical data and characteristics were collected in the consultation before initiating treatment with *Clostridium histolyticum* collagenase. The results related to the response to treatment were collected by telephone call to the patients since, in the context of a

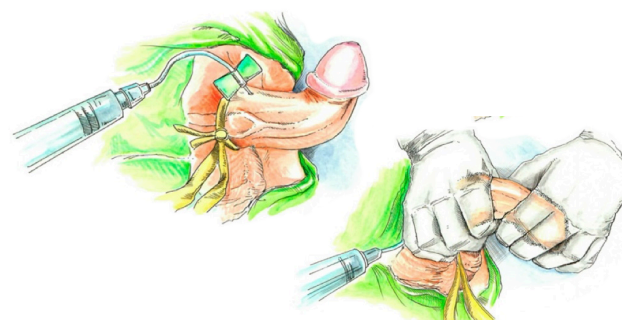
**Figure 2:** Image taken from "Guía para el médico en el tratamiento de la Enfermedad de Peyronie con Xiapex".



**Figure 3:** Image taken from "Guía para el médico en el tratamiento de la Enfermedad de Peyronie con Xiapex".



**Figure 4:** Modeling process. Image taken from <https://tratamientopeyronie.com/fase-cronica/tratamiento-no-invasivo-inyectable/>



COVID-19 pandemic, it was not possible to proceed to the appointment of the patients at the hospital center for the measurement and collection of results.

Initially, variables related to clinical aspects were analyzed, such as age at the onset of symptoms and at the beginning of treatment, risk factors (Diabetes Mellitus, arterial hypertension and vascular diseases) and if they had received previous medical treatment and what it had been. Likewise, variables related to the characteristics of the penis deformity were obtained, such as the angle of curvature, its direction, the length and width of the plate and, finally, whether they presented penile pain associated with the erection.

Regarding the information on the result of the treatment, collected through individualized telephone calls to the treated patients, questions were asked about general satisfaction with the treatment, reduction of the angle of curvature, modification of penile length, penile pain, if they would repeat the treatment and if would recommend it.

The application of the treatment was carried out following the indications that appear in the "Product Sheet", respecting the inclusion criteria: palpable plaque and curvature deformity of at least 30 degrees at the beginning of the treatment and less than 90 degrees. Patients with ventral deformity, "hourglass" or calcified plaque were excluded.

The complete treatment consists of applying 4 cycles. Each of them consists of 2 injections of the drug and an in-office penis modeling process. It should be between 1 to 3 days between the first injection and the second. Modeling is done 1 to 3 days after the second injection. The cycles should be approximately 6 weeks apart. In the time that elapses between one cycle and the next, the patient must perform penis modeling activities on a daily basis. (See **figure 2, 3**)

Penis modeling is a manual procedure that helps reduce curvature deformity and straighten the shaft of the penis. In addition to this in-office penis modeling procedure, patients were instructed to perform penis modeling at home.

It can be administered up to a total of 4 treatment cycles, that is, 8 injections and 4 modeling procedures. A smaller number of cycles may be applied if the urology physician so indicates, if the patient refuses to continue with the treatment or, as indicated in the Technical Data Sheet, if the angle of the curvature after the first, second or third cycle is less 15 degree. (See **figure 4**).

## Results

The treatment was applied in 22 male patients whose mean age was 59.3 years (range 39-73 years). Regarding



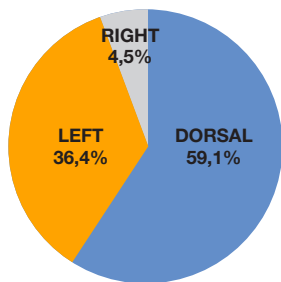
cardiovascular risk factors, 18,2% had Diabetes Mellitus (DM) and 27.3% had Arterial Hypertension (HT).

40.9% of the patients had previously received treatment with Auxin®, a drug that is used orally whose components are Vitamin A and E. In 4 of the patients who had received this treatment, other drugs such as Tamoxifen (3 cases) and Verapamil (1 case).

The time elapsed between the onset of symptoms related to Peyronie's disease and the start of treatment with Xiapex® was, on average, 1.2 years (range 0-3 years).

Regarding the clinical characteristics of the disease, the mean penile curvature angle was 61.4 degrees with a dorsal direction in 59.1% of the cases; in only 1 case there was curvature towards purely right laterality, the rest being to the left laterality. (See **figure 5**).

**Figure 5.** Direction angle curvature.



The mean length of the fibrotic plaque was 2.3 cm (range 0.5-4 cm) and the mean width was 1.2 cm (range 0.5-2.5 cm). 27.3% of the patients had pain before starting treatment.

In 77.27% of the patients, 4 treatment cycles were applied, that is, 8 injections of Xiapex®. In two cases, treatment could not be continued because patients did not continue to attend check-ups. In 1 case it was suspended due to not obtaining results and in another due to the appearance of another additional fibrotic plaque. Finally, treatment could not be continued in 1 case due to the European shortage of Xiapex®.

The evaluation of the results was carried out by means of a telephone call to the patients. 40.91% of them (9 patients) reported improvement after completing the treatment, and 31.82% (7 cases) indicated that they had perceived a decrease in the angle of penile curvature.

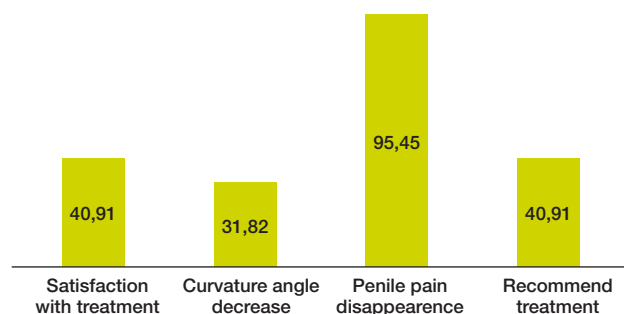
It is curious that 3 of the patients who claimed to have improved after treatment reported not having improved in the angle of curvature. This leads us to think that the improvement to which they referred was related to the

disappearance of pain and the stabilization of the process, as well as to their overall psychological satisfaction.

On the other hand, one patient reported having noticed a decrease in the angle of curvature but, nevertheless, he considered that this fact alone did not improve his initial clinical situation.

Regarding penile pain, especially related to erection, 95.45% of the patients answered that it had disappeared. One of the main problems of penile curvature correction is penile shortening, 50% (11 cases) of the patients reported this fact. Finally, 40.91% of the patients would recommend Xiapex® treatment to other patients. (See **figure 6**).

**Figure 6.** Main variables taken from the telephone questionnaire (%).



As adverse reactions, only mention 1 case that presented severe but self-limited hematoma that did not require surgical drainage.

## Discussion

Peyronie's disease has been studied for more than 260 years since François de la Peyronie described it in 1743. Despite this, epidemiological data that are currently available are limited. According to Chung et al<sup>1</sup>, the disease presents a prevalence rate between 0.4-20.3%, with a higher prevalence among patients with Diabetes Mellitus and erectile dysfunction. As can be seen, the range provided in the different studies is still very wide. In the USA, Stuntz et al<sup>2</sup>, (2016) carried out a survey in which 0.7% confirmed cases were determined compared to 11% of probable cases, which suggests that it is an underdiagnosed disease.

The age of onset of the disease is usually between 50-60 years, although more and more cases are reported in younger patients. In our study, the mean age coincides with that usually referred to, however, it can be stated that there are cases of younger patients, such as a 39-year-old patient who participated in the study.

The most commonly associated risk factors are diabetes, high blood pressure and vascular diseases, as can be seen in the results obtained in the present study.

In general, the first symptom usually referred to is penile deformity (52-94%) followed by penile pain (20-70%), as described in their study by Pryor<sup>3</sup> and Ralph<sup>4</sup>. The appearance of fibrotic plaques is reported as an initial symptom in 39% of patients with a dorsal deviation as the most frequent; as in the current study where it was present in 59% of the cases.

It is a disease with an important psychological affectation. Validated questionnaires and multiple studies, as reported by Nelson et al<sup>5</sup>, show that around 50% of patients with Peyronie's disease have moderate or severe depression.

Classically, the management of the disease was based on the choice of surgical treatment over conservative treatment. Regarding the conservative, the studies are often contradictory and with very unsuccessful results. They mainly consisted of oral treatments such as pentoxifylline, vitamin E, tamoxifen, procarbazine, potassium paraminobenzoate (POTABA), among others.

One of the most used had been potassium paraaminobenzoate (POTABA); It is a drug with an anti-inflammatory and fibrinolytic effect due to the activation of monoamine oxidase. It was the only oral drug approved by the American drug agency for Peyronie's disease based on studies that concluded that, although it does not reduce penile curvature, it could prevent its progression.

Another option was colchicine, which could be given in combination with vitamin E. Colchicine is an alkaloid that inhibits the formation of microtubules and the secretion of collagen by fibroblasts. The second is an antioxidant with an effect on tissue regeneration. It was claimed that they could reduce pain, the size of the plaque and, in some cases, the degree of curvature.

Although, in routine medical practice a very insignificant effect was evidenced with these drugs. Even in the absence of adverse events, it was found that treatment with these could delay the approach to the disease in a more effective way.

Treatment with Xiapex® for all this was a before and after, it was not just another treatment option, it was the only non-surgical option that initially offered very promising results. Many centers, after being approved in Europe and despite the high economic cost involved, began to apply it to be able to offer patients with Peyronie's disease what was supposed to be an effective and non-surgical treatment.

The approval of this drug came after the IMPRESS I and II clinical trials. These are randomized, double-blind, placebo-controlled trials. Overall, a mean improvement in curvature of 34% was demonstrated compared to 18.2% in the placebo group. It was established that the greatest probability of improvement in curvature was in

plaques curved between 30 and 60°, longer duration of the disease and absence of calcification. However, it is curious that 18.2% improved in the placebo arm.

One of the most controversial aspects has been the reference to the number of cycles. Many groups have studied the influence of the number of cycles on the results obtained. Anaissie et al<sup>6</sup> in a retrospective review of 77 patients treated between April 2014 and March 2016 concludes that the therapeutic benefit decreases from the third cycle, not finding significant differences from this in the results obtained. In the same study, the researchers wanted to find predictive response factors, finding that only the response that the patient presents after the first cycle can serve to predict the final response. They consider that the 4-cycle protocol proposed in the product sheet is not being applied in most of the centers due to its high cost and difficulty in compliance with care. In most centers, alternative protocols are applied with fewer injections and adding traction mechanisms.

In the present study, the number of cycles indicated in the product sheet has been maintained. Although in some cases no significant improvement was observed after the application of the injections, the initial idea was maintained in order to be able to homogenize results and make a more objective comparison. Two patients who decided not to continue applying the treatment should be highlighted.

As mentioned above, in the period that elapses between cycle and cycle, penile modeling must be applied. This fact has also been questioned or valued in different studies. Ziegelmann et al<sup>7</sup> conducted a study in which 51 patients who completed the 4 cycles of collagenase treatments were analyzed and were divided into 2 groups: 35 patients underwent mechanical traction modeling and the rest without said device. No statistically significant differences were found in curvature reduction or penetration ability. Differences were reported, although with no degree of significance, in penis length, being + 0.4cm on average in the group that used the traction device. In the study by Raphl et al<sup>4</sup> include 2 groups in which a vacuum device is used with the difference that, in one of them, in addition, classical modeling is performed. It reports that there are no significant differences in the reduction of the angle of curvature. On the contrary, Fernández-Pascual et al<sup>8</sup> carry out a study in which they include 50 patients who undergo aggressive modeling and traction device and 94 patients to whom only aggressive modeling is applied. It concludes that the former achieve a 36% improvement in curvature compared to 28% for the latter. He adds that, in the first group, there is an increase in adverse effects such as ecchymosis, bruising and pain.

Another aspect to take into account is the overall satisfaction of the patient. In a study led by Ziegelmann et al<sup>9</sup> from the Mayo Clinic in Minnesota analyzed in 69



patients treated with Xiapex®, among other things, the degree of subjective improvement. They conclude that patients reported a sensation of improvement in curvature with each cycle of collagenase injections. This study can be considered as the first to prospectively assess patient satisfaction not only with objective data but also subjective data provided by themselves with significant improvement in the ability to restore penetrative intercourse.

In the present study, up to 40% of the patients considered the treatment satisfactory, regardless of the reduction in the angle of curvature. Therefore, it must be valued that the assessment of this disease must be global, both in clinical objective aspects and in the patient's own experience. The implication of the psychological sphere cannot be forgotten and the evolution of the patient must also be considered in this area.

In relation to this issue, the study carried out by Anaissie et al<sup>10</sup> derives from the same conclusion. It also addresses the satisfaction of the partners, collecting a satisfaction of the patient and the partner of 67% and 71%, respectively. It establishes significant predictive factors of partner satisfaction such as previous sexual intercourse, the improvement in the ability to maintain relationships with the treatment and the absence of hypoesthesia in the glans after it. It therefore assumes that the injection of Xiapex® in patients with Peyronie's disease achieves a significant benefit for the sexual health of the couples.

With regard to safety problems and adverse effects, according to various studies, patients undergoing collagenase injections experience at least a mild or moderate reaction localized to the penis. Most frequently, according to Carson et al<sup>11</sup>, is the appearance of hematoma (50.2%), penile pain (33.5%), inflammation (28.9%) and pain at the injection site (24.1%).

The analyzed patients presented mild reactions that were not specifically reported except for 1 case that presented penile hematoma and associated pain. In any case, they were all resolved spontaneously.

Analyzing the results globally, it can be considered that in our center the results were lower than those expected or those reflected in the literature. A possible cause could be a defect in the form in the application of the treatment but, probably, this should not be the main reason for this as it is a relatively simple technique, which does not require a long learning curve and has been carried out scrupulously following the indications given by the manufacturer.

However, the scientific method requires criticism and assessment of possible biases that may have occurred. The main one would be the low number of patients undergoing treatment. It is a generalized bias, already commented and pointed out in the vast majority of studies as in many of those previously referenced. Although this

fact is understandable since it is a disease that, although it has a higher prevalence than what is really known, as previously indicated, the number of patients who consult for it is lower than the one who actually suffers from it in the population.

Another aspect that can skew the sample is the appropriate selection of patients. Although the inclusion criteria specified by the manufacturer have been followed, various studies have observed how there are factors that could influence the patient's response to treatment. These would be: plaque length, its calcification, long evolution time, among others. Although the mean time from the onset of symptoms to the use of treatment is 1 year, it could be, as supported by various studies, that the application in the acute phase, at the onset of symptoms, could favor the patient's response. The use of Doppler ultrasound should also be considered for an expanded study of the characteristics of the fibrosis plaque and to confirm the absence of calcification in the plaque.

Finally, another bias to be pointed out is the lack of validity of the questionnaire made to the patients to analyze the results obtained. This would have been more objective if the pertinent measurements and analyzes could have been carried out in a medical consultation with an adequate anamnesis and examination of the patients.

These aspects have also been valued in other works. Müller and Mulhall<sup>12</sup> in a study based on another 26 published in the last 15 years on Peyronie's disease conclude that most of the published trials have lacked a control group and only a few have had any randomization, which constitutes a relevance bias. Even a consensus conference is considered necessary to share and establish guidelines for the definition, adequate duration of treatment and follow-up.

It can be considered that there are two aspects related to the treatment of the disease through the injection of collagenase that need further analysis and study. On the one hand, the placebo effect that the treatment has; As reflected in the studies that led to the approval of the drug, approximately 20% of those who were treated with placebo in the clinical trial presented significant improvement in the disease. This fact reveals an important psychological component in the disease, mentioned above, and, therefore, a questioning of the results collected in all the studies that address the treatment of Peyronie's disease. On the other hand, the fact that since December 31, 2019 the drug has been out of circulation in Europe is of special importance and significance. According to the American laboratory that produces it, it has decided to withdraw it from all over Europe for strictly economic reasons. Producing this drug is very expensive and the price in Europe is lower than in the US, being already high in our continent, assuming an outlay of between € 5000-7000 for 4

complete treatment cycles per patient. All of this can lead to questioning that it could be about more than price. Currently, there are drugs for benign diseases that involve a large financial outlay and that, nevertheless, remain available. Therefore, it can be deduced that the results initially reported in the studies cannot be extrapolated to those that most urologists assess in the patients they treat.

Other questionable considerations are the large number of studies that modify the initial protocol proposed by the manufacturer; some add traction or vacuum devices instead of the described modeling and others question the number of cycles. All this casts doubt on the very favorable result of the treatment of Peyronie's disease with Xiapex® collected in the literature.

Finally, as shown in most studies, the expectations created during the treatment approach to the patient take on special relevance in relation to the results that they assess. A clash between expectation versus reality that can further lead to frustration and psychological worsening of patients must be avoided.

It is important to know the difficulty that currently exists to present studies that start a path against the current, but it is a moral obligation to inform the scientific community of the results obtained in this study, with all the biases and objections mentioned, to collaborate in favor of medicine being able to continue advancing, and giving a reference to those centers that, like our case, the results obtained have not been as expected.

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## Conclusions

It can be concluded that treatment with *Clostridium histolyticum* collagenase is a safe and established treatment for Peyronie's disease. It seems that the evidence leads to think that it can affect the progression of the disease in the active phase, although there is an important placebo effect. It should also be taken into account that it is essential to associate a traction or modeling effect, intense and persistent over time, for which additional devices can be used.

The scientific evidence regarding the use of Xiapex® is extensive but variable, so it can be deduced that they are not easily reproducible.

There are multiple studies that report modified protocols, but they have a small number of patients and are largely uncontrolled. The results obtained are highly variable and depend on the selection criteria of the patients and multiple other predisposing factors that have not been fully clarified. Therefore, patients should be counseled on the efficacy of collagenase and the high cost of treatment. New clinical trials, with a better design, would be necessary to provide the drug with objective and reproducible results.

The withdrawal by the pharmaceutical company of the drug in Europe represents a burden to be able to advance in favor of greater scientific evidence and to achieve a non-surgical solution for patients affected by Peyronie's disease. We will have to wait for new studies, mainly from the US, to value the effectiveness of the product and demand, if necessary, its redistribution throughout our continent.

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# The effect of Familact probiotic supplement in patients with diabetes (Evaluation of Blood Glucose Parameters, Lipid Profile)

*El efecto del suplemento probiótico Familact en pacientes con diabetes  
(Evaluación de glucosa en sangre y perfil lipídico)*

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## Abstract

Due to the increasing prevalence of this disease, the present study was performed to investigate the effect of pure probiotic supplement Familact on fasting blood glucose and insulin indices and lipid profile of patients with type 2 diabetes. This double-blind randomized controlled clinical trial was performed on 60 patients with type 2 diabetes. The subjects in the Moore intervention group received 7 probiotic capsules containing 7 strains of a mixture of *Lactobacillus* and *Bifidobacterium*, at a dose of 10<sup>10</sup> CFU, and the placebo group received 4 placebo capsules containing magnesium stearate daily for 6 weeks. Dietary intake, anthropometric indices including weight, body mass index, waist circumference, waist circumference and hip circumference, along with biochemical indices including fasting blood sugar, fasting blood insulin and lipid profile were measured and evaluated at the beginning and end of the study. Statistical analysis was performed using SPSS software using chi-square test, t-test and analysis of variance. Finally, it was observed that the mean fasting blood sugar in the probiotic group was significantly lower than before the intervention ( $P = 0.001$ ). Also, at the end of the study, the amount of HDL cholesterol in the probiotic group was significantly increased compared to the amount before the intervention ( $P = 0.006$ ), but this increase was not significant in comparison between groups. Minor and intragroup increases in blood insulin and cholesterol, decrease in LDL cholesterol and insulin and triglyceride resistance were also not significant.

**Keywords:** Probiotics, type 2 diabetes, fasting blood sugar, insulin, *Lactobacillus*.

## Resumen

Debido a la creciente prevalencia de esta enfermedad, el presente estudio se realizó para investigar el efecto del suplemento probiótico puro Familact en los índices de glucosa e insulina en sangre en ayunas y en el perfil lipídico de los pacientes con diabetes de tipo 2. Este ensayo clínico controlado y aleatorizado a doble ciego se realizó en 60 pacientes con diabetes de tipo 2. Los sujetos del grupo de intervención Moore recibieron 7 cápsulas de probióticos que contenían 7 cepas de una mezcla de *Lactobacillus* y *Bifidobacterium*, a una dosis de 10<sup>10</sup> UFC, y el grupo de placebo recibió 4 cápsulas de placebo con estearato de magnesio al día durante 6 semanas. Al principio y al final del estudio se midieron y evaluaron la ingesta dietética, los índices antropométricos, como el peso, el índice de masa corporal, el perímetro de la cintura y el perímetro de la cadera, y los índices bioquímicos, como la glucemia en ayunas, la insulina en ayunas y el perfil lipídico. El análisis estadístico se realizó con el programa informático SPSS mediante la prueba de chi-cuadrado, la prueba t y el análisis de la varianza. Se observó que la media de azúcar en sangre en ayunas en el grupo probiótico era significativamente menor que antes de la intervención ( $p = 0.001$ ). Asimismo, al final del estudio, la cantidad de colesterol HDL en el grupo probiótico aumentó significativamente en comparación con la cantidad anterior a la intervención ( $P = 0.006$ ), pero este aumento no fue significativo en la comparación entre grupos. Tampoco fueron significativos los aumentos menores e intragrupo de la insulina y el colesterol en sangre, la disminución del colesterol LDL y la resistencia a la insulina y los triglicéridos.

**Palabras clave:** Probióticos, diabetes de tipo 2, glucemia en ayunas, insulina, *Lactobacillus*.

## Introduction

Today, diabetes is one of the most common diseases in the world, affecting half of the world's population. Treatment for diabetes varies depending on the type<sup>1-3</sup>. In type 1 diabetes, the main treatment is based on insulin intake as the most important chemical drug, while in type 2 diabetes, due to environmental factors, the main and various treatments, including drug and chemical treatments, lifestyle changes (increased physical activity, reduced stress)<sup>4-6</sup>. And smoking cessation) and diet changes and natural remedies. Therefore, in type 2 diabetes, today, most efforts are made to reduce the number of chemical drugs used in this disease by using lifestyle changes and natural remedies, including herbal remedies and other natural substances<sup>7-10</sup>.

Even prevented the disease. Because doctors are increasingly emphasizing the diagnosis of latent diabetes as a way to prevent type 2 diabetes, as well as its complications, including cardiovascular, eye and kidney problems<sup>11-13</sup>. For this reason, in this study, the effect of probiotics as a natural substance that can have therapeutic or preventive properties on type 2 diabetes is investigated. Therefore, in this project, in order to investigate the possibility of prescribing probiotics as a dietary supplement to help treat or reduce the complications of diabetes along with diet and medication and in the next stage, its use in the production of probiotic dairy and non-dairy products, its effect on patients with type 2 diabetes will be discussed. research questions:

1. Is the mean and difference of mean lipid profile (plasma concentration of HDL-C, LDL-C, Total Cholesterol, TG) between the two groups of patients with type 2 diabetes receiving probiotics and placebo, before and after the intervention and in each Are the two groups different before and after the intervention?
2. Are the mean and mean differences in fasting plasma glucose levels between the two groups of patients with type 2 diabetes receiving probiotics and placebo, before and after the intervention, and in each of the two groups, before and after the intervention?
3. Is the mean and mean difference in plasma insulin levels between the two groups of patients with type 2 diabetes receiving probiotics and placebo, before and after the intervention, and in each of the two groups, before and after the intervention?

## Research Hypotheses

1. Mean and difference of mean lipid profile levels (plasma concentrations of HDL-C, LDL-C, Total Cholesterol, TG), between two groups of patients with type 2 diabetes receiving probiotics and placebo, before and after the intervention and in each It differs from the two groups.

2. Mean and difference of mean fasting plasma glucose levels between the two groups of patients with type 2 diabetes receiving probiotics and placebo, before and after the intervention and in each of the two groups, before and after the intervention.
3. Mean and difference of mean plasma insulin level between the two groups of patients with type 2 diabetes receiving probiotics and placebo, before and after the intervention and in each of the two groups, before and after the intervention. Mean and difference of mean level of insulin resistance index (HOMA-IR) between the two groups of patients with type 2 diabetes receiving probiotics and placebo, before and after the intervention and in each of the two groups, before and after the intervention. Mean and difference of mean level of anthropometric indices (weight, body mass index, abdomen circumference and hip circumference) between two groups of patients with type 2 diabetes receiving probiotics and placebo, before and after the intervention and in each of the two groups, before is different from after the intervention.

## A review of Studies

Despite the wide range of animal studies around the world, most of the studies conducted in Iran to measure the effect of probiotics on blood parameters of people with type 2 diabetes have been human studies<sup>14-18</sup>.

However, these studies make up the bulk of human studies available worldwide. The first human study conducted in Iran was conducted in Shiraz in 2010 by Ms. Mazloun et al in their study, they examined the effect of daily intervention on a number of probiotic capsules containing 1,500 mg of *L. bacteria. acidophilus* *L. bulgaricus*, *L. bifidum*, and *L. casei* or placebo capsules, containing 1000 mg of magnesium stearate, in 40 diabetic patients (20 in the intervention group and 20 in the placebo group), for 6 weeks they paid. Finally, despite a significant reduction in waist circumference of the intervention samples compared to the placebo group, no significant changes were observed in the patients' blood sugar, blood insulin and other blood factors<sup>19-22</sup>.

It should be noted that after measuring the quality of human articles through the Jadad scale, this study did not obtain a sufficient and desirable score in terms of the quality of clinical studies<sup>23</sup>.

Ms. Ijtihad et al. they intervened. Unlike Mazlum et al, they were able to report a significant reduction in fasting blood sugar levels as well as glycosylated hemoglobin in patients. But they did not see a significant difference in blood insulin levels and insulin resistance at the end of their study<sup>24-28</sup>.

In several animal studies, the effect of probiotics on the control of fasting blood sugar has been found to have

a significant effect on lowering fasting blood sugar in various ways<sup>29-31</sup>.

Most of the strains used were *Lactobacillus* and *Bifidobacterium*. The intervention period of these studies varied from 3 to 8 weeks, except for two studies that Hsieh et al<sup>32</sup> and Andersson et al<sup>33</sup> respectively. Finished at 14 and 18 weeks. The results of this study showed significant and positive effects on lowering blood sugar in diabetic rats without any side effects. It is also noteworthy that these significant effects were seen from the sixth week of the study until the end of the intervention period.

The results of a study by Honda et al in 2012<sup>34</sup> showed that the use of *Lactobacillus* GG strain in diabetic rats in 2 stages of 3 and 6 weeks, separately and in daily doses of  $y\%$  and 0.5%, respectively. 0% of the solution enriched with the mentioned bacteria causes a significant decrease in the levels of *Nasha* blood sugar, 5-hour blood sugar and glycosylated hemoglobin, while the use of *Lactobacillus Bulgaricus* strain during these two stages showed a significant result in none of the indicators. This study showed that *Lactobacillus* GG was more potent than *L. Bulgaricus* and had a greater effect on intestinal metabolic activity. In a 2013 study, Huang et al examined the effect of *Lactobacillus Plantarum* on four distinct groups. These 4 groups included the control group, the group receiving *Lactobacillus Plantarum* pure and at a daily dose of 108 CFU, the group receiving fermented vegetables and the group receiving a mixture of *Lactobacillus Plantarum* and fermented vegetables. They found that the intervention of these substances separately for 8 weeks significantly reduced blood sugar and significantly increased blood insulin in diabetic rats<sup>35</sup>.

In fact, this study showed that probiotics can play a positive role in glycemic control even without the presence of prebiotics as a carrier and adjuvant effect. In a study, Tomaro et al found that high doses of *L. fermentum* (1010 CFU) significantly decreased blood sugar levels in diabetic and hypercholesterolemic mice only 11 days after the start of the intervention, which remained at the end of 8 weeks of intervention. Remaining and even at the end of this period, fasting blood sugar levels of diabetic rats showed a significant decrease.

However, they did not report significant changes in blood insulin levels, insulin resistance, and glycosylated hemoglobin<sup>36</sup>.

On the other hand, Hsieh et al in a study conducted in 2013, stated that the daily intervention of high dose of *L. reuteri* (109 2 CFU) for 14 weeks increases the beneficial intestinal flora of *Bifidobacteria* (*Lactobacilli*) and *Lactobacilli* and reduces harmful bacteria. It becomes the gastrointestinal tract (*Clostridia*) and therefore can have beneficial effects on controlling blood sugar in diabetics. At the end of their intervention period, they observed a

significant decrease in fasting blood sugar, glycosylated hemoglobin, 5-hour blood sugar and insulin resistance, as well as a significant increase in blood insulin levels in diabetic mice receiving probiotics compared to the healthy or placebo group<sup>37</sup>.

Alsalami et al. Also stated that daily intervention of a mixture of *L. acidophilus*, *L. rhamnosus* and *B. lactis* at a dose of 75 mg / kg body weight in mice for only 3 days had significant hypoglycemic effects, especially in early-stage diabetes develops in diabetic rats and decreases serum glucose sub-graph (AUC)<sup>38</sup>. In this study, they showed that probiotic intervention after administration of glycilazide (a hypoglycemic drug) increased the bioavailability of this drug in mice with type 1 diabetes compared to non-diabetic mice.

## Research Method

In the simple random method of this study, the Balanced Block method is also used to reduce possible errors. In fact, in this method, patients are divided into  $m$  groups and in each group, they are randomly selected so that they are randomly assigned to treatment A and B and finally each group is randomly selected. This method assigns the same treatment to each group.

## Research Community

With inclusion and exclusion criteria Patients with type 2 diabetes referred to Metabolism Research Center who were willing to cooperate were invited to study. Main criteria for inclusion in the study and sample selection: Patients whose blood sugar is defined according to WHO or ADA index (non-insulin dependent diabetes due to non-response of the body to secretory insulin and often due to obesity and inactivity) and less It takes 15 years for them to develop diabetes and they will be in the age range of 25 to 65 years. Selected individuals, in addition to taking medications prescribed by a physician, should be at a controlled level in terms of blood sugar and lipids, and during the study can use these drugs without changing the previous dosage<sup>39</sup>.

Selected individuals should not use hormone replacement therapy or vitamin supplements. Also, people who smoke and drink alcohol or who have chronic kidney, liver, lung, and chronic or acute inflammatory diseases (especially acute pancreatitis and endocarditis), heart valve disease, short bowel syndrome, and allergies. People with low immune systems (autoimmune) and pregnant and lactating women were removed from the list of eligible people. (These conditions were confirmed by the clinical consultant in the case of the subjects).



## Exclusion Criteria

People who become allergic to probiotic or placebo capsules during the study, or become pregnant during the study, or develop one of the above-mentioned diseases, or have to change the dosage of medications, will be excluded from the study. Also, people who took less than 10% of probiotic capsules or placebo were excluded from the study.

Sampling method and sample size: Specifications of the studied samples (entry and exit criteria) along with sampling method and sample size.

*Determining the sample size and how to calculate it (Sample Size):* Considering  $\alpha$  error of 0.05 and 80% power and considering Total Cholesterol = 0.25 = mmol / yr, standard deviation of 2.5 and variance of 6.25, and considering drop of 32 samples per drop in each group and in total 64 people were studied. The sample size is calculated based on the above assumptions and using STATA software and considering the equal volume in each of the groups. Formula 1, the desired formula in calculating the sample size.

$$n = \frac{2\delta^2 \left( Z_{1-\frac{\alpha}{2}} + Z_{1-\beta} \right)^2}{(\mu_1 - \mu_2)^2}$$

$$\begin{array}{lll} Z_{1-\alpha/2} = 96/1 & 95\% & \delta = 2.5 \\ Z_{1-\beta} = 84/0 & 80\% & \mu_1 - \mu_2 = 2 \end{array}$$

## Sampling

After approval by the Medical Ethics Committee and determination of 64 patients (32 in each group) participants who were willing to cooperate and signed the informed consent, using the Balanced Block method (division of individuals into equal groups) with the same size, random selection of individuals in each group to receive capsule or placebo treatment) were included in the study and were divided into one of two intervention groups (probiotic capsule recipient) and placebo (placebo recipient). Data were collected through interviews, anthropometrics, and biochemical tests.

## Data Collection Tools

The questionnaire used in collecting and evaluating the food consumed by the participants was a 24-hour feed questionnaire as well as a food frequency

questionnaire (FFQ) and a questionnaire to measure their physical activity, each before and after the intervention by the facilitator and separately for each participant was completed. After collecting the food information of the participants, the average information in the mentioned questionnaires was converted into home units in terms of grams and entered into the Nutritionist 4 software to calculate the calories consumed micronutrients and macronutrients.

Then, the information obtained from this software along with other information and findings were entered into EndNote software version 16 for final analysis of information. To measure the weight before and after the intervention of each participant from a digital scale with an accuracy of 1.0 kg. The German model "Ska" was used. Also, to measure their height, a portable height gauge with an accuracy of 0.1 cm (Ska model, made in Germany) was used. The size of their breasts, abdomen and hips were also measured using a plastic meter before and after the intervention. For the intervention capsules, the strains used per gram of Familact supplement capsules are as follows:

- Lactobasillus caseai 2×10<sup>8</sup> cfu/g
- Lactobasillus Acidofilus 2×10<sup>8</sup> cfu/g
- Lactobasillus Bulgarigus 2×10<sup>9</sup> cfu/g
- Lactobasillus rhamnosus 3×10<sup>8</sup> cfu/g
- Bifidobacterium Breve 2×10<sup>8</sup> cfu/g
- Bifidobacterium Longum 1×10<sup>9</sup> cfu/g
- Streptococcus Thermophilus 3×10<sup>8</sup> cfu/g

The total dose for each of the 7 strains is 1010 CFU per. Capsule. Patients' venous blood samples at the beginning and end of the study, which were taken at a rate of 5 cc each time for 10 hours of fasting, were poured into 1 ml microtubes and stored in a freezer at -70 ° C until the experiments. Serum glucose, total cholesterol and triglyceride levels were measured by calorimetric method (based on enzymatic method (Glucose Kit, Pars Azmoun Company, USA-France), Alcyon Iran) and autoanalyzer 300 (HDL and LDL cholesterol concentration using Photometric method, Pars Azmoun Company, USA-France and Alcyon Iran) and autoanalyzer (300 were measured).

**Table 1:** Summary of application descriptions.

Name of equipment or materials	Consumption or non-Consumption	Required number
Familact probiotic supplement	Consumption	210
Placebo capsules	Consumption	150
single-use glove	Consumption	100
Microtube 1.5 cc	Consumption	25
5 ml blood collection syringe	Consumption	140
Insulin Kit	Consumption	1
Glucose kit and lipid profile	Consumption	y
Blue sampler head	Consumption	3
Yellow sampler head	Consumption	3000
Disposable test tube	Consumption	280
Micro tube	Consumption	3
Micro tube	Consumption	1000
Small packet milk	Consumption	144

## Data Analysis Method

Numerical indicators and frequency tables were used to display the data, so that the data is shown as an average (standard deviation) for quantitative variables and as a frequency (percentage) for qualitative variables. In general, SPSS software version 16 was used for data analysis. The normality of data distribution was assessed using Kolmogorov-Smirnov test. Chi-square test and Fisher's exact test were used to analyze the qualitative data. T test and paired T test were used to compare quantitative traits. Analysis of variance with repetitive data was used to investigate the trend of quantitative trait changes between the two groups by controlling confounders. Significant level was considered  $P < 0.05$  in all cases.

## Place and Time of Study

The sampling of this project started, from patients with type 2 diabetes, and having the conditions for inclusion in the study mentioned in the proposal, was performed from Taleghani Hospital. On Saturday, Tuesday, and Wednesday, patients were referred to Taleghani Hospital in Tehran, Velenjak St., Yemen St.

Therefore, the facilitator was present at the hospital on the mentioned days and talked orally with the patients about the free probiotic supplement intervention plan and its possible benefits in reducing the complications of diabetes.

Some people participated in the project voluntarily. Patients referred to clinic department, on Sundays,

**Table II:** Basic information of individuals.

*P	Medicine	Probiotic group	
P=0/020	61,3± 5,2	57/3±7/5	Age (years)
P=0/438	Woman (n=14) 46%/7 man (n=16) 53%/3	Woman (n=13) 43%/3 man (n=17) 56%/7	man / Woman (percentage and number)
P=0/612	154/4 ±9/08	164/89 ±9/29	Height (Sunni meters)
P=0/697	5/8 ±2/8	6/16 ±3/05	Period of diabetes (year)
P=0/712	7% 47% 46%	5% 45% 50%	Physical activity (percentage) Top · medium · Low
P=0/186	41%/7 53%/3 5%	44%/7 51%/3 4%	Medications received (percentage) · Matt Fermin · Glybine glamide · Other medicines

**Table III:** Comparison of the effect of probiotics on biochemical parameters before and after the intervention in the groups.

The value of P between groups †	Placebo group			Probiotic group			Variables
	Intragroup p value*	After the intervention	Before intervention	Intragroup P value*	After the intervention	Before intervention	
P=0/610	P=0/910	147/0±36/3	146/8±35/1	P=0/001	132/7±33/6	146/5±4/3	Fasting blood sugar (mg / dl)
P=0/811	P=0/713	153/8±38/5	155/1±33/9	P=0/510	151/4±35/y	149/3±36/3	Total cholesterol (mg/dl)
P=0/521	P=0/423	153/8±38/5	151/1±33/9	P=0/100	135/3±61/3	141/8±62/3	Triglyceride (mg/dl)
P=0/823	P=0/832	44/5±6/9	44/6±6/0	P=0/006	46/3±10/8	44/y±11/7	HDL cholesterol (mg/dl)
P=0/715	P=0/810	81/9±36/0	82/5±31/1	P=0/812	77/5±31/y	79/4±39/y	LDL cholesterol (mg/dl)
P=0/412	P=0/931	10/5±5/0	10/5±5/6	P=0/913	10/5±5/1	10/3±6/5	Fasting insulin μU/ml
P=0/410	P=0/641	3/7±1/8	3/6±1/7	P=0/112	3/3±1/6	3/7±2/7	Insulin resistance (IU/ml/mg/dl)

HDL: High Density of Lipoprotein

LDL: Low Density of Lipoprotein

\*Comparison within the group at the end of the intervention, the value of P is less than 0.05. (Fasting blood glucose unit is mg / dL.) The mean fasting blood glucose before intervention in the probiotic group was 146.5 43 43.6 mg / dL and after the intervention in this group was 33.6, reached 132.7 mg / dL. While the mean of this index before and after the intervention in the placebo group was 146.8 35 35.1 and 147 36 36.3 mg / dl, respectively.

Based on analysis of variance, comparing the mean blood sugar after the intervention, it was found that the mean of this index decreased in the probiotic group compared to the placebo group, but this decrease did not reach a significant level ( $P = 0.1$ ).

In contrast, the intragroup comparison showed that this decrease reached a significant level after the probiotic intervention compared to before the intervention in the intervention group. Also, the mean HDL cholesterol before the intervention in the probiotic group was 44.1 1 1.1 mg / dl and the mean in this group after the intervention reached 46.29 mg/dl.

While the mean of this index before and after the intervention in the placebo group was 44.6 0 0.6 and 45.4 9 6.9 mg / dl, respectively. Based on analysis of variance, comparing the mean HDL cholesterol after the intervention, it was found that the mean of this index increased in the probiotic group compared to the placebo group, but this increase did not reach a significant level ( $P = 0.4$ ). In contrast, the intragroup comparison showed that this increase reached a significant level after the probiotic intervention compared to before the intervention in the intervention group.

\*Comparison within the group at the end of the intervention, the value of P is less than 0.05. (The unit of fasting blood sugar is milligrams per deciliter.) Comparison between group and intragroup other biochemical parameters showed that blood insulin levels and insulin resistance in the probiotic group compared to the placebo group did not change significantly. Also, in relation to lipid profile levels, no significant intragroup and intergroup changes were reported for total cholesterol, triglyceride and LDL cholesterol indices.

Regarding anthropometric variables, weight, waist circumference and body mass index in both groups decreased slightly compared to the initial value, which did not reach a significant level. Also, changes in waist circumference and hip circumference did not change significantly. (All values are  $P < 0.05$ ).

Mondays, and sometimes Tuesdays. The day before each patient was referred, I reminded them by phone of the date of the test. The number of patients referred per day varied from 2 to 5 (due to limited admission conditions). The duration of intervention was 6 weeks for each patient.

The difference between groups at the beginning of the study was significant in terms of  $P < 0.05$ . Numbers are expressed as "standard deviation mean" and "percentage". Measurement of basal food intake (before intervention) including energy intake, micronutrients and macronutrients in the intervention subjects, which was measured by N4 software, showed that there was no significant difference between the energy intake of individuals before the intervention. This comparison also showed that there was no significant difference between micronutrients and macronutrients, except for sodium, vitamin D, vitamin E, and PUFA, sodium and selenium intake ( $P < 0.05$ ).

Therefore, to compare the mean of the variables after the intervention, the distorting effect of age nodes, sodium, selenium, vitamin D and vitamin E intake and PUFA intake were adjusted. After comparing the diets of patients before and after the intervention, it was shown that there was no significant difference between energy intake, and all macronutrients and micronutrients ( $P < 0.05$ ). **Table III** lists the information related to the analysis of food intake of individuals before and after the intervention.

## Discussion

### *The effect of probiotics on fasting blood sugar*

The present experimental study showed that the intervention of probiotics without changing the diet or applying a special diet in individuals, can significantly reduce fasting blood sugar (FPG) in the probiotic group, while this reduction in comparison between the probiotic group and the placebo group Did not reach a significant level. Positive effects of probiotics on the control and improvement of FPG were seen in many animal studies. In several animal studies that examined only fasting blood sugar, significant effects on FPG reduction were observed within the intervention group and between the placebo and intervention groups. However, there are many differences between the study method and the groups tested in these studies. In fact, this effect was significant in the probiotic intervention group compared to the diabetic and non-diabetic groups receiving placebo<sup>40</sup>, the fat-rich diet group<sup>41</sup>, Huang et al or fructose<sup>42</sup>, (used to cause diabetes), the group receiving a diet rich in fermented vegetables<sup>43</sup>, and the group receiving skim milk<sup>44</sup>, has been seen. In the present study, from 7 different strains including 3 species of *Lactobacillus* (*Lactobasillus casei*, *Lactobasillus acidophilus*, *Lactobasillus Bulgarigus*, *Lactobasillus rhamnosus*), 2 strains of *Bifidobacterium*

(*Bifidobacterium Brev*, *Bifidobacterium longum*) and 10 strains of *Cocene 1 Each*. Capsule was used. In all of these animal studies, a class of the same bacteria called *Lactobacillus* was used.

However, different species such as *L. reuteri* GMNL-, *L. plantarum*, *L. fermentum*, *Lactococcus lactis*, *L. rhamnosus* GG and *L. bulgaricus* have been used in each study; In only two studies, a mixture of *L. fermentum*, *L. acidophilus* and *Bifidobaterium lactis* or a mixture of *Lactobacillus casei* and *Lactococcus lactis* biovar *diacetylactis* was used. The dose of probiotics intervened in these studies varies from 108 to 1010 CFU per gram of body weight or ml of gavage per sample per day. The time of intervention also varied from 3 to 8 weeks, except for a study conducted by Hsieh et al at 14 weeks<sup>44</sup>, and Andersson et al at 20 weeks<sup>45</sup>.

In the study of Honda et al., 2 intervention sessions were performed separately; They believed that the effect of probiotics on diabetes control was not due to cellular components and immune factors, but to the type of bacterial strain and its ability to be active in the gut. In conclusion, in this study, they examined the effect difference between *L.GG* and *L. bulgaricus* for 6 weeks and at a daily dose of 0.5% and also the effect difference between active *L. GG* and *L. ggar* heated for 3 weeks and at a daily dose of 2%, and found that only active *L. GG* significantly reduced blood sugar in both groups<sup>46</sup>.

In general, animal studies show that *Lactobacillus* strain intervention can have positive effects on lowering blood sugar in diabetic animals; Also, although in these studies the minimum daily dose of 108 CFU was reported in each mouse and the minimum duration of intervention was 3 weeks, it is not possible to determine the effects of the effective dose and period of intervention and the effect of different species.

However, the results of the present study also showed a significant decrease in the probiotic group before and after the intervention, although this reduction was not significant compared to the placebo group. In contrast, very few human studies are correct. Similar to the present study, among the 4 human studies that examined the effect of probiotics on FPG, **y** the study reported a significant difference in FPG levels and Andreasen et al. As well as the oppressed and colleagues could not achieve significant results<sup>47</sup>.

Ijtihad and his colleagues in Tabriz used enriched yogurt with a daily dose of 300 g and  $106 \times 600$  CFU per gram of *L. acidophilus* La and *B. lactis* Bb strains for 6 weeks and a significant reduction in group comparison. And reported between groups<sup>48</sup>.

While Asemi et al in Kashan, similar to the present study, used capsules containing seven strains of bacteria (*L.*

acidophilus, *L. casei*, *L. rhamnosus*, *L. Bulgaricus*, *B. breve*, *B. longum* and *Streptococcus thermophilus*) with this difference. The dose and duration of the present study was 10<sup>10</sup> CFU per day for 6 weeks, but in their study, one capsule containing 14 10<sup>10</sup> CFU per day for 8 weeks<sup>49</sup>.

The important point in Asemi et al study is that the level of FPG in the placebo group has increased, while the level of this index in the probiotic group after the intervention was equal to its value before the intervention and had a very small increase and their study showed that Probiotic intervention significantly prevented fasting blood glucose compared with the placebo group, and they did not report any FPG-lowering effects in their study.

However, the present study reported a significant decrease in the probiotic group after the intervention of pure probiotic capsules, so the result of the present study is more important and more indicative of the therapeutic and prophylactic effects of probiotics.

In general, in previous studies, significant differences were found in the improvement of FPG levels in both high-dose interventions of several probiotic strains and interventions with lower doses of 2 bacterial strains at 6 and 8 weeks, respectively; From these results, it can be inferred that the duration of effective intervention, which is approved and effective by the FDA up to 8 weeks<sup>50</sup>.

It is more important depending on the type of strain or their dose, because Andreasen et al. Intervened with *L. acidophilus* at a daily dose of 10<sup>10</sup> CFU per tablet but for only 4 weeks and did not achieve significant results. Also, Mazlum and his colleagues, who used *L. acidophilus*, *L. longum*, *L. bifidum* and *L. casei* strains in a daily dose of 1500 mg in their study in Shiraz, did not report any decrease in fasting blood sugar in patients.

Honda and colleagues stated that preventing glucose uptake into the intestine through probiotic intervention is the main mechanism of 2-hour hypoglycemia and that the anti-diabetic effect of probiotics is due to this mechanism. The main mechanism of this finding was the reduction of hepatic glycogen storage and the changes in the 2K-dependent osteocalcin pathway by *B. fragilis*. In fact, they found that osteocalcin levels were significantly and positively correlated with *B. fragilis* levels in diabetic rats<sup>60</sup>; Osteocalcin is a protein dependent on vitamin 2K<sup>51</sup>, and *B. fragilis* is one of the major bacteria producing vitamin K<sup>52</sup>.

On the other hand, it has been shown that vitamin K together with osteocalcin can play an important role in improving the state of diabetes in humans, so this mechanism may indicate the cause of 2-hour hypoglycemia in diabetic rats.

## Effect of probiotics on lipid profile

The present study also showed that probiotic intervention could lead to a significant increase in HDL levels in the intervention group, although this increase was not significant compared to the placebo group. In this regard, 5 animal studies also reported a significant increase in HDL and intragroup and HDL levels. Among these studies, El-khamisi et al stated that the mixed intervention of *L. acidophilus* and *B. lactis* has a better and more favorable effect on increasing the level of HDL than the intervention of each species alone.

They used a daily gavage dose of 10<sup>8</sup> CFU for 6 weeks. In their 3 studies, Yadav et al. Used a probiotic mixture called "Dahi" which includes 3 species of *Lactobacillus* in similar doses (73 8 10<sup>8</sup> CFU per day) but in different intervention periods of 8 weeks, 6 weeks and 4 weeks and in each Three studies showed a significant increase in HDL levels in diabetic rats compared to the control group at the end of the intervention, although they did not show any significant increase within the group.

Very few human studies have examined the effect of probiotics on HDL levels. Out of 3 human studies that have evaluated the effect of probiotics on HDL index so far, 2 studies reported a significant increase in the level of this index. Asemi et al in Kashan, who tested supplements containing probiotic strains, stated that HDL levels in the intervention group increased significantly compared to the placebo group after 8 weeks of 108-day daily intervention of bacteria. Similar to this study, the present study also evaluated the net supplementation of probiotic-like strains, with the difference that the interventional dose was higher at about 10<sup>10</sup> CFU but for a shorter period of time (due to the absence of possible complications from the high dose of the intervention) and at about 6 weeks and only a significant increase within the group was reported. It is possible that increasing the time intervened will affect the outcome of the work.

In Another study conducted by Ijtihad et al. In Tabriz and Mohammad Shahi et al in Ahvaz, probiotic-enriched yogurt was used at a daily dose of 300 mg for 6 and 8 weeks of intervention. Out of these 2 studies, which had the same working method and only different intervention time, only Mohammad Shahi et al were able to report a significant increase after the intervention period in the probiotic group compared to the initial values in this group, which is similar to the present study. From these results, it can be inferred again that increasing the intervention period if lower doses can be used can have a positive effect on improving the results.

However, due to the limited human results in this field, more clinical studies are needed. Regarding other lipid profile indices, the present study did not achieve significant results at TG, LDL-C, and TC levels. In fact, a



very small decrease was observed in TG and LDL levels, but did not reach a significant level.

Studies have also reported contradictory effects in this regard. Studies that achieved significant results often used high doses or longer intervention periods. For example, Hsieh et al. [53], and Huang et al. [4], after 14 and 8 weeks of intervention, respectively, had a significant reduction in the total cholesterol of the group receiving 10<sup>10</sup> 2 daily CFU probiotics. For each mouse of *L. reuteri* GMNL<sup>5</sup>, and the group receiving daily received one ml containing 10<sup>9</sup> × 1 CFU probiotic per mouse of *L. plantarum* K<sup>10</sup>. These studies also reported a significant decrease in LDL and TG levels. However, a study by Andersson et al. On diabetic mice using a daily probiotic dose of about 40 g/kg body weight of *L. plantarum* DSM showed only a significant reduction in TC levels despite high doses. And their high intervention time did not report a decrease in other lipid parameters. On the other hand, in human studies conducted in this field, which is limited to 3 studies, different results have been expressed. Unlike the present study, Asemi et al who used probiotic capsules (mentioned above) were also able to report a significant decrease in LDL levels, although they did not report a significant change in TC levels similar to the present study?

On the other hand, Mohammad Shahi et al as well as Ijtihad et al. could not mention a significant change in LDL level after the intervention of yogurt enriched with probiotics. In the meantime, only Ijtihad et al. Mentioned a significant decrease in TC level both at the intra-group and inter-group level. Many mechanisms have been suggested in these animal studies due to the reduction of probiotics on the lipid profile. Adherence of bacterial cell wall to cholesterol in the gastrointestinal tract, deconjugation of bile ducts, and production of short-chain fatty acids are important in these studies. However, this work needs more research.

## Effect of Probiotics on Insulin

In the present study, no significant change in blood insulin levels was reported in diabetics. However, two animal studies measuring serum insulin alone reported both significant reductions in blood insulin levels after intervention with probiotics<sup>20</sup>. While 4 animal studies achieved significant results in reducing the level of this index<sup>11</sup>, These significant results (at serum and blood plasma insulin levels) in the intervention with high doses of probiotics (daily 10<sup>8</sup>, 10<sup>9</sup>, 10<sup>10</sup> CFU 50, per mouse, 1 mg or 2% of probiotics to for each mouse) was obtained in the medium duration (6, 8 69, and 9 weeks) or long (14 weeks).

*Lactobacillus* species were used in all significant studies, except for two studies in which a mixture of *Lactobacillus*

and *Bifidobacterium* was used in one and *Lactococcus* in the other. The effect of probiotic reduction on blood insulin is debatable in human studies. Because among human studies, 3 studies examined the effect of probiotics on plasma insulin index and serum insulin and only Asemi et al despite the high intervention of several species of bacteria (*L. acidophilus*, *L. casei*, *L. rhamnosus* *L. bulgaricus*, *Bifidobacterium breve*, *B. longum*, *Streptococcus thermophilus*) for a long time (8 weeks)<sup>9</sup>, or Andreassen et al. by intervening a bacterial species (*L. acidophilus*) for a short time (4 weeks), were able to increase Report significance at the level of this factor only in their intragroup comparison<sup>7</sup>, and similar to the present study, no significant intergroup results were reported in all three studies. Most of the reported results on the effect of probiotics on plasma insulin are based on animal studies, and since there are few reports from human studies, more human studies are needed to better conclude on the effect of probiotics on insulin in diabetic specimens.

The role of probiotics in insulin resistance (IR) Chronic hyperglycemia in diabetics is usually due to long-term insulin resistance and is one of the most important pathophysiological factors in type 2 diabetes<sup>18</sup>.

The present study could not report a significant change in the level of this index after 6 weeks of intervention of a mixture of beneficial bacteria. In this regard, despite the similarity of the results of other studies, some animal studies mentioned a significant change. Two studies reported a significant effect on improving insulin resistance<sup>22</sup>, In these two studies, approximately equal doses of *L. reuteri* (10<sup>9</sup> 2 F CFU per mouse) and *L. plantarum* (10<sup>9</sup> × 1 CFU per mouse) were used for 14 and 8 weeks of intervention, respectively. However, Tomaro et al did not achieve significant results with the intervention of *L. fermentum* NCIMB at a daily gavage dose of 10<sup>10</sup> CFU per mouse for 8 weeks in hyperlipidemic and hyperglycemic mice<sup>27</sup>.

Tanida et al. Also used the method of injection of *Lactobacillus casei* Shirota at a dose of 10<sup>8</sup> × 1 CFU for less than 150 minutes before blood insulin test, and did not achieve significant results<sup>32</sup>. Human studies did not show significant results along with the present study. Ijtihad et al. (2012) and Asemi et al evaluated insulin resistance using the HOMA-IR method, but did not observe any significant change in the level of this factor. Andreassen et al in their study also intervened with *L. acidophilus* NCFM at high dose (10<sup>10</sup> CFU) for only 4 weeks, and also examined the insulin resistance index by measuring HOMA-IR<sub>2</sub>, but as a result No significance was reached at the level of this index<sup>33</sup>. Despite these results, these studies suggest that proinflammatory factors and hyperlipidemia play an important role in regulating insulin resistance and are themselves affected by probiotic intervention; Therefore, the use of probiotics



in interventions can have important effects on improving insulin resistance. The number of studies in this field is very limited and contradictory, so that human studies have not achieved significant results and among animal studies, only 2 studies using *Lactobacillus* strain for a long time were able to achieve the desired and significant results, using different subspecies. Bacteria and in different doses may be the reason for the different results. Therefore, due to the limited amount of animal and especially human information available, we need to conduct this group of studies with more accurate methods to obtain better results.

## Effect of probiotics on body weight and food intake

The present study showed that receiving probiotics without changing the diet had no effect on weight loss after the intervention period. Most studies in this regard have been animal. Twenty animal studies<sup>9-16</sup>, and only two human studies to date have examined changes in body weight of the test specimens<sup>22-28</sup>, of which only 12 animal studies reported food intake along with weight changes. Although the present study did not report a significant change in the weight change process, but a slight weight loss trend was observed in the subjects after the intervention period.

Contrary to the present study, some studies have suggested that probiotic intervention increases the weight of diabetic specimens. Four studies more accurately stated that probiotics-maintained body weight in the samples and resulted in a continuous weight loss process induced by streptozotocin<sup>3-6</sup>, or alloxan<sup>4</sup>, high fat diet (HFD)<sup>9</sup>, or high fructose diet (HFrD)<sup>2</sup>. This weight gain was demonstrated in the study of Marraza et al with the intervention of *L. rhamnosus* CRL along with soy milk. They found that soy milk intervention improved and increased the weight lost in streptozotocin-induced diabetic mice, and increased this amount with the intervention of *L. rhamnosus* CRL.

They stated that giving streptozotocin to mice caused DNA damage, protein loss, and hypoinsulinemia, which in turn prevented carbohydrates from being consumed as energy in the mice and ultimately reduced their weight.

On the other hand, the intervention of soy milk and probiotics, due to having micronutrients and macronutrients, proteins and salts, as well as isoflavone aglycone, leads to increasing and compensating for their lost weight. Marraza et al did not correlate this weight gain with energy intake because, similar to the present study, they did not observe a significant change in rat food intake<sup>47</sup>. Matsuzaki et al also reported a significant increase in weight after *L. casei* intervention compared to the control group in alloxan-induced diabetic rats and

stated that probiotic intervention prevented the alloxan-induced weight loss process, which had an effect in the higher dose *L. casei* (0.1% daily) is more sensitive than lower dose (0.05% daily)<sup>42</sup>.

Bejar et al<sup>39</sup>, as well as Davari et al<sup>16</sup>, initially observed weight loss in diabetic mice, while after intervention *L. plantarum* TN<sup>52</sup>, and A mixture of *L. acidophilus* and *B. lactis*, *L. fermentum*<sup>46</sup>, prevented continued weight loss and significant weight gain was seen in the probiotic group compared to the control group. Andersson et al. Stated that *L. plantarum* DSM intervention after HFD diet increased lean mass in the probiotic and HFD group compared to the control group (HFD recipient), which in turn led to greater glucose excretion and ultimately lower sugar. Samples and their body weight gain. They also cited another reason for weight gain as increased colonization due to *L. plantarum* DSM intervention, which leads to increased adipose tissue and improved functional mucosa of the gastrointestinal tract of mice. They stated that this significant increase in the weight of the samples was not due to the increase in energy intake<sup>5</sup>. While Huang et al attributed the weight loss and prevention of weight gain to the HFFrD diet by *L. plantarum* intervention and its association with probiotics, bacterial polypidemic properties, or changes in adiponectin and leptin levels in the mice<sup>1</sup>.

Zhang et al who studied the preventive and therapeutic effect of *L. casei* Zhang on type 2 diabetes in two different groups, observed that *L. casei* Zhang caused significant weight loss only. In the *L.* group, it was preventive compared to the control group, and had no effect on the *L.* treatment group. They attributed the weight loss to its association with the glucagon-like peptide (GLP-2) and said that the intervention of this bacterium reduced the effect of GLP-2 and reduced the absorption of carbohydrates and fats in mice and their body weight. They are also reduced<sup>3</sup>.

In another experiment after the intervention of *Lactobacillus casei*, Matsuzaki and colleagues reported weight loss and prevention of continued weight gain in samples of mice with non-insulin dependent diabetes, and even declared this effect to be dose-dependent and stated that the rate of reduction Weight in the higher dose group (2 mg per day) was higher than in the lower dose group (0.05%). However, Matsuzaki and colleagues reported no change in the dietary intake of the samples, and therefore the main cause of weight loss was unknown and probably due to a change in their immune system after probiotic intervention. Among the 12 animal studies that examined changes in energy intake<sup>36-42</sup>, all studies similar to the present study, except for two studies<sup>3-9</sup>, reported no significant change in changes in this index.

In fact, only Bejar et al 40, and Yun et al 10, both observed a significant decrease in food intake of the

probiotic group compared to the control group after the intervention. In contrast, very few human studies have been done in this regard. So, that only Asemi et al<sup>7</sup> and Ijtihad et al<sup>9</sup>, measured the amount of weight changes and similar to the present study, both no group and intergroup changes in the weight of the samples after the intervention Announced. Therefore, more studies are needed in this field.

## Conclusion

Overall, the present study stated that the intervention of a dietary supplement containing a pure mixture of probiotics for 6 weeks can lead to a decrease in fasting blood sugar in people with type 2 diabetes and also significantly improve their good cholesterol (HDL) cholesterol levels. Increase, so that other lipid parameters, especially total cholesterol in patients, do not change. However, it can be argued that both of these effects may prevent the progression of diabetes and its complications, such as insulin resistance and hyperglycemia, or prevent other comorbidities such as cardiovascular disease, hyperlipidemia, or heart attack and stroke. Be. Past animal studies have shown that safe and high-dose intervention in these strains

over a relatively long and effective period of time can have beneficial effects on improving fasting blood sugar levels, lipid profiles, blood insulin, and even lowering the level of proinflammatory markers. Increased levels of antioxidant markers in diabetic patients. As mentioned, the type of probiotic intervention is very effective in changing the results because the present study used a probiotic dietary supplement, while some human studies used probiotic carriers that have macronutrients and micronutrients such as carbohydrates, protein and calcium. They are interfering agents and have a distinct role in lowering blood sugar or other biochemical parameters, even without probiotics. Therefore, the use of probiotic supplements as adjunctive therapy will be discussed separately from the consumption of probiotic carriers. However, other existing studies have shown that it does not significantly affect the blood counts of diabetes, although there may be an error in the method of their research or a difference in these methods has been used by the user. These supplements are used in some diseases such as autoimmune diseases including thyroid disease, type 1 diabetes, allergies and especially gastrointestinal diseases such as short bowel syndrome, or other diseases such as acute pancreatitis, chronic kidney and liver disease, heart valve disease.

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# La intervención de los poderes públicos frente a las pseudoterapias: posibilidades y límites

*The intervention of public authorities against pseudotherapies: possibilities and limits*

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## Resumen

El desafío para la salud pública planteado por la proliferación de las pseudoterapias legitima e incluso exige la intervención de los poderes públicos en este contexto. Tras una caracterización inicial en la que se destaca la variedad y muy diversos grados de peligrosidad de este tipo de prácticas, así como los distintos instrumentos para intentar atajar sus efectos (entre ellos, una normativa administrativa quizás desaprovechada), se hace hincapié en los límites que constriñen la intervención estatal, relativos a los derechos fundamentales de los ciudadanos que las adoptan y los de quienes las defienden y difunden. Dentro ya del campo sancionador, se analiza con mayor detalle el juego del Derecho penal en este contexto, a través de figuras como la estafa, el intrusismo, la publicidad engañosa o los delitos contra la salud pública relacionados con los medicamentos no autorizados.

**Palabras clave:** Pseudoterapias, intrusismo, estafa, salud pública, libertad de expresión, publicidad engañosa.

## Abstract

The recent proliferation of pseudotherapies undoubtedly poses a challenge to public health which not only legitimizes but even requires public action in this field. After an initial characterization of this phenomenon, which highlights the variety and very different degrees of dangerousness entailed by this type of practices, this paper goes over the various legal instruments which enable a better control by the state, not loosing sight, on the other hand, of constitutional constraints on state intervention imposed by the fundamental rights of the citizens who decide to adopt them and the freedom of speech of those who defend and disseminate them. Within the sanctioning field, the paper analyzes the role of criminal law in this context through criminal offenses such as fraud, professional intrusion, misleading publicity or drug-related crimes against public health.

**Keywords:** Pseudotherapies, professional intrusion, public health, freedom of speech, misleading publicity.



## Introducción. La multiplicidad de planos de la intervención estatal sobre un grupo heterogéneo de prácticas.

En los últimos años, los poderes públicos de distintos países vienen intensificando su intervención para hacer frente a la creciente difusión y práctica de las hasta hace no mucho tiempo conocidas como “terapias alternativas y complementarias”, que –en una mutación cargada de significado por la connotación mucho más peyorativa de la nueva terminología–, en la actualidad han pasado a designarse con mayor frecuencia como “pseudoterapias”<sup>1</sup>. En lo que a España se refiere, las intenciones más recientes de avanzar en esta línea por parte del Gobierno se plasmaron en el año 2018 en un Plan para la Protección de la Salud frente a las Pseudoterapias de los Ministerios de Sanidad, Consumo y Bienestar Social, y Ministerio de Ciencia, Innovación y Universidades<sup>2</sup>, en el que se trazaban una serie de líneas de actuación y modificaciones normativas que –presumiblemente por los nuevos tiempos impuestos por la pandemia– no han podido todavía llevarse a término en su totalidad.

Hablar de pseudoterapias –asumida su definición como “sustancia, producto, actividad o servicio con pretendida finalidad sanitaria que no tenga soporte en el conocimiento científico ni evidencia científica que avale su eficacia y su seguridad”<sup>3</sup> – supone, para empezar, referirse a un grupo numeroso y muy heterogéneo de prácticas, que no deben ser tratadas sin más como un todo indistinto. La diversidad es notable en todos sus aspectos: su origen ideológico (las hay totalmente desprovistas de tal componente frente a algunas en las que este sí parece con claridad, como las llamadas “terapias de conversión” de la orientación sexual, o aquellas otras, más próximas las teorías conspirativas, cargadas de un confuso tinte supuestamente contestatario contra la alianza del “poder”, la ciencia convencional y la industria farmacéutica<sup>4</sup> (notas que permean al menos una parte del movimiento antivacunas). La diversidad es también notable por lo que se refiere a su apariencia de seriedad científica (encontramos desde el curanderismo más elemental a otras revestidas de solvencia científica

y tecnológica, como las recientes ofertas por empresas norteamericanas de costosos tratamientos preventivos y curativos del Covid con células madre<sup>5</sup>), o, en fin, y esto es lo más relevante, en lo que atañe a su grado de peligrosidad para la salud. En relación con este último factor, que es el que aquí más nos interesa, existe un amplio rango de propuestas, del mismo modo que – en el plano más general de las “pseudociencias”– poco tienen que ver en cuanto a su dañinidad, por ejemplo, el creacionismo y las doctrinas negacionistas del cambio climático, estas sí extremadamente peligrosas para la supervivencia del planeta. Pseudoterapias directamente inocuas por más que de eficacia no (o cuando menos no todavía) científicamente validada, que además conviven sin problema con la terapia convencional, coexisten con otras claramente peligrosas per se en tanto promueven el consumo de productos con consecuencias adversas para la salud, sea porque disminuyen los efectos terapéuticos del tratamiento convencional, sea por su propia toxicidad (así, el caso reciente del MMS, el preparado de clorito sódico conocido como Suplemento Mineral Milagroso, que se presenta como medicamento curativo del trastorno autista y muchas otras dolencias)<sup>6</sup>; a ello se añade, como factor de peligrosidad especialmente relevante, la cuestión de si, con independencia del contenido de la práctica en sí misma, se induce al abandono de la terapia científicamente contrastada (como ocurre con algunas de las dirigidas a pacientes oncológicos). Que la implantación firme de prácticas de este último tipo –como sucede en Estados Unidos– pueden generar un verdadero problema de salud pública lo evidencian cada vez más estudios científicos comparativos de los índices de supervivencia de pacientes oncológicos que recurren a unos u otros medios para afrontar su enfermedad<sup>7</sup>.

En el concreto caso español, la implicación de los poderes públicos en esta materia no solo se legitima por el deber de tutela de la salud pública que el art. 43 de la Constitución de 1978 les asigna, sino que enlaza con la creciente implicación de numerosos colectivos –de profesionales médicos, de pacientes o en defensa de los intereses de estos, de científicos<sup>8</sup>, etc.– que insisten en demandar una actuación más enérgica al Estado en la

1. Sobre la importancia de la terminología en la construcción del discurso sobre el tema, con un recorrido sobre su utilización en la esfera de las políticas públicas en los últimos años en España, LOPERA PAREJA, «El debate político sobre las terapias alternativas y complementarias en España en la interfaz entre ciencia, política y sociedad (1979-2018)», *Perspectivas de la Comunicación* 2019, Vol 12, Nº 2, pp. 155-193.

2. 20181108 Plan Protección frente pseudoterapias VF

3. Plan Nacional 2018, p. 3.

4. La imbricación de la falsa información con el aspecto conspirativo las hace especialmente difíciles de contrarrestar, puesto que como muy bien explican SUNSTEIN y VERMEULE (“Conspiracy Theories”, University of Chicago Public Law & Legal Theory Working Paper No. 199, 2008) las teorías conspirativas son en esencia autoconfirmatorias (los intentos de desmontarlas por los poderes públicos son percibidos y presentados precisamente como una persecución y por ende la confirmación de su acierto).

5. «Preying on Public Fears and Anxieties in a Pandemic: Businesses Selling Unproven and Unlicensed “Stem Cell Treatments” for COVID-19», *Cell Stem Cell* 26, June 4, 2020.

6. La presentación como tratamiento del trastorno autista resulta especialmente peligrosa, en la medida en que los quienes lleguen a ingerirlo no serán personas competentes (por más que mal informadas), sino a menudo menores a los que les será suministrado por sus progenitores.

7. Skyler B. Johnson, Henry S. Park, Cary P. Gross, James B. Yu, “Use of Alternative Medicine for Cancer and Its Impact on Survival”, *JNCI J Natl Cancer Inst* (2018) 110(1): djx145.

8. Así, la carta firmada por medio centenar de científicos *Contra las pseudociencias y las artes mágicas*, EL PAÍS 14-2-2015.

protección de la salud de los ciudadanos, especialmente de quienes, en situación de especial vulnerabilidad por padecer una enfermedad seria, pueden ser más propensos a esperanzarse con cualquier promesa de curación. También en el tratamiento del tema por parte de los medios de comunicación, que en tantas ocasiones se han prestado a jugar el papel de difusores de este tipo de prácticas (a través de entrevistas, reportajes, o simplemente acogiendo publicidad), puede empezar a detectarse un cierto cambio de actitud en los últimos tiempos, más acorde con su papel de pieza capital en la conformación de una opinión pública informada<sup>9</sup>.

Las iniciativas planteadas por el Plan para la Protección de la Salud frente a las Pseudoterapias de 2018 no han podido todavía concretarse en el proyectado nuevo Real Decreto de Protección de la Salud frente a las Pseudoterapias. En estas páginas haremos alusión a algunas de las medidas propuestas, pero también a los instrumentos normativos con los que ya cuenta nuestro ordenamiento, que abarcan por cierto una escala de intensidad muy diversa. De mayor a menor, como veremos, pueden entrar aquí en juego figuras delictivas como la publicidad engañosa, la estafa o el intrusismo; por otro lado, en un sector tan intervenido por el Derecho Administrativo como es el sanitario se cuenta como es lógico con una regulación detallada de la fabricación, publicidad y venta de medicamentos<sup>10</sup>, e incluso con una regulación específica perfectamente aplicable a las pseudoterapias (el Real Decreto 1907/1997, sobre publicación y promoción comercial de productos, actividades o servicios con pretendida finalidad sanitaria) que probablemente podría ser más utilizada en nuestro contexto de lo que lo ha sido hasta el momento, y que permite el ejercicio de la potestad sancionadora de la Administración. Más allá de este aspecto puramente sancionatorio, a los poderes públicos les cabe también por supuesto actuaciones más sencillas, como denegar solicitudes de uso de locales e infraestructuras de titularidad pública (por ejemplo, municipales) para celebrar encuentros en los que vayan a difundirse este tipo de terapias, prohibir expresamente

su práctica en centros sanitarios públicos<sup>11</sup>, no prestarles cobertura dotándolas de revestimiento científico (aspecto en el que cobra especial importancia el papel de las Universidades)<sup>12</sup>, o liderar campañas informativas en la red dirigidas al conjunto de los ciudadanos<sup>13</sup>.

## Los límites infranqueables a la intervención tuitiva del Estado

Al margen de la concreta modalidad de intervención estatal en juego, la misma se topa necesariamente con una serie de límites impuestos por nuestro propio marco constitucional en tanto derivados del respeto a los derechos fundamentales, sean los de los enfermos convencidos de los efectos curativos de una pseudoterapia, sean los de quienes la defienden o difunden. El primero de tales límites –que ni siquiera para preservar su salud o incluso su vida puede el Estado imponer una actuación terapéutica a un ciudadano, tampoco en el supuesto de que su rechazo a la terapia basada en la evidencia se asiente en informaciones falsas- hace tiempo que ha sido asumido con claridad por nuestro ordenamiento jurídico y asimilado por los profesionales; el segundo –el juego en este contexto del derecho fundamental a la libertad de expresión- pertenece igualmente al núcleo de nuestro sistema jurídico, pero sus contornos pueden resultar más complejos.

1. La especial peligrosidad de las pseudoterapias (o al menos de aquellas que conducen al rechazo por el paciente de las terapias basadas en la evidencia en casos de dolencias graves) se relaciona estrechamente con la imposibilidad jurídica de imponer una terapia convencional al ciudadano que libremente la rechaza. Lo que convierte estos casos en especialmente complicados y frustrantes para el profesional que en primera instancia ha atendido al paciente es el hecho de que no pueda hablarse en puridad de un “rechazo informado”, en la medida en que la negativa se basa en asunciones falsas sobre datos relevantes para su elección, relativas a la aptitud curativa de otros cursos de acción preferidos por él (la

9. Numerosos trabajos recientes dan cuenta del papel de los medios en este ámbito. Entre otros, CORTIÑAS-ROVIRA/MOYA-ARRABAL, “La falsaciencia (pseudociencia) als mitjans de comunicació. Estudi de les estratègies discursives d’inserció social a la premsa espanyola (2011-2016)”, *Communication Papers –Media Literacy & Gender Studies–* Vol.7 - No13 (2018), pp. 129-144.

10. Real Decreto 1416/1994, de 25 de junio, por el que se regula la publicidad de los medicamentos de uso humano, o el Real Decreto Legislativo 1/2015, de 24 de julio, por el que se aprueba el texto refundido de la Ley de garantías y uso racional de los medicamentos y productos sanitarios.

11. Por ejemplo, Instrucción 22/2017 de la Conselleria de Salut de la Generalitat Valenciana, que en los centros sanitarios de titularidad pública de la Comunidad Valenciana no autoriza la publicidad, promoción, presencia o práctica de cualquier actividad no oficialmente reconocida e incluye una lista ejemplificativa de las en aquel momento consideradas como pseudociencias por el Ministerio.

12. Comunicado de la Conferencia de Rectores de las Universidades Españolas de febrero de 2021, <https://www.crue.org/2021/02/comunicado-contra-pseudoterapias/>

13. En el marco del ya citado Plan para la Protección de la Salud frente a las Pseudoterapias, de noviembre de 2018, el Gobierno ha creado la página [www.conprueba.es](http://www.conprueba.es), en la que entre otras iniciativas se van publicando los informes emitidos por la Red Española de Agencias de Tecnologías Sanitarias y Prestaciones del SNS (RedETS) sobre concretas técnicas. En febrero de 2021, por ejemplo, se han publicado las conclusiones sobre las prácticas conocidas como magnetoterapia estática, la dieta macrobiótica, el masaje tailandés y la sanación espiritual activa, todas las cuales han sido consideradas pseudoterapias. Cuestión distinta, como señala PIGGLIUCI, (“How to behave virtuously in an irrational world”, *Disputatio. Philosophical Research Bulletin*, Vol. 9, No. 13, Jun. 2020, pp. 4 y ss. acudiendo a la tríada aristotélica, es la importancia persuasiva que, más allá de la presentación de la evidencia o lógica (logos), despliegan el factor autoridad (ser percibido como fuente fiable, ethos) y, sobre todo, el de la conexión emocional con el destinatario del mensaje (pathos), un aspecto explotado por los difusores de las pseudoterapias y que las campañas informativas dirigidas a la generalidad de los ciudadanos no deben desatender.

imposición de manos, el MMS, la magnetoterapia estática o cualesquiera otros). A diferencia del ya clásico supuesto del Testigo de Jehová (que conoce perfectamente la necesidad de la transfusión y el riesgo que su negativa a consentirla puede comportar), en los supuestos que nos ocupan cabe cuando menos poner en duda si el paciente, a la hora de prestar o negar su consentimiento informado, ha aprehendido realmente las consecuencias de su opción, y si su decisión de rechazar el tratamiento indicado por la *lex artis* hubiera sido la misma de haber sabido que la milagrosa terapia no era tal (que no es así lo evidencia la frecuencia con la que pacientes ya muy graves terminan acudiendo a la Medicina convencional una vez comprobada la ineficacia de la terapia engañosa). Con todo y con ello, incluso aun tratándose de decisiones no verdaderamente “informadas”, el grado de invasividad de los derechos fundamentales que la imposición de un tratamiento comporta obliga a respetar la decisión del paciente también en estos casos<sup>14</sup>. Solo cuando se encuentra en juego la salud de terceros puede plantearse algún tipo de compulsión, que incluso aquí suele encontrar límites: por ejemplo, cuando se propugna la vacunación obligatoria (por motivos no ya de salud individual sino pública) no se trata de autorizar su administración coercitiva al ciudadano, sino de terminar induciéndole a consentir a través de la amenaza de sanciones pecuniarias (como recientemente ha dispuesto, de modo excepcional en un contexto general de voluntariedad de la vacuna contra el COVID-19, la Ley 8/2021 de la Comunidad Autónoma de Galicia, por cierto recurrida ante el Tribunal Constitucional por el Gobierno español)<sup>15</sup>.

**2.** Desde un punto de vista jurídico, resulta esencial diferenciar la mera *difusión* de ideas, creencias o construcciones teóricas de la *efectiva práctica de la actividad* como servicio; en un terreno intermedio entre ambas se sitúa, por su parte, la *publicidad* de dicha actividad comercial. Por lo que se refiere al primer estadio –y me limito ahora a las construcciones más claramente falsas y peligrosas dentro de la amplia escala a la que antes me refería– planteamos las posibilidades de intervención estatal suscita una controversia de importante calado jurídico, con un componente de legitimidad o límites (cómo controlar la difusión de informaciones objetivamente falsas y peligrosas para

bienes jurídicos relevantes sin invadir la esfera de los derechos fundamentales de quien las emite) y un segundo componente, este ya de orden fáctico, relativo a las posibilidades reales de ejercer un control de contenidos en esta nueva era tecnológica en la que la información ha dejado de difundirse a través de los medios clásicos de comunicación de masas para pasar a transmitirse en gran parte a través de las redes sociales y en general, de internet.

Que la mera difusión de este tipo de construcciones (previa a su efectiva práctica pero también incluso a su oferta o publicidad) entraña ya un riesgo para la salud pública parece claro: es la consulta de blogs, páginas web, canales de Youtube, etc. en los que se ponderan las ventajas de unas determinadas terapias frente a la medicina convencional lo que convence y motiva a personas enfermas a acudir a determinados servicios. Puede también sostenerse, por otra parte, que la difusión de esta información falsa queda excluida del amparo del derecho fundamental a “comunicar o recibir libremente información”, que el art. 20.1.d de la Constitución Española condiciona a que se trate de información “veraz”. Sin embargo, aun siendo cierto lo anterior, la posibilidad de prohibir (con sanciones penales o administrativas) la mera difusión o circulación de este tipo de construcciones o teorías se enfrenta a enormes dificultades. La primera de ellas es que en este contexto resulta muy complejo delimitar la mera información (falsa) de la expresión y difusión de “los pensamientos, ideas y opiniones mediante la palabra, el escrito o cualquier otro medio de reproducción”, que sí se encuentra protegido como derecho fundamental por el art. 20.1a CE –especialmente, aunque no solo, cuando revista un sesgo ideológico, por absurdo o rechazable que pueda parecer (“la medicina convencional aliada con la gran industria farmacéutica solo persigue el lucro en detrimento de la salud de los ciudadanos; por eso solo la medicina natural garantiza ...”); un derecho que según el texto constitucional, no puede restringirse “mediante ningún tipo de censura previa”, y que según el Tribunal Constitucional acoge sin duda también las ideas más molestas o rechazables<sup>16</sup>. En el caso de celebración de encuentros, congresos, etc., se halla en juego, además, el derecho fundamental de reunión del art. 21 CE, cuyo ejercicio

14. En este mismo sentido se pronuncia desde hace ya décadas la doctrina claramente dominante; así, p. ej., BROCK, *Life and Death, Philosophical essays in biomedical ethics* Cambridge University Press, Cambridge, Mass., 1993, p. 80; ENGELHARDT, *Los fundamentos de la Bioética*, Paidós, Barcelona/Buenos Aires/Méjico, 1996, p. 330, o HAKSAR, *Equality, Liberty and Perfectionism*, Oxford University Press, New York, 1979, p. 240.

15. Por supuesto, desde el punto de vista jurídico existe una importante diferencia entre la negativa a la propia vacunación y la que afecta directamente a personas incompetentes para decidir por sí mismas, cuando es la persona que ha de prestar el consentimiento por representación (padre de un menor, tutor de una persona judicialmente incapacitada) la que se niega a otorgarlo. A este respecto, el Tribunal Europeo de Derechos Humanos ha considerado compatible con el Convenio de Europeo de Derechos Humanos las sanciones y restricciones de derechos derivadas del incumplimiento del deber de vacunar a los hijos (puede consultarse el resumen de esta reciente jurisprudencia en el apunte de CLIMENT DURAN, <https://idibe.org/tribuna/tehd-avala-las-sanciones-restricciones-derechos-derivadas-del-incumplimiento-del-deber-vacunar-los-hijos/>).

16. El mismo derecho fundamental ampara, por descontado, la crítica acerva a las pseudoterapias peligrosas; de ahí que los tribunales hayan desestimado todas las demandas civiles de protección del derecho al honor interpuestas en los últimos años contra distintas voces críticas –periodistas y comunicadores científicos, directivos de la Asociación para Proteger a los Enfermos de Terapias Pseudocientíficas (APETP) o el propio Ministro de Ciencia– por personas o asociaciones –Asamblea Nacional de Homeopatía (ANH), la Asociación de los Profesionales y Autónomos de las Terapias Naturales (COFENAT), la Fundación de Terapias Naturales (FTN) o la Fundación Salud y Naturaleza (FSN)– que las defienden o ejercen. Puede verse, por ejemplo, la STS 57/2020, de 4 de febrero, que desestima una demanda contra el Ministro de Ciencia.

“no necesitará autorización previa”. Así pues, iniciativas que en los últimos años pretenden de la Administración la prohibición de la celebración de encuentros de este tipo están destinadas al fracaso<sup>17</sup>; cosa distinta es que se dirijan a los organizadores advertencias de posibles sanciones administrativas (que como después veremos podrían ser incluso penales) para el caso de que en el curso de dichas actividades se incurriera en concretas conductas prohibidas, como la publicidad engañosa.

Es precisamente en ese campo fronterizo entre la mera difusión de las ideas y la promoción y publicidad de productos y métodos pretendidamente sanitarios donde se sitúa el Real Decreto de 1997 antes citado y también algunas de las líneas de actuación del Plan de 2018 que pretende reformarlo, en concreto para “incluir en la regulación los actos o encuentros” y “la utilización de internet o redes sociales” cuando unos u otra “conlleven la publicación o promoción comercial de productos, actividades o servicios con pretendida finalidad sanitaria”. Como con toda razón apunta Álvarez Rubio<sup>18</sup>, no termina de entenderse, en realidad, qué novedad aportaría la reforma apuntada, pues lo cierto es que las prohibiciones del RD 1907/1996 se formulan con carácter general y por tanto ya abarcan tanto la promoción o publicidad realizada en actos o encuentros como la que tiene lugar a través de internet o en redes; cosa distinta es que cualquier actuación en este último caso enfrenta enormes dificultades desde el punto de vista práctico<sup>19</sup>. En los últimos años, de hecho, la Generalitat de Catalunya ha hecho uso de esta normativa en varias ocasiones para multar a los organizadores de encuentros en los que se ha promocionado el consumo del MSS<sup>20</sup>.

## Responsabilidades penales

El Código penal no contempla expresamente, como delito en sí mismo, la publicidad o la práctica de pseudoterapias, por peligrosas que estas puedan resultar para la salud. Ello no es óbice, sin embargo, para que en ciertos casos puedan resultar aplicables determinadas figuras delictivas: el intrusismo, la estafa, la publicidad de medicamentos y la publicidad engañosa; mayores dificultades plantea en cambio la aplicación de las lesiones o el homicidio.

Repasaremos brevemente las dificultades de encaje que a menudo se plantean y el modo en que las han abordado los tribunales.

**1. Estafa.** El delito de estafa del art. 248 del CP, que en cualquier caso únicamente atiende a una dimensión parcial del problema (la protección del patrimonio), ha sido aplicado en ocasiones a las prácticas que nos ocupan, si bien su encaje no está exento de dificultades.

En primer lugar, la propia idea de “engaño” requiere la conciencia de la falsedad de lo que se afirma, un elemento muy evidente en algunos casos pero no tanto en otros. En segundo término, dado que la estafa se define como utilizar “engaño bastante para producir error en otro, induciéndolo a realizar un acto de disposición en perjuicio propio o ajeno”, el delito no puede aplicarse (según una interpretación muy consolidada) si tal concatenación de sus factores no se produce en el orden descrito; la estafa requiere un engaño que produzca un error y que como consecuencia de este se lleve a cabo la disposición patrimonial, de tal manera que los supuestos conocidos como de “error preexistente” –cuando el luego perjudicado demanda un servicio ya previamente convencido de sus ventajas- no darán lugar al delito (como ocurre, por ejemplo, con quien previamente persuadido de las facultades de un echador de cartas o del sanador por imanes, acude a él y le paga por su servicio). A pesar de esta dificultad inicial, sí es posible sancionar por estafa cuando en momentos posteriores el sujeto “añade” elementos engañosos más allá del inicial convencimiento erróneo de la víctima, que consiguen el pago de cantidades más allá de las iniciales<sup>21</sup>.

La tercera dificultad de aplicación de la estafa en estos contextos tiene que ver con el elemento del engaño “bastante”, que durante al menos unos años había sido interpretado por el Tribunal Supremo en el sentido de excluir la estafa en los engaños muy burdos o extremadamente elementales (en los que se daría una cierta vulneración del deber de autoprotección del propio engañado), que no satisfarían así el requisito de ser “objetivamente bastantes”. Con todo, el propio Tribunal ha ido matizando enormemente esta idea, que él mismo reconoce que no puede servir para excluir la protección de las personas más crédulas por razón de su edad, formación cultural, o circunstancias que las hacen especialmente vulnerables, elementos todos ellos que han de incluirse en el parámetro de la valoración del carácter bastante del engaño. De ahí que (incluso siendo engaños muy burdos) suela condenarse en casos próximos a los que aquí nos interesan (propiamente esoterismo con supuestos

17. <https://www.lavanguardia.com/politica/20180111/434219528876/el-colegio-de-medicos-de-barcelona-pide-prohibir-congreso-contra-oncologos.html>

18. “Problemas (y paradojas) actuales de la regulación publicitaria en el ámbito sanitario”, *LEX* N° 26 - AÑO XVIII - 2020-II, pp. 177-204, pp. 190-191.

19. De hecho, el art. 4 del RD 1907/1996 contiene prohibiciones que se ajustan perfectamente a determinadas pseudoterapias. Así, “queda prohibida cualquier clase de publicidad o promoción directa o indirecta, masiva o individualizada, de productos, materiales, sustancias, energías o métodos con pretendida finalidad sanitaria”, entre otros casos, cuando “se destinen a la prevención, tratamiento o curación de enfermedades transmisibles, cáncer y otras enfermedades tumorales, insomnio, diabetes y otras enfermedades del metabolismo”, o cuando “atribuyan carácter superfluo o pretenda sustituir la utilidad de los medicamentos o productos sanitarios legalmente reconocidos”. Lo mismo sucede con la Ley de Competencia Desleal, cuyo art. 23.3, reputa como desleal, por engañoso, “proclamar, falsamente, que un bien o servicio puede curar enfermedades, disfunciones o malformaciones”.

20. <https://www.lavanguardia.com/local/leida/20181026/452557092851/salud-sanciona-josep-pamies-pseudociencia.html>: [https://elpais.com/sociedad/2018/10/22/actualidad/1540229352\\_723272.html](https://elpais.com/sociedad/2018/10/22/actualidad/1540229352_723272.html)

21. Ver el razonamiento en tal sentido, por ejemplo, en la STS 407/2005 de 23 de marzo.



fines curativos de enfermedades)<sup>22</sup> y que solo se haya absuelto en supuestos de víctimas con cierta formación cultural<sup>23</sup> (e incluso aquí resulta muy discutible, puesto que la especial vulnerabilidad derivada del deseo o incluso la desesperación por recobrar la salud también debe ser tomada en consideración; de hecho, el propio TS así lo hace en otras sentencias en las que se condena por estafa en engaños burdos a pesar de que los enfermos no necesariamente presentan un muy bajo nivel cultural). Junto a ello se sitúan condenas por estafa en prácticas más claramente catalogables como pseudoterapias, revestidas de una pátina de cierta mayor credibilidad<sup>24</sup>. Ha de apuntarse, en cualquier caso, que los supuestos que llegan a los tribunales suelen caracterizarse por un perjuicio patrimonial elevado y son, en todo caso, puntuales.

**2. Publicidad engañosa.** Si la estafa sólo puede entrar en juego cuando la práctica engañosa en cuestión ha tenido lugar y ha producido un efectivo perjuicio patrimonial, el delito de publicidad engañosa puede aplicarse a lo que podríamos considerar el “estadio previo” a la estafa. El art. 282 Cp sanciona con una pena de prisión de seis meses a un año o multa de 12 a 24 meses a los “fabricantes o comerciantes que, en sus ofertas o publicidad de productos o servicios, hagan alegaciones falsas o manifiesten características inciertas sobre los mismos, de modo que puedan causar un perjuicio grave y manifiesto a los consumidores, sin perjuicio de la pena que corresponda aplicar por la comisión de otros delitos”. Se trata en cualquier caso un delito orientado a la protección genérica de los consumidores y sus intereses como tales, y no a la tutela de la salud pública. De hecho, ha sido muy escasamente aplicado en el contexto de las pseudoterapias (quizás, entre otros factores, por la dificultad de encuadrar a quienes las practican en el término legal de “comerciantes”), aunque sí existe alguna sentencia<sup>25</sup>. Quizás una modificación del precepto o la introducción de un subtipo específico concebido expresamente para las pseudoterapias engañosas más peligrosas (en términos similares a los del ya citado Real Decreto 1907/1997, sobre publicación

y promoción comercial de productos, actividades o servicios con pretendida finalidad sanitaria) podría resultar un instrumento adecuado para la intervención penal, pues carece de sentido que se tipifiquen como merecedoras de sanción penal conductas engañosas aptas para poner en peligro los intereses (patrimoniales) de los consumidores y no se haga lo propio con la publicidad de prácticas fraudulentas peligrosas para un bien jurídico de primer rango como es la salud.

**3. Intrusismo.** Esta figura delictiva del art. 403 CP sí ha sido ocasionalmente aplicada a la práctica de pseudoterapias, las más de las veces en concurso de delitos con la estafa (convivencia posible en la medida en que cada uno de los delitos protege un bien jurídico distinto y por tanto sanciona una dimensión diferente de la conducta)<sup>26</sup>. El encaje bajo el intrusismo, definido (en la modalidad que aquí nos interesa) como ejercer “actos propios de una profesión sin poseer el correspondiente título académico expedido o reconocido en España de acuerdo con la legislación vigente”, solo resulta posible, empero, si efectivamente se realizan dichos actos propios de la profesión, reservados por ello a quienes acreditan la formación académica requerida. El núcleo de la cuestión reside, por tanto, en clarificar cuáles son exactamente tales actos en el caso del ejercicio de la medicina (la jurisprudencia los agrupa en “profilaxis”, “diagnóstico” y “tratamiento” de la enfermedad), y en perfilar de qué modo han de llevarse a cabo para que la conducta pueda considerarse intrusiva; en este último sentido, suele considerarse que si se emplea un cauce completamente ajeno a lo científico (diagnosticar mediante adivinación, revisión de la dentadura o empleo de imanes, o tratar a través de la meditación, el masaje o la imposición de manos, por ejemplo) no podrá decirse que se practica un *acto propio* de la profesión<sup>27</sup>. De ahí que los actos del curanderismo más burdo (cercanos al esoterismo o la brujería) no perfeccionen el delito, como tampoco lo hacen per se numerosas técnicas encuadrables en la categoría de las pseudoterapias<sup>28</sup>. Ha de reconocerse,

22. Por ejemplo, STS 831/2015, de 29 diciembre, o STS 833/2016 de 3 noviembre.

23. STS 89/2007 de 2 febrero (los dos engañados por un curandero, que perseguían la curación del cáncer padecido por su padre, eran administrativo y auxiliar de clínica).

24. STS 1612/2002 de 1 abril, conocida por la enorme entidad del caso: médico no especialista en oncología, que se presentaba como tal, y que entre otras conductas vendió a precio elevadísimo a centenares de pacientes un producto no autorizado compuesto de urea y suero fisiológico, supuestamente curativo del cáncer; STS 407/2005, de 23 de marzo, relativa a un sujeto que se presenta como “doctor y especialista en Biocibemética cuántica holográfica y medicina neurofocal” que diagnostica y/o trata enfermedades por, entre otros, el procedimiento de pasar un cilindro por el cuerpo y prescribir la extracción de toda o parte de la dentadura a numerosos pacientes (a los que, además, induce a abandonar el tratamiento convencional), inyectarles procaína o recetarles una “medicación embrionaria”, por todo lo cual cobró importantes cantidades.

25. En nuestro país ha sido aplicado por el Juzgado de lo Penal núm. 18 de Madrid en su sentencia 268/2014, de 4 de julio, en el caso del medicamento Biobac; en Francia es interesante la condena por este delito publicitario dictada en 2015 por el tribunal correccional de Montpellier contra un defensor de la doctrina de la “biología total” (según la cual toda enfermedad, incluido el cáncer, es el resultado de un conflicto interior no resuelto), que inducía a sus seguidores al abandono completo de los tratamientos convencionales (<https://france3-regions.francetvinfo.fr/occitanie/herault/montpellier-metropole/montpellier/fondateur-biologie-totale-condamne-2-ans-ferme-montpellier-844921.html>).

26. Por ejemplo SAP Cádiz 166/2010, de 23 de abril o, muy recientemente, STS 167/2020, de 19 de mayo.

27. En este sentido recientemente, por todos, GONZÁLEZ URIEL, «Medicina alternativa y delito de intrusismo. Algunas consideraciones al hilo de la STS 167/2020», *La Ley Penal* nº 147, nov-dic 2020, p. 10.

28. De ahí la absolución en el caso, de gran repercusión mediática, de un joven estudiante universitario valenciano aquejado de leucemia linfoblástica aguda que acude a un naturópata, el cual, según los hechos probados de la sentencia (37/2018 de 29 enero, del Juzgado de lo Penal núm. 10 de Valencia) se limita a recetarle complementos vitamínicos y una dieta, aconsejándole que consultara con los profesionales su compatibilidad con el tratamiento de quimioterapia.



en cualquier caso, que existe una zona gris en la que no resulta tan sencillo determinar si el método empleado para el diagnóstico (pensemos, por ejemplo, en una entrevista con el paciente sobre sus síntomas) puede considerarse medianamente “científico” y dar por ello lugar al intrusismo.

Una precisión relevante atañe a la prescripción de sustancias: siguiendo la anterior línea de razonamiento, cuando se trate de medicamentos autorizados y que requieran receta médica, nos encontraremos sin duda ante intrusismo (como acto de “tratamiento”); no será así, en cambio, si se prescriben o suministran sustancias no autorizadas (lo que en su caso podrá suponer un delito contra la salud pública de los que trataremos enseguida *infra*), plantas supuestamente medicinales o complementos vitamínicos inocuos.

Conviene tener presente, por otro lado, que el mero uso de términos que implícita o explícitamente remitan a la profesión titulada (“clínica”, “consulta”, “médico osteópata, homeópata, naturópata”, etc.) no realiza por sí mismo el delito (por más que pudieran contribuir al engaño a efectos del delito de estafa), que solo se perfecciona, como se ha explicado, si se realizan los actos propios de la profesión; comprobado esto último, la atribución pública del título que no se posee (“licenciado en medicina”) sí da lugar a un tipo agravado, con un incremento notable de la pena (de la multa del tipo básico se pasa a prisión de seis meses a dos años), la misma que se impone en caso de que la actividad se ejerciere “en un local o establecimiento abierto al público en el que se anunciare la prestación de servicios propios de aquella profesión”<sup>29</sup>.

**4. Delito contra la salud pública en relación a medicamentos.** El art. 362 del Código penal castiga, entre otras conductas, la de fabricar, suministrar, comercializar u ofrecer “medicamentos (...) que carezcan de la necesaria autorización exigida por la ley”, siempre que “con ello se genere un riesgo para la salud o la vida de las personas”; la pena asignada es de seis meses a tres años de prisión, multa e inhabilitación especial. A diferencia de la estafa o la publicidad engañosa, que como se ha señalado atienden al aspecto más patrimonial de la conducta -y en puridad, más secundario a los efectos que aquí interesan-, el art. 362 y los que le siguen sí se orientan claramente al bien jurídico relevante en el contexto de las pseudoterapias (la salud pública), si bien solo puede aplicarse, como es obvio, a los casos en los que de alguna manera se utilicen productos a los que se atribuyen propiedades curativas –cosa que no

sucede, ni mucho menos, en todas las prácticas–.

Nos encontramos ante lo que en Derecho penal se denomina un “delito de peligro”, en el que la sanción de la conducta no requiere la producción de un resultado lesivo (lesiones, muerte); la razón de la sanción se encuentra, ya en un momento previo, en su peligrosidad para el bien jurídico. Tal peligrosidad, eso sí, ha de resultar probada, como elemento del delito que es -precisamente en ella reside, además, la frontera entre el ilícito meramente administrativo y el penal, pues si comercializar un producto no autorizado por las autoridades sanitarias competentes siempre constituye ilícito sancionable por la Administración, hacerlo cuando con ello se pone en riesgo la salud de sus eventuales consumidores se considera ya merecedor de sanción penal. Precisamente esta falta de peligrosidad explica las absoluciones recaídas en casos en que se trataba de sustancias inocuas<sup>30</sup>; ello no es óbice, sin embargo, para que sí se aprecie el delito cuando, aun tratándose de una sustancia inocua per se, se suministre de modo que *sustituya* el tratamiento convencional, que sería donde residiría la verdadera peligrosidad para la salud<sup>31</sup>.

**5. Lesiones u homicidio.** En los casos de prácticas de pseudoterapias que han llegado a los tribunales penales en nuestro país son absolutamente infrecuentes las acusaciones por delitos de lesiones u homicidio. Existen, sin embargo, supuestos en los que podría sostenerse la responsabilidad por tales resultados (no entraremos aquí en si a título de mera imprudencia o de dolo eventual), como sucede si se prescriben o venden productos con efectos perjudiciales probados; el que el paciente los hubiera ingerido voluntariamente no impediría la imputación si lo hizo desconociendo sus efectos adversos. Tampoco ofrecerían dificultades los casos de suministro de sustancias dañinas a pacientes incompetentes para decidir, como menores con trastorno autista. Por otra parte, en la jurisprudencia existe algún caso puntual en el que resulta del todo sorprendente que la Fiscalía no acusara por lesiones; me refiero en concreto al caso antes citado de la STS 407/2005, de 23 de marzo, en el que se llegaron a producir extracciones de toda la dentadura a algunos pacientes. Ha de recordarse que el nuestro ordenamiento penal el hecho de que las lesiones sean consentidas solo supone una atenuación de la pena según el art. 155 Cp, y en casos como este, a mi juicio, ni siquiera tal cosa, puesto que el consentimiento solo despliega tales efectos atenuatorios si es emitido de forma “válida” y “libre”, lo que entiendo que no sucede

29. No da lugar a intrusismo, en cambio, la realización de los actos propios de una determinada especialidad médica que no se posee, si se acredita la titulación básica (razón por la cual se absuelve de intrusismo en la ya citada STS 1612/2002 de 1 abril: el sujeto, que se presentaba como oncólogo sin serlo, sí era licenciado en medicina).

30. Así por ejemplo en el conocido como caso Acobiomol, juzgado por la SAP Cádiz 166/2010, de 23 de abril (sí se condenó, en cambio, por estafa e intrusismo).

31. El TS dejó clara esta interpretación en la ya citada STS 1612/2002, de 1 de abril; en aplicación de esta idea, el Juzgado de lo Penal núm. 18 de Madrid en su sentencia 268/2014, de 4 de julio, que entendió del conocido como caso *Biobac* absuelve del delito por entender que, además de ser inocuo, el producto no se suministró como sustitutivo de las terapias convencionales.

cuando el sujeto actúa bajo engaño pensando que tales lesiones van a conseguir curarle de otras afecciones.

El problema más complejo de calificación penal se presenta en los supuestos en los que la responsabilidad por lesiones u homicidio no pretende derivarse de un método o producto lesivo per se, sino del hecho de haberse inducido al abandono del tratamiento convencional. Esto fue precisamente lo que sucedió, según los hechos probados de la sentencia, en el supuesto enjuiciado por la STS 1612/2002, en el que, además de proporcionarse un producto en principio inocuo a cientos de pacientes oncológicos, se les indujo al abandono de cualquier otro tratamiento; a pesar de que según la sentencia, la gran mayoría de aquellos habían fallecido, ni Fiscalía ni la acusación particular acusaron por homicidio, lo que impidió al Tribunal Supremo pronunciarse sobre ello (aunque sí tuvo en cuenta el factor mencionado para sancionar por delito contra la salud pública). Más de quince años después, dicha acusación sí se formula (por el padre del joven fallecido) en el caso resuelto por el JP núm. 10 Valencia 37/2018, 29 enero, que en cambio absuelve de estos cargos al no haber resultado probado que se indujera a dicho abandono<sup>32</sup>.

Lo cierto es, en cualquier caso, que incluso habiéndose probado la inducción al abandono de la medicación o el tratamiento, la imputación de responsabilidad por el resultado de muerte se enfrenta a múltiples dificultades. No resultará sencillo probar, para empezar, hasta qué punto este abandono incidió en el curso de una enfermedad que ya de entrada podía encontrarse muy

avanzada; por otra parte, en un sistema penal como el español, en el que no se sanciona el inducir a otra persona a una conducta peligrosa para su salud y ni siquiera el inducir a una autolesión (solo se pena la inducción al suicidio en el art. 143.1 del Código penal), la única manera de imputar el resultado de muerte al responsable de la pseudoterapia pasaría por considerar que el paciente se encontraba absoluta y totalmente engañado, que era un mero instrumento ciego en manos de quien le manipulaba por completo. No es fácil trazar la frontera entre esta situación y aquella otra en la que el paciente, habiendo sido informado por sus médicos de lo absurdo y peligroso de su decisión, simplemente elige no creerles –esto es, asume libremente lo que otros le han intentado hacer ver que es una conducta peligrosa para su salud–; y ello explica quizás la reticencia de la Fiscalía a acusar por homicidio o lesiones incluso en estos casos que intuitivamente se perciben como muy graves.

## Conclusiones

Este breve repaso ha permitido mostrar los instrumentos legales con los que ya cuentan los poderes públicos de nuestro país para hacer frente a las pseudoterapias que representan un mayor peligro para la salud de los ciudadanos. Un arsenal normativo quizás incompleto y siempre limitado por determinadas exigencias constitucionales, pero que probablemente podría ser aprovechado y rendir más frutos de los que hasta el momento ha permitido cosechar.

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32. Se considera probado, de hecho, lo contrario: que el acusado recomendó al paciente la continuación de la quimio, y que consultara con sus médicos la compatibilidad entre esta y los suplementos vitamínicos aconsejados (consulta que aparece reflejada en la historia clínica). La sentencia insiste en que el joven paciente ya era muy reacio a someterse a quimioterapia antes de acudir a los servicios del acusado, entre otros factores por haber recibido dicha influencia en su propia familia.

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1. Sobre la importancia de la terminología en la construcción del discurso sobre el tema, con un recorrido sobre su utilización en la esfera de las políticas públicas en los últimos años en España, LOPERA PAREJA, «El debate político sobre las terapias alternativas y complementarias en España en la interfaz entre ciencia, política y sociedad (1979-2018)», *Perspectivas de la Comunicación* 2019, Vol 12, Nº 2, pp. 155-193.

1. 20181108 Plan Protección frente pseudoterapias VF

1. Plan Nacional 2018, p. 3.

1. La imbricación de la falsa información con el aspecto conspirativo las hace especialmente difíciles de contrarrestar, puesto que como muy bien explican SUNSTEIN y VERMEULE ("Conspiracy Theories", *University of Chicago Public Law & Legal Theory Working Paper* No. 199, 2008) las teorías conspirativas son en esencia autoconfirmatorias (los intentos de desmontarlas por los poderes públicos son percibidos y presentados precisamente como una persecución y por ende la confirmación de su acierto).

1. «Preying on Public Fears and Anxieties in a Pandemic: Businesses Selling Unproven and Unlicensed "Stem Cell Treatments" for COVID-19», *Cell Stem Cell* 26, June 4, 2020.

# Cyto-histological correlation in cervical cytology

*Correlación cito-histológica en la citología cervical*

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## Abstract

The results obtained with the use of a follow-up sheet of cytological results sent to the clinicians responsible for the patients are presented. 81% of responses have been obtained, which has allowed verifying that the rates of final histological diagnoses have maintained an excellent correlation with the initial cytological results, especially in the essential diagnosis, the high-grade intraepithelial lesion of the cervix, a 67% specificity, a percentage within the expected range.

**Keywords:** Cytology, quality.

## Resumen

Se presentan los resultados obtenidos con la utilización de una hoja de seguimiento de resultados citológicos enviada a los clínicos responsables de las pacientes. Se ha obtenido un 81% de respuestas, lo que ha permitido comprobar que las tasas de diagnósticos histológicos finales han mantenido una excelente correlación con los resultados citológicos iniciales, especialmente en el diagnóstico esencial, la lesión intraepitelial de alto grado del cuello uterino, una especificidad del 67%, un porcentaje dentro de lo esperado.

**Palabras clave:** Citología, calidad.

## Introduction

Since its description by GN Papanicolaou<sup>1</sup>, cervical cytology (CvC) has played a decisive role in the prevention of cervical cancer (CC). The impacts on its incidence and mortality have been attributed to the systematic application of cytology from first well-executed and documented experiences, especially the so-called Walton Report<sup>2</sup>, which laid the groundwork for the first cytological population screening to prevent CC. Subsequently and to this day, its use has continued applying well-established protocols agreed upon by the main regulatory entities, supranational<sup>3</sup> or national<sup>4</sup>.

The fundamental objective of CVC is to enable the histological diagnosis of pre-cancerous lesions of the cervix. Knowing well the natural history of CC makes it possible. In this regard, it is necessary to clarify important differences related to squamous cell carcinoma and adenocarcinoma, the two most common histological forms of CC, in a ratio of 8 to 2 in terms of frequency<sup>5</sup>. The LAST report<sup>6</sup> established the idea of a binary classification of squamous pre-cancerous

lesions, low and high grade lesions, suppressing the "moderate" ones, a source of diagnostic problems and, consequently, therapeutic ones<sup>7</sup>. This is the dichotomous classification in use in the healthcare environment that we share, and is, consequently, the one used in this work, always bearing in mind that the low grade represents the histological response to HPV infection<sup>8</sup>, with little capacity for progression to a high-grade, authentic pre-neoplastic lesion. On the other hand, we do not know well the natural history of cervical adenocarcinoma. We know that in a high percentage it also responds to the infectious aggression of HPV 5 but we have not been able to safely identify its other possible primary causes or its pre-neoplastic phases, if there are<sup>9</sup>, leading to all of this to an ineffectiveness of the CvC in detecting adenocarcinomas<sup>10</sup>, causing that, as we have known for some years<sup>11</sup>, the decrease that in the population registries of tumors is registered of the CC, thanks to the population-based screening programs - not the opportunists<sup>12</sup> - affects only to the squamous variety.

Figure 1

<b>FOLLOW-UP SHEET</b>			DOCTOR:
NAME			AGE:
			CLINIC HISTORY:
CYTOLOGICAL			RESULT:
			DATE:
COLPOSCOPY*			RESULT:
(NO)	(YES)		
BIOPSY			RESULT:
(NO)	(YES)		
HPV DETERMINATION			RESULT:
(NO)	(YES)		

\* IFCCP Colposcopy Nomenclature. Available on <https://ifcpc.org/medical-professionals/ifcpc-nome>

This high effectiveness of CvC has been unequivocally related with the quality of the cytological take, it means the existence of a good representation of the endocervical and / or metaplastic glandular epithelia, a recognized marker of the quality of the cell sample taken<sup>13</sup>.

In the Laboratory where the authors work, an action has been judged of great interest and has been introduced into our quality control protocols of our work: monitoring the follow-up of reports issued as positive to some degree –following the conceptual terminology recommended by the Bethesda System<sup>14</sup>– by referring a file to the clinician responsible for the patient (**Figure 1**), with a double objective: to facilitate him the follow-up of the case and avoid its loss, and at the same time obtain from him the information about its final resolution in order to validate the quality of our reports, thus allowing the proper closure of the case.

In this article we present and discuss the results of this control procedure that were obtained between November 2019 and November 2020.

## Material and method

Between November 30, 2019 and November 30, 2020, our Laboratory issued 17,731 CVC reports. The distribution of anomalous results was as follows during this period:

• AGC	11	0.06%
• ASC-H	19	0.11%
• ASC-US	49	0.27%
• LG-SIL	71	0.40%
• HG-SIL	31	0.17%
• Ca	0	
Total	181	1.01%

AGC: Atypia in Glandular Cells

ASC-H: Atypia in Squamous Cells, intraepithelial lesion is not ruled out

ASC-US: Atypia in Squamous Cells of Uncertain Significance

LG-SIL: Low Grade Squamous Intraepithelial Lesion

HG-SIL: High Grade Squamous Intraepithelial Lesion

Ca: Carcinoma

Consequently, 181 follow-up files have been sent to the gynecologists.

## Results

147 responses out of 181 files sent have been received: 81.2% responses, according to the following distribution by initial cytological results:

- AGC 9 of 11
- ASC-H 13 of 19
- ASC-US 41 of 49
- LG-SIL 58 of 70
- HG-SIL 27 of 31

In the cases reported as ASC-US, following recommended clinical procedures 4, to perform an HPV determination was recommended. In the cases reported as ASC-US / Human Papilloma Virus (HPV) (+) - 15 out of 41, a 36.6% - or as AGC, ASC-H, LG-SIL and HG-SIL, it was recommended to perform colposcopy<sup>4</sup>.

According to the information contained in the files received, biopsies were taken in 73 cases, distributed as follows by initial cytological results:

- AGC 5 of 9
- ASC-H 11 of 13
- ASC-US/HPV + 13 of 15
- LG-SIL 21 of 58
- HG-SIL 23 of 27

That is, 73 biopsies taken out of a total of 122 positive cytology follow-up reports received.

The final histological results, distributed by initial cytological result, have been the following:

- AGC: 5 biopsies taken 2 Adenocarcinoma  
2 Chronic Cervicitis  
1 Endocervical Hyperplasia
- ASC-H: 11 biopsies taken 6 HG-SIL  
2 LG-SIL  
1 Chronic Cervicitis  
2 Squamous Metaplasia
- ASC-US/HPV+: 13 biopsies taken 1 HG-SIL  
8 LG-SIL  
3 Squamous Metaplasia  
1 Chronic Cervicitis
- LG-SIL: 21 biopsies taken 3 HG-SIL  
16 LG-SIL  
2 Chronic Cervicitis
- HG-SIL: 23 biopsies taken 1 Squamous Carcinoma  
20 HG-SIL  
2 LG-SIL

## Discussion

A 1,01% of abnormal cytological results (ACR) is a low percentage if we relate it to a historical reference, not subsequently modified, which evaluated the rate of ACR in a sample carried out at 14 reference Spanish Cytology Laboratories<sup>15</sup>, with a sample of 409,443 cytological results that contained 3,56% abnormal results. The reason is in all probability that our Laboratory receives samples from annual reviews, not triennial reviews, as recommended by the Spanish Gynecology Society<sup>12</sup>. Annual review yes, but of gynecological health, including all its aspects; It is even possible that certain situations of gynecological pathology require more frequent controls, but the annual preventive control of cervical cancer represents an unjustified over-control<sup>16</sup>. We believe that this explanation is at the base of the low percentage of cytological positivities that we are here reporting.

We have received 81% of responses to the follow-up files sent, a percentage that we believe can be considered highly satisfactory and which, in our opinion, indicates the interest that this control initiative has for clinicians who send cytological samples to our Laboratory. This level of response obliges us to continue in this line of collaboration.

73 biopsies have been taken from among the 122 cases of positive cytologies in some degree, 60%, related with complete certainty to the percentage of positive colposcopies carried out from the cytological results, following the recommendations formulated by the Scientific Societies and established in good clinical practice<sup>12</sup>. It is a percentage that can be considered within expectations: in a cervical pathology unit the rate of cervical biopsies is around this figure<sup>17</sup>. As has been reflected when previously we have facilitated the results of our work, by taking these 73 biopsies the following lesions have been diagnosed:

- 28 LG-SIL
- 30 HG-SIL
- 2 Adenocarcinoma
- 1 Squamous carcinoma

In addition to:

- 1 Endocervical Hyperplasia
- 6 Squamous Metaplasia
- 6 Chronic Cervicitis

Analyzing each of these results in terms of the specificity of our cytological results, we can deduce the following comments:

➤ 28 cases of LG-SIL have been diagnosed, based on the following cytological results:



- > 2 cases of ASC-H
- > 8 cases of ASC-US / HPV +
- > 16 cases of LG-SIL
- > 2 cases of HG-SIL

Consequently, the specificity for this final diagnosis has been 57%, an acceptable figure 4, since we are talking about very initial cytological modifications that can overlap with indeterminate atypia, as in our case, 10 out of 28.

> The 30 cases of histological HG-SIL finally diagnosed arise from:

- > 1 case of ASC-US / HPV +
- > 3 cases of LG-SIL
- > 20 cases of HG-SIL

Consequently, the specificity for this final diagnosis has been 67%, absolutely within the expected range<sup>4</sup>. The cytological result of ASC-H has been the starting point to reach the diagnosis of HG-SIL in 6 cases, an expected situation given the cytological characteristics of this result<sup>14</sup>.

We think it is interesting to comment that, as specified, 27 HG-SIL cytological results were issued, but 23 biopsies were taken, since in two cases the colposcopy showed grade 1 changes<sup>18</sup>, the clinician deciding not to take a biopsy, as was the case. It occurred in the other two cases, with grade 2 changes<sup>18</sup> in women under 25 years of age in whom strict follow-up of the case was decided, following current recommendations<sup>4</sup>.

> We note that two cases of adenocarcinoma have been diagnosed based on AGC results, of which five

were issued, with also two cases of chronic cervicitis and one case of endocervical hyperplasia as final results. Having detected these two cases of adenocarcinomas represents good news, even if it is based on a lower-range cytological result.

> In the set of the cyto-histological correlation that we are presenting and commenting on, there is a cytological over-diagnosis in thirteen cases. Chronic cervicitis can cause cytological changes that are difficult to demarcate with intraepithelial lesions, as well with a very active initial processes of cervical re-epithelialization, squamous metaplasia. In the first cases and based on these results presented here, requesting post-treatment cyto-colposcopic control of cervicitis that we initially report is a procedure that we follow in our Laboratory, always supported by a personalized contact with the responsible clinician, commenting on the case.

## Conclusions

A follow-up card for cytological results shared with the clinician responsible for the patient has shown its usefulness to establish cyto-histological correlations. This evaluation has shown that the issued results corresponded with the expected cervical intraepithelial lesions diagnostic rates.

Establishing mechanisms for monitoring and controlling the cytological reports issued by a Cytology Laboratory is an essential mechanism to assess the quality of its work and to establish the necessary collaboration with the clinical in charge of the patients.

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# Genotypic and phenotypic assessment of antibiotic resistance of MRSA bacteria isolated from food stuffs

*Evaluación genotípica y fenotípica de la resistencia a los antibióticos de bacterias MRSA aisladas de alimentos*

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## Abstract

**Background:** Methicillin-Resistant *Staphylococcus aureus* (MRSA) bacteria are one of the chief causes of food-borne diseases. This survey was done to assess the genotypic and phenotypic profiles of antibiotic resistance of MRSA isolates of foodstuffs.

**Methods:** Forty MRSA strains isolated from foodstuffs were selected. Isolates were approved using the biochemical tests and also cefoxithin and oxacillin susceptibility testing. MRSA isolates were phenotypically assessed by the disk diffusion. DNA was extracted from the MRSA isolates and subjected to PCR identification of antibiotic resistance genes.

**Results:** MRSA bacteria harbored the high prevalence of resistance toward penicillin (100%), tetracycline (95.00%), erythromycin (90.00%), and gentamicin (87.50%) antibiotics. *AacA-D* (55.00%), *ermA* (50.00%), and *tetK* (50.00%) had the highest distributions amongst examined antibiotic resistance genes.

**Conclusion:** Foodstuffs may be sources of resistant-MRSA, which pose a hygienic threat concerning their consumption. Nevertheless, further investigations are compulsory to understand additional epidemiological and molecular features of MRSA in foodstuffs.

**Keywords:** Methicillin-resistant *Staphylococcus aureus*, phenotype, genotype, antibiotic resistance, foodstuffs.

## Resumen

**Antecedentes:** La bacteria *Staphylococcus aureus* resistente a la meticilina (SARM) es una de las principales causas de enfermedades transmitidas por los alimentos. Este estudio se realizó para evaluar los perfiles genotípicos y fenotípicos de la resistencia a los antibióticos de los aislados de SARM de los alimentos.

**Métodos:** Se seleccionaron 40 cepas de SARM aisladas de productos alimenticios. Los aislados se aprobaron mediante pruebas bioquímicas y también de susceptibilidad a la cefoxitina y la oxacilina. Los aislados de SARM se evaluaron fenotípicamente mediante la difusión en disco. Se extrajo el ADN de los aislados de SARM y se sometió a la identificación por PCR de los genes de resistencia a los antibióticos.

**Resultados:** Las bacterias MRSA presentaron una alta prevalencia de resistencia a los antibióticos penicilina (100%), tetraciclina (95,00%), eritromicina (90,00%) y gentamicina (87,50%). *AacA-D* (55,00%), *ermA* (50,00%) y *tetK* (50,00%) tuvieron las distribuciones más altas entre los genes de resistencia a los antibióticos examinados.

**Conclusión:** Los productos alimenticios pueden ser fuentes de SARM resistentes, lo que supone una amenaza higiénica en relación con su consumo. No obstante, es necesario realizar más investigaciones para conocer otras características epidemiológicas y moleculares del SARM en los alimentos.

**Palabras clave:** *Staphylococcus aureus* resistente a la meticilina, fenotipo, genotipo, resistencia a los antibióticos, productos alimenticios.

## Introduction

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a Gram-positive and catalase-positive bacterium and common phenomena of the *S. aureus* bacteria resistant to penicillins and cephalosporins<sup>1</sup>. The bacterium is responsible for severe cases of human clinical infections, including respiratory tract, urinary tract, blood, soft tissues, wound, burn and cerebrospinal infections with high distribution amongst hospitalized patients<sup>2, 3</sup>. It is responsible for about 100,000 infectious cases and 20-30% mortality in the United States<sup>4</sup>. MRSA bacteria are also substantial causes of food-borne diseases known with several clinical signs, particularly short incubation period and weakness vomiting, nausea, abdominal cramps, and toxic shock syndrome<sup>5, 6</sup>.

MRSA bacteria caused more complicated diseases for a more extended period compared to other bacteria. Most of their isolates harboured complete resistance toward diverse classes of antibiotic agents, particularly fluoroquinolones, aminoglycosides, tetracyclines, macrolides, cephalosporins, penicillins, and carbapenems<sup>7</sup>. Genetically, diverse antibiotic resistance genes are mainly responsible for the occurrence of antibiotic resistance in MRSA bacteria. *TetK*, *ermA*, *gyrA*, *dfiA*, *rpoB*, and *aacA-D* were considered as the most important antibiotic resistance genes in the MRSA isolates<sup>8</sup>.

Rendering the high MRSA importance as a food-borne pathogen, the present survey was performed to assess the genotypic and phenotypic patterns of resistance of MRSA bacteria isolated from numerous ready-to-eat food samples.

## Materials and methods

### MRSA isolates

The present research was performed according to the Canadian Guidelines of Research Ethics in Medical Sciences. Through the summer of 2020, 40 MRSA isolates of numerous food samples were selected for this survey. Isolates were identified using Gram staining, hemolytic activity on sheep blood agar (Merck, Germany), catalase test, coagulase test (rabbit plasma), oxidase test, OF glucose test, bacitracin sensitivity test (0.04 U), mannitol fermentation on Mannitol salt agar (Merck, Germany), urease activity, nitrate reduction, phosphatase, deoxyribonuclease (DNase, Merck, Germany) test, Voges-Proskauer (VP) (Merck, Germany) test and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation tests<sup>9</sup>. MRSA identification was performed using ceftioxin and oxacillin susceptibility testing<sup>10</sup>.

### Phenotypically survey of antibiotic resistance

Mueller-Hinton agar (Merck, Germany) and principles

of the Clinical Laboratory Standard Institute (CLSI) were applied for this purpose<sup>11</sup>. Phenotypic resistance of MRSA bacteria was assessed toward gentamicin (10 µg/disk), ciprofloxacin (5 µg/disk), clindamycin (2 µg/disk), erythromycin (15 µg/disk), penicillin (10 µg/disk), and tetracycline (30 µg/disk) (Oxoid, UK)<sup>12, 13</sup>.

### Genotypically survey of antibiotic resistance

DNA was extracted from MRSA isolates of food samples using DNA extraction kit (Thermo Fisher Scientific, St. Leon-Rot, Germany)<sup>14-16</sup>. Quality and quantity of extracted DNA were then checked<sup>17-19</sup>. Antibiotic resistance genes were detected using the Polymerase Chain Reaction (PCR) (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany). **Table I** shows the PCR circumstances for antibiotic resistance genes detection<sup>20-22</sup>. Electrophoresis was done according to standard procedure<sup>23, 24</sup>.

## Results

All 40 MRSA isolates were assessed by the disk diffusion and PCR for phenotypically and genotypically assessment of antibiotic resistance, respectively. **Table II** shows the phenotypic resistance pattern of MRSA isolates. Disk diffusion showed that MRSA bacteria harbored the high prevalence of resistance toward penicillin (100%), tetracycline (95.00%), erythromycin (90.00%), and gentamicin (87.50%) antibiotics. The lowest resistance rate was obtained against ciprofloxacin (62.50%) and clindamycin (50.00%).

**Table III** shows the genotypic resistance pattern of MRSA isolates. *AacA-D* (55.00%), **ermA** (50.00%), and *tetK* (50.00%) had the highest distributions. However, *gyrA* (25.00%) and *rpoB* (30.00%) had the lowest distribution.

**Table IV** shows the simultaneous presence of antibiotic resistance encoding genes amongst the examined MRSA isolates. Majority of isolates harboured *aacA-D* + *ermA* (25.00%), *aacA-D* + *tetK* (25.00%), and *aacA-D* + *rpoB* (25.00%) antibiotic resistance encoding genes.

## Discussion

MRSA is a nosocomial pathogen with both healthcare and community sources. It is also considered as a foodborne pathogen responsible for severe morbidity globally<sup>25</sup>. Contaminated food stuffs, especially those with animal origin, are determined as one of the likely causes of MRSA transmission to human<sup>26</sup>. Thus, MRSA in food stuffs has two paramount importance: food-borne diseases and transmission of antibiotic resistance.

The present survey was addressed the MRSA genotypic and phenotypic patterns of antibiotic resistance. MRSA

isolates of foodstuffs harboured the maximum resistance rate against penicillin (100%), tetracycline (95.00%), erythromycin (90.00%), and gentamicin (87.50%). Additionally, they harboured *aacA-D* (55.00%), *ermA* (50.00%), and *tetK* (50.00%) antibiotic resistance genes. Thus, phenotypic pattern of resistance was approved by the genotypic pattern. Irregular and indiscriminate antibiotic prescription are the main reasons for the high prevalence of resistance amid the MRSA isolates. Similarly, high MRSA resistance rate toward penicillin, tetracycline, erythromycin, and gentamicin was also reported previously. Safarpour Dehkordi et al. (2017)<sup>27</sup> reported that the *S. aureus* resistance rate toward penicillin, ceftaroline, gentamicin, amikacin, kanamycin, azithromycin, erythromycin, tetracycline, doxycycline, ciprofloxacin, levofloxacin, clindamycin, trimethoprim-sulfamethoxazole, chloramphenicol and rifampin antibiotic agents were 100%, 10%, 81.08%, 70.27%, 43.24%, 59.45%, 86.48%, 100%, 81.08%, 48.64%, 43.24%, 48.64%, 83.78%, 29.72%, and 35.13%, respectively. They also showed that the distribution of *aacA-D*, *tetK*, *tetM*, *msrA*, *ermA*, *ermC*, and *linA* antibiotic resistance genes were 62.16%, 72.97%, 27.02%, 64.86%, 72.97%, 27.02%, and 43.24%, respectively. Findings of the present survey and those of Safarpour Dehkordi et al. (2017)<sup>27</sup> were similar to those of Fowoyo and Ogunbanwo (2017)<sup>28</sup> and Akanbi et al.

(2017)<sup>29</sup>. High distribution of antibiotic resistance genes was also reported in surveys conducted in Algeria<sup>30</sup>, China<sup>31</sup>, and Egypt<sup>32</sup>.

Rendering our results, only some of the MRSA bacteria, which harboured resistance to a particular group or a specific antibiotic carried the specific antibiotic resistance encoding gene to that particular antibiotic agent. This matter also found for other antibiotic agents and resistance genes. This finding is maybe because the presence of antibiotic resistance genes is only one of the known procedures for the occurrence of antibiotic resistance in bacteria<sup>33-35</sup>.

## Conclusion

In conclusion, MRSA isolates of foodstuffs harboured high prevalence of resistance and distribution of antibiotic resistance genes. This finding may show the role of food stuffs in the transmission of MRSA bacteria to human populations. Using high-quality raw materials, proper hygienic circumstances in food processing, adequate cooking of food samples, cross-contamination prevention, and antibiotic prescription rendering the outcomes of disk diffusion can diminish the risk of MRSA.

**Table 1:** Primers and following conditions of the PCR<sup>20</sup>.

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50µL)
<i>AacA-D</i>	F: TAATCCAAGAGCAATAAGGGC R: GCCACACTATCATAACCCACTA	227	1 cycle: 94 °C ----- 5 min. 25 cycle: 94 °C ----- 60 s 55 °C ----- 70 s 72 °C ----- 60 s	5 µL PCR buffer 10X 1.5 mM MgCl <sub>2</sub> 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.25 U Taq DNA polymerase (Fermentas) 2.5 µL DNA template
<i>ermA</i>	F: AAGCGGTAACCCCTCTGA R: TTCGCAATCCCTTCTCAAC	190	1 cycle: 72 °C ----- 10 min	
<i>tetK</i>	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360	1 cycle: 94 °C ----- 10 min. 25 cycle: 94 °C ----- 20 s 52 °C ----- 20 s 72 °C ----- 50 s 1 cycle: 72 °C ----- 5 min	
<i>gyrA</i>	F: AATGAACAAGGTATGACACC R: TACGCGCTTCTAGTATAACGC	223	1 cycle: 94 °C ----- 2 min. 30 cycle: 94 °C ----- 60 s 50 °C ----- 60 s 72 °C ----- 60 s 1 cycle: 72 °C ----- 5 min	5 µL PCR buffer 10X 2 mM MgCl <sub>2</sub> 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
<i>dfpA</i>	F: CTCACGATAACAAGAGTCA R: CAATCATTGCTTCGTATAACG	201	1 cycle: 94 °C ----- 5 min. 32 cycle: 94 °C ----- 60 s 56 °C ----- 45 s 72 °C ----- 60 s 1 cycle: 72 °C ----- 10 min	5 µL PCR buffer 10X 2 mM MgCl <sub>2</sub> 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
<i>rpoB</i>	F: ACCGTCGTTTACGTTCTGTA R: TCAGTGATAGCATGTGTATC	460	1 cycle: 94 °C ----- 5 min. 32 cycle: 94 °C ----- 60 s 56 °C ----- 45 s 72 °C ----- 60 s 1 cycle: 72 °C ----- 10 min	5 µL PCR buffer 10X 2 mM MgCl <sub>2</sub> 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template

**Table II:** Phenotypic resistance pattern of MRSA isolates.

N. MRSA Isolates	N (%) isolates resistant to each antibiotic					
	P10*	Gen	Ert	Tet	Cip	Clin
Food stuffs (40)	40 (100)	35 (87.50)	36 (90.00)	38 (95.00)	25 (62.50)	20 (50.00)

\*P10: penicillin (10 µg/disk), Gen: gentamicin (10 µg/disk), Ert: erythromycin (15 µg/disk), Tet: tetracycline (30 µg/disk), Cip: ciprofloxacin (5 µg/disk), Clin: clindamycin (2 µg/disk).

**Table III:** Genotypic resistance pattern of MRSA isolates.

N. MRSA Isolates	N (%) isolates harbored each antibiotic resistance genes					
	<i>aacA-D</i>	<i>ermA</i>	<i>tetK</i>	<i>gyrA</i>	<i>dfrA</i>	<i>rpoB</i>
Food stuffs (40)	22 (55.00)	20 (50.00)	20 (50.00)	10 (25.00)	15 (37.50)	12 (30.00)

**Table IV:** Simultaneous presence of antibiotic resistance encoding genes amongst the examined MRSA isolates.

N. MRSA Isolates	N (%) isolates harbored each antibiotic resistance genes										
	<i>aacA-D</i> + <i>ermA</i>	<i>aacA-D</i> + <i>tetK</i>	<i>aacA-D</i> + <i>gyrA</i>	<i>aacA-D</i> + <i>dfrA1</i>	<i>aacA-D</i> + <i>rpoB</i>	<i>ermA</i> + <i>tetK</i>	<i>ermA</i> + <i>gyrA</i>	<i>ermA</i> + <i>dfrA1</i>	<i>ermA</i> + <i>rpoB</i>	<i>tetK</i> + <i>gyrA</i>	<i>tetK</i> + <i>dfrA1</i>
Food stuffs (40)	10 (25.00)	10 (25.00)	5 (12.50)	8 (20.00)	6 (15.00)	10 (25.00)	5 (12.50)	7 (17.50)	6 (15.00)	6 (15.00)	8 (20.00)

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## ORIGINAL

# Antibiotic resistance pattern of methicillin-resistant *Staphylococcus aureus* isolates from powdered packaged medicinal plants and bottle herbal distillates

*Patrón de resistencia a los antibióticos de los Staphylococcus aureus aislados resistentes a la meticilina procedentes de plantas medicinales envasadas en polvo y destilados de hierbas en botella*

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## Abstract

**Background:** Human involvement in the medicinal plant's and herbal distillate's processing caused a potential risk of microbial contamination, particularly with *Staphylococcus aureus*. The present research was performed to assess the prevalence and antibiotic resistance of methicillin-resistant *S. aureus* bacteria isolated from diverse kinds of powdered packaged medicinal plant and bottle herbal distillate samples.

**Methods:** Three-hundred different powdered packaged medicinal plant and bottle herbal distillate samples were collected and examined by the culture method. MRSA strains were identified using oxacillin and cefoxitin disk diffusion. The pattern of antibiotic resistance of MRSA isolates was examined using disk diffusion.

**Results:** Eighteen out of 300 (6.00%) examined samples were contaminated with MRSA. *Rosa damascene* medicinal plant (13.33%) and *Alhagi maurorum* bottle distillate (10%) had the highest MRSA bacteria contamination rate. Bottle herbal distillate samples had a higher contamination rate of MRSA ( $P < 0.05$ ). MRSA isolates harbored the highest prevalence of resistance toward trimethoprim-sulfamethoxazole (100%), cefixime (100%), ampicillin (100%), tetracycline (88.88%), erythromycin (88.88%), and rifampin (83.33%) antibiotic agents.

**Conclusion:** Medicinal plants and herbal distillates were potential sources of antibiotic-resistant-MRSA bacteria.

**Keywords:** Medicinal plants, herbal distillates, methicillin-resistant *Staphylococcus aureus*, antibiotic resistance.

## Resumen

**Antecedentes:** La participación del hombre en el procesamiento de plantas medicinales y destilados de hierbas provocó un riesgo potencial de contaminación microbiana, en particular con *Staphylococcus aureus*. La presente investigación se llevó a cabo para evaluar la prevalencia y la resistencia a los antibióticos de las bacterias *S. aureus* resistentes a la meticilina aisladas de diversos tipos de muestras de plantas medicinales envasadas en polvo y de destilados de hierbas en botella.

**Métodos:** Se recogieron 300 muestras diferentes de plantas medicinales envasadas en polvo y de destilados de hierbas en botella y se examinaron mediante el método de cultivo. Las cepas de SARM se identificaron mediante difusión en disco de oxacilina y cefoxitina. El patrón de resistencia a los antibióticos de los aislados de SARM se examinó mediante difusión en disco.

**Resultados:** Dieciocho de las 300 (6,00%) muestras examinadas estaban contaminadas con SARM. La planta medicinal *Rosa damascena* (13,33%) y el destilado de botella *Alhagi maurorum* (10%) presentaron el mayor índice de contaminación por bacterias SARM. Las muestras de destilado de botella de hierbas tenían un mayor índice de contaminación por SARM ( $P < 0,05$ ). Los aislados de SARM presentaron la mayor prevalencia de resistencia a los agentes antibióticos trimetoprim-sulfametoxazol (100%), cefixima (100%), ampicilina (100%), tetraciclina (88,88%), eritromicina (88,88%) y rifampicina (83,33%).

**Conclusión:** Las plantas medicinales y los destilados de hierbas fueron fuentes potenciales de bacterias SARM resistentes a los antibióticos.

**Palabras clave:** Plantas medicinales, destilados de hierbas, *Staphylococcus aureus* resistente a la meticilina, resistencia a los antibióticos.

## Introduction

Medicinal plants and herbal distillates are rich sources of therapeutic agents with high beneficial effects on human health. They have a comprehensive role in Iranian folk medicine<sup>1, 2</sup>. Diverse kinds of medicinal plants including *Zataria multiflora* (*Z. multiflora*), *Satureja bachtiarica* (*S. bachtiarica*), *Aloysia citrodora* (*A. citrodora*), *Rosa damascene* (*R. damascene*), *Lavandula angustifolia* (*L. angustifolia*), *Alhagi maurorum* (*A. maurorum*), *Cichorium intybus* (*C. intybus*), *Melissa officinalis* (*M. officinalis*), *Mentha piperita* (*M. piperita*), and *Fumaria officinalis* (*F. officinalis*) are extensively used as antimicrobial, antioxidant, anticancer, anti-neoplasia, food additive, anti-inflammation, wound healing, antiseptic, anti-diabetic, diuretic, expectorant, stimulating the central nervous system, digestive, anti-mutagenic, sedative, analgesic, etc. agents among people all-around the world<sup>3-10</sup>. They also have an export aspect. Thus, it is essential to ensure the quality and safety of these products.

Human involvement in the packaging, powdering, and further procedures of medicinal plants and preparation of bottle herbal distillates caused their unintentional microbial contamination, particularly with foodborne pathogens<sup>11-17</sup>. *Staphylococcus aureus* (*S. aureus*) is a foodborne pathogen that originated from the skin and upper respiratory tract. The bacterium is responsible for severe nosocomial and community-acquired infections, foodborne diseases, and food poisoning. Food-related diseases and disorders caused by *S. aureus* are mainly known by abdominal cramps, nausea, vomiting, weakness, diarrhea, and toxic shock syndrome (TSS)<sup>18</sup>. Methicillin-resistant *S. aureus* (MRSA) is an antibiotic-resistant strain with simultaneous resistance toward cephalosporins and penicillins groups of antibiotics<sup>10</sup>. MRSA bacteria mainly harbored the *mecA* gene responsible for the occurrence of resistance toward penicillins<sup>19</sup>. MRSA bacteria harbored higher resistance toward commonly used antibiotic agents, particularly penicillins, cephemis, glycopeptides, aminoglycosides, macrolides, and tetracyclines, fluoroquinolones, nitrofurantoin, lincosamides, folate pathway antagonists, phenols, ansamycins, and even streptogramins<sup>20-23</sup>.

According to MRSA's uncertain role as a foodborne bacterium in medicinal plants and herbal distillates, the present survey was performed to assess MRSA isolates' prevalence and antibiotic resistance from powdered packaged medicinal plants and bottle herbal distillates.

## Materials and methods

### Samples

From May 2019 to January 2020, a total of 300 diverse kinds of powdered packaged medicinal plants including *Z. multiflora* (n= 30), *S. bachtiarica* (n= 30), *A. citrodora*

(n= 30) and *R. damascene* (n= 30) and bottle herbal distillates including *L. angustifolia* (n= 30), *A. maurorum* (n= 30), *C. intybus* (n= 30), *M. officinalis* (n= 30), *M. piperita* (n= 30) and *F. officinalis* (n= 30) were randomly collected from shopping centers, Tehran, Iran. A total of 50 g samples were collected from each powdered packaged medicinal plant and bottle herbal distillate sample using a sterile laboratory tube. All bottle herbal distillates were produced conventionally in small traditional producing units. Additionally, all collected powdered packaged medicinal plants were dried, powdered, and packed conventionally in traditional production units. Specifications about samples were recorded according to their labels. All samples were directly transferred to the laboratory at 4°C.

### Isolation and identification of *S. aureus*

Twenty-five grams of each collected powdered packaged medicinal plant and bottle herbal distillate samples were blended with 225 mL of buffered peptone water (Merck, Germany). At that time, solutions were homogenized using Stomacher (Interscience, Saint-Nom, France). At that point, five milliliters of the achieved solution was transferred into 50 mL Trypticase Soy Broth (TSB, Merck, Germany) supplemented with 10% NaCl and 1% sodium pyruvate and incubated for 18 h at 35°C. At that moment, a loopful of the culture was transferred into Baird-Parker agar supplemented with egg yolk tellurite emulsion (Merck, Germany) and incubated at 37°C for about 24 h. Black shiny colonies enclosed with significant zones identified using biochemical tests including Gram staining, oxidase test, catalase activity, resistance to bacitracin (0.04 U), coagulable test (rabbit plasma), urease activity, glucose O/F test, Voges-Proskauer (Merck, Germany) test, nitrate reduction, phosphatase, deoxyribonuclease (DNase, Merck, Germany) test, mannitol fermentation, hemolysis activity on blood agar (Merck, Germany) and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation tests<sup>23</sup>.

### Identification of MRSA isolates

MRSA bacteria were recognized using cefoxitin (30 µg) and oxacillin (1 µg) susceptibility test<sup>24</sup>. Principles of the Clinical and Laboratory Standards Institute (CLSI) was applied<sup>24</sup>.

### Antibiotic resistance of MRSA isolates

The phenotypic pattern of antibiotic resistance of MRSA bacteria isolated from powdered packaged medicinal plant and bottle herbal distillate samples was assessed using the disk diffusion method using the Mueller-Hinton agar (Merck, Germany) medium. A 0.5 McFarland standard concentration of MRSA was prepared and used for the disk diffusion. Ideologies of Clinical and Laboratory Standards Institute (CLSI) were used for this goal<sup>24</sup>. Diverse kinds of antibiotic groups

including tetracycline (30 µg/disk), doxycycline (30 µg/disk), trimethoprim-sulfamethoxazole (1.25/23.75 µg/disk), erythromycin (15 µg/disk), kanamycin (30 µg/disk), cefixime (30 µg/disk), chloramphenicol (30 µg/disk), rifampin (5 µg/disk), imipenem (10 µg/disk), and ampicillin (10 µg/disk) were applied for this goal (Oxoid, UK)<sup>25</sup>. The method was performed using the way designated previously<sup>23</sup>. Media were incubated aerobically at 37°C for about 24 h. Afterward, the diameter of the bacteria's growth inhibition zone was measured and compared with those recorded by the CLSI. MRSA BAA 2313 was prepared from the Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, and used as control.

#### DNA extraction and quality examination

MRSA isolates were sub-cultured on TSB media (Merck, Germany) and incubated for 48 h at 37°C. Genomic DNA was extracted from MRSA colonies using the DNA extraction kit (Thermo Fisher Scientific, St. Leon-Rot, Germany). Guidelines of the producing company were performed for this purpose. The purity (A260/A280) of extracted DNA was examined by the NanoDrop device (NanoDrop, Thermo Scientific, Waltham, MA, USA)<sup>26-28</sup>. The extracted DNA quality was examined using electrophoresis on 2% agarose gel<sup>29-31</sup>.

#### Detection of the methicillin-resistance encoding gene

The *mecA* gene in the MRSA isolates was examined using polymerase chain reaction (PCR). **Table I** shows the PCR conditions used for the detection of the *mecA* gene<sup>32</sup>. All PCR and electrophoresis ingredients were prepared commercially (Thermo Fisher Scientific, Germany). Eppendorf Mastercycler (Hamburg, Germany) device was applied in PCR. All runs included a negative DNA control of sterile PCR grade water (Thermo Fisher Scientific, Germany) and positive DNA control consisting of positive DNA of each target gene. Ten microliters of PCR products were examined by electrophoresis in a 2% agarose gel in 1× TBE buffer at 90 V for 30-40 min, stained with SYBR Green (Thermo Fisher Scientific, Germany). MRSA (BAA 2313) (Faculty of Veterinary Medicine, University of Tehran) and PCR-grade water (Thermo Fisher Scientific, Germany) were used as positive and negative controls, respectively.

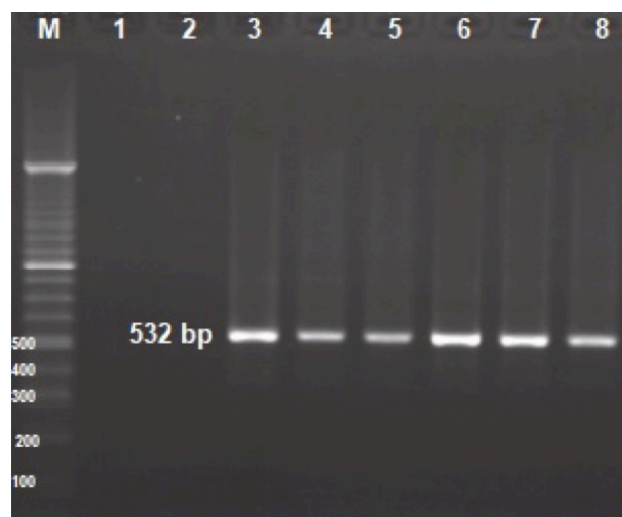
#### Statistical analysis

Statistical analysis was done using the SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact two-tailed test were used to assess any significant relationship between the data achieved in this survey. P-value <0.05 was considered a significant statistical level<sup>33, 34</sup>.

## Results

The presence of the *mecA* gene was confirmed in all of the MRSA isolates (**Figure 1**).

**Figure 1:** PCR gel electrophoresis of the *mecA* gene in the MRSA isolates. M: Marker (100 bp, Thermo Fisher Scientific, Germany), 1: Negative control (PCR-grade water, Thermo Fisher Scientific, Germany), 2: Negative sample, 3-7: Positive samples for the *mecA* gene (532 bp), 8: Positive control (MRSA ATCC BAA2313).



**Table II** shows MRSA bacteria's prevalence in diverse kinds of powdered packaged medicinal plant and bottle herbal distillate samples. Eighteen out of 300 (6.00%) powdered packaged medicinal plant and bottle herbal distillate samples were contaminated with MRSA. *R. damascene* powdered packaged medicinal plant (13.33%) and *A. maurorum* bottle herbal distillate (10%) had the highest prevalence of MRSA bacteria. Statistically significant difference was obtained for the prevalence of MRSA between powdered packaged medicinal plants and bottle herbal distillates ( $P < 0.05$ ).

**Table III** shows MRSA bacteria's antibiotic resistance pattern isolated from diverse powdered packaged medicinal plant and bottle herbal distillate samples. MRSA isolates showed the highest prevalence of resistance against trimethoprim-sulfamethoxazole (100%), cefixime (100%), ampicillin (100%), tetracycline (88.88%), erythromycin (88.88%), and rifampin (83.33%) antibiotic agents. The prevalence of resistance toward imipenem (27.77%) and doxycycline (44.44%) was lower than other examined antibiotic agents. A statistically significant difference was obtained between the type of samples and the prevalence of antibiotic resistance ( $P < 0.05$ ).



**Table I:** PCR conditions used for the detection of the *mecA* gene<sup>32</sup>.

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50µL)
<i>mecA</i>	F: AAAATCGATGGTAAAGGTTGGC R: AGTCTGCAGTACCGGATTTC	532	1 cycle: 94 °C ----- 2 min. 30 cycles: 94 °C ----- 30 s 55 °C ----- 30 s 72 °C ----- 30 s 1 cycle: 72 °C ----- 4 min	5 µL PCR buffer 10X 2 mM MgCl <sub>2</sub> 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template

**Table II:** Prevalence of MRSA bacteria in examined samples.

Types of samples		N. samples collected	N. samples contaminated with MRSA (%)	Distribution of <i>mecA</i> gene in MRSA isolates (%)
Medicinal plants	<i>M. piperita</i>	30	1 (3.33)	1 (100)
	<i>Z. multiflora</i>	30	1 (3.33)	1 (100)
	<i>S. bachtiarica</i>	30	1 (3.33)	1 (100)
	<i>A. citrodora</i>	30	1 (3.33)	1 (100)
	<i>R. damascene</i>	30	4 (13.33)	4 (100)
	<b>Total</b>	<b>150</b>	<b>8 (5.33)</b>	<b>8 (100)</b>
Herbal Distillates	<i>L. angustifolia</i>	30	2 (6.66)	2 (100)
	<i>A. maurorum</i>	30	3 (10)	3 (100)
	<i>C. intybus</i>	30	2 (6.66)	2 (100)
	<i>M. officinalis</i>	30	1 (3.33)	1 (100)
	<i>F. officinalis</i>	30	2 (6.66)	2 (100)
	<b>Total</b>	<b>150</b>	<b>10 (6.66)</b>	<b>10 (100)</b>
<b>Total</b>	<b>300</b>	<b>18 (6.00)</b>	<b>18 (100)</b>	

**Table II:** Antibiotic resistance pattern of MRSA bacteria isolated from examined samples.

Types of samples (N. MRSA)		N. samples contaminated with MRSA (%)									
		Tet*	Dox	Tr-sol	Ert	Kan	Cef	C30	Rif	Imp	Am
Medicinal plants	<i>M. piperita</i> (1)	1 (100)	-	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	-	1 (100)
	<i>Z. multiflora</i> (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
	<i>S. bachtiarica</i> (1)	1 (100)	-	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	-	1 (100)
	<i>A. citrodora</i> (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	-	1 (100)
	<i>R. damascene</i> (4)	3 (75)	2 (50)	4 (100)	3 (75)	3 (75)	4 (100)	2 (50)	2 (50)	2 (50)	4 (100)
	<b>Total (8)</b>	<b>7 (85.50)</b>	<b>4 (50)</b>	<b>8 (100)</b>	<b>7 (85.50)</b>	<b>7 (85.50)</b>	<b>8 (100)</b>	<b>6 (75)</b>	<b>6 (75)</b>	<b>3 (37.50)</b>	<b>8 (100)</b>
Herbal Distillates	<i>L. angustifolia</i> (2)	2 (100)	1 (50)	2 (100)	2 (100)	-	2 (100)	1 (50)	2 (100)	1 (50)	2 (100)
	<i>A. maurorum</i> (3)	2 (66.66)	1 (33.33)	3 (100)	2 (66.66)	2 (66.66)	3 (100)	1 (33.33)	2 (66.66)	1 (33.33)	3 (100)
	<i>C. intybus</i> (2)	2 (100)	1 (50)	2 (100)	2 (100)	-	2 (100)	1 (50)	2 (100)	-	2 (100)
	<i>M. officinalis</i> (1)	1 (100)	-	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	-	1 (100)
	<i>F. officinalis</i> (2)	2 (100)	1 (50)	2 (100)	2 (100)	-	2 (100)	1 (50)	2 (100)	1 (50)	2 (100)
	<b>Total (10)</b>	<b>9 (90)</b>	<b>4 (40)</b>	<b>10 (100)</b>	<b>9 (90)</b>	<b>3 (30)</b>	<b>10 (100)</b>	<b>5 (50)</b>	<b>9 (90)</b>	<b>3 (30)</b>	<b>10 (100)</b>
<b>Total (18)</b>	<b>16 (88.88)</b>	<b>8 (44.44)</b>	<b>18 (100)</b>	<b>16 (88.88)</b>	<b>10 (55.55)</b>	<b>18 (100)</b>	<b>11 (61.11)</b>	<b>15 (83.33)</b>	<b>5 (27.77)</b>	<b>18 (100)</b>	

\*Tet: tetracycline (30 µg/disk), Dox: doxycycline (30 µg/disk), Tr-sol: trimethoprim-sulfamethoxazole (1.25/23.75 µg/disk), Ert: erythromycin (15 µg/disk), Kan: kanamycin (30 µg/disk), Cef: cefixime (30 µg/disk), C30: chloramphenicol (30 µg/disk), Rif: rifampin (5 µg/disk), Imp: imipenem (10 µg/disk), Am: ampicillin (10 µg/disk).

## Discussion

*S. aureus* and MRSA are bacteria that reside on the skin and respiratory tract, contamination of powdered packaged medicinal plant and bottle herbal distillate samples occurs unintentionally. Additionally, manipulating medicinal plants and herbal distillates during their processing in the manufacturing units is crucial for their contamination.

The present survey was performed to assess MRSA bacteria's prevalence and antimicrobial resistance isolated from powdered packaged medicinal plant and bottle herbal distillate samples. The prevalence of MRSA in examined samples was 6.00%. Isolates showed higher resistance toward trimethoprim-sulfamethoxazole, cefixime, ampicillin, tetracycline, erythromycin, and rifampin antibiotic agents. Some of the examined

samples, such as *Z. multiflora* and *S. bachtiarica* medicinal plants and *M. officinalis*, and *M. piperita* herbal distillates, had a lower contamination *S. aureus* rate. One of the probable reasons for this finding is the high antimicrobial effects of *Z. multiflora*, *S. bachtiarica*, *M. officinalis*, *M. piperita*, and *C. intybus* against diverse kinds of bacteria<sup>35-38</sup>. Thus, *S. aureus*' growth and survival have been decreased and even stopped in these medicinal plants and their derived products.

*S. aureus* is most expected to originate from herbal product's contact with food handlers throughout harvesting, processing, and storage, and its absence reflects acceptable hygiene practices. Our findings also revealed that herbal distillates had a higher contamination rate with MRSA than medicinal plants. The probable reason



is maybe the extinction of MRSA bacteria during medicinal plants' drying process. Additionally, the processing of herbal distillates requires more human involvement and manipulation. Thus, MRSA bacteria's transmission from the infected staff and workers producing units to the herbal distillates may be another reason for MRSA's high prevalence in these samples. Despite the high importance of the topic, many limited surveys have been conducted in this field. A survey concocted by Sousa Lima et al. (2020)<sup>39</sup> disclosed that the prevalence of *S. aureus* bacteria amongst the homemade and commercial herbal medicine samples (*Lippia alba*, *Peumus boldus* Molina, *Cymbopogon citratus*, *Carapa guianensis*, *Copaifera langsdorffii*, *Stryphnodendron adstringens*, *Costus spicatus*, and *Arrabidaea chica*) was 88.50% and 23.50%, respectively. Kaume et al. (2012)<sup>40</sup> described that the prevalence of *S. aureus* amongst the medicinal plants marketed to patients who suffered from the HIV infection in Kenya was 71.40%, which was entirely higher than our findings. Esimone et al. (2007)<sup>41</sup> also reported that the prevalence of *S. aureus* amongst the medicinal plants sold in Nigeria was 8.70%. Similarly, a high contamination rate of herbal products with *S. aureus* and other Staphylococcal species has been reported previously from Bangladesh<sup>42</sup>, Korea<sup>43</sup>, Germany<sup>44</sup>, Sudan<sup>45</sup>, and Saudi Arabia<sup>46</sup>. Species of examined medicinal plants, methods of examination, the hygienic condition of producing factories, and their staff are essential factors that affected the prevalence of staphylococcal contamination in medicinal plants and herbal distillates.

Irregular and unauthorized prescription of antibiotics is the main reason for the high prevalence of antibiotic resistance in MRSA isolates. Ngemenya et al. (2019)<sup>47</sup> described that

the *S. aureus* strains isolated from herbal remedies in Cameroon were resistant against five classes of examined antibiotic agents (amikacin, cefotaxime, cefuroxime, imipenem, trimethoprim, and ceftriaxone). Braide et al. (2013)<sup>48</sup> stated that the *S. aureus* bacteria isolated from herbal remedies were susceptible to ofloxacin, chloramphenicol, gentamicin, pefloxacin, ciprofloxacin, and erythromycin antibiotic agents. Presence of infectious agents other than MRSA has also been reported in different investigations conducted in Iran<sup>49-54</sup>.

## Conclusion

As it showed, medicinal plants and herbal distillates samples were potential sources of antibiotic-resistant-MRSA bacteria. It seems that their consumption may cause foodborne infections caused by MRSA bacteria, which pose an essential public health threat. The present survey is the first report of the prevalence of antibiotic-resistant-MRSA isolated from *Z. multiflora*, *S. bachtiarica*, *A. citrodora*, and *R. damascene* powdered packaged medicinal plants and *L. angustifolia*, *A. maurorum*, *C. intybus*, *M. officinalis*, *M. piperita* and *F. officinalis* bottle herbal distillates, globally. Bottle herbal distillates harbored a higher prevalence of MRSA isolates and also higher antibiotic resistance.

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# Effect of estrogen and insulin sensitivity due to exercise training with dual intensities in female rats with estradiol valerate-induced PCOS

*Efecto de la sensibilidad a los estrógenos y la insulina debido al entrenamiento con ejercicios de intensidad dual en ratas hembras con SOP inducido por valerato de Estradiol*

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## Abstract

**Background:** Exercise training can improve polycystic ovary syndrome (PCOS). However, the effect of various intensities of exercise training is unclear, hence the purpose of this study was to evaluate the effect of exercise training with dual intensities on estrogen and insulin sensitivity in female rats with estradiol valerate-induced PCOS.

**Methods:** In this semi experimental study, forty adult (Wistar rats weight:  $180 \pm 20$  gr) after induction of PCOS, were divided into four groups (n=10): healthy control (HC), polycystic control (PC), low intensity training (LI) and moderate intensity training (MI) groups. Exercise training consisted of eight-week exercise with 50-55% maximal oxygen consumption (20m/min speed) and 70-75% maximal oxygen consumption (28m/min speed), 3 days a week for 60 minutes. Data analysis was performed using SPSS software (version 16.0, SPSS). One-way ANOVA test was used for data analysis. The level of significance was set at  $p < 0.05$ .

**Results:** Significant difference was observed between glucose concentration of MI and PC ( $p = 0.039$ ), also there was significant difference between LI and PC ( $p = 0.005$ ). Insulin significantly redreased in MI compared to PC ( $p = 0.002$ ). Also, significant difference was found between LI and PC ( $p = 0.005$ ).

**Conclusion:** It seems that exercise training with low and moderate intensity may reduce insulin and glucose level. Low intensity may improve insulin sensitivity, and can be considered as a non-pharmacological treatment method to regulate glucose hemostasis in polycystic ovary syndrome patients.

**Keywords:** Polycystic ovary syndrome, exercise training, insulin sensitivity, estrogen.

## Resumen

**Antecedentes:** El entrenamiento con ejercicios puede mejorar el síndrome de ovario poliquístico (SOP). Sin embargo, el efecto de varias intensidades de entrenamiento no está claro, por lo tanto, el propósito de este estudio fue evaluar el efecto del entrenamiento con intensidades duales en los estrógenos y la sensibilidad a la insulina en ratas hembras con SOP inducido por valerato de estradiol.

**Métodos:** En este estudio semiexperimental, cuarenta ratas adultas (peso de las ratas Wistar:  $180 \pm 20$  gr) tras la inducción del SOP, fueron divididas en cuatro grupos (n=10): control sano (HC), control poliquístico (PC), entrenamiento de baja intensidad (LI) y entrenamiento de intensidad moderada (MI). El entrenamiento consistía en ocho semanas de ejercicio con un 50-55% de consumo máximo de oxígeno (velocidad de 20 m/min) y un 70-75% de consumo máximo de oxígeno (28m/min de velocidad), 3 días a la semana durante 60 minutos. El análisis de los datos se realizó con el software SPSS (versión 16.0, SPSS). Para el análisis de los datos se utilizó la prueba de ANOVA de una variable. El nivel de significación se fijó en  $p < 0,05$ .

**Resultados:** Se observó una diferencia significativa entre la concentración de glucosa de MI y PC ( $p = 0,039$ ), también hubo una diferencia significativa entre LI y PC ( $p = 0,005$ ). La insulina se redujo significativamente en el IM en comparación con el CP ( $p = 0,002$ ). También se encontró una diferencia significativa entre el IM y el CP ( $p = 0,005$ ).

**Conclusiones:** Parece que el entrenamiento con ejercicios de baja y moderada intensidad puede reducir el nivel de insulina y glucosa. La baja intensidad puede mejorar la sensibilidad a la insulina, y puede considerarse como un método de tratamiento no farmacológico para regular la hemostasia de la glucosa en pacientes con síndrome de ovario poliquístico.

**Palabras clave:** Síndrome de ovario poliquístico, entrenamiento con ejercicios, sensibilidad a la insulina, estrógenos.

## Introduction

It is obvious that infertility is one of the main problems that have been increased since 1955 and also 10%-15% of individuals are suffering from it. Polycystic ovary syndrome (PCOS) is one of the causes of infertility. PCOS is the most common endocrine abnormality in premenopausal women. It seems that genetic etiology plays an important role in this disorder<sup>1</sup>. The most prevalent characteristic of PCOS are irregular menstrual cycles, imbalance of sex hormones and polycystic ovarian morphology. It is reported that metabolic disorders, such as insulin resistance is associated with PCOS. Severity and incidence of PCOS is characterized by life style<sup>2</sup>. So the result frightening the life is consists of Breast cancer risk, cardio vascular disease and type two diabetes<sup>1</sup>. Diabetes mellitus is also associated with central obesity and increased level of incomplete insulin functions of pancreatic cells and increased level of leptin<sup>3, 4</sup>. In accordance to previous researches 50-70 percent of females have some level of PCO and resistance to insulin. This resistance maybe due to obesity or hyperandrogenism<sup>5</sup>. Previous studies reported that PCOS is not the malfunction of some specific part of body because recent findings show that hormonal changes might play role in this syndrome such as increased level of androgens, 17 alpha hydroxyprogesterone and estrogen<sup>6</sup>. Also increased serum level of LH is prevalent among female with PCOS. The increased release of LH is associated with free Estradiol and estrogen<sup>7</sup>. Nevertheless, there is a specific relationship between serum level of estrogen and biochemical index of insulin resistance in PCOS females. Scientists believe that regular exercise training is a safe method of treatment without regarding clinical actions<sup>2</sup>. Jedel et al. (2011) conducted a research to investigate the effect of exercise training on hyperandrogenism. 84 PCOS females participated in a period of long term training and finally results revealed that estrogen and androgen reduced due to exercise<sup>8</sup>. Also literatures studying PCOS showed that exercise training change level of sexual hormone such as estrogen without regarding weight change or insulin sensitivity<sup>9</sup>. In addition to hormonal disorders, PCOS are faced with metabolic disturbance such as insulin resistance<sup>4</sup>. Its mechanism in PCOS is different and about 60 percent of PCOS patients are struggling with insulin resistance too. It is reported that the mean number of PCOS patient are more than healthy people especially in the obese individuals<sup>10</sup>. Also exercise training is recommended for prevention and treatment of these patients with insulin sensitivity. Doing exercise training regularly accompanied with dietary regimen are those elements that lead to weight reduction and hormonal adjustment in females<sup>11</sup>. In this field, exercise training ensue reduction in insulin levels and improvement in glucose usage and insulin sensitivity which could decrease body fat<sup>12, 13</sup>. Researchers believe that not high intensity but probably regular exercise is a natural healthy cure. In this direction, Benrick et al. (2013)

reported that exercise training led to significant reduction of glucose comparing to PCOS control<sup>14</sup>. Qui and et al. 2009 investigated the effect of training on insulin resistance in 20 wistar rats undertaken PCOS. The rats swam 120 min for 2 weeks. The final result showed that serum level of insulin decreased after exercise training but no significant change was observed in plasma glucose compared to control group<sup>15</sup>. Since there is a contradiction about the intensity of exercise training and the effect of exercise intensity in PCOS have not been clearly determined regarding the influence of exercise training in improvement of hormonal imbalance and glucose hemostasis, in the aim of the present study was to investigate changes of estrogen and insulin sensitivity due to exercise training with dual intensities in female rats with estradiol valerate-induced PCOS.

## Material and methods

### Animals

Forty female Wistar rats were ( $180 \pm 20$  g) were selected and kept in animal house. They had every 2 to 3 consecutive estrous cycles during 12 to 14 day. Their cages were clarified three times per week with alcohol and adequate water in appropriate container was catered.

### Approval

This research was confirmed by ethical committee of Jahrom University of Medical Sciences and morality was considered.

### Induction of PCOS

A variety of hormonal and non-hormonal methods to PCO phenotype, consisting long-term use of light, testosterone, dehydroepiandrosterone (DHT), adrenocorticotrophic and estradiol valerate. In this study, we utilized estradiol Valerate. 30 female rats were randomly singled out from forty. 4 mg of estradiol valerate dissolved in 0.2 mg of Sesame oil was injected (IM) in thigh area<sup>16</sup>.

### Protocol design

Initially, subjects were divided into control ( $n = 10$ ) and polycystic ( $n = 30$ ) groups that were overtaken PCOS by intramuscular estradiol valerate injection following 60 days. The PCOS subjects were split into 3 groups of polycystic control (PC) ( $n = 10$ ), low-intensity exercise (LI) ( $n = 10$ ) and moderate intensity exercise (MI) ( $n = 10$ ). Training was executed 1 h/day, 6 days/week for eight weeks. Moderate intensity: ( $28$  m/min-70%-75% $VO_2$ Max), and Low intensity ( $20$  m/min-50%-55% $VO_2$ Max) running were performed at 0 slope.

### Exercise training protocol

The LI and MI were trained on a rodent motor-driven treadmill at a 0° slope. For induction of adaptation, rats



exercised at Treadmill velocity of 10 m/min for 15min during the 1st work of training. The treadmill velocity and exercise duration elevated gradually until the animals ran for 1 h/day during the 2<sup>nd</sup> and 3<sup>rd</sup> work of training. The treadmill speed and exercise duration were constant. Frequency (6d/work) and duration (1 h/d) were constant during the study.

### Vaginal smears and blood sampling

microscopic analysis of the predominant cell types qualified estrus cycle stage obtained through daily vaginal smears<sup>16</sup> Vaginal smear test was done in two month for certitude of PCOS induction within subjects. After the test, we selected those having 2 or 3 regular estrous cycle within 12-14 days. Blood sample was directly taken from their heart through a 5 cc syringe following 32 hours after the last exercise bout. After isolation of blood serum, dependent variables were assessed.

### Blood collection and tissue preparation

To minimize the effect of last exercise session, the subjects were finally anesthetized with diethyl ether and sodium pentobarbital (50 mg/kg, intraperitoneal injection) after a12-h fasting and 32 h after the last exercise session and their blood was obtained from the heart. Tubes containing plasma sample aliquots were kept frozen at -80°C until being analyzed<sup>16</sup>.

### Measurement

Estrogen was assessed by ELISA kit (monobind e2). Insulin sensitivity was calculated by quantitative insulin sensitivity check index (QUICKI) formula:  $1 / (\log (\text{fasting insulin } \mu\text{U/mL}) + \log (\text{fasting glucose mg/dL}))$ . ELISA kits specific for the rat were used to determine plasma insulin (BioVendor Shibayagi, Japan), and serum glucose was measured via spectrophotometry method (spectrophotometer wavelength was adjusted in 650 nm).

### Analyzing method

A Shapiro-wilk test was applied to determine normality of distribution which was found to be normally distributed. A one-way analysis of variance (ANOVA) was performed to determine the differences in a parameter among the groups. Significant differences were identified using a least significant difference (Bonferroni) post-hoc test.

## Results

**Table I** shows the indicates biochemical blood parameters in groups (mean±S.D).

**Table I:** Indicates biochemical blood parameters in groups (mean±S.D).

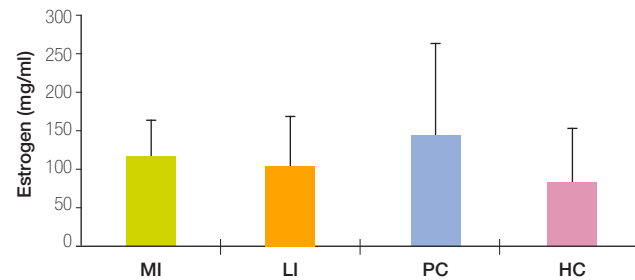
Groups	Estrogen (ng/ml)	Glucose (mg/dl)	Insulin (μU/ml)	Insulin sensitivity
MI	119.01±50.56	157.08±31.7 *	0.56±0.25 *	0.54±0.06
LI	104.84±66.22	145.02±22.8 *	0.41±0.3 *	0.6±0.14 *
PC	144.79±121.53	203.2±52.9	1.5±0.75	0.41±0.04
HC	86.98±67.99	94.7±17.5	0.65±0.52	0.63±0.1

Values are mean ± SD \* p < 0.05 vs. PC

### Estrogen

No significant difference was observed between MI (119.01±50.56) and PC (144.79±121.53) also, there was no statistical change between LI (104.84±66.22) and PC, although both MI and LI showed reduction comparing to PC. No significance reduction was in LI compared to PC. Comparing HI and PC showed a significant difference, (**Figure 1**).

**Figure 1:** Glucose level of groups after 8 week.

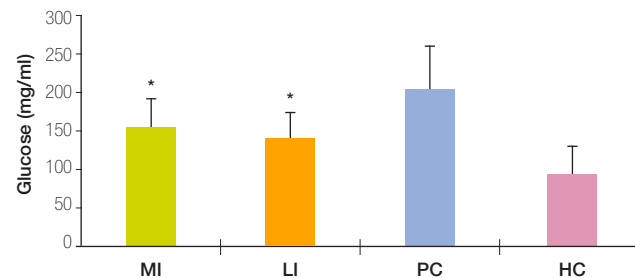


\*significant comparing to PC (p<0.05)

### Glucose

According to finding illustrated in the **table I**, significant reduction was observed between glucose concentration of MI(157.08±31.7) and PC(203.2±52.9) (p=0.039), also there was significant decrease between LI (145.02±22.8) and PC (p=0.005) but no significant change was seen between MI and LI, (**Figure 2**).

**Figure 2:** Glucose level of groups after 8 week.

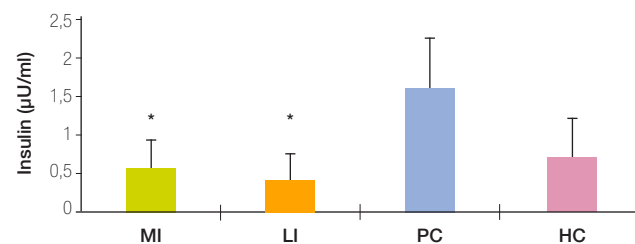


\*significant comparing to PC (p<0.05)

### Insulin

After 8 week exercise training, serum level of insulin did significantly change in MI (0.41±0.3) comparing to PC (1.5±0.75) (p=0.002). Also statistically significant reduction was observed between LI (0.566±0.25) and PC (p=0.00). There was no significant change between MI and LI, (**Figure 3**).

**Figure 3:** Insulin level of groups after 8 week.

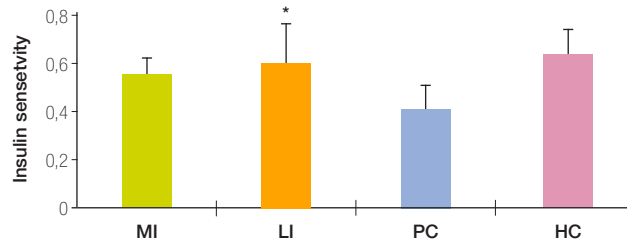


\*significant comparing to PC (p<0.05)

## Insulin sensitivity

Base on **table I**, a significant elevation was observed between LI ( $0.6 \pm 0.14$ ) and PC ( $0.41 \pm 0.04$ ), ( $p=0.003$ ), but no significant change was between MI ( $0.54 \pm 0.06$ ) and PC, Also no statistical difference was found between MI and LI, (**Figure 4**).

**Figure 4:** Insulin level of groups after 8 week.



\*significant comparing to PC ( $p < 0.05$ )

## Discussion

The aim of this study was to examine the effect of eight weeks exercise training with different intensities on levels of estrogen and insulin, glucose and insulin sensitivity due to exercise training with dual intensities in female rats with polycystic ovary syndrome. The results of the present investigation showed that the eight-week exercise training induced a significant change in glucose level in low intensity group in comparison to PC (**Figure 2**). This finding was supported by previous researches<sup>17,18</sup>. However, there is a controversy in the results obtained by Holm and colleagues (2010)<sup>19</sup>. Benrick et al. (2013) conducted a research on rats with polycystic ovary syndrome, and finally concluded that exercise training leads to the a significant declined in glucose level in these rats, moreover, exercise can lead to decrease in Insulin resistance in rats with polycystic disease<sup>18</sup>. Qiu et al. (2009)<sup>15</sup> examined the effect of exercise on improvement of Insulin resistance in 21 day wistar rats with polycystic disease for 120 minutes of swimming per day for two weeks, concluded that testosterone and insulin levels in practice was significantly lower than control group. But, they did not observe the significant differences among groups in glucose and progesterone values. Among the mechanisms that lead to decrease glucose level after aerobic exercise, included the increased of post-insulin recipient's message<sup>20</sup>; increase in expression of transfer protein of glucose, GLUT-4<sup>21</sup>; increasing the activity of glycogen synthase hexokinase<sup>22</sup>; Reduction of secretion and elevation of clarification of free fatty acids; glucose uptake from the blood to muscle can be due to the increase of the muscle capillaries and alteration in muscle structure in order to increase intake of glucose<sup>23</sup>. Therefore, one of the methods of reducing insulin resistance and risk of cardiac disease, type II diabetes, is aerobic exercise particularly in overweight and PCOS patients. One of the limitation of this research was that these models do not reproduce exactly what happens in the real syndrome which may affect our findings.

In non-inflammation situation, TNF-alpha is derived from adipose tissue and its plasma level is associated with body fat mass. TNF-a opposites by insulin Message forming through signaling reduction by means of phosphorylation serine<sup>24</sup>. In this research it was revealed that insulin level was significantly decreased -%73 in LI and -%62 in MI comparing to PC. This finding was supported by previous investigations<sup>17, 18</sup>. However, there is a controversy in the results obtained by Holm and colleagues (2010)<sup>19</sup>. Fasting hyperinsulinemia is abundant in obese PCOS patients and this is, in part, secondary to increased basal insulin secretion rates<sup>25</sup>. Insulin responses to an oral glucose load are elevated in lean and obese PCOS patients, but acute insulin responses to an intravenous glucose load (AIRg), first-phase insulin secretion, are similar to weight-matched control women. When the relationship between insulin secretion and sensitivity is examined lean and obese PCOS women fall below the relationship in weight-matched control women, and the disposition index is significantly decreased by PCOS as well as by obesity<sup>26</sup>. In this study, training period was considered as 8-weeks. In addition, the training was performed with two intensities (low and moderate) for 60 minutes. Furthermore, recently some studies showed that training with low to moderate-intensity training can cause weight loss and body fat percent loss and increased fat oxidation<sup>27, 28</sup>. Hence, the low intensity exercise, is a common type exercise in researches due to the decline of weight and considered as appropriate intensity. In the study, we observed no significant change in estrogen level in PCOS groups. Gonadotropin is the glycoprotein that is released in response to gonadotropin-releasing hormone (GnRH) from the pituitary. The sinus mood release of GnRH causes production of estrogen concentrations. Response of estrogen levels to exercise depends on menstrual cycle which complicates sexual hormones response to physical activity. Another aspect of this complexity are reduction of body fat that can induce some changes in estrogen secretion<sup>29</sup>. On the other hand, physical activity can increase hormone of beta endorphin which cause reduction in GnRH, followed by decrease in estrogen levels<sup>30</sup>. The results obtained from this study imply the role of intensity/volume of training on insulin sensitivity which are considered as crucial variables that can determine healing process of PCOS<sup>31, 32</sup>.

## Conclusion

As a result, according to this result, Low and moderate intensity exercise may be effective in reduction of glucose and insulin level. Low intensity could elevate insulin sensitivity, and could be considered as a non-pharmacological treatment method to regulate glucose hemostasis in polycystic ovary syndrome patients. Although, due to the lack of research evaluating the influence of different exercise intensities on patients with Polycystic syndrome future studies are recommended for more clarification.

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## ORIGINAL

# Evaluation of risk factors, risk assessment and health-related quality of life in patients with dyslipidemia in Kempegowda Institute of Medical Sciences (Kims) Hospital and Research Centre, Bangalore, India

*Evaluación de los factores de riesgo, valoración del riesgo y calidad de vida relacionada con la salud en pacientes con dislipidemia en el Hospital y Centro de Investigación del Instituto Kempegowda de Ciencias Médicas (Kims), Bangalore, India*

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## Abstract

**Background:** The research aims to evaluate the risk factors, risk assessment, and health-related quality of life in patients with dyslipidemia.

**Methods:** This is an observational study conducted for 3 months in a tertiary care hospital setting. All the patients meeting the selected criteria were identified in the outpatient clinic and inpatient wards of the Department of General Medicine. The patients or the caregivers were briefed about the study, and consent was obtained to be recruited.

**Results:** A total of 200 patients were enrolled in the study based on the inclusion and exclusion criteria, out of which 55% (n=111) were male, and 45% (n=89) were female. The mean age of our study subjects was found to be 55.5±12.1 years. The health-related quality of life of the patient is a combined assessment of the physical component summary (PCS), and mental component summary (MCS). The average of PCS and MCS was 37.1 and 47.3, respectively, indicating an overall average of HRQOL as 42.2, which represents low HRQOL in patients with dyslipidemia.

**Conclusion:** The result of our study show age, gender, lack of physical activity, overweight, HTN, DM, consumption of alcohol, and smoking were risk factors for dyslipidemia. The present study highlights that patients with dyslipidemia have poor HRQOL as the overall PCS and MCS scores were low. Focus on dyslipidemia management is urgently required in India to halt the rising tide of coronary heart disease.

**Keywords:** Risk factors, risk assessment, quality of life, dyslipidemia.

## Resumen

**Antecedentes:** La investigación tiene como objetivo evaluar los factores de riesgo, la evaluación del riesgo y la calidad de vida relacionada con la salud en pacientes con dislipidemia.

**Métodos:** Se trata de un estudio observacional realizado durante 3 meses en un entorno hospitalario de atención terciaria. Se identificó a todos los pacientes que cumplían los criterios seleccionados en la consulta externa y en las salas de hospitalización del Departamento de Medicina General. Se informó a los pacientes o a los cuidadores sobre el estudio y se obtuvo el consentimiento para ser reclutados.

**Resultados:** Un total de 200 pacientes fueron incluidos en el estudio en base a los criterios de inclusión y exclusión, de los cuales el 55% (n=111) eran hombres, y el 45% (n=89) eran mujeres. La edad media de los sujetos del estudio fue de 55,5±12,1 años. La calidad de vida relacionada con la salud del paciente es una evaluación combinada del resumen del componente físico (PCS), y del resumen del componente mental (MCS). La media de PCS y MCS fue de 37,1 y 47,3, respectivamente, lo que indica una media general de CVRS de 42,2, que representa una CVRS baja en los pacientes con dislipidemia.

**Conclusiones:** El resultado de nuestro estudio muestra que la edad, el sexo, la falta de actividad física, el sobrepeso, la HTA, la DM, el consumo de alcohol y el tabaquismo fueron factores de riesgo de dislipidemia. El presente estudio pone de manifiesto que los pacientes con dislipidemia tienen una mala CVRS, ya que las puntuaciones globales de PCS y MCS fueron bajas. Es necesario centrarse urgentemente en el tratamiento de la dislipidemia en la India para detener la creciente ola de enfermedades coronarias.

**Palabras clave:** Factores de riesgo, evaluación del riesgo, calidad de vida, dislipidemia.

## Introduction

Despite the high advances in the medical sciences, some diseases caused morbidity and mortality<sup>1-10</sup>. Dyslipidemia is considered a major cause of death in developed and developing countries, according to the estimates by the World Health Organization (WHO), dyslipidemia is associated with more than half of the cases of coronary artery disease and more than four million deaths per year, globally. Lipid abnormalities, including total cholesterol and a high level of low-density lipoprotein cholesterol (LDL-C) and low level of high-density lipoprotein cholesterol (HDL-C), are the most important risk factors for cardiovascular diseases.<sup>11</sup> It is considered a major risk factor for cardiovascular complications. Many studies such as those conducted by the World Health Organization and Framingham have established the association of plasma cholesterol level with the risk of cardiovascular disease. This association also confirmed with the results of a meta-analysis of 10 cohort studies, which stated that for a reduction of 0.6 mmol/L of serum total cholesterol in adults aged over 60 years, the risk of fatal cardiovascular disease decreased by 27%.<sup>13, 14</sup>

The Indian Council of Medical Research (ICMR) surveillance project reported a prevalence of dyslipidemia of 37.5% among adults aged 15-64 years, with an even higher prevalence of dyslipidemia 62% among young male industrial workers.<sup>11</sup> Various studies also reported greater triglyceride levels in rural and urban populations associated with low HDL cholesterol levels.<sup>12, 16</sup>

An important shortcoming of Indian epidemiological studies is the lack of large studies with details of patterns of dyslipidemia. Compared with Western populations, Indians and migrant South Asians tend to have higher triglyceride levels and lower HDL cholesterol while total cholesterol levels are lower than in the US and the UK. Only a few extensive studies have reported the prevalence of different forms of lipid abnormalities among Indians. India Heart Watch study carried out among urban middle-class subjects in 11 cities of India with fasting lipid estimation revealed alarming facts that India is undergoing a rapid epidemiological transition with increasing population, economic prosperity, urbanization, and ageing with associated risk factor transition. An increase in cardiovascular risk and hypercholesterolemia is also associated with increased adverse lifestyles such as greater smoking and tobacco use, change in nutritional habits with greater intake of unhealthy diets, and increasingly sedentary lifestyle. All the risk mentioned above factors have contributed to the sharp rise in the burden of non-communicable diseases, especially coronary heart disease (CHD). Even in rural areas of India, non-communicable and chronic diseases have become the leading causes of death.<sup>12-16</sup>

Health-related quality of life (HRQOL) includes the physical, mental, and social aspects of health and is an essential outcome measure that has gained much attention in recent years. HRQOL specifically focuses on an individual's perception of health and life satisfaction that may be linked with the World Health Organization (WHO) definition of health as "the state of complete physical, mental, and social wellbeing, and not merely the absence of disease and infirmity."<sup>15</sup> HRQOL was assessed by the SF-36, a short form of the Medical Outcome Study. It contains 36 questions. The SF-36 assesses eight health dimensions: physical functioning, role physical limitation, bodily pain, general health, social functioning, role emotional limitation, mental health, and vitality. From these eight scales, it is possible to calculate two summary scores, physical component summary (physical functioning, role physical limitation, bodily pain, and general health) and mental component summary (social functioning, role emotional limitation, mental health, and vitality), and also the overall HRQOL. Certain studies conducted on the impact of health-related quality of life of the patients with dyslipidemia have established a direct association of dyslipidemia with low HRQOL. The major contributing factors identified were rigid dietary prescriptions, medication side effects, the need for regular medical care, and psychological effects.<sup>12</sup> Improvement of HRQOL would be very important in the management of patients with dyslipidemia. The expanding pattern in patients with dyslipidemia makes this assessment exceptionally applicable, to more readily oversee dyslipidemia patients and therefore improve their QOL. The alarming rise in the global prevalence and disease burden of dyslipidemia makes the reports of these studies highly relevant for the effective management of dyslipidemia patients and consequently improve their HRQOL.<sup>13,17</sup> Therefore, this study is proposed with the primary objective to concentrate on identifying various risk factors, risk assessment associated with dyslipidemia, and measuring its impact on patient's HRQOL. Secondary objectives are to evaluate the drug-related problem associated with dyslipidemia management and assess the knowledge, attitude, and practice of dyslipidemia patients towards their illness and treatment.

## Materials and methods

The study was conducted at Kempegowda Institute of Medical Sciences (Kims) Hospital and Research Centre, a 500 bedded hospital with state-of-the-art facilities for patients. This study was conducted on patients drawn from the Outpatient clinic and Inpatient wards of the Department of General Medicine.

Patients' meeting with inclusion and exclusion criteria were included in the study. The purpose of the study was explained to the patient and consent was taken. A self-designed case report form was used to collect the patients'



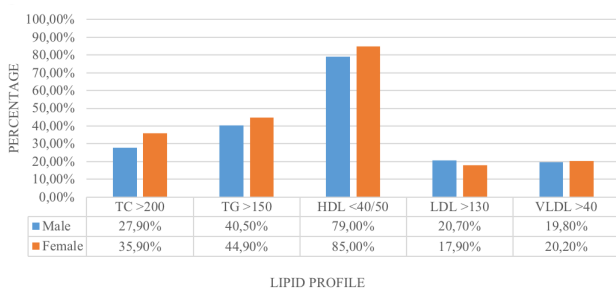
demographic data, medical issues, past medical history, laboratory data, and other relevant information collected by interviewing and referring to their medical records in the hospital (both inpatient and outpatient) documented. Also, the SF-36 (Short form survey) questionnaire was used to interview the patient to assess the patient's health status. The knowledge, attitude, and practice of dyslipidemia patients towards their illness were assessed using the KAP questionnaire. JBS-3, a risk calculator was used to assess the risk factors and risk assessment, both 10-year risk and lifetime risk of CVD in all individuals. This is done by entering the laboratory values obtained during interviewing/ data collection in the JBS-3 risk calculator. PCNE v8.01 is a classification tool used to evaluate drug-related problems by using the data obtained during interviewing/ data collection. All the data collected were entered into Microsoft Excel and an appropriate statistical analysis tool was applied. Categorical variables were presented using tables and graphs. Continuous variables were presented as mean +/- SD. Descriptive statistical methods were used to measure the mean, median, and standard deviation.

## Result and discussion

This study included a total of 200 patients drawn from the Outpatient clinic and Inpatient wards of the Department of General Medicine in Kempegowda Institute of Medical Sciences (Kims) Hospital and Research Centre, Bengaluru. The study was conducted from January 2018 to March 2018. Out of the 200 sets of the population enrolled for the study 55.00% were male (n=111), 45.00% were female (n=89). Among the 200 patients, about 60 patients i.e.30%, had habits such as smoking, alcohol, and tobacco use, and the remaining 70% had none. Out of 60 patients' highest number were among the use of both alcohol and smoking and the least were the use of all three. Prevalence of abnormal total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), and very-low-density lipoprotein (VLDL) in women was higher than in men whereas the level of (LDL) was higher in men, as shown in **figure 1**.

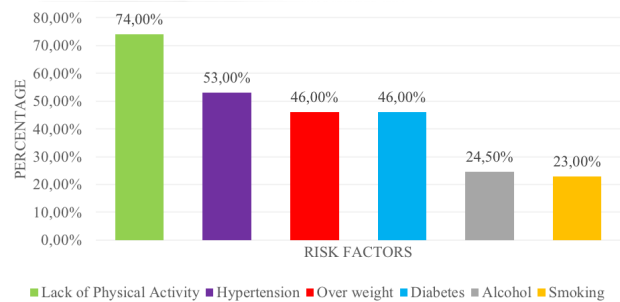
Out of the 200 patients involved in the study, most of the patients were found to be between the age group of 55-64 (31.5%), thus as the age increases there is a proportionate increase in the risk of acquiring dyslipidemia.

Figure 1: Prevalence of dyslipidemia.



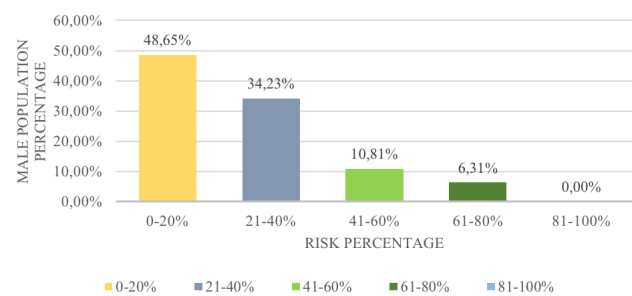
Prevalence of the disease was seen more in the male population 55% (n=111), than in the female population 45% (n=89). **Figure 2** shows the risk factors of patients.

Figure 2: Risk factors of a patient.



The majority of patients in the study i.e. 74.00% (n=148) were found to have lack of physical activity as a chief risk factor and the least percentage of risk factor was among patients who were smoking i.e. 23.00% (n=46), and other risk factors included hypertension, overweight, diabetes, and alcohol consumption as shown in **figure 3**.

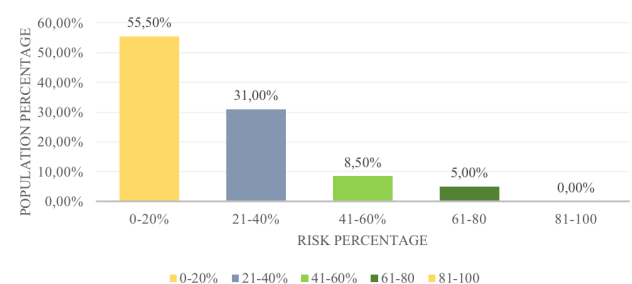
Figure 3: Risk assessment in the male population.



Charlson Co-Morbidity Index (CCI) scoring was done for all the 200 patients in the study to assess the ten-year mortality for a patient who may have a range of comorbid conditions, and the highest average of 2.53 was seen in patients between the age group of 55-64.

**Risk Assessment:** Joint British Societies-3 (JBS-3) risk score analysis revealed that 55.5% of the study population have the risk of 0-20% for developing CVD in the next 10 years and the least of 5% study population is in between 61-80, as shown in **figure 4**.

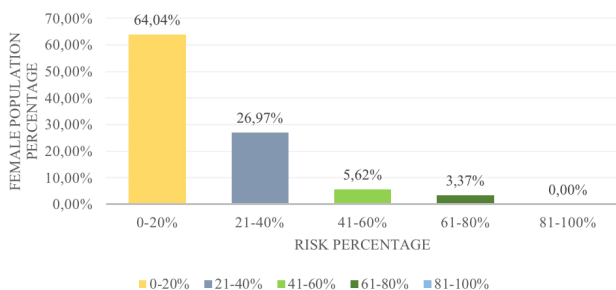
Figure 4: Risk assessment of patient.



**Risk Assessment in Male Population:** JBS-3 risk score analysis to evaluate the risk assessment in the male population revealed 48.6% have risk between 0-20% for developing CVD in the next 10 years, as depicted in **figure 3**.

**Risk Assessment in Female Population:** JBS-3 risk score analysis to evaluate the risk assessment in the female population revealed 64.04% have risk between 0-20% for developing CVD in the next 10 years, as depicted in **figure 5**.

**Figure 5:** Risk assessment in the female population.

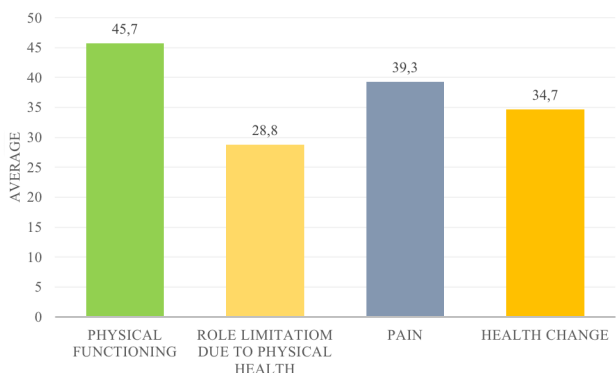


A risk assessment done on a study population of 200, have shown variation by 13.8 years in heart age from chronological age. The average chronological and heart age was  $55.5 \pm 12.1$  and  $69.3 \pm 17.1$  years respectively.

**Health-Related Quality of Life Assessment:**

**Physical component summary (PCS):** Analysis of HRQOL in the study population using SF-36 revealed the average of physical functioning (PF), role limitation due to physical health (RLPH), pain, and health change to be 45.7, 28.8, 39.3, and 34.7 respectively. The physical functioning dimension had a better average and the least average was seen in role limitation due to physical health, as shown in **figure 6**.

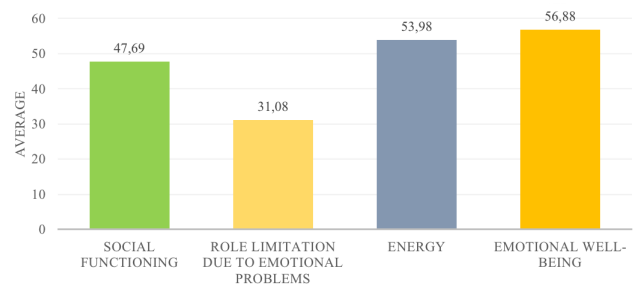
**Figure 6:** Physical component summary.



**Mental component summary (MCS):** Analysis of mental component revealed the average of social functioning, role limitation due to emotional problems (RLEP), energy,

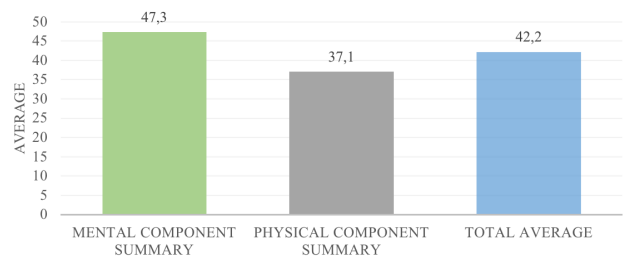
and emotional well-being to be 47.6, 31, 53.9, and 56.8, respectively. Emotional well-being had a better average and the least average was seen in role limitation due to emotional problems, as shown in **figure 7**.

**Figure 7:** Mental component summary.



**Physical and mental component summary:** The total average of four dimensions each under MCS and PCS were 47.3 and 37.1 respectively and the overall average of both PCS and MCS was 42.2, as shown in **figure 8**.

**Figure 8:** Physical and Mental component summary.



**Drug-Related Problems:** The drug-related problems identified in our study population includes an inappropriate combination of drugs, the patient uses/takes less drug than prescribed or does not take the drug at all, patient stores drug inappropriately and inappropriate timing or dosing intervals, as shown in **table I**.

**Table I:** Drug-related problems of the patient.

Drug-Related Problems	Number of patients
C1.4*	05
C7.1	05
C7.6	04
C7.7	04
Total number of DRP	18

\*C1.4: Inappropriate combination of drugs or drugs and herbal medication  
 C7.1: Patient uses/takes less drug than prescribed or does not take the drug at all  
 C7.6: Patient stores drug inappropriately  
 C7.7: Inappropriate timing or dosing intervals

Similar researches have been performed in this field. Farhat et al. (2016)<sup>18</sup> reported a lower overall QOL score among dyslipidemic patients compared with controls (57.9% and 76.5%, respectively;  $p < 0.001$ ). Waterpipe smoking (adjusted odds ratio ( $OR_a$ ) = 4.113, 95% confidence interval (CI): 1.696-9.971,  $p = 0.002$ ), hypertension ( $OR_a$  = 3.597, 95% CI: 1.818-7.116,  $p < 0.001$ ), diabetes ( $OR_a$  = 3.441, 95% CI: 1.587-7.462,  $p = 0.002$ ),

cigarette smoking ( $OR_a = 2.966$ , 95% CI: 1.516–5.804,  $p = 0.001$ ), and passive smoking ( $OR_a = 2.716$ , 95% CI: 1.376–5.358,  $p = 0.004$ ) were significantly associated with dyslipidemia in individuals older than 30 years. A higher overall QOL score ( $p = 0.013$ ) was observed in patients treated with statins compared to other lipid-lowering medications. In a survey of Tziallas et al. (2012)<sup>19</sup>, Three hundred and fifty-nine subjects were involved of whom 206 (57.4%) met the diagnostic criteria for the MetSyn (“cases”) and 153 (42.6%) did not (“comparator group”). Comparisons of SF-36 scores between patients with and without MetSyn revealed statistically significant differences except bodily pain subscale. A predominance of anxiety (60%) and depressive symptoms (67%) was observed among subjects with MetSyn. Patients with MetSyn undergoing a therapeutic approach showed no improvement in its general and mental health ( $p$ : NS). Similar findings were reported in surveys conducted in Brazil<sup>20</sup>, Iran<sup>21</sup>, Romani<sup>22</sup>, and USA<sup>23</sup>.

## Conclusion

The study was conducted in a tertiary care teaching hospital in urban premises of Bengaluru comprising of patients who met the inclusion and exclusion criteria. The majority of the patients enrolled in the study were males, the age distribution seen highest in the age group between 55-64.

The results of our study show age, gender, lack of physical activity, overweight, HTN, DM, consumption

of alcohol, and smoking were found. The result of risk assessment revealed 55.5% of the study population has a risk of  $\leq 20\%$  and 44.4% of the study population have a risk  $\geq 21\%$  of developing CVD in the next 10 years. Comparing actual age and heart age data has shown variation by 13.8 years in heart age from the actual age. The average actual age and heart age were  $55.5 \pm 12.1$  and  $69.3 \pm 17.1$  years, respectively.

The present study highlights the fact that patients with dyslipidemia have poor HRQOL as the overall PCS and MCS scores were low. KAP assessment revealed out of the 200 study population that most of the population lacks knowledge about the disease, but in case of practice and attitude towards the disease, the majority of the population has a positive response. Evaluation of DRPs showed a majority of them were potential drug interactions and poor medication adherence.

Hence, these results highlight the pivotal role of the pharmacist as a health care team provider in the management of dyslipidemia through routine screening programs for blood lipid levels and appropriate intervention programs aimed at risk factor reduction, improve the HRQOL and to reduce the risk percentage for developing CVD in the future, and also improve the knowledge about the disease to the patients, as more information about the disease and its management will empower patients to manage their conditions better. Focus on dyslipidemia management is urgently required in India to halt the rising tide of coronary heart disease.

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## ORIGINAL

# *Helicobacter pylori* distribution in dental plaque specimens collected from individuals referred to dental clinics

*Distribución de Helicobacter pylori en muestras de placa dental recolectadas de individuos remitidos a clínicas dentales*

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## Abstract

**Background:** According to documented thesis, dental plaques may be sources of *Helicobacter pylori* in the oral cavity. The present research was performed to assess the distribution of *H. pylori* in dental plaque samples collected from individuals referred to dental clinics.

**Methods:** Four hundred patients referred to the dental clinics for routine check-ups were assessed in this survey. Dental plaque presence is the prominent inclusion factor. All patients who had received antimicrobial options or antibacterial mouthwashes three months before the experiment were excluded from the research. Two hundred and fifty dental plaque samples were collected from included patients. Samples were taken from the gingival crevice at the most profound pocket reading and removed from the clinical site using a sterile universal curette. Samples were cultured, and suspected colonies were confirmed using the biochemical tests and the polymerase chain reaction (PCR).

**Results:** The mean age of the included population was 54.5 year, with a male to female ratio of 140/110. Molar (45.2%) dental plaque was the most commonly identified, followed by premolar (35.8%). *H. pylori* was detected in 42 out of 250 (16.8%) dental plaque specimens. Molar teeth plaque specimens (19.4%) had the highest distribution of *H. pylori*, while incisor teeth (12.0%) harboured the lowest.

**Conclusion:** The role of dental plaques, particularly in the molar and premolar areas as *H. pylori* reservoirs, was determined. Oral hygiene observation and proper antimicrobial uses can diminish the *H. pylori* distribution.

**Keywords:** *Helicobacter pylori*, diagnosis, distribution, dental plaque.

## Resumen

**Antecedentes:** Según tesis documentadas, las placas dentales pueden ser fuentes de *Helicobacter pylori* en la cavidad oral. La presente investigación se llevó a cabo para evaluar la distribución de *H. pylori* en muestras de placa dental recogidas de individuos remitidos a clínicas dentales.

**Métodos:** En este estudio se evaluaron 400 pacientes remitidos a las clínicas dentales para revisiones rutinarias. La presencia de placa dental es el principal factor de inclusión. Se excluyeron de la investigación todos los pacientes que habían recibido opciones antimicrobianas o colutorios antibacterianos tres meses antes del experimento. Se recogieron 250 muestras de placa dental de los pacientes incluidos. Las muestras se tomaron de la hendidura gingival en la lectura más profunda de la bolsa y se extrajeron del sitio clínico utilizando una cureta universal estéril. Las muestras se cultivaron y las colonias sospechosas se confirmaron mediante las pruebas bioquímicas y la reacción en cadena de la polimerasa (PCR).

**Resultados:** La edad media de la población incluida fue de 54,5 años, con una relación hombre/mujer de 140/110. La placa dental molar (45,2%) fue la más comúnmente identificada, seguida de la premolar (35,8%). Se detectó *H. pylori* en 42 de los 250 (16,8%) especímenes de placa dental. Las muestras de placa de los dientes molares (19,4%) presentaban la mayor distribución de *H. pylori*, mientras que los dientes incisivos (12,0%) albergaban la menor.

**Conclusiones:** Se determinó el papel de las placas dentales, especialmente en las zonas molares y premolares, como reservorios de *H. pylori*. La observación de la higiene oral y el uso adecuado de antimicrobianos pueden disminuir la distribución de *H. pylori*.

**Palabras clave:** *Helicobacter pylori*, diagnóstico, distribución, placa dental.



## Introduction

The human body includes  $10^{13}$  somatic cells and  $10^{14}$  normal or commensal microbes<sup>1</sup>. These commensal bacteria reside on the surfaces of teeth or prostheses within complex ecosystems termed biofilm. Dental plaque is the host-associated biofilm. Supragingival and subgingival plaque provide an optimal aerophilic and microaerophilic environment for the survival of these microorganisms. Approximately six billion microbes representing 300-500 reside in these environments in the oral cavity.

An association between oral infections and systemic diseases has been suspected for centuries. Assyrians proposed the effect of oral health on the rest of the body in the 7<sup>th</sup> century BC. In the 18<sup>th</sup> century, a Pennsylvanian physician<sup>2</sup> named Benjamin Rush was quoted as remarking that arthritis could be treated in some people after infected teeth were extracted.

Over the past decade, a growing body of scientific evidence suggests an exquisite association between oral infection (e.g., viruses, bacteria, yeast) and systemic diseases (e.g., atherosclerosis, cardiovascular disease, cerebrovascular disease, premature, low birth weight, and pulmonary diseases and disorders), and also between systemic diseases (e.g., arthritic, diabetic, HIV, and osteoporotic) and oral, dental, and craniofacial disorders<sup>3</sup>.

It has long been speculated that dental plaque might harbour *Helicobacter pylorus* (*H. pylori*) and, therefore, might be a source of reinfection of the gastric mucosa<sup>4</sup>. *H. pylori* is a microaerophilic and Gram-negative spiral coccoid bacterium known as a causative agent for gastric adenocarcinoma, peptic ulcer disease, duodenal ulcer, type B gastritis, and B-cell lymphoma<sup>5</sup>. The area around the dental plaque has a low oxidation potential promoting the growth of facultative anaerobes. Newman<sup>6</sup> suggested that bacteria fermenting carbohydrates in food produce a low pH in the dental plaque, and this microaerophilic acidic environment with an average oral temperature of 35-37°C can be ideal for the growth of *H. pylori*.

About 50% of the subjects living in developed and developing countries are affected by *H. pylori*. These infections are complicated to eradicate, and it has been postulated that a sanctuary or sanctuaries which allow them to evade antimicrobial therapy much exists. Desai and Majmudar<sup>7</sup> suggested that recrudescence of infection following cessation of therapy may occur, owing to the recolonization of the stomach from the *H. pylori* present in dental plaque are unaffected by the antimicrobial treatment. Knowledge of this pathogenic organism will permit a target for therapeutic procedures and a monitoring tool for therapy efficacy and learn about the various transmission routes.

According to the high importance of bacteria and the absence of epidemiological surveys in this field, the present research was performed to assess the *H. pylori* distribution in dental plaque specimens collected from individuals referred to dental clinics.

## Materials and methods

### Ethics

All personal information of individuals included in the study were kept secret. Written informed consent was taken from all individuals. The study protocol was ethically approved by the University of Traditional Medicine of Armenia.

### Inclusion and exclusion criteria

A total of 400 patients referred to the Armenia dental clinics for routine check-ups were assessed in this survey. All patients with dental plaque samples were included in this survey. Dental plaque presence is the prominent inclusion factor. All patients who had received antimicrobial options or antibacterial mouthwashes three months before the experiment were excluded from the research. All of the selected patients were non-smokers.

### Dental specimens

From January to April 2021, 250 male and female patients with dental plaque will different age were included in the study. A dental plaque sample was taken from the gingival crevice at the most profound pocket reading and removed from the clinical site using a sterile universal curette. The curette tip was inserted into the depths of the crevice/pocket, moved coronally while in contact with the tooth surface to remove both sub and supragingival plaque.

### *H. pylori* isolation and identification

The dental plaque sample from each patient was cultured into a sterile tube containing 5% sheep blood agar, chocolate agar and a selective medium and transported to the microbiology laboratory to be incubated microaerophilically (5% oxygen, 85% nitrogen, and 10% CO<sub>2</sub>) using the MART system (MART system, Lichtenvoorde, The Netherland) at a temperature of 37°C for seven days. Culture media were supplemented with 5% of horse serum (Sigma, St. Louis, MO, USA), nalidixic acid (30 mg/L), vancomycin (10 mg/L), cycloheximide (100 mg/L), and trimethoprim (30 mg/L) (Sigma, St. Louis, MO, USA)<sup>8-14</sup>. Suspected colonies were then identified using Gram stain, motility, colony morphology, and biochemical tests such as urease, oxidase, and catalase tests<sup>15</sup>. For comparison, a reference strain of *H. pylori* (ATCC 43504) was employed.

### Polymerase Chain Reaction (PCR) identification of bacterial isolates

PCR was used to definitely identified the *H. pylori* isolates<sup>16</sup>. For this purpose, genomic DNA was extracted

using a DNA extraction kit (Thermo Fisher Scientific, St. Leon-Rot, Germany). The technique was performed rendering to the factory guidelines<sup>17,18</sup>. Purity (A260/A280) and concentration of extracted DNA were then plaid (NanoDrop, Thermo Scientific, Waltham, MA, USA), and the DNA quality was scrutinized by electrophoresis<sup>19-22</sup>. PCR was accompanied using a PCR thermal cycler (Eppendorf Co., Hamburg, Germany) rendering to the described procedure<sup>23-25</sup>. *H. pylori* (26695) was positive, while sterile PCR grade water (Thermo Fisher Scientific, Germany) was used as negative controls. Electrophoresis was performed using 2% agarose gel stained with ethidium bromide run in a 90 V for about 30 min<sup>26-28</sup>. Briefly, Ten microliters of PCR product were exposed to electrophoresis in a 2% agarose gel in 1X TBE buffer at 80 V for 30 min, stained with SYBR Green. The UVI doc gel documentation systems (Grade GB004, Jencons PLC, London, UK) were applied to analyze images<sup>29-33</sup>.

### Data analysis

Data were subjected to Microsoft Office Excel (version 15; Microsoft Corp., Redmond, WA, USA). The statistical analysis was performed employing the SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact two-tailed test were applied to measure any significant relationship. *P*-value <0.05 was considered as a significant numerical level<sup>34-36</sup>.

## Results

### Demographic characters

**Table I** shows the demographic characters of the study population. As shown, the mean age of the studied population was 54.5 year, with a male to female ratio of 140/110. Totally, 20.8% of the study population had a history of alcohol. Molar (45.2%) dental plaque was the most commonly identified, followed by premolar (35.8%).

**Table I:** The study population of the present survey.

Demographic characters	Individuals (250 people)
Mean age (SD)	54.5 (15.2)
Sex (M/F)	140/110
Mean weight (SD)	68.3 (12.6)
Mean BMI (SD)	24.2 (3.9)
Alcohol (%)	20.8
Dental plaque location	
Incisor teeth (%)	25 (10.0)
Canine teeth (%)	30 (12.0)
Premolar teeth (%)	82 (32.8)
Molar teeth (%)	113 (45.2)

### *H. pylori* distribution

PCR procedure was used to detect *H. pylori* in dental plaque specimens. **Table II** shows the *H. pylori* distribution amongst examined dental plaque specimens. Forty-two out of 250 (16.8%) dental plaque specimens were positive for *H. pylori*. Molar teeth plaque specimens (19.4%) harboured the highest distribution

of *H. pylori*, while incisor teeth (12.0%) harboured the lowest. Statistically, a significant difference was obtained between the site of dental plaque samples and *H. pylori* distribution (*P* <0.05)

**Table II:** *H. pylori* distribution amongst examined dental plaque specimens.

Study groups	N. collected specimens	N. specimens positive for <i>H. pylori</i> (%)
Dental plaque position	Incisor teeth	3 (12.0)
	Canine teeth	4 (13.3)
	Premolar teeth	13 (15.8)
	Molar teeth	22 (19.4)
Total	250	42 (16.8)

## Discussion

Despite high advances in medical sciences, diverse issues have been kept complicated in this field of science<sup>38-40</sup>. In this regard, *H. pylori* have become an essential public health issue in the last century<sup>41</sup>. Several investigations have been focused on finding the exact route of *H. pylori* infection. Some researchers found that the human dental plaques can be the reservoir of *H. pylori*<sup>42</sup>. Others showed that the oral cavity (dental plaque, tongue, saliva, root canals, tonsil tissue, oral mucosa) are essential sources of *H. pylori* other than the gastric mucosa<sup>43</sup>. In the present survey, *H. pylori* was detected in 16.8% of the dental plaque samples, with a higher distribution amongst the plaques collected from molar teeth. Food accumulation in the molar teeth and difficult access to cleaning and brushing can probably lead to *H. pylori* growth in this area.

Diverse researches have been conducted to determine the role of dental plaques as sources of *H. pylori* infections<sup>44,45</sup>. Chitsazi et al. (2006)<sup>46</sup> stated that *H. pylori* was detected in 34.1% of dental plaque specimens. The *H. pylori* prevalence of infection in dental was 31.8% and 36.4% in patients with and without gastric infection. Medina et al. (2010)<sup>47</sup> mentioned that *H. pylori* was detected in 18.3% of oral samples and 88.3% of gastric biopsies. Saudia authors<sup>48</sup> showed that 65.0% of patients had *H. pylori*-positive dental plaque, and more than 50% harboured the bacteria in their stomach. They also showed that periodontitis patients had a significantly higher *H. pylori* percentage in their dental plaque (79.0% versus 43.0%; *P* <0.05) and the stomach (60.0% versus 33.0%; *P* <0.05) than those without periodontitis. Even though *H. pylori* may be detected in the stomach of about 50% of the world's population, its individual to individual transmission mechanisms are not yet identified. *H. pylori* transmission could occur through faecal-oral and oral-oral routes. The bacterium may be transmitted orally and detected in dental plaque and saliva<sup>49</sup>. High distribution of *H. pylori* in dental plaque has been reported in surveys conducted in Mexico<sup>50</sup>, Iran<sup>51</sup>, Japan<sup>52</sup>, and Morocco<sup>53</sup>. An Iranian survey<sup>54</sup> revealed that the frequency of detection of *H. pylori* in the dental plaque samples were

44% (20/45), 66.67% (30/45) and 77.78% (35/45) using PCR, loop-mediated isothermal amplification (LAMP) and positivity for both tests, respectively. In the current survey, all *H. pylori* isolates were identified by biochemical tests and definitely PCR. due to the high sensitivity and specificity of applied diagnostic tests, results have a high confidence level.

The present survey was preliminary research on the distribution of *H. pylori* in the dental plaque samples. It is limited to the lack of the study of the nutritional and gastrointestinal diseases of the examined population and their relations with the *H. pylori* distribution. Additionally,

the absence of results about the role of alcohol consumption in *H. pylori* distribution among patients is another limitation.

## Conclusion

This survey showed the relatively high *H. pylori* prevalence amongst the dental plaque specimens. The role of dental plaque as a source of *H. pylori* was determined in this survey. Oral hygiene observation, dental plaques removal, mouthwash, and regular brushing can reduce the *H. pylori* distribution.

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# Comparative study of clinical symptoms, laboratory and radiological findings of COVID-19 patients admitted to ICU ward with internal ward of Forghani Hospital in Qom: a case series

*Estudio comparativo de síntomas clínicos, hallazgos de laboratorio y radiológicos de pacientes COVID-19 ingresados en la sala de UCI con sala interna del Hospital Forghani en Qom: una serie de casos*

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## Abstract

This analytical study was performed in Forqani Hospital of Qom from April to June of the year with the aim of comparing laboratory and radiological findings in patients with coronavirus 2020. Laboratory and radiological findings were performed between 50 patients with coronary artery hospitalization in the inpatient ward and 50 patients admitted to the ICU of Qom. After collecting information, data analysis was performed with SPSS24 software. "In patients admitted to the inpatient ward, the age of patients was  $54 \pm 2.29$  years and 54% were male and the mean age of patients admitted to intensive care units was  $60 + 2 + 01$  years and 64% were male. Based on radiological findings in patients admitted to the inpatient ward and ICU, bilateral lung involvement was 68% and 84%, respectively ( $p < 0.05$ ). The finding of grand glass in the lungs in patients admitted to the inpatient ward was significantly higher than the ICU patients and also the finding of consolidation in the lungs in patients admitted to the ICU was significantly more than the patients admitted to the inpatient ward ( $P < 0.05$ ). In laboratory findings, there was a significant difference between WBC, lymphocyte, BUN and creatinine and CRP in patients admitted to the ICU with patients admitted to the inpatient ward ( $P < 0.05$ ). The results of this study show that in patients with coronavirus diagnosis, there was a significant difference in radiological and laboratory findings in patients with COVID-19 admitted to the inpatient and intensive care units such as blood oxygen levels, WBC, lymphocyte platelets, CRP, creatinine. There was a significant difference between urea and lung CT scan findings and diagnosis of lung consolidation in patients admitted to the ICU compared with patients admitted to the ward.

**Keywords:** Coronavirus infection, SARS CoV-2, critical care, clinical features.

## Resumen

Este estudio analítico se realizó en el Hospital Forqani de Qom de abril a junio del año con el objetivo de comparar los hallazgos de laboratorio y radiológicos en pacientes con coronavirus 2020. Se realizaron hallazgos de laboratorio y radiológicos entre 50 pacientes con coronavirus ingresados en la sala de hospitalización y 50 pacientes ingresados en la UCI de Qom. Tras recoger la información, se realizó el análisis de los datos con el software SPSS24. En los pacientes ingresados en la sala de hospitalización, la edad de los pacientes era de  $54 \pm 2/29$  años y el 54% eran hombres y la edad media de los pacientes ingresados en las unidades de cuidados intensivos era de  $60 \pm 2 \pm 01$  años y el 64% eran hombres. Según los hallazgos radiológicos en los pacientes ingresados en la sala de hospitalización y en la UCI, la afectación pulmonar bilateral fue del 68% y del 84%, respectivamente ( $p < 0,05$ ). El hallazgo de gran vidrio en los pulmones en los pacientes ingresados en la sala de hospitalización fue significativamente mayor que en los pacientes de la UCI y también el hallazgo de consolidación en los pulmones en los pacientes ingresados en la UCI fue significativamente mayor que en los pacientes ingresados en la sala de hospitalización ( $p < 0,05$ ). En cuanto a los resultados de laboratorio, hubo una diferencia significativa entre el recuento de glóbulos blancos, los linfocitos, el BUN, la creatinina y la PCR en los pacientes ingresados en la UCI y en los pacientes ingresados en la sala de hospitalización ( $P < 0,05$ ). Los resultados de este estudio muestran que en los pacientes con diagnóstico de coronavirus hubo una diferencia significativa en los hallazgos radiológicos y de laboratorio en los pacientes con COVID-19 ingresados en la sala de hospitalización y en las unidades de cuidados intensivos, como los niveles de oxígeno en sangre, los glóbulos blancos, los linfocitos, las plaquetas, la PCR y la creatinina. Hubo una diferencia significativa entre los hallazgos de la urea y la tomografía computarizada de pulmón y el diagnóstico de consolidación pulmonar en los pacientes ingresados en la UCI en comparación con los pacientes ingresados en la sala.

**Palabras clave:** Coronavirus infection, SARS CoV-2, critical care, clinical features



## Introduction

SARS-CoV-2 (The Severe Acute Respiratory Syndrome Coronavirus 2), formerly known as the New Coronavirus (2019-Ncov), is a new virus that appeared in December 2019 and causes COVID-19<sup>1</sup>. The virus causes a syndrome that in some cases can lead to a dangerous respiratory condition, which will require specialized management of the disease in the ICU<sup>2</sup> It is also known as an intensive care unit. It is the seventh known coronavirus to infect humans. Other beta-coronaviruses have already caused other epidemics in the last two decades in Asia, such as SARS-COV from 2002 to 2003 in China and later from 2012 to 2013 in Saudi Arabia as MERS-COV<sup>3</sup>. Similarities and differences observed in epidemiology, clinical manifestations and treatment of SARS, MERS and COVID, although the clinical form of SARS, MERS and COVID-19 is similar, differences have been observed in the initial reports<sup>4</sup>. Preliminary research has shown patterns in chest CXR and CT. For example, an early prospective study in Wuhan showed 98% (40 out of 41 cases) of bilateral CT chest involvement among patients, with consolidation being the most common finding in this study<sup>5</sup>. Other researchers have examined chest CT in patients, the appearance of grand-glass and consolidation was common among patients<sup>6</sup>.

Covid-19 is an emerging disease and its clinical, laboratory and radiological characteristics are unknown and there are many differences in different studies. Human-to-human transmission is common in this disease. Respiratory droplets and human-to-human communication are the main routes of transmission of the virus. In the early stages of the disease, symptoms of acute respiratory infection are observed, and a number of patients quickly develop ARDS and other severe symptoms, which eventually lead to organ damage. Historically, coronaviruses have been formally identified as a new viral family in the 1960s following the discovery of several new human respiratory pathogens. These viruses are known to have structures on their surface called spikes. Almost 40 years after the identification of this group of viruses and in late 2002 and early 2003, a coronavirus caused severe respiratory complications in humans known as SARS (COAR) or Acute Respiratory Syndrome<sup>7</sup>. The sudden emergence of SARS led to new research to understand the main mechanisms of reproduction and pathogenicity of members of this viral family with the aim of controlling them worldwide. The outbreak of SARS-COV infected 8096 people and killed 794 people and the mortality rate of this virus was 9.8%. Then in 2012, another virus of this family broke out again in the Middle East, especially in Saudi Arabia, called MERS (MERS-COV), infecting a total of 2.260 people and causing 35.5% human deaths. Seven years after the onset of Morse disease, the onset of COVID 19 disease by RNA - SARS-CoV-2 virus in December 2019 in Wuhan City, Hui Province, China, was associated

with symptoms of acute respiratory syndrome and widespread and rapid outbreak. The moon reached the stage of a global epidemic<sup>8</sup>. In late December 2019, a series of unexplained cases of pneumonia were reported in Wuhan, China. The government and health researchers in China took swift action to control the epidemic, and began etiological research<sup>10</sup>. On January 12, 2020, the WHO temporarily named the new virus as the New Coronavirus-2019. In the present study, the same phrase "New Coronavirus-2019" has been used<sup>11</sup>. On January 30, 2020, the WHO announced the New Coronavirus Epidemic 2019 as an International Public Health Emergency (PHEIC)<sup>12</sup>. On February 11, 2020, the WHO officially named the disease caused by the New Coronavirus Disease 2019 (COVID-19). On the same day, the International Virus Classification Committee (CSG) Study Group named it Severe Acute Respiratory Syndrome, and on February 23, 2020, 77041 cases of Covid-19 infections were confirmed in China. This number of infections has exceeded the prevalence of Acute Respiratory Syndrome (SARS) in China in 2002<sup>13</sup>.

Structure of Coronavirus-2019 Coronaviruses are enveloped, non-segmented viruses with single-stranded, Positive-sense RNA of animal origin and belong to the family Corona. The size of the virus genome is between 26 and 32 kb, which is one of the largest RNA viruses. These viruses have two different types of surface proteins and get their name from this appearance. The family of coronaviruses is serologically divided into four genera: alpha, beta, gamma and delta<sup>14</sup>. Approximately 30 types of coronavirus have been identified in humans, mammals and birds. Human coronaviruses are caused by alpha and beta genera. Coronaviruses are one of the most common viruses, with 30 to 60 percent of the population having antibodies against it<sup>15</sup>. New Coronavirus-2019 (SARS-COV-2) is a beta-coronavirus coronavirus. Covid-19 is the third known animal coronavirus disease after SARS and MERS Middle East Respiratory Syndrome, both of which belong to the beta-coronavirus category<sup>16</sup>. Origin of New Coronavirus-2019 an epidemiological study of early cases of modern coronavirus pneumonia-2019 showed that many cases were exposed to the Hanan seafood market in Wuhan, China<sup>17</sup>. The WHO report also states that the new Coronavirus-2019 has been detected in environmental samples collected from Hanan seafood markets<sup>18</sup>. But it is not yet clear what specific species of animals will carry the new coronavirus-2019. 33 of the 585 environmental samples available in the Hanan Seafood Market were positive for New Coronavirus-2019. Some of these include a variety of live animals, such as hedgehogs, badgers, snakes, turtles, birds, and possibly anteaters, but bats do not<sup>19</sup>. Therefore, bats were not likely to have direct contact with humans and did not have direct transmission to humans, and direct transmission of the virus from bats to humans seems unlikely<sup>20</sup>. A study by Ji et al. Showed that the New Coronavirus-2019 is a chimeric virus between bat coronavirus and coronavirus

of unknown origin. Compared to other animals, they found that snakes are very likely to be the reservoir of the new coronavirus-2019<sup>21</sup>. A study by Benvenuto et al. Showed that the New Coronavirus 2019 is closely related to closely isolated coronaviruses of a particular type of Chinese bat<sup>22</sup>. Their research supports the theory that the chain of transmission from bats to humans has begun. Chan et al. confirmed that the new coronavirus-2019 was a new coronavirus highly associated with the SARS bat coronavirus<sup>23</sup>. Recently, Zhou et al. And Wu et al. found that the sequence similarity between New Coronavirus 2019 and SARS coronavirus is 79.5%. They also found that the new coronavirus-2019 is highly homologous to bat coronavirus. Thus, current evidence strongly supports that New Coronavirus-2019 was derived from bats, although the intermediate or intermediate hosts of New Coronavirus-2019 are not yet known<sup>24</sup>.

The study by Wang et al. showed that from January 10 to 24, 2020, the number of people infected with Covid-19 infection in China increased 31.4 times. On February 23, 2020, the number of patients with Covid-19 in China was 1879, equal to January 10, 2020. They estimated the mortality rate of Covid-19 based on the number of patients at 2.84%. The researchers also found that the male-to-female mortality rate was 3.25 to 1, the median age of death was 75 years, the median time from the first symptoms to death was 14 days, and the median time from initial symptoms to death in people 70 years of age and older (5/11 days) is shorter than people under 70 (20 days). These findings suggest that the disease may progress faster in adults than in young people<sup>25</sup>. The study by Li et al. Reported that the mean age of 425 patients infected with modern coronavirus-2019 was 59 years, of which 56% were male, the mean incubation period was 5.2 days, and approximately half of adult patients were 60 years and older. In the early stages, the number of infected patients doubled every 7.4 days. The rate of disease transmission from the infected person was 2.2. Although 55% of the first patients infected with Covid-19 were related to the Hanan seafood market, the number of unrelated cases has increased logarithmically since late December 2019<sup>26</sup>. Of the 41 patients with Covid-19 infection in their study, Huang et al. Showed that 73% of patients were male and 32% of patients had underlying diseases including diabetes (8 patients), hypertension (6 patients), and diseases. Were cardiovascular (6 patients). He was 49 years old. Of the 41 patients, 27 were related to the Hanan seafood market. The mortality rate of coronavirus patients in this study was reported to be 15%<sup>27</sup>. The study by Wu et al. estimated the transmission rate of infected patients to be 0.3. The mortality rate of patients with coronavirus in this study was reported to be 14%<sup>28</sup>. Preliminary studies have shown that people with underlying diseases are at higher risk for complications and mortality from Covid-19 disease. Approximately 50% of hospitalized patients suspected of having a new coronavirus have

other chronic diseases, and about 40% of hospitalized patients with confirmed new SARS-COV-2 coronavirus infection have cardiovascular or cerebrovascular disease. They are vascular. The researchers also found large differences in mortality by age group, with Guan et al. Reporting 1,099 cases of Covid-19 infection. They found that fever of 87.9% and cough of 67.7% were the most common symptoms. Diarrhea 3.7% and vomiting 5% were rare. Abnormalities in chest CT images were observed in 96% of patients infected with Covid-19 and in 82.1% of them lymphopenia was recorded<sup>29</sup>. In the study of the radiological findings of 81 patients with COVID-19 in Wuhan, China: A descriptive study of 81 patients with COVID-19, in which 42 men and 30 women participated and the mean age of the company they are equal to 49.5 years. The average number of lung segments involved is 10.5. The most common patterns of bilateral involvement were 79%, peripherals 54%, grand glass 65%, and the lower lobe of the lung 27%. Symptoms are as follows: 73% fever, shortness of breath 70%, cough 59%, sputum 19%, weakness 9%, vomiting 5%, headache 6%, dizziness 2%, diarrhea 4%. The mean of laboratory findings is also reported as follows: WBC 8100, Lymphocyte 1100, Platelet 212200, Hemoglobin 12.3, CRP 6.47, ALT 2.46, AST 40.8, D-dimer 6.5. In Wang et al.'s study entitled Frequency and Distribution of Chest Radiographic Findings in COVID-19 Patients, 64 patients were studied. The study involved 26 men and 38 women, and the average age of patients was 56 years. 31% of patients had normal CXR. 59% of patients had consolidation and 41% had left lung involvement. 63% of patients had significant lower lung involvement and none of the patients had upper lung involvement. PE was also observed in 2% of patients, pulmonary nodules were not observed in any of the patients<sup>30&33</sup>.

In a study by Burnheim et al. Entitled Chest CT Findings in Coronavirus Disease: Relationship to Duration of Infection, 121 symptomatic patients were studied. In this study, 61 men and 60 women were studied, the average age of the participants was 45.3 years. The radiological appearance of 22% of patients was normal, 34% of patients with radiological changes had only a grand glass view, 2% did not have a consolidation view, and the rest of the patients had both a grand glass view and consolidation. 15% of patients had single lobe involvement, 12% had two lobe involvement, 9% had 3 lobe involvement, 15% had 4 lobe involvement, and 27% had 5 lung involvement<sup>34</sup>. In a study by Ming Yin et al. Entitled COVID-19 infection imaging profile: radiological findings and review of sources, 21 patients were examined, of which 13 were male and 8 were female. 86% of patients had grand glass facade, 19% had grand glass nodules and 62% had consolidation. The two patients had no chest radiological changes. 86% of patients with peripheral involvement had 1 pre-hilar involvement. 90% of patients had fever, 48% cough, 15% sputum, 10% sore throat, 10% diarrhea and

5% chest pain. The mean of laboratory results was as follows: hemoglobin 13.8, WBC 5.3, neutrophils 3.33, lymphocytes 1.29, and platelets 169, PT 36, D-dimer 0.4, Na 139, K 3.86, and Urea 45/4. In a study by Xavi et al. Entitled Clinical Findings in a Group of COVID-19 Patients outside Wuhan, China: A retrospective case study, 62 COVID-19 patients were studied, 35 were female and 27 were male. The mean of laboratory findings was as follows: WBC 4.7, neutrophil 2.9, lymphocyte 1, hemoglobin 13.7, platelet 176, D-dimer 0.2, also 52 patients had bilateral lung involvement<sup>35&36</sup>.

## Research methods

The present study is an analytical study. In this study, 100 hospitalization cases related to Covid-19 were reviewed. Of these 100 patients, 50 were hospitalized in the inpatient ward and 50 were hospitalized in the ICU. Patients were selected from those whose diagnosis of COVID-19 was confirmed based on diagnostic methods and with the opinion of a pulmonologist. After selecting patients, information about clinical signs, laboratory and radiological findings are recorded in a researcher-made checklist. The prepared form had 3 sections to record the required information, the first part included demographic information of patients such as age, gender, duration of symptoms, and length of hospital stay, ward and the second part included laboratory findings such as WBC number, platelet count, ESR level, CRP, and the fourth part included radiological findings such as unilateral or bilateral pulmonary involvement, the presence of consolidation, grand glass view, etc.

## Calculate the sample size

Considering alpha (first study error) as 0.05, d (study accuracy) as 0.1, P as 0.8, 100 people enter the study

$$N = \frac{[P(1-P)] \times (Z_{1-\alpha/2})^2}{(d)^2} \rightarrow N = \frac{(0.54 \times 0.3) \times 5}{(0.1)^2} \rightarrow N = 50 \quad (1)$$

## Data analysis method

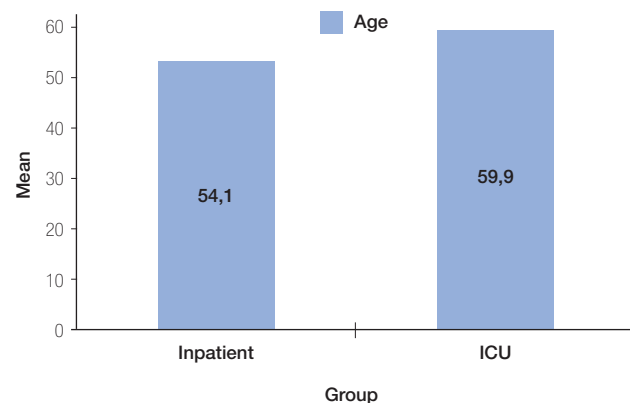
The collected data were analyzed using SPSS24 statistical software and T-test at the significant level of P value <0.05.

## Research results

In the present study, clinical symptoms, laboratory and radiological findings in patients with coronary artery in the ICU and the internal ward were compared, and the findings were presented in two descriptive and analytical sections. Age distribution of patients with coronary

artery disease, the mean age of patients with coronary hospitalization in the inpatient ward was 54 + 2.29 years and the mean age of patients admitted to the intensive care unit was 60 + 2 + 01 years. The mean age of patients admitted to the ICU was higher than that of patients admitted to the ICU, but this difference was not significant (P = 0.06).

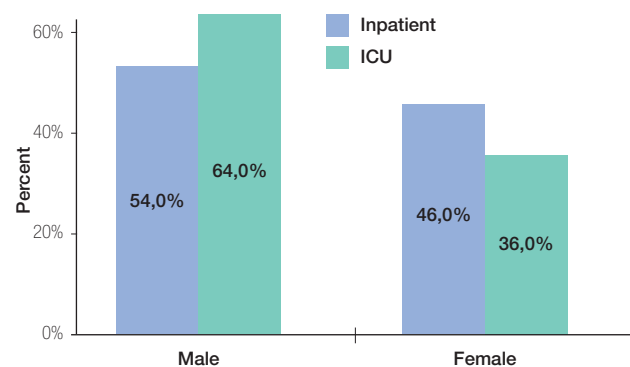
Figure 1: Comparison of the mean age of patients with coronary artery inpatient and ICU.



## Gender distribution of patients with coronary artery disease

In patients with coronary hospitalization in the internal ward, 54% of patients were male and 46% female and in patients with coronary artery in the ICU, gender was 64% male and 36% female. Frequency of male patients admitted to the ICU compared to hospitalized patients It was higher in the internal part but this difference was not significant (P = 0.31).

Figure 2: Percentage of relative frequency of gender in patients with coronary artery in two groups.



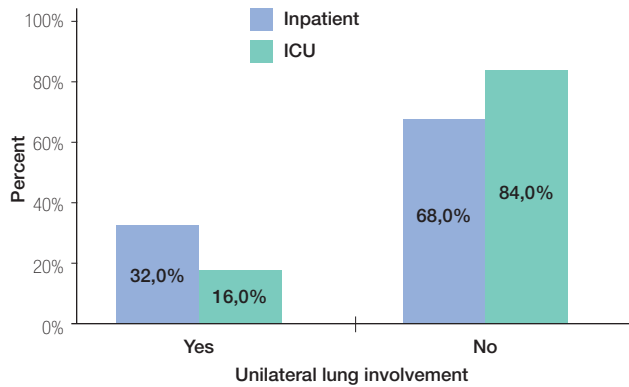
## Radiological findings

### Frequency of unilateral lung involvement in patients with coronary artery disease

In patients with coronary hospitalization in the internal ward, unilateral lung involvement was diagnosed in 32%

of patients and in patients with coronary hospitalization in the ICU, unilateral lung involvement was diagnosed in 16% of patients. The frequency of unilateral lung involvement in patients admitted to the inpatient ward was higher compared to patients admitted to the intensive care unit, which was a significant difference ( $P = 0.05$ ).

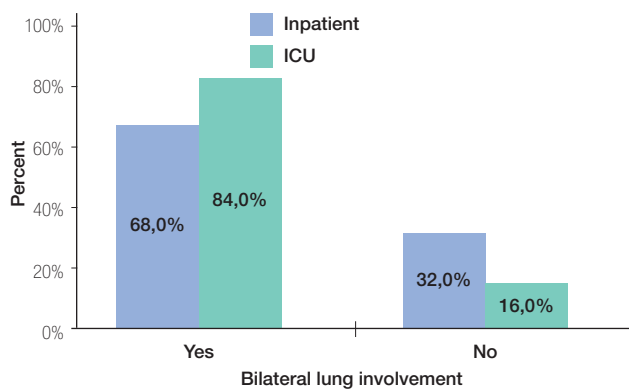
**Figure 3:** Percentage of relative frequency of unilateral involvement in coronary artery disease in the two groups.



**Frequency of bilateral lung involvement in patients with coronary artery disease**

Bilateral lung involvement was diagnosed in 68% of patients with coronary hospitalization in the internal ward and bilateral lung involvement was diagnosed in 72% of patients admitted to the ICU. The frequency of bilateral lung involvement in patients admitted to the intensive care unit was significantly higher compared to patients admitted to the intensive care unit ( $P = 0.048$ ).

**Figure 4:** Percentage of relative frequency of unilateral involvement in patients with corona in the two groups.

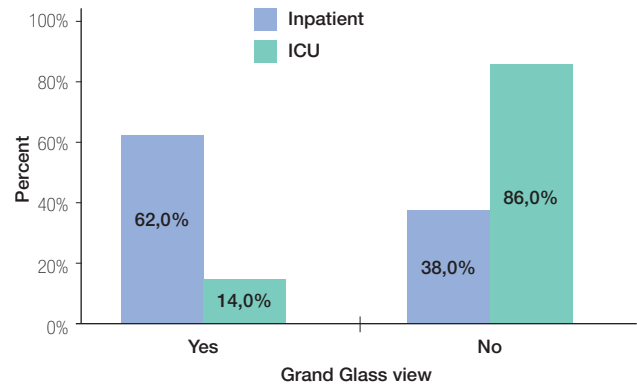


**Frequency of grand-glass, consolidation and nodules in the lungs of patients with coronavirus**

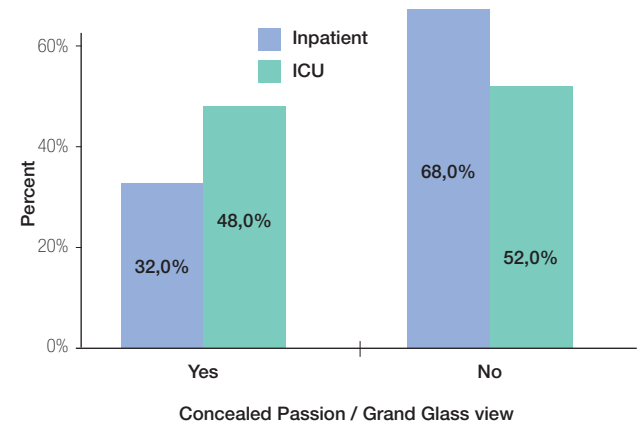
In patients with coronary hospitalization in the inpatient ward, the diagnosis of grand-glass in 62%, grand glass / consolidation 32% and consolidation alone in 4% of patients and in patients with coronary hospitalization in the ICU diagnosis of grand glass lung in 14%, Grand -

Glass / consolidation was 48% and consolidation alone in 38% of patients. In general, radiological findings of grand glass in patients admitted to the inpatient ward were higher than patients in the intensive care unit ( $P = 0.0001$ ) and in patients admitted to intensive care units, more radiological findings were grand-glass / consolidation and consolidation alone. ( $0.001/0 = P$ ).

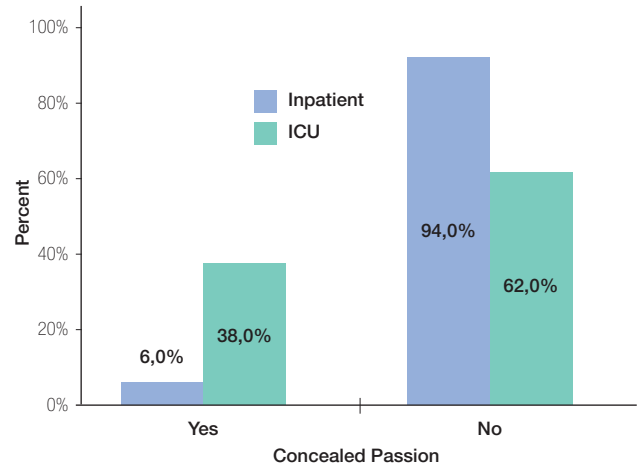
**Figure 5:** Relative frequency of Grand-Glass diagnosis in patients with coronary artery disease in the two groups.



**Figure 6:** Relative frequency of Grand-Glass/Consolidation diagnosis in patients with coronary artery disease in the two groups.



**Figure 7:** The relative frequency of the diagnosis of consolidation in patients with coronary artery disease in the two groups.

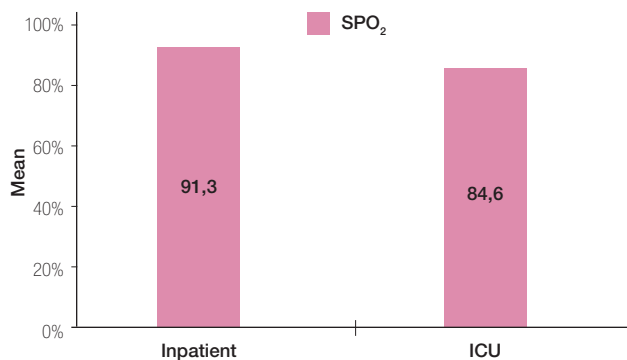


## Laboratory findings

### Comparison of blood oxygen levels in patients with coronary heart disease

In patients with coronary hospitalization in the inpatient ward, the mean SPO<sub>2</sub> was 91.3 + 5.9% and in patients admitted to the intensive care unit was 84.6 + 10.7%. As it is known, blood oxygen level in patients with coronary artery in the ICU is significantly lower than patients in the inpatient ward (P = 0.0001).

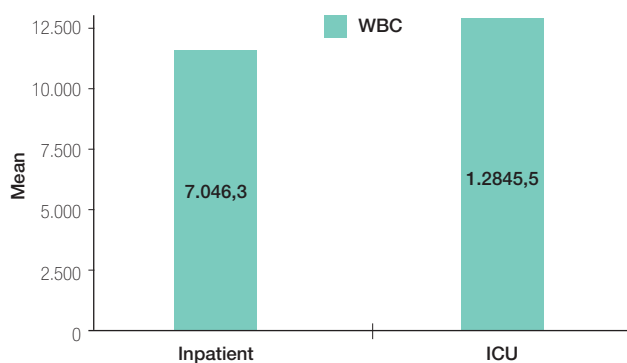
Figure 8: Comparison of mean SPO<sub>2</sub> in hospitalized patients.



### Comparison of WBC changes in patients with coronary artery disease

In patients with coronary artery hospitalization in the inpatient ward, the mean of WBC was 7046.3 + 554.16 and in patients admitted to the intensive care unit was 12845 + 29.58 + 12845. As it is known, the number of WBCs in patients with coronary artery in the ICU was significantly higher than the patients in the inpatient ward (P = 0.045), which in most of these patients was leukocytosis.

Figure 9: Comparison of mean white blood cell count in patients with coronary artery disease in the study groups.

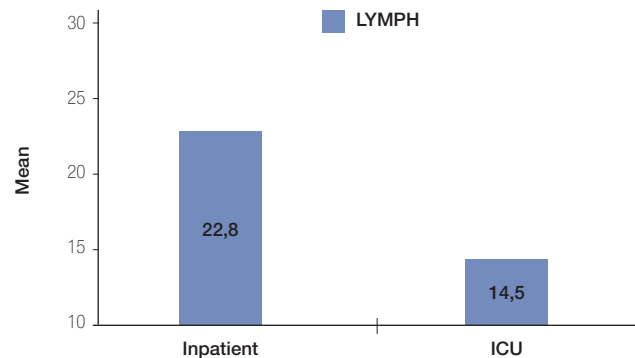


### Comparison of lymphocyte changes in patients with coronary artery disease

In patients with coronary artery hospitalization, the mean lymphocyte was 22.8 + 1.61 and in patients admitted to the intensive care unit was 14.5 + 1.56. As it is known, the number of lymphocytes in patients with coronary

hospitalization in the ICU was significantly lower than patients admitted to the inpatient ward (P = 0.0001).

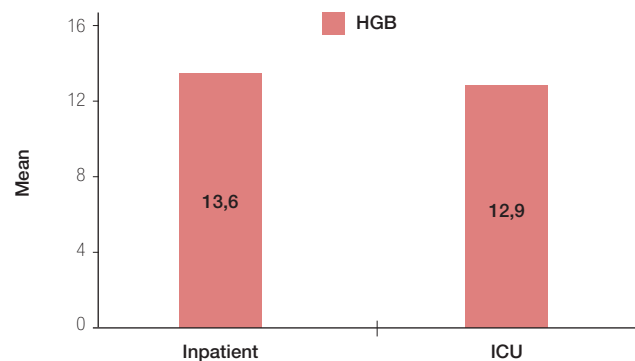
Figure 10: Comparison of the mean number of lymphocytes in patients with coronary artery disease in the study groups.



### Comparison of hemoglobin changes in patients with coronary artery disease

In patients with coronary hospitalization in the inpatient ward, the mean hemoglobin was 13.6+ 1.53 and in patients admitted to the intensive care unit was 12.9 + 2.06. As it is clear that no significant difference was observed between serum hemoglobin levels in the two groups of patients (P = 0.07).

Figure 11: Comparison of mean hemoglobin in patients with coronary artery disease in the study groups.

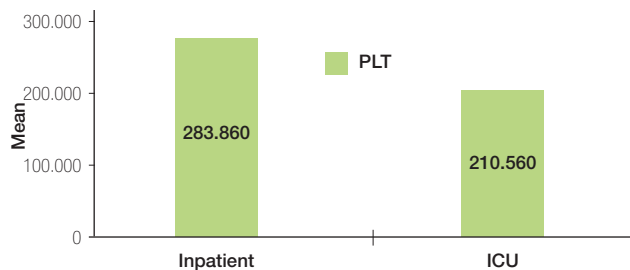


### Comparison of platelet changes in patients with coronary artery disease

In patients with coronary artery hospitalization in the internal ward, the mean platelet count was 283860 + 66364.19 and in patients admitted to the intensive care unit was 210560 + 9901/48. As it is clear that no significant difference was observed between platelet levels in the two groups of patients (P = 0.27). However, the mean platelet count was higher in the inpatient group compared to the ICU patients, which could be due to the administration of anticoagulants in the ICU patients.



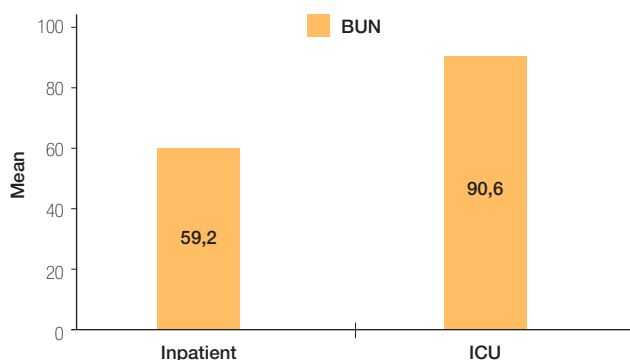
**Figure 12:** Comparison of mean platelets in patients with coronary artery disease in the study groups.



**Comparison of changes in urea nitrogen (BUN) in patients with coronary artery disease**

In patients with coronary hospitalization in the inpatient ward, the mean serum level of BUN was 59.2 + 64.88 and in patients admitted to the intensive care unit was 90.6 + 81.02. As it is known, there is a significant difference between serum BUN levels in the two groups of patients (P = 0.035). The mean BUN in the group of patients admitted to the inpatient ward was significantly lower compared to patients admitted to the ICU.

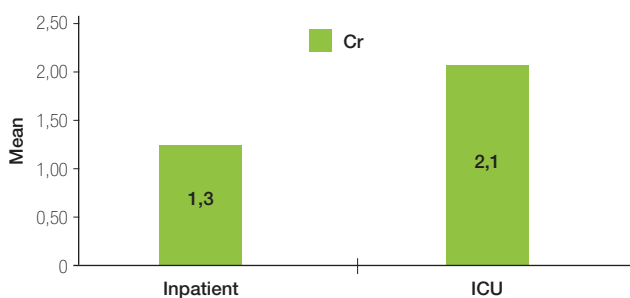
**Figure 13:** Comparison of mean BUN in patients with coronary artery disease in the study groups.



**Comparison of creatinine (Cr) changes in patients with coronary artery disease**

In patients with coronary hospitalization in the internal ward, the mean serum level of Cr is 1.32 + 0.21 and in patients admitted to the intensive care unit is 2.1 + 0.21. As it is known, there is a significant difference between serum creatinine levels in the two groups of patients (P = 0.014). The mean Cr in the group of patients admitted to the inpatient ward was significantly lower compared to patients admitted to the ICU.

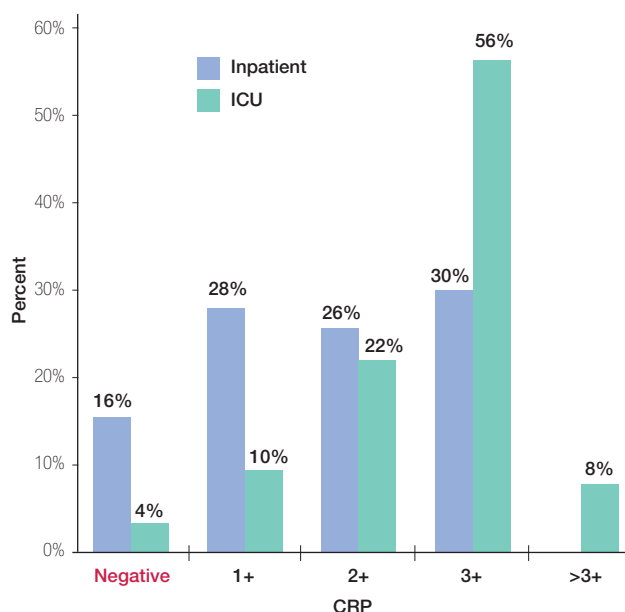
**Figure 14:** Comparison of mean creatinine in patients with coronary artery disease in the study groups.



**Comparison of CRP index in patients with coronary artery disease**

In patients with coronary hospitalization in the inpatient ward, CRP (qualitative) index in 16% (8 cases) negative, 28% (14 cases) +1, in 26% (13 cases) +2 and in 30% (15 cases) +3 and in patients admitted to the intensive care unit in 4% (2 cases) negative, in 10% (5 cases) +1, in 22% (11 cases) +2, in 56% (28 cases) +3 and in 8% (4 cases) was more than +3. As it is known, there is a significant difference between the inflammatory index of CRP in the two groups of patients (P = 0.003). So that this index was significantly lower in the group of patients admitted to the inpatient ward compared to patients admitted to the ICU.

**Figure 15:** Percentage of CRP inflammatory index in patients with coronary artery disease in the study groups.



**Discussion**

In this study, which compared the laboratory and radiological findings of patients with COVID-19 admitted to the inpatient and intensive care units of Forghani Hospital in Qom, patients in two groups of 50 people were studied. According to the findings of this study, in patients admitted to the inpatient ward, the age of patients was 54.2 ± 2.29 years and 54% were male patients and the mean age of patients admitted to intensive care units was 60 ± 2.01 years and 64% of patients were male. The overall mean age and mortality of male patients in the ICU ward were higher (P < 0.05). In patients admitted to the inpatient ward, unilateral lung involvement 32% of patients 68% bilateral involvement, and in patients with coronary hospitalization in ICU 16% of patients had unilateral lung involvement and 84% of patients had bilateral lung involvement (p < 0.05). In patients admitted to the inpatient ward, the mean SPO2 was 91.3 ± 5.9% and in patients admitted to the intensive care unit was 84.6 ± 10.7%. (0.001/0 = P). In patients admitted to the inpatient department and ICU, respectively, the mean

of WBC was  $7046.3 \pm 555.16$  and  $12845 \pm 29.08 + 0845$  ( $P = 0.045$ ), in most of these patients leukocytosis was present. The mean lymphocytes in patients admitted to the inpatient and ICU wards were  $22.8 \pm 1.61$  and  $14.5 \pm 1.56$ , respectively ( $P = 0.0001$ ).  $+1$  was  $6/13$  and  $6/02 +$  was  $12.9$  ( $P = 0.07$ ). The mean platelet count was  $283860 \pm 66364.19$  and in patients admitted to the intensive care unit was  $210560 \pm 9901/48$  ( $P = 0.27$ ). However, the mean platelet count was higher in the inpatient group compared to the ICU patients, which could be due to the administration of anticoagulants in the ICU patients. In patients admitted to the inpatient and intensive care units, the mean serum level of BUN was  $59.2 \pm 64.88$  and  $90.6 \pm 81.02$ , respectively. As it is known, there is a significant difference between serum BUN levels in the two groups of patients ( $P = 0.035$ ). In patients admitted to the inpatient ward and ICU, the mean serum level of Cr is  $1.32 \pm 0.21$  and  $2.1 \pm 0.21$ , respectively. As it is known, there is a significant difference between serum creatinine levels in the two groups of patients ( $P = 0.014$ ). The mean of Cr in the group of patients admitted to the inpatient ward was significantly lower compared to patients admitted to the ICU. Also, there was a significant difference between the inflammatory index of CRP in the two groups of patients ( $P = 0.003$ ). So that this index in the group of patients admitted to the inpatient ward was significantly lower compared to patients admitted to the ICU. In the study of colleagues and colleagues, which was conducted on 72 patients, 42 men and 30 women participated

and the average age of the company. The laboratory findings included: WBC 8100, lymphocyte 1100, platelet 212200, hemoglobin 12.3, CRP 6.47, ALT 2.46, AST 40.8, D-dimer 6.5 (68). In my study, the laboratory findings of hemoglobin, platelets, and lymphocytes were consistent with the findings of this study, but the mean age of the patients in our study was higher than the mean age of the patients in this study.

## Conclusion

Based on the findings of this study, there was a significant difference in radiological and laboratory findings in patients with COVID-19 hospitalized in the inpatient and intensive care units, such as blood oxygen levels, WBC, lymphocyte platelets, CRP, creatinine and urea and related findings. There was a significant difference between CT scan of the lung and diagnosis of lung consolidation in patients admitted to the ICU compared with patients admitted to the ward. Due to the significant difference between laboratory and radiological findings in patients with coronary artery, inpatient hospitalization with patients admitted to the ICU is recommended. If laboratory and radiological findings in patients with this study match, to provide medical services and care. Patients in the decision-making of hospitalization of patients in special wards, decisions should be made that due to the widespread epidemic of this disease, special beds can be managed in medical centers.

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## ORIGINAL

# Direct instruction and inquiry-based method in teaching endodontics among preclinical dentistry students

*Instrucción directa y método basado en la indagación en la enseñanza de la endodoncia entre los estudiantes de odontología preclínica*

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## Abstract

**Background:** As part of the education and training of preclinical students in endodontics, traditional lectures on the content are normally delivered, and laboratory exercises on extracted tooth specimens are carried out. Innovative methods to improve the effectiveness and quality of teaching dental students have been proposed, such as inquiry-based teaching. This study aimed to compare the effectiveness of direct instruction and inquiry-based teaching on the performance in root canal treatment exercises of preclinical students.

**Methods:** A total of 49 students enrolled in the preclinical course in Endodontics during the first semester of 2016-2017 in a Dentistry school in the Philippines agreed to participate in the study. The students were first exposed to direct instruction then followed by inquiry-based teaching. The performance of the students in their root canal treatment exercise in both methods was recorded. The performance of the students using the two methods was compared using the t-test.

**Results:** Based on the students' scores in the exercise after direct teaching, a greater percentage failed, while after the inquiry-based method, their scores showed that the percentage of students who passed has markedly increased. There is a significant difference in the students' performance using direct teaching only and their performance after the inquiry-based teaching at 0.05 level of significance.

**Conclusion:** Therefore, inquiry-based teaching is effective in improving the preclinical students' performance in endodontics.

**Keywords:** Direct instruction, inquiry-based teaching, performance, root canal treatment exercise.

## Resumen

**Antecedentes:** Como parte de la educación y la formación de los estudiantes preclínicos de endodoncia, normalmente se imparten conferencias tradicionales sobre el contenido y se realizan ejercicios de laboratorio con especímenes de dientes extraídos. Se han propuesto métodos innovadores para mejorar la eficacia y la calidad de la enseñanza de los estudiantes de odontología, como la enseñanza basada en la indagación. El objetivo de este estudio es comparar la eficacia de la instrucción directa y la enseñanza basada en la indagación sobre el rendimiento en los ejercicios de tratamiento del conducto radicular de los estudiantes preclínicos.

**Métodos:** Un total de 49 estudiantes matriculados en el curso preclínico de Endodoncia durante el primer semestre de 2016-2017 en una escuela de Odontología en Filipinas aceptaron participar en el estudio. Los estudiantes fueron expuestos primero a la instrucción directa y luego a la enseñanza basada en la indagación. Se registró el rendimiento de los estudiantes en su ejercicio de tratamiento de conductos en ambos métodos. Se comparó el rendimiento de los estudiantes con los dos métodos mediante la prueba t.

**Resultados:** Según las puntuaciones de los estudiantes en el ejercicio después de la enseñanza directa, un mayor porcentaje fracasó, mientras que después del método basado en la indagación, sus puntuaciones mostraron que el porcentaje de estudiantes que aprobaron ha aumentado notablemente. Existe una diferencia significativa en el rendimiento de los estudiantes utilizando sólo la enseñanza directa y su rendimiento después de la enseñanza basada en la indagación a un nivel de significación de 0,05.

**Conclusión:** Por lo tanto, la enseñanza basada en la indagación es eficaz para mejorar el rendimiento de los estudiantes preclínicos de endodoncia.

**Palabras clave:** Enseñanza directa, enseñanza basada en la indagación, rendimiento, ejercicio de tratamiento de conductos.

## Introduction

Endodontic treatment is performed in a major portion of dental emergencies worldwide, and there is an increasing need to educate and train dental students in this specialty, such that they are prepared to perform better and predict treatment outcomes<sup>1</sup>. The emphasis on inquiry-based teaching is especially important in laboratory courses, as these are the courses in which students apply the process of science<sup>2</sup>. Over the past decade, repeated calls have been made to incorporate more active teaching and learning in undergraduate biology courses<sup>3</sup>.

The twenty-first century's onset and its challenges require developing a learning community where accomplished teachers make sure that both teachers and students meet today's challenges. In this regard, Arends and Kilcher<sup>4</sup> stressed that a true learning community had been created for teachers and for their students, one that helps them meet the many and varied challenges of teaching in twenty-first-century schools.

Teaching practices in the twenty-first century are guided by several learning theories, the most popular of constructivism. This theory stresses that the students/learners reconstruct their conceptions in active, meaningful experiences. Thus, teaching is student-centered in the constructivist viewpoint<sup>5</sup>.

The direct instruction model is straightforward. It is designed to promote mastery of skills (procedural knowledge) and factual knowledge (declarative knowledge)<sup>6</sup>.

Direct instruction aims at accomplishing two major learner outcomes: mastery of well-structured knowledge and acquisition of varied skills<sup>7</sup>.

Teacher use of direct instruction, particularly the lecture method, comprises many classroom time in traditional classrooms. However, the primary criticism of direct instruction is that it is teacher-centered and emphasizes teacher-talk<sup>8</sup>.

Subject areas in dental education embody scientific concepts and skills. As a result, dental science instruction has become highly constructive, such that it has shifted from an emphasis on acquiring content knowledge to constructing scientific understanding. This has led to applying constructivist teaching and learning strategies such as the discovery approach, inquiry approach, integrative teaching, and cooperative learning. In this current study, the researchers applied an inquiry-based approach as a type of student-initiated model.

Inquiry-based teaching is a constructivist model conceived to help students understand how phenomena work and the processes used to investigate these phenomena<sup>9</sup>.

Inquiry-based teaching requires a high degree of interaction among the learner, the teacher, the materials, the content, and the environment. The most crucial aspect of the inquiry teaching model is that it allows both student and teacher to become persistent seekers, askers, interrogators, questioners, and ponderers<sup>10</sup>.

The instructional learner outcomes of inquiry-based teaching are as follows: acquire an understanding of the focus problem; develop thinking skills that underlie scientific reasoning; develop dispositions and commitment to scientific processes; and develop metacognitive skills and learner autonomy<sup>11</sup>.

Overall, the inquiry-based model promotes an inquisitive and investigative mindset among the learners. Moreover, since the inquiry-based model is student-centered, it promotes active learning on the part of the students.

In this study, two teaching models are compared as regards effectiveness: direct instruction and inquiry-based teaching.

There are studies conducted to measure the performance of preclinical dental students in performing root canal treatment exercises. These studies utilized other methods of instruction.

According to Friedlander and Anderson<sup>12</sup>, the teaching of advanced endodontic courses at the predoctoral level is common, but it cannot be easy to assess teaching effectiveness. Advanced modules placed later in the dental curriculum provide the opportunity to introduce a new topic, revisit and reinforce concepts learned previously, and instill the notion of lifelong learning. At any level, the introduction of new techniques to novices must be based on recognition of their prior knowledge and experience and their need for explicit direction, stepwise instruction, and comprehensive feedback. Assessment of students' performance should provide insights into what they know and can do and steer them towards desired outcomes. In addition, assessment can provide valuable feedback on teaching effectiveness.

A module was piloted for inclusion in the University of Otago (New Zealand) fourth-year dental curriculum<sup>13</sup>. This involved the use of tapered hand and rotary nickel-titanium files for root canal preparation and was taught through a didactic program (lectures and problem-based learning seminars) and a series of preclinical hands-on sessions. Findings from formative and summative assessments and student, peer, and self-evaluation indicated that the module's objectives were met and that it was effective in both providing students with the basic skills for using this type of instrumentation and increasing their understanding and enthusiasm for endodontics.

In a previous study, endodontics students had access to simulated models, and endodontic emergency care



practice also included feedback sessions after each clinical session. It was found that the intervention gave significantly improved their grades, which support the implementation and further evaluation of similar interventions in other areas of teaching and learning dentistry subjects<sup>14</sup>.

This study's major purpose was to determine the comparative effectiveness of direct instruction and inquiry-based teaching on the learning performance of preclinical dental students in root canal treatment exercises.

Specifically, this study aimed to determine the following:

1. The performance of the preclinical dental students in root canal treatment exercise after direct instruction;
2. The performance of the preclinical dental students in root canal treatment exercise after inquiry-based teaching; and
3. The comparison of the performance of the preclinical dental students in root canal treatment exercise in the two methods of teaching and if the difference is significant.

## Materials and methods

This experimental study involved a single group exposed to two methods of teaching. A total of 49 students enrolled in the preclinical course in Endodontics during the first semester of 2016-2017 agreed to participate in the study. Ethical issues were thrashed out, and the study's purpose was explained to the participants, including the nature of their participation. The students were first exposed to direct instruction then followed by inquiry-based teaching. The exercise was done on extracted tooth specimens which were checked and approved by the instructor. The root canal exercise involved the sequence of steps from access preparation, determination of working length, biomechanical preparation, and obturation. The performance level of the students in their root canal treatment exercise in the two methods of teaching was recorded. In addition, the performance of the students using the two methods was compared using the t-test.

## Results and discussion

The mean score of the students in the root canal exercise after being exposed to the direct instruction method is 93.35 out of the highest possible score of 135. Based on a passing score for this exercise which is 94.50, the mean score of the students is considered as failed. Considering the number of students who passed, only 48.98% of the students got scores within and above the passing mark. Although almost half of the students passed, this result can be explained by observing that direct instruction emphasizes more on teacher talk<sup>15</sup>. Hence the students were not actively involved.

The preclinical students had a mean score of 113.39 in their exercise after being exposed to inquiry-based teaching. This means that the students passed the exercise. Also, the number of students who passed the exercise comprised 95.92%, showing a marked increase in the passing rate. The students' higher scores using inquiry-based teaching show that a student-centered approach can help improve students' performance in a laboratory course<sup>16</sup>. The t-test result shows that the computed value is greater than the tabular value at alpha .05 level of significance ( $12.22 > 2.021$ ), which fails to accept the null hypothesis. There is a significant difference between the mean scores of the students in the two methods ( $p < .05$ ). Therefore, the preclinical students performed better after being exposed to the inquiry-based method. Similar results were found in a previous study where students' grades in endodontics improved after an intervention using simulation and feedback sessions<sup>17</sup>.

## Conclusions

The preclinical students performed better in the endodontics exercise after being exposed to the inquiry-based method than their performance in the direct instruction method. The marked improvement in their scores shows that a student-centered method that leads to active student involvement helps students learn better in a laboratory course such as endodontics. The difference between the two methods was found to be significant therefore, based on the findings of this study, the inquiry-based method is more effective in teaching endodontics compared to direct instruction. Therefore, the inquiry-based method is appropriate for laboratory courses in the dental curriculum and other fields.

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# Enterotoxigenic profiles of *Staphylococcus aureus* strains isolated from sweet samples

*Perfiles enterotoxigénicos de cepas de Staphylococcus aureus aisladas en muestras de dulces*

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## Abstract

**Background:** Enterotoxigenic *Staphylococcus aureus* is considered to be one of the major cause of foodborne diseases worldwide. The present investigation was done to study the enterotoxigenic genes distribution of *S. aureus* strains isolated from sweet samples.

**Methods:** Sixty *S. aureus* strains were isolated from sweet samples using culture. *S. aureus* strains were identified using the biochemical tests. Enterotoxigenic gene profile of the isolates was studied using the PCR.

**Results:** *Sec* (10.0%) harbored the highest distribution amongst examined isolates, while *seb* (1.0%) and *sed* (1.0%) harbored the lowest. Among all isolates of all examined sweet brands, brand C isolates harbored the highest distribution of *sea* (8.0%), *sec* (12.0%), and *sed* (4.0%) enterotoxigenic genes. Brand B isolates harbored the highest distribution of the *seb* (5.0%) enterotoxigenic gene.

**Conclusion:** Enterotoxigenic genes distribution was related to the types of samples. Simultaneous presence of several enterotoxigenic genes in some isolates showed an important public health issue.

**Keywords:** Prevalence, enterotoxigenic genes, *Staphylococcus aureus*, sweet.

## Resumen

**Antecedentes:** El *Staphylococcus aureus* enterotoxigénico se considera una de las principales causas de enfermedades de transmisión alimentaria en todo el mundo. La presente investigación se llevó a cabo para estudiar la distribución de los genes enterotoxigénicos de las cepas de *S. aureus* aisladas de muestras de dulces.

**Métodos:** Se aislaron sesenta cepas de *S. aureus* de muestras de dulces mediante cultivo. Las cepas de *S. aureus* se identificaron mediante pruebas bioquímicas. Se estudió el perfil genético enterotoxigénico de los aislados mediante la PCR.

**Resultados:** *Sec* (10,0%) albergó la mayor distribución entre los aislados examinados, mientras que *seb* (1,0%) y *sed* (1,0%) albergaron la menor. Entre todos los aislados de todas las marcas de dulces examinadas, los aislados de la marca C albergaron la mayor distribución de genes enterotoxigénicos *seb* (8,0%), *sec* (12,0%) y *sed* (4,0%). Los aislados de la marca B albergaron la mayor distribución del gen enterotoxigénico *seb* (5,0%).

**Conclusión:** La distribución de los genes enterotoxigénicos estaba relacionada con los tipos de muestras. La presencia simultánea de varios genes enterotoxigénicos en algunos aislados puso de manifiesto un importante problema de salud pública.

**Palabras clave:** Prevalencia, genes enterotoxigénicos, *Staphylococcus aureus*, sweet.

## Introduction

*S. aureus* is a Gram-positive and cocci-shaped bacterium responsible for nosocomial and community-acquired infections and foodborne diseases<sup>1-3</sup>. Foodborne diseases caused by *S. aureus* are characterized by nausea, vomiting, weakness, abdominal cramps, diarrhea, and toxic shock syndrome<sup>4,5</sup>.

*S. aureus* bacteria secrete a group of extracellular enzymes, ease tissue extinction and dispersal, and membrane damaging toxins that cause catalytic effects on host cells and tissue damage<sup>6</sup>. They are low-molecular-mass and single-chain proteins with 23 different major types (SEA to SEV). *Sea*, *seb*, *sec*, and *sed* are the most commonly detected types in the cases of foodborne diseases and food poisonings<sup>7</sup>.

Rendering the high importance of enterotoxigenic *S. aureus* and the absence of epidemiological surveys on its prevalence, the current research was done to study the distribution of enterotoxigenic genes of the *S. aureus* strains isolated from sweet samples.

## Materials and methods

### Isolates

Sixty *S. aureus* isolates of traditional sweet samples (three different brands) were included in this study. Isolates were cultured in Trypticase Soy Broth (TSB, Merck, Germany) supplemented with 10% NaCl and 1% sodium pyruvate (incubated at 37°C for 24 h). In addition, *S. aureus* isolates were identified using cultured into Baird-Parker agar supplemented with egg yolk tellurite emulsion (Merck, Germany) and Gram staining, hemolytic activity on sheep blood agar (Merck, Germany), catalase activity, coagulated test (rabbit plasma), oxidase test, glucose O/F test, resistance to bacitracin (0.04 U), mannitol fermentation on Mannitol salt agar (Merck, Germany), urease activity, nitrate reduction, phosphatase, deoxyribonuclease (DNase, Merck, Germany) test, Voges-Proskauer (Merck, Germany) test and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation tests<sup>8</sup>.

### DNA extraction

*S. aureus* isolates were sub-cultured on Tryptic Soy Broth media (TSB, Merck, Germany) and further incubated for 48 h at 37 °C<sup>9</sup>. Genomic DNA was extracted from bacterial colonies using the DNA extraction kit (Thermo Fisher Scientific, Germany), according to the manufacturer's instruction. After extraction, the DNA samples were quantified (NanoDrop, Thermo Scientific, Waltham, MA, USA), their purity checked (A260/A280), and their concentrations adjusted to 50 ng/μL. The integrity of the DNA was evaluated on a 2% agarose gel stained with ethidium bromide (0.5 μg/mL) (Thermo Fisher Scientific, Germany). The DNA concentration was also estimated by spectrophotometric absorbance at 257 nm (Hach, DR5000, USA). The DNA was stored at -20°C pending subsequent PCR analysis<sup>10-17</sup>.

### Enterotoxigenic genes identification

**Table I** represents the oligonucleotide primers and PCR conditions used to amplify enterotoxigenic genes<sup>18</sup>. A programmable DNA thermo-cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used in all PCR reactions. All runs included a negative DNA control consisting of sterile PCR grade water (Thermo Fisher Scientific, Germany) and positive DNA control consisting of positive DNA of each target gene. Fifteen microliters of amplified PCR products were subjected to electrophoresis in a 2% agarose gel in 1× TBE buffer at 90 V for 30-40 min, stained with SYBR Green (Thermo Fisher Scientific, Germany)<sup>19-22</sup>.

### Statistical analysis

Statistical analysis was done using the SPSS 25.0 statistical software (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact two-tailed test were used to assess any significant relationship between data collected in this survey. P-value <0.05 was considered as a statistical significant level<sup>23-27</sup>.

## Results

### Enterotoxigenic gene distribution based on isolates

A total of 60 *S. aureus* isolates were assessed in the present study to found their enterotoxigenic gene profiles. **Table II**

**Table I:** PCR conditions used for enterotoxin genes detection<sup>18</sup>.

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50μL)
<i>Sea</i>	F: TTGGAACGGTTAAAACGAA R: GAACCTTCCCATCAAAAACA	120	1 cycle: 94°C ----- 2 min.	5 μL PCR buffer 10X 2 mM MgCl <sub>2</sub> 150 μM dNTP 0.75 μM of each primers F & R 1.5 U Taq DNA polymerase 3 μL DNA template (Thermo Fisher Scientific)
<i>Seb</i>	F: TCGCATCAAAGTACAAACG R: GCAGGTACTCTATAAGTGCC	478	30 cycles: 94°C ----- 120 s	
<i>Sec</i>	F: GACATAAAAGCTAGGAATTT R: AAATCGGATTAACATTATCC	257	55°C ----- 120 s 72°C ----- 60 s	
<i>Sed</i>	F: CTAGTTTGGTAATATCTCCT R: TAATGCTATATCTTATAGGG	317	1 cycle: 72°C ----- 8 min	

characterizes the enterotoxigenic gene profile of *S. aureus* strains isolated from sweet samples. *Sec* (10.0%) harbored the highest distribution amongst examined isolates, while *seb* (1.0%) and *sed* (1.0%) harbored the lowest.

**Table II:** Enterotoxin genes distribution amongst *S. aureus* strains.

Samples N (N <i>S. aureus</i> )	(%) isolates harbor each each enterotoxigenic gene			
	<i>sea</i>	<i>seb</i>	<i>sec</i>	<i>sed</i>
Sweet samples (60)	3 (5.0)	1 (1.6)	6 (10.0)	1 (1.6)

### Enterotoxigenic gene distribution based on brands of sweet samples

**Table III** assesses the *S. aureus* enterotoxigenic gene profiles according to the brand of sweet samples. Among all isolates of all examined sweet brands, brand C isolates harbored the highest distribution of *sea* (8.0%), *sec* (12.0%), and *sed* (4.0%) enterotoxigenic genes. Brand B isolates harbored the highest distribution of the *seb* (5.0%) enterotoxigenic gene.

**Table III:** Enterotoxigenic genes profile of *S. aureus* isolates according to the brands of sweet samples.

Samples N (N <i>S. aureus</i> )	N (%)isolates harbor each enterotoxigenic gene			
	<i>sea</i>	<i>seb</i>	<i>sec</i>	<i>sed</i>
Brand A (15)	-	-	1 (6.6)	-
Brand B (20)	1 (5.0)	1 (5.0)	2 (10.0)	-
Brand C (25)	2 (8.0)	-	3 (12.0)	1 (4.0)
Total (60)	3 (5.0)	1 (1.6)	6 (10.0)	1 (1.6)

## Discussion

Several kinds of infectious diseases caused significant disorders globally<sup>28-31</sup>. In this survey, the role of enterotoxigenic *S. aureus* as an important cause of foodborne diseases was determined. *S. aureus* is one of the most frequent causes of food poisoning. The main food poisoning etiologic agents are staphylococcal enterotoxins. There are diverse SE kinds; types A (SEA) and B (SEB) are the most clinically significant enterotoxins. In this survey, sweet samples were the sources of enterotoxigenic *S. aureus*. *S. aureus* was

also detected in sweet samples assessed in previous investigations conducted in Brazil (12.00%)<sup>32</sup>, Pakistan (6.70%)<sup>33</sup>, Spain (6.10%)<sup>34</sup> and Japan (19.40%)<sup>35</sup>.

Some of probable reasons for the presence of enterotoxigenic genes amongst the *S. aureus* isolates were contamination of raw materials used to make sweets such as milk, eggs and even flour with the *S. aureus*, use of insufficient temperature to produce sweet samples, sweet maintenance at high temperatures and humidity, which has led to the enterotoxin production, and finally transmission of enterotoxin-producing strains from contaminated hands and nose of producing staffs during sweet production.

Heat stable SEs produced by enterotoxigenic *S. aureus* strains are considered as major cause of food poisoning. Findings showed that *sec* and *sea* were the most commonly detected enterotoxigenic genes. Song et al. (2015)<sup>36</sup> stated that 5.60% of *S. aureus* bacteria carried the *sea*, and 3.50% harbored the *seb* enterotoxins. Zhang et al<sup>37</sup> signifies that the *sea* gene was obtained in 24.10% and the *seb* gene in 4.20% of *S. aureus* bacteria. They also described that 20.50% of *S. aureus* isolates carried the *sed* gene, 6.80% carried the *sec* gene, and 0.60% carried the *see* gene. Kroning et al<sup>32</sup> described the high prevalence of *sea* (33.4%) and *seb* (16.6%) enterotoxigenic genes.

## Conclusions

Sweet samples were considered as sources of enterotoxigenic *S. aureus*. Type A and C enterotoxigenic genes were identified as the most abundant. Simultaneous presence of enterotoxigenic gene in some *S. aureus* isolates must be considered as serious health hazard regarding the consumption of Iranian sweet samples. Proper time and temperature for cooking of sweet samples is important, but preventing cross-contamination is the most effective ways to prevent occurrence of *S. aureus* in sweet samples.



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# Prescriptions analysis for various effects of polypharmacy in Kempegowda Institute of Medical Sciences (Kims) Hospital and research centre, India

*Análisis de prescripciones para diversos efectos de la polifarmacia en el Hospital y centro de investigación del Instituto de Ciencias Médicas de Kempegowda (Kims), India*

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## Abstract

**Background:** To assess the polypharmacy and appropriateness of prescriptions in patients in Kempegowda Institute of Medical Sciences (KIMS) Hospital and Research Centre, India.

**Methods:** This investigation was completed on 1000 patients of age both males and females. Data was gathered through the review of case sheets. Polypharmacy was noticed as dependent on admission and discharge prescriptions. The prescription components, drug utilization behavior, and prescribing compliance to hospital formulary were noted and subjected for Analysis of prescriptions for various impacts of polypharmacy.

**Result:** A total of 1.000 patients, 530 (53%) were males and 470 (47%) were females. Polypharmacy was present in 300 prescriptions (30%). The highest prevalence of polypharmacy was found in the 70-79 years age group compared to the other groups and it was measurably significant. and the average number of drugs per patient was 7. According to 1000 prescriptions analyzed, 142 prescriptions comprised of potential drug interactions and it was found that 235 drug interactions were present. From drug interactions, aspirin/clopidogrel and clopidogrel/atorvastatin were the most common drug interaction pairs observed among prescribed medications. Out of the 235 interventions proposed, the most incessant recommendation was on observing for adverse effect (44.01%) followed by dose adjustment (15.81%).

**Conclusion:** As the population ages, polypharmacy increases. The elderly often require multiple medications to treat multiple health-related conditions. The demographic details of the study population show that 70 % of polypharmacy occurred in elderly people. From the current investigation, we can conclude that polypharmacy prompts more potential drug-drug interactions. To improve drug safety in this high-risk population, appropriate prescribing is vital.

**Keywords:** Polypharmacy, prescription, elderly, drug interactions, adverse drug interaction.

## Resumen

**Antecedentes:** Evaluar la polifarmacia y la idoneidad de las prescripciones en los pacientes del Kempegowda Institute of Medical Sciences (KIMS), India.

**Métodos:** Esta investigación se llevó a cabo en 1.000 pacientes de ambos sexos. Los datos se recogieron mediante la revisión de las hojas de los casos. Se observó que la polifarmacia dependía de las prescripciones al ingreso y al alta. Los componentes de la prescripción, el comportamiento de utilización de los fármacos y el cumplimiento de la prescripción en el formulario del hospital se anotaron y se sometieron a un análisis de prescripciones para diversos impactos de la polifarmacia.

**Resultados:** De un total de 1.000 pacientes, 530 (53%) eran varones y 470 (47%) eran mujeres. La polifarmacia estaba presente en 300 prescripciones (30%). La mayor prevalencia de polifarmacia se encontró en el grupo de edad de 70-79 años, en comparación con los demás grupos, y fue notablemente significativa, y el número medio de fármacos por paciente fue de 7. Según las 1.000 prescripciones analizadas, 142 prescripciones contenían posibles interacciones farmacológicas y se descubrió que había 235 interacciones farmacológicas. En las interacciones farmacológicas, aspirina/clopidogrel y clopidogrel/atorvastatina fueron los pares de interacciones farmacológicas más comunes observados entre medicamentos prescritos. De las 235 intervenciones propuestas, la recomendación más incesante fue la de observar los efectos adversos (44,01%), seguida del ajuste de la dosis (15,81%).

**Conclusiones:** A medida que la población envejece, la polifarmacia aumenta. Los ancianos suelen necesitar varios medicamentos para tratar múltiples de salud. Los detalles demográficos de la población estudiada muestran que el 70% de la polifarmacia se produjo en personas mayores. A partir de la presente investigación, podemos concluir que la polifarmacia provoca más interacciones farmacológicas potenciales. Para mejorar la seguridad de los medicamentos en esta población de alto riesgo, es vital una prescripción adecuada.

**Palabras clave:** Polifarmacia, prescripción, ancianos, interacciones farmacológicas, interacción farmacológica adversa.

## Introduction

Polypharmacy is most usually characterized as the use of five or more medications day by day by a person. A few studies additionally by and large characterize polypharmacy as the utilization of different simultaneous medications or synchronous long haul utilization of different medications by the equivalent individual.<sup>1-5</sup> The prevalence of polypharmacy is estimated to be somewhere in the range of 10% and 90% relying upon the definition utilized, the age group examined, and the geographic area.<sup>6</sup>

Polypharmacy isn't foolish, yet in numerous cases can prompt negative results or helpless treatment viability, frequently being more harmful than helpful or presenting too much risk for too little advantage. Therefore, health professionals consider it a situation that requires monitoring and review to validate whether all of the medications are still necessary. Concerns about polypharmacy include increased adverse drug reactions, interactions of drugs, prescribing cascade, and higher costs. Polypharmacy is often associated with a diminished quality of life, including decreased mobility and cognition.<sup>7, 8</sup>

In this day and age where medical decision-making is frequently affected and sometimes controlled by financial considerations, it is usually for specialists to become disappointed and regularly resentful when cost turns into an issue in deciding the fitting treatment for a patient. The most well-known situation for such a response is the point at which a physician is informed that there is an elective treatment that is nearly as great and significantly more affordable. When the third parties interject themselves into the specialist-patient relationship in this way, it is simple for a physician to feel that the financial requirements and not nature of consideration are leading American drugs in the 21st century. Despite all this, however, the cost must be considered as an issue for determining the treatment plan. Once again, if the expense of a specific blend of medications makes it unthinkable for patients to get the medications, they won't take them, and all things considered, the prescription regimen will be incapable. Indeed, even the most liberal protection plans have impediments or copy on medications. It is significant while thinking about polypharmacy to think about the financial impact at least on the patient, if not on the healthcare system as a whole. The more medications recommended, the higher the expense and the more intricacies are made as a result.<sup>9</sup> For all the above cases, prescriptions Analysis for various effects of polypharmacy in a south Indian tertiary care hospital is so significant and Need for study.

## Materials and methods

Our study was led in the Department of Pediatrics, Kempegowda Institute of Medical Science (KIMS) Hospital

and Research Centre, Inida. It is 1200 beds tertiary care teaching and super specialty hospital, with an Outpatient and Inpatient facility. The hospital provides specialized healthcare services to all strata of people in and around Bangalore. This study was carried out on 1000 patients of age both males and females. In this strategy, the inpatient review of case sheets and medicines was screened for prescriptions Analysis for various effects of polypharmacy on a consistent schedule. All the recommended drugs alongside different prescriptions and significant data were noted in a modified information accumulation structure to discover polypharmacy and others. The investigation patients were pursued day by day until their release. The Micromedex, Medscape, articles, and relevant reference books were used as tools to review the collected data. The prescribed medication was checked for their existence in the hospital and also the relevant dosing calculation and polypharmacy. Check for any error in prescription such as doses, frequency, and route of administration, and analysis of prescription for any polypharmacy, etc. The prescription components, drug utilization conduct, and endorsing consistency to hospital formulary were noted and oppressed for Analysis of prescriptions for different impacts of polypharmacy. Polypharmacy was observed based on admission and discharge prescriptions.<sup>10, 11</sup>

## Result and discussion

### Drug usage

In our study population out of 300 patients' prescriptions contains polypharmacy, which that 159 were male and 141 were female and in the pediatric population 53% were male and 47% were female (**Table I**).

**Table I:** Number of drug usage by study patients.

Number of Drug Dispensed	Male		Female		Total	
	N	%	N	%	N	%
1,2,3	7	2.25	4	1.43	11	3.67
4,5,6	46	15.51	35	11.63	81	27.14
7,8,9	106	35.31	101	33.88	207	69.18

N=300

A total of 300 drugs were prescribed, and thus the average number of drugs per patient was 7. Among studied patients, 3.67% were using 1-3 medications followed by 27.14% of patients using 4-6 medications. According to table 2,3 Extensive (70%), polypharmacy was observed in the study population.

in our study various classes of drug-like Pantoprazole, Aspirin, Paracetamol, Zincovit, Levocetirizin, Atorvastatin, etc. Were prescribed. Out of which 53% males, 47 % females have prescribed Pantoprazole which was highest among monotherapy. A different class of medications is endorsed in our study populace dependent on the patient condition and comorbidities .out of which 87males,

47 females are prescribed atorvastatin drugs which were the highest followed. Totally 20.74% atorvastatin was prescribed. 16.41% of patient used Pantoprazole, 10.22 H. Actapid, 6.81% Zicovit, 6.5% Ceftriaxone, 5.27 Paracetamol.

### Potential Drug-Drug Interaction

Concerns about polypharmacy include increased adverse drug reactions, drug interactions, Pharmacists Intervention, prescribing cascade, and higher costs. Polypharmacy is often associated with decreased quality of life, including decreased mobility and cognition (Table II).

**Table II:** Potential drug-drug interaction inpatient.

Parameters		Total	
		N	%
Severity	Major	101	40.59
	Moderate	145	58.11
	Minor	6	1.28
Pharmacodynamic Interaction		147	57.26
Pharmacokinetic Interaction		91	36.75
Unknown Mechanism		18	5.98
Management	Monitoring	185	73.93
	Dose adjustment	38	13.67

### Drug interaction frequency

Out of 300 prescriptions analyzed, 142 prescriptions comprised of potential drug interactions and it was found that 235 drug interactions were present. The incidence of potential drug interaction was 63.64%. Among 235 drug interactions, 90 types of interaction combinations were identified. The studied prescription comprised 58.11% moderate interaction, 40.59% major drug interactions, and 1.28 minor drug interactions. Among them, 57.26% were pharmacodynamic drug interactions followed by 36.75% of pharmacokinetic interaction and 5.98% of unknown mechanism interactions (Table III).

**Table III:** Frequency of drug interaction in the study population.

Frequency of pDDI	Male		Female		Total	
	N	%	N	%	N	%
1	52	37.14	23	16.42	75	53.57
2	25	17.85	18	12.14	43	30
3	14	10	5	3.57	19	13.57
4	2	1.42	0	0	2	1.42
5	2	1.42	0	0	2	1.42

N=142

In most patients, the cases of one potential drug interaction were identified with a median of 1.67 potential drug-drug interactions. Among them, 30% of prescriptions had two potential drug-drug interactions.

### Pharmacists Intervention

Out of the 235 interventions proposed, the most frequent suggestion was on monitoring for adverse effect (44.01%) followed by dose adjustment (15.81%). 25.64% of interventions were accepted and therapy was changed. Various interventions provided by the pharmacist and their result are presented in table IV.

**Table IV:** Types of pharmacist intervention to prevent pDDI.

Types of intervention	Total	
	N	%
Substitution	24	10.25
Stop/avoid/dose adjustment	37	15.81
Monitoring	103	44.01
No change	70	29.91

The pharmacist's role concerning clinical results of different adverse events is very significant as pDDIs are a noteworthy factor for the hospitalization of patients. A clinical pharmacist can help in the improvement of pharmacotherapy. A clinical pharmacist can discover factors that may result in irrational prescriptions. Such factors are called "drug-related problems" and may change the ideal impacts of medications. The role of the pharmacist in the created world is all around perceived but this profession is not well established in the developing nations including India. The absence of an appropriate role of the pharmacist in less-created nations is driving patients with a higher ratio of drug-related problems. This can be reduced by the clinical pharmacist's active participation in direct patient care.<sup>12-14</sup>

The incidence rate of adverse drug interactions was found to be 20%. This rate is similar to the study conducted in Iran<sup>12</sup>. Another study reported 17.53% of observed drug interaction which is lower than this study. The most common drug interaction pair resulting in adverse drug reactions was aspirin/clopidogrel (5). Bleeding was the most important interaction in 8 cases followed by hypoglycemia (4) and QT-interval prolongation (4). The most common objective drug is aspirin and the precipitant drug is clopidogrel. Similarly, Bleeding was the most common clinical effect of observed drug interaction in a South Indian study.<sup>10-13</sup> (Tables V, VI).

**Table V:** Observed adverse drug-drug interaction.

Object Drug	Precipitant Drug	No. of adverse drug-drug interaction	Adverse outcome
Enalapril	Spirolactone	2	Hyperkalemia
Aspirin	Clopidogrel	6	GI bleeding
Amiodarone	Atorvastatin	1	Muscle pain
Clopidogrel	Acenocoumarol	2	bleeding
Venlafaxine	Ivabradine	1	QT prolong
Furosemide	Hydrocortisone	2	Hypokalemia
Aspirin	Acenocoumarol	3	Bleeding
Domperidone	Cilnidipine	2	QT prolong
Insulin	Aspirin	1	Hypoglycemia
Aspirin	Heparin	1	bleeding
Clopidogrel	Atorvastatin	2	thrombocytopenia
Aspirin	Telmisartan	1	Increase creatinine
Insulin	Nebivolol	1	hypoglycemia
Domperidone	Atorvastatin	1	QT prolong
Amiodarone	Nebivolol	1	bradycardia
Spirolactone	Aspirin	1	hyperkalemia
Metformin	Ramipril	2	hypoglycemia

### Conclusion

As the population ages, polypharmacy increases. The elderly often require multiple medications to treat multiple health-related conditions which requires more cautions



**Table VI:** Details of observed drug interaction (adverse drug interaction).

Interacting drug	No.	Effect	WHO causality	Naranjo Causality	Severity
Enalapril/Spironolactone	2	Hyperkalaemia	probable	Probable	Mild
Aspirin/Clopidogrel	66	bleeding	probable	Probable	Major
Amiodarone/atorvastatin	1	Muscle pain	possible	Possible	Mild
Clopidogrel/Acenocoumarol	2	bleeding	probable	Probable	Moderate
Venlafaxine/ivabradine	1	QT prolong	possible	Possible	Moderate
Furosemide/Hydrocortisone	2	Hypokalaemia	probable	Probable	Mild
Aspirin/Acenocoumarol	3	Bleeding	possible	Probable	Moderate
Domperidone/Cilnidipine	2	QT prolong	possible	Possible	Mild
Insulin/aspirin	1	Hypoglycaemia	probable	Possible	Moderate
Aspirin/Heparin	1	bleeding	possible	Possible	Moderate
Aspirin/Telmisartan	1	Increase creatinine	possible	Probable	Mild
Insulin/nebivolol	1	hypoglycaemia	probable	Probable	Moderate
Domperidone/Atorvastatin	1	QT prolong	possible	Probable	Mild
Amiodarone/nebivolol	1	bradycardia	possible	Probable	Moderate
Spironolactone/aspirin	1	hyperkalaemia	probable	Probable	Moderate
Metformin/Ramipril	2	hypoglycaemia	possible	Possible	Mild

particularly on renal function. The demographic details of the study population showed that 70 % of polypharmacy occurred in elderly people because they use 6 or more medicine. This study shows that DDIs are frequent among hospitalized cardiac patients. The majority of interactions were pharmacodynamic, having moderate severity. Anti-

platelets and anti-coagulants were commonly implicated in many PDDIs in this study and therefore require intensive monitoring during therapy. From the current study, we can reason that polypharmacy leads to more potential drug-drug interactions. To improve drug safety in this high-risk population, appropriate prescribing is vital.

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# Evaluation of arch bar fixation without surgical aid in jaw fracture treatment

*Evaluación de la fijación de barras de arco sin ayuda quirúrgica en el tratamiento de fracturas de mandíbula*

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## Abstract

**Background:** In traumatic patients, head, neck, and facial bone fractures are common. Inter-maxillary fixation (IMF) in jaw fracture surgery helps to improve the position of the maxilla and mandible, as well as the use of elastics in the postoperative period of fractures. IMF is performed with the use of wires applied directly on the teeth, or with quick-fix screws or with arch bars attached to them. Arch bar is the most complete technique for jaw fixation. This technique is time-consuming and manipulation of the wires lead to the increasing risk of glove puncture and needle stick injury to the surgeon or assistants. These problems lead to inventing alternative techniques.

**Materials and Methods:** This study was a clinical trial in which 36 patients require arch bar fixation were selected. They were randomly divided into treatment and control groups by cluster randomization. The first group treated with arch bar in the new method and the second group treated with the classic method of arch bar placement. For the new method the surgeon prebend the wires like U and pass their all trough interdental spaces by his own. These two groups were compared in terms of the time of placing arch bars, its vertical mobility after one week and one month, and the number of glove perforations. Finally, the data were analyzed using SPSS (version 22) software.

**Result:** A total number of 36 patients were included in this study, results showed that the average time for arch bar wiring in the classical and experimental groups was 40/39 ±3/071 and 29/33±2/567 minute respectively (p0.135). There was a statistically significant difference in the number of glove perforations between the classic and the experimental group (p0.024). In the present study, 61/1% of gloves in the experimental group were without holes, but in the classic group, 33/3% of gloves were without holes. In the classic group, 44/4% of gloves had 2 holes and 5/6% of gloves had 3 holes. None of the gloves in the experimental group had 3 holes. One week after wiring arch bar, there was no significant difference between those groups in the looseness rate (p 0.560). But after 1 month, a significant difference was found between groups because the average looseness rate in the experimental group was higher (p 0.016).

**Conclusion:** With due attention to statistical data, presented method had more efficiency due to less working time, fewer glove perforations, and simpler arch bar opening. Even there is no need for any professional assistants.

**Keywords:** Arch bar, jaw fractures, Intermaxillary fixation.

## Resumen

**Antecedentes:** En los pacientes traumatizados, las fracturas de cabeza, cuello y huesos faciales son frecuentes. La fijación intermaxilar (FMI) en la cirugía de fracturas de mandíbula ayuda a mejorar la posición del maxilar y la mandíbula, así como el uso de elásticos en el postoperatorio de las fracturas. La FMI se realiza con el uso de alambres aplicados directamente sobre los dientes, o con tornillos de fijación rápida o con barras de arco unidas a ellos. La barra de arco es la técnica más completa para la fijación de la mandíbula. Esta técnica requiere mucho tiempo y la manipulación de los alambres conlleva un riesgo creciente de perforación del guante y de lesiones por pinchazo de aguja para el cirujano o los asistentes. Estos problemas llevan a inventar técnicas alternativas.

**Materiales y métodos:** Este estudio fue un ensayo clínico en el que se seleccionaron 36 pacientes que requerían la fijación de la barra del arco. Se dividieron aleatoriamente en grupos de tratamiento y de control mediante aleatorización por grupos. El primer grupo fue tratado con la barra de arco en el nuevo método y el segundo grupo fue tratado con el método clásico de colocación de la barra de arco. En el nuevo método, el cirujano predobló los alambres en forma de U y los pasó por completo a través de los espacios interdientales. Estos dos grupos se compararon en cuanto al tiempo de colocación de las barras del arco, su movilidad vertical después de una semana y un mes, y el número de perforaciones del guante. Por último, los datos se analizaron mediante el programa informático SPSS (versión 22).

**Resultados:** Un número total de 36 pacientes fueron incluidos en este estudio, los resultados mostraron que el tiempo medio de cableado de las barras de arco en los grupos clásico y experimental fue de 40/39 ±3/071 y 29/33±2/567 minutos respectivamente (p 0.135). Hubo una diferencia estadísticamente significativa en el número de perforaciones del guante entre el grupo clásico y el experimental (p 0.024). En el presente estudio, el 61/1% de los guantes del grupo experimental no tenían perforaciones, pero en el grupo clásico, el 33/3% de los guantes no tenían perforaciones. En el grupo clásico, el 44/4% de los guantes tenían 2 agujeros y el 5/6% de los guantes tenían 3 agujeros. Ninguno de los guantes del grupo experimental tenía 3 agujeros. Una semana después de alambrear la barra del arco, no hubo diferencias significativas entre esos grupos en la tasa de alojamiento (p 0.560). Pero después de 1 mes, se encontró una diferencia significativa entre los grupos porque la tasa media de alojamiento en el grupo experimental fue mayor (p 0.016).

**Conclusión:** prestando la debida atención a los datos estadísticos, el método presentado fue más eficaz debido al menor tiempo de trabajo, el menor número de perforaciones del guante y la apertura más sencilla de la barra del arco. Incluso no hay necesidad de ningún asistente profesional.

**Palabras clave:** Barra de arco, fracturas de mandíbula, fijación intermaxilar.

## Introduction

The treatment of jaw fractures has a long history, from ancient Egypt to the present. Historical insight improves understanding of current techniques and provides the basis for the development of new methods. Since ancient times, physicians have described many different techniques for treating mandibular fractures, the principle of which has always been repositioned and immobilization of the bone fragments. However, during the past 50 years, an improvement of anesthetic and radiographic techniques, the introduction of antibiotics, specially designed instruments, and advances in biomaterial have allowed maxillofacial surgeons to improve outcomes while reducing morbidity. The main objective in all treatments for treating fractures is to obtain proper occlusion and stable joint movements by appropriate insertion of broken parts<sup>1</sup>. Recently, typical surgical methods for the treatment of mandibular fractures is the arch bar method. Arch bar is composed of a hooked, stainless steel wire called Arch and ligated to teeth with stainless steel wires. However, arch bars can damage the teeth and periodontal tissue and tend to be uncomfortable for patients during the fixation period. Moreover, daily maintenance of oral hygiene is difficult for patients with an arch bar<sup>2</sup>. Surgeons are also exposed to the risk of blood-transmitted diseases through skin punctures by wires when affixing these devices. The speed of action in arch wiring is very important during surgery<sup>2</sup>. Arch bar is a simple and an initial treatment for most jaw fractures, especially for the mandible. Treatment by the arch bar is also necessary in some cases where the treatment plan includes insertion and rigid fixation. In some cases, it can be as a final treatment without the need for a hard fixation<sup>3,4</sup>. There are several types of the arch bar for IMF, such as Erich, Winters, handmade, and plastic / polymeric<sup>4,5</sup>. IMF helps maxilla-mandibular positioning in orthognathic surgery, and the use of elastics in the postoperative period of fractures or orthognathic surgery. The most complete IMF procedure comes with the arch bar. However, it has been performed with the use of wires applied directly on the teeth, or with arch bars wired to them<sup>6</sup>. These procedures are time-consuming, and manipulation of the wires lead to the increasing risk of glove puncture and needle stick injury to the surgeon or assistants. These problems lead to inventing alternative techniques. Therefore, aim of the present study was the evaluation of arch fixation without surgical aid in jaw fracture treatment.

## Materials and methods

Thirty six patients with mandible fractures who were referred to Poursina Hospital and needed to Arch bar were selected. The patients were divided into two groups. Group I, consisted of 18 patients who were randomly treated with arch bar fixation without an assistant. Group

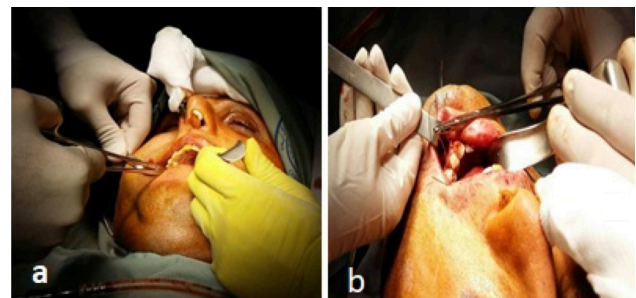
II consisted of 18 patients treated with arch bar fixation with an assistant as a control group. Then, two groups were compared in terms of the time of the wiring arch bar and its vertical mobility after one week and one month, and the number of glove perforations. Arch bar wires used were stainless steel. This new technique consist of following steps; while starting surgery, arch bar and prepared wires are placed on the patient's chest. The wires are prepared in the shape of U, that one arm is longer than another arm (**Figure 1**).

The right-handed surgeon stands on the right side of the patient's head. Then, the assistant only holds the retractor and suction in his hands. Overall during this technique the assistant's role is just retracting and do suction. In this step, the surgeon choose a quadrant (upper right) and holds a farabeuf retractor in one hand and wire holder forceps in the other hand. Also the assistant hold a tongue retractor (Minnesota retractor) and suction (**Figure 2**), then the surgeon begin to pass the U shape wire across the interdental space from the patient's posterior upper jaw teeth one by one to anterior teeth. The surgeon should catch on U wires by a wire holder from the longer arm and pass it through lingual side of each interdental tooth space. After the long arm of U wire passed through the distal interdental of each tooth, the surgeon takes another arm on lingual side by wire holder and pass it through mesial interdental space of the same tooth. When

**Figure 1:** U-shaped wires with a longer arm to fix the arch bar in the study method.



**Figure 2:** (a) surgical instruments holding technique at the beginning of surgery by the surgeon and his assistant. (b) the opposite lower quadrant.





the short arm of the wire appears in the Buccal area the surgeon takes both arms of U wire on the buccal side and pull it out until the wire fix beyond the tooth. This process continues for each tooth from posterior to anterior of the quadrant. After that the surgeon goes to another quadrant (its better the upper left side quadrant) and does the steps similar to process that done in the previous quadrant. At this stage, the surgeon immediately gives the farabeuf to his assistant and takes the Minnesota retractor from him for retracting tongue. Then the process continues as above while the assistant retract the buccal with farabeuf and suctioning (**Figure 2**).

The surgeon passes wires from lingual distal and mesial interdental spaces to buccal side and fix it by pulling out and continues this process for each tooth from posterior to anterior. After passing all the wires in the upper jaw and bending them, it is time to place the arc bar between arms in the buccal side and ties wires around arch bar (**Figure 3**).

**Figure 3:** Arch bar insertion after passing wires around the upper jaw.



After finishing the upper arch bar the surgeon all process as previous for the lower jaw arch bar from right to left and posterior to anterior on each quadrant. Finally, after the surgery, the surgeon recorded the operation time, and then pulled out his gloves in the scrub room. Then, he fills his gloves (MAXTER®) with normal saline to find any perforations (**Figure 4**).

In the control group, the conventional wiring method was used. With a professional assistant that mainly helps for fixing arch bars.

The postsurgical evaluation of the patients arch bars was done in two steps, one in the first week and one

in the first month for measurement the arch bar vertical displacement, looseness rate, by counting vertical displacement in three sites of each quadrant (posterior left, posterior right and anterior). Data were analyzed using SPSS software. Mann Whitney test was used to compare the non-parametric variables between two groups.

**Figure 4:** Saline filled surgical gloves at the end of operation for punctures.



## Results

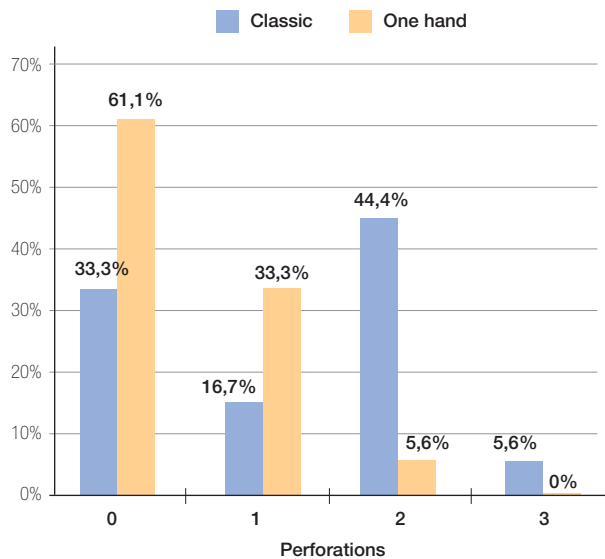
In the present study, we have evaluated the arch fixation method between two groups. They were compared in terms of the time of the wiring arch bar, the number of glove perforations and the looseness rate of the arch. Shapiro-Wilk analysis showed that, the data follow the normal distribution, except for the number of glove perforations variable (**Table I**).

**Table I:** Determination of variables normality.

P value	Statistic	variable
168/0	984/0	Time of wiring arch bar (minute)
179/0	951/0	Time of wiring arch bar (second)
112/0	061/0	Number of glove perforations
237/0	994/0	Looseness rate of the arch after one week (mm)
102/0	950/0	Looseness rate of the arch after one month (mm)

Therefore, Mann Whitney test was used to compare the number of glove perforations between two groups and independent t-test was used for other variables. There was a statistically significant difference in the number of glove perforations between the classic and the experimental group ( $p=0/024$ ). We observed that, 61/1% of gloves in the experimental group were without holes, but in the classic group, 33/3% of gloves were without holes. In the classic group, 44/4% of gloves had 2 holes and 5/6% of gloves had 3 holes. None of the gloves in the experimental group had 3 holes (**Figure 5**).

**Figure 5:** Frequency and number of glove perforations in control and experimental groups.



One week after wiring arch bar, there was no significant difference between those groups in the looseness rate ( $p_{0/560}$ ). But after 1 month, a significant difference was found between groups because the average looseness rate in the experimental group was higher ( $p_{0/016}$ ).

## Discussion

According to the study of Chhabr, one of the criteria for success in fixing arches in jaw fractures is a smaller incidence of surgical glove perforations<sup>7</sup>. In the present study, the lowest number of glove perforations was seen in the experimental group. Despite the progress made in the fixation of jaw fractures, the IMF is still considered as

a necessity. Therefore, it is necessary to achieve a more appropriate, faster and more complete method to fixing an arch bar that requires a little time. The minimal risk of infection transmission is one of the most suitable criteria for treating jaw fractures and IMF. Prolonged surgical time, aggressive treatment and the use of sharp instruments during surgery can lead to failure of routine infection control<sup>8</sup>. The method mentioned in this study reduced the surgical time, reduced the risk of infection transmission and the penetration of sharp instruments into the surgeon's hands. Fixation of the arch bar is actually the most dangerous part of treating jaw fractures. The purpose of the present study was not only to present a new method for fixing arch bar as a safe method, but also was to compare the new method with the classic method, which was successful in this regard. Patients requiring arch bar are exposed to high HIV risk<sup>9</sup>. In the present study, the risk of transmission of infection was lower compared to the classical method. Reaching a more appropriate and more effective method to fixing the arch bar has been studied by some researchers<sup>10,11</sup>. The arch bar fixing time in our presented method was shorter than the classic method for 11 minutes. The shorter fixation time in our study compared to the study conducted by Abassi<sup>11</sup> and Lagvankar<sup>10</sup>, indicates the importance of the one handed method.

## Conclusion

The results of this study showed that the placement of the arch bar without the help of a professional assistant is possible. According to statistical data, presented method had more efficiency due to less working time, fewer glove perforations, and simpler arch bar opening. This technique was called the One Hand Arch bar Placement method by the authors that could be used by surgeons in the future.

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# The efficacy of Cognitive-behavior therapy based parenting for anxious children with cancer

*La eficacia de la crianza basada en la terapia cognitivo-conductual para los niños ansiosos con cáncer*

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## Abstract

**Aim:** Cancer is one of the most distressing and life-threatening diseases. In many cases, people with cancer experience many psychological problems. Therefore, the design and implementation of psychotherapy interventions in this group is very important.

**Method:** This study was a randomized clinical trial with two CBT-based groups, plus pharmacotherapy and pharmacotherapy alone. Twenty-seven subjects participated in the present study.

**Result:** The results of repeated measure analysis indicated a significant difference between the two groups of intervention and treatment as usual in the severity of anxiety after post-test ( $p < 0.001$ ).

**Conclusion:** It seems that group CBT based parenting has a significant effect on reducing the severity of anxiety in children with cancer. Future studies are suggested to designing appropriate interventions to meet the needs of children with cancer.

**Keywords:** Cognitive-behavior therapy based parenting, anxiety, cancer.

## Resumen

**Objetivo:** El cáncer es una de las enfermedades más angustiosas y amenazantes para la vida. En muchos casos, las personas con cáncer experimentan muchos problemas psicológicos. Por lo tanto, el diseño y la implementación de intervenciones psicoterapéuticas en este grupo es muy importante.

**Método:** Este estudio fue un ensayo clínico aleatorio con dos grupos basados en terapia cognitivo conductual (TCC), más farmacoterapia y farmacoterapia sola. Veintisiete sujetos participaron en el presente estudio.

**Resultados:** Los resultados del análisis de medidas repetidas indicaron una diferencia significativa entre los dos grupos de intervención y tratamiento habitual en la gravedad de la ansiedad después de la prueba ( $p < 0,001$ ).

**Conclusión:** Parece que la crianza grupal basada en la TCC tiene un efecto significativo en la reducción de la gravedad de la ansiedad en niños con cáncer. Se sugieren futuros estudios para diseñar intervenciones adecuadas a las necesidades de los niños con cáncer.

**Palabras clave:** Crianza basada en la terapia cognitivo-conductual, ansiedad, cáncer.

## Introduction

Cancer is a growing chronic disease, and it is expected that by 2030 more than 3 million people in the UK will be diagnosed with cancer<sup>1</sup>. The prevalence of cancer in people aged 0-19 in the United States is 16,592 per million people<sup>2</sup>. Also, in Iran, the prevalence of cancer in children was estimated at 48-112 girls and 51-144 boys per million in 2010<sup>3</sup>. However, in recent decades, scientific advances have made significant contributions to cancer treatment and the likelihood that people will survive; the studies suggest that about greater than 80% of children with cancer will have a chance of 5-year survival<sup>4,5</sup>. As a result, many children in the world have experienced cancer and been successfully treated in the future. Therefore, the child has gone through this crisis physically. However, the negative psychological effects of cancer will remain in the child for the next years<sup>6-8</sup>. Anxiety is one of the major problems that people with cancer will experience even years after cancer treatment. Moreover, this anxiety will negatively affect their quality of life<sup>9,10</sup>. Studies indicate that anxiety in cancer survivors is from 9 % to 33%<sup>11-13</sup>.

In addition, cancer and its treatment procedure may also lead to pain disabilities<sup>14,15</sup>. Therefore, it may people with cancer not be able to participate in psychotherapy and appropriate interventions. Therefore parent-focused treatment without children's contribution is more helpful in this group of children.

Many studies indicated that the most effective treatments for anxiety in children are CBT-based approaches<sup>16-18</sup>. Therefore, many studies have examined appropriate and adapted alternative approaches in children. Rapee et al. implemented the CBT approach on parents to reduce children's anxiety and indicated that parents-focused programs could indirectly reduce children's anxiety<sup>16</sup>.

Similarly, Cartwright et al. designed a parenting-based group intervention known as "from timid to tiger" for anxious young children. This intervention is a CBT-based parenting program<sup>19</sup>. The results of a study found that the intervention can help reduce children's anxiety (18). The present study investigated the effect of this program on anxious children with cancer.

This program is derived from two broad categories of cognitive-behavioral therapy and parental behavioral training<sup>19</sup>. The efficacy of CBT approaches in adult anxiety is well established, and several meta-analyses support the efficacy of CBT in anxiety disorders<sup>20, 21</sup>. On the other hand, many parenting behavioral training programs have been designed. These programs are widely used in the treatment of many childhood disorders, especially externalization problems. Parenting programs have been used for decades, and there is ample evidence of their efficacy in anxiety disorders<sup>22-25</sup>.

CBT helps patients identify cognitive distortions for reality testing, and learn new skills to challenge irrational thoughts<sup>26</sup>. In the present intervention, the CBT techniques are taught to the parents, and the children are transferred. Furthermore, many studies show that inappropriate parenting has a significant positive relationship with anxiety disorders. Many components, including parental punishment and anger<sup>27</sup>, rejection<sup>28</sup>, and neglect<sup>29</sup>, have been identified in children's anxiety. Improving this environment and educating parents can play an important role in reducing children's anxiety. The present study aimed to perform "from timid to tiger" in anxious children with cancer.

## Materials and methods

### Ethics

This study was approved by the Iran University of medical sciences Ethics Committee (IR.IUMS.REC.1398.851) and confirmed in the Iranian Registry of Clinical Trials (IRCT20200728048237N1).

### Participants

Thirty-eight children and their caregiver were recruited through the regular treatment of children referred to the Ali-Asghar hospital for any type of cancer and high level of anxiety. The children were between 6 and 10 years old. Inclusion criteria for a child's participation in the study were: at least six months had passed since the diagnosis of cancer and the acute period of the disease, and the child score on the CBCL scale internalization scale was above clinical cutoff. Exclusion criteria also included: the child or parent had moderate to severe learning disabilities, and the child had bipolar disorder or psychotic disorders.

### Procedure

The present study was a randomized controlled trial. Both groups were assessed in four stages through pre-test, fifth session, post-test, and follow-up. In the present study, all children received the same dose of medication to treat their anxiety. After one month of medication, individuals were randomly divided into two groups of medication and medication with the present intervention. The intervention group used a parenting-based group intervention for anxious children (based on the treatment guide of Cartwright et al. (2010))<sup>19</sup>.

### Measures

#### Parent Report. Child Behavior Checklist (CBCL)

This scale is one of the assessment instruments in the family and was designed to assess children's emotional-behavioral problems and abilities<sup>30</sup>. It measures three dimensions of internalizing problems, externalizing problems, and general problems. In this study, the symptoms related to internalizing were considered. The CBCL has excellent internal consistency, test-retest reliability, and validity<sup>31</sup>.

### Spence Children's Anxiety Scale

This questionnaire was prepared to assess the severity of children's anxiety. The Spence Anxiety Scale is a self-report scale and consists of 44 items and six subscales. In the original version of the internal consistency questionnaire, this scale was 0.92 for general anxiety and 0.60 to 0.82 for other subscales. Reliability results of the retest were reported to be 0.63 for the whole scale and 0.51 to 0.75 for the subscales during 12 weeks<sup>32</sup>. This instrument has good psychometric properties in children aged 6-12 years<sup>33</sup> in Iran.

## Results

The results of this study indicated that there is no significant difference between the studied groups in gender ( $\chi^2 = 0.068$ , sig = 0.795), mother (sig = 0.441,  $\chi^2 = 9.99$ ) and father education level (sig = 0.452,  $\chi^2 = 11.925$ ).

The age range of the study participants was between 6-10 years, and their birth order was 1-3. Also, the mean and standard deviation of the total age of the participants were 8.92 and 1.16, respectively. There was no significant difference between the two groups in the age variable. Descriptive characteristics of anxiety severity are presented in **table I**.

**Table I:** Descriptive characteristics of anxiety severity in children.

Group	Variable	Time	Mean	SD
Intervention		Pre-test	33.17	6.91
		Fifth-session	29.25	6.70
		Post test	23.58	6.21
		Follow-up	22.42	4.19
Treatment as usual		Pre-test	32.73	9.85
		Fifth-session	30.46	9.87
		Post test	29.87	8.59
		Follow-up	27.27	8.54

In order to analyze the effectiveness of the present intervention in reducing the level of anxiety in children, repeated measures analysis of variance was used. Before using this test, Leven's test, Box's test of equality of covariance, and Mauchely's Test of Sphericity were used, and none of them were significant. The results of tests of within-subjects effects are presented in **table II**.

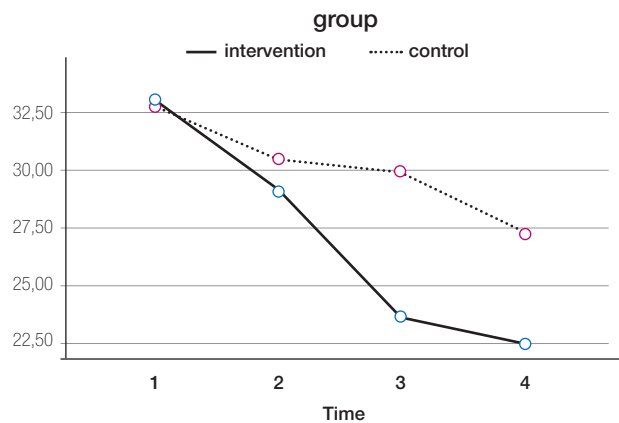
The above table results indicate that the effect of time has a significant effect on the scores of children's anxiety symptoms. Therefore, regardless of the type of group, there was a significant difference between the pre-test, fifth session, post-test, and follow-up scores. The

**Table II:** Results of repeated-measures analysis of variance for within-subjects effects.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Effect size	Power
Time	1017.238	3	339.079	23.395	0.001	0.483	1.00
Time*group	194.461	3	64.820	4.472	0.006	0.152	0.863
Error	1087.021	75	14.494	-	-	-	-

interaction between time and treatment group is also significant. Therefore, there is a significant difference between the two groups over time. **Figure 1** shows the difference between the two groups in the four stages of assessing child anxiety.

**Figure 1:** Comparison of changes in child anxiety symptoms in four stages.



## Discussion

The study results indicated that the present intervention effectively reduces anxiety symptoms in the post-test evaluation phase in the intervention group, and there is a significant difference between the two groups. The researcher did not find a study that specifically examines the efficacy of the present intervention in the anxiety of children with cancer, and previous studies have examined the present intervention only on the anxiety of non-cancerous children<sup>18</sup>. However, the results of this study were consistent with studies that have examined this intervention in children with anxiety disorders<sup>16</sup>. Also, in the other studies, the parent-based intervention reduced the children's anxiety. For example, Ginsburg & Schlossberg<sup>34</sup> study, a parent-centered CBT-based therapy, was able to help reduce children's anxiety. Thompson et al.<sup>35</sup>. Also performed a parent-centered intervention on children; the results showed that the present intervention could improve the internalization symptoms of children. Finally, the manual of present intervention claims that through parenting, the same effects of cognitive-behavioral therapy can be created in children<sup>19</sup>. According to the researcher's data, there was no inconsistency with the current finding.

On the other hand, many studies on pediatric cancer studies have examined the effectiveness of cognitive-

behavioral psychotherapy. For example, previous research has shown that cognitive-behavioral play therapy can help improve the symptoms of depression and anxiety in children with cancer<sup>36</sup>. In this intervention, the first important technique of the first session is to secure the parent-child relationship through play therapy<sup>19</sup>, and the findings of this study are in line with previous research.

In explaining these findings, paying attention to the underlying approaches of the present intervention can be very helpful. This intervention is based on the cognitive-behavioral model and parenting training<sup>19</sup>. In the cognitive-behavioral model, it is assumed that there is a relatively stable set of dysfunctional beliefs in anxiety disorders. Moreover, challenging with these thoughts can help reduce anxiety<sup>37</sup>. In addition, the goal of parenting programs is to change the parenting environment<sup>38</sup>, and when the environment changes, child problematic behaviors improve<sup>39</sup>. In the first session of the present intervention, the fundamental beliefs of a self-confident and healthy child are introduced to the parents. Furthermore, all techniques work to create these beliefs<sup>19</sup>.

Studies show that CBT interventions that directly treat the child are not very effective<sup>19</sup>. As in cognitive-behavioral therapy, clients are encouraged to specialize in their minds. In this program, parents are encouraged to be specialists in their child's anxiety. Therefore, when new problems arise, parents can provide a solution without consulting a psychologist confidently. Thus, in this treatment, important components of cognitive-behavioral therapy were passed on to children through parents<sup>19</sup>.

On the other hand, in this intervention, parental behavioral training takes place. Parental behavioral education is also based on the behavioral approach and social learning theory. Many studies have investigated the effectiveness of this approach and shown effective results in improving children's problems<sup>19</sup>. Another issue that could be explained in this finding was that many parents said that reporting children's cancer was very harmful to them and their children during the sampling. Moreover, often the parents did not receive special psychological support at the center after receiving cancer diagnosis. Some parents even said that sometimes parents were reprimanded for diagnosing cancer and being late. It seems that one of the reasons for the effectiveness and active participation of parents in the present treatment was the strong need for such an environment where parents could receive the necessary psychological support and talk to other parents. Numerous studies have shown that support and being in a group can be effective on their own<sup>40</sup>. Part of the treatment also introduced a technique for parents to hide their unpleasant feelings from their children<sup>19</sup>. In this technique, parents were told not to say anything or show any emotion if they could not control themselves. The parents reported that the technique

was very helpful during the child's physical treatment, such as injections and chemotherapy. Therefore, the transmission of parental anxiety to children was prevented. This finding can be justified by social learning theory, which believes that imitation plays a key role in many disorders<sup>41</sup>. Furthermore, many studies have found the role of imitation and maternal Psychological Control in the development of anxiety disorders and have shown that anxious parents are more likely to raise anxious children<sup>42,43</sup>.

Finally, the present treatment was expressed to parents in simple language with metaphor and example. In recent years, many studies had shown that metaphors and stories increase the efficacy of treatments<sup>44</sup>. The most important metaphors in the intervention were related to fight and flight response and avoidance. Many parents stated that children showed signs of anxiety before the present intervention (such as heart rate, screaming, etc.) during physical therapy for cancer. Parents were afraid that this anxiety would lead to a recurrence and worsening of cancer. However, parents felt more comfortable when the fight and flight response was normal and safe. They acknowledged that anxiety is an inevitable part of life and that children who can cope with their fears can better manage their anxiety in the future.

On the other hand, sometimes children used to say "you do not love me" or "I do not love you" and so on during the cancer treatment procedure. When told to parents, these phrases are very common in young children (even those without cancer) and are completely meaningless; they could more easily use the technique of ignoring these sentences and felt better emotions.

## Conclusion

In this intervention, the principles of cognitive-behavioral therapy were transferred to children through parenting components. The results of this study also confirm the claim of the underlying theory of the present intervention. Furthermore, the present intervention was able to reduce anxiety symptoms in children. The present study had several limitations that warrant consideration. Firstly, Due to the prevalence of coronavirus pandemic, dropout was unavoidable. Secondly, In this study, self-report tools were used, which if possible, it is better to use objective tools in future studies. Thirdly, the follow-up period was two months due to time and commuting limitations. Naturally, longer follow-up periods can increase the assurance of treatment stability. Additionally, further efforts are suggested to adjusting a new intensive and specific version of the current manual for reducing anxiety in children with cancer. Furthermore, in pandemic situations, designing online parenting programs that target children with cancer is more suitable for children with disabilities and diseases.

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# Seckel syndrome associated dental anomaly; case report

*Anomalía dental asociada al síndrome de Seckel; reporte de un caso*

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## Abstract

Seckel syndrome is a rare autosomal recessive with severe growth retardation, bird-headed profile, and microcephaly. It is characterized by short stature, skeletal defects, mental retardation, and characteristic facial features such as microcephaly, micrognathia, and a bird-head appearance. Dental findings include hypodontia, enamel hypoplasia, crowding, and Class II malocclusion. Craniofacial features may include facial asymmetry, down-slanting palpebral fissures, lobeless ears, and dental abnormalities, including enamel hypoplasia, hypodontia, and microdontia in the patients with SS may be at high risk of developing acute myeloid leukemia. The purpose of the paper is to report a case of the seckel syndrome with a dental anomaly.

**Keywords:** Seckel syndrome, dental, anomaly, case report.

## Resumen

El síndrome de Seckel (SS) es una rara enfermedad autosómica recesiva que cursa con un grave retraso del crecimiento, un perfil de cabeza de pájaro y microcefalia. Se caracteriza por baja estatura, defectos esqueléticos, retraso mental y rasgos faciales característicos como microcefalia, micrognatia y apariencia de cabeza de pájaro. Los hallazgos dentales incluyen hipodoncia, hipoplasia del esmalte, apiñamiento y maloclusión de clase II. Características craneofaciales pueden incluir asimetría facial, fisuras palpebrales inclinadas hacia abajo, orejas sin lóbulos y anomalías dentales, incluyendo hipoplasia del esmalte, la hipodoncia y la microdoncia. Los pacientes con SS pueden tener un alto riesgo de desarrollar leucemia mieloide aguda. El propósito de este trabajo es informar de un caso de síndrome de Seckel con una anomalía dental.

**Palabras clave:** Síndrome de Seckel, dental, anomalía, reporte de caso.

## Introduction

Seckel syndrome is an autosomal recessive condition without any sex predilection, with an incidence reported of 1:10,000 live-born children<sup>1,2</sup>.

The syndrome is characterized by severe intrauterine growth retardation, severe short stature, severe microcephaly, bird-headed profile, receding chin and forehead, sizeable beaked nose, mental retardation, and other congenital anomalies. Rudolf Virchow introduced the term "bird-headed dwarf" in the context of proportionate dwarfism with low birth weight, mental retardation, a pointed nose, and micrognathia<sup>3</sup>.

Polyarthritis nodosa affects multiple organ systems, most commonly kidneys, gastrointestinal tract, nervous system, muscles, and soft tissue<sup>4</sup>.

In one-half of cases, head circumference is more retarded than height, while it is as retarded as height for the remainder. Other systemic manifestations associated with SS include Fanconi anemia, leukemia, chronic nephritis, dysgenesis of the cerebral cortex, corpus callosum, and a vast spectrum of skeletal defects<sup>7</sup>.

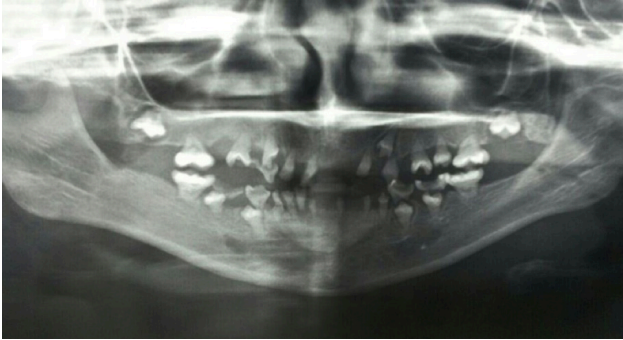
• Small brain with small gyri. The cerebral hemispheres' volume can be reduced to one-third of routine, particularly in the cerebral cortex<sup>8</sup>. Dental findings include hypodontia, enamel hypoplasia, crowding, and Class II malocclusion<sup>9</sup>.

This paper's purpose was to report the case of a female patient with Seckel syndrome and describe her dental manifestations.

## Case report

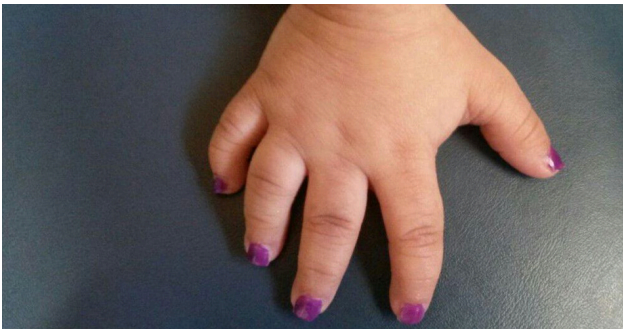
A nine-year-old female child reported to Oral Medicine and Radiology department with a chief complaint of noneruptive teeth and micro dent (Figure 1). History revealed that a child with severe growth retardation.

**Figure 1:** Panoramic view of noneruptive teeth and micro dent were presented in a child.



The patient had proportional short stature, weight, height, and head circumference below the third percentile. On physical examination, she had a short finger (Figure 2).

**Figure 2:** A girl case with a short finger.



The patient's general appearance was characterized by a small, prominent forehead, slightly beaked nose, midface hypoplasia, hypertelorism, high hair line with thin hair, and eyebrow (Figure 3).

**Figure 3:** The general appearance of a case patient.



Investigation of the orthopantomogram and panoramic radiographs (Figure 1) revealed that maxillary permanent left central incisors and right second premolar and mandibular permanent second molar were congenitally missing. The teeth appeared to have enamel hyperplasia with short roots, partially obliterated pulp. The permanent first molar had a bulbous crown.

On laboratory examinations, results of complete blood revealed low-level hemoglobin (10.9 g/dl, normal range:12-15 g/dl) and MCV(65/9, normal range 77-95), while the levels RDWC (18/3, normal range 11-14) and platelet count (588 1000/ $\mu$ l, normal range 150-450 1000/ $\mu$ l), D.H.E.A So4 (311 micg/dl, normal range 2/8-85/2). The range of 25OH VitD was insufficient.

## Discussion

Seckel syndrome (Online Mendelian Inheritance in Man database Number 210600) is a rare form of primordial dwarfism, which was initially described in 1960.<sup>10</sup>

The syndrome as severe intrauterine growth retardation, severe short stature, severe microcephaly, bird-headed profile, receding chin and forehead, sizeable beaked nose, mental retardation, and other congenital anomalies. Rudolf Virchow introduced the term "bird-headed dwarf" in the context of proportionate dwarfism with low birth weight, mental retardation, a pointed nose, and micrognathia<sup>3</sup>.

Presented herein is a child with classic Seckel phenotype, who had both an open and a closed-lip schizencephaly, resulting in global developmental delay and bilateral sensorineural deafness<sup>11</sup>.

Synostosis of cranial sutures occurs in approximately 50%. Other craniofacial features may include facial asymmetry, down-slanting palpebral fissures, lobeless ears.<sup>12</sup> Dental abnormalities include enamel hypoplasia, hypodontia, microdontia, taurodontic root morphology. Several cases have demonstrated a high-arched palate, and cleft palate has also been reported<sup>10</sup>.

This patient had enamel hypoplasia, hypodontia, and microdontia, but Cleft palate was not observed in the present cases. Regen reported the patient with Seckel syndrome type II and described her oral manifestations. She presented interesting dental findings, including gingival hyperplasia, recession and ulceration, significant crowding, and early exfoliation of the primary dentition with the permanent dentition's accelerated eruption.<sup>9</sup>

The dentition has been described sporadically in SCKL, focusing on enamel hypoplasia malocclusion microdontia, dentin dysplasia, and taurodontism<sup>13</sup>.

De Coster reported a 14-year-old boy is presented with brain hypoplasia, pachygyria, hydrocephaly, enamel hypoplasia and root dysplasia in the temporary dentition, and oligodontia, severe microdontia, and delayed eruption of the permanent dentition<sup>13</sup>.

About 170 cases of Seckel syndrome have been reported worldwide, and there is considerable heterogeneity in their clinical characteristic<sup>11</sup>.

Hematopoietic disorders have been reported in approximately 15% of patients with SS<sup>14</sup>.

The authors suggested that the patients with SS may be at high risk of developing acute myeloid leukemia. However, the risk of chronic myeloid leukemia was noted following the aspiration biopsies of the bone marrow of both patients, and the relevant specialists planned routine controls of the patients<sup>10</sup>.

Kilic et al. reported a case with low-level vitD and osteomalacia<sup>15</sup>. Our patient had 25OH VitD insufficient. Vitamin D plays an essential role in skeletal development, bone health maintenance, and neuromuscular functioning<sup>16</sup>. Clinical features include growth failure, hypotonia, rachitic bones, and enamel hypoplasia<sup>17</sup>.

The craniofacial features of Seckel syndrome allow differentiation from other syndromes of growth deficiency with microcephaly, such as *Dubowitz syndrome*, *fetal alcohol syndrome*, *trisomy 18*, *de Lange syndrome*, *Bloom syndrome*, *Nijmegen breakage syndrome*, and *Fanconi syndrome*<sup>12</sup> also SS should be differentiated from closely resembling *Cockayne syndrome*, *progeria*, *Hallermann-Streiff syndrome* (HSS) *Dyggve-Melchior-Clausen* (DMC) syndrome<sup>3</sup>.

- Fanconi anemia is clinically defined by pancytopenia in the first decade of life and complicated by leukemia, pigmentary changes in the skin, cardiac, kidney, and limb (radius aplasia) malformations. Affected adults are also at high risk for non-hematologic malignancies. Mild microcephaly (often without intellectual disability) is observed in 10%-25% of individuals<sup>8</sup>.

- Cockayne's syndrome is a rare, autosomal recessive disorder characterized clinically by cachectic dwarfism, cutaneous photosensitivity, loss of adipose tissue, mental retardation, skeletal and neurological abnormalities, and pigmentary degeneration of the retina<sup>18</sup>.

While progeria is a rare premature aging syndrome characterized by retarded physical development, abnormal facies, skeletal abnormalities, and early onset of scleroderma<sup>19</sup>.

## Conclusion

The knowledge of symptoms and risk factors of Seckel syndrome is essential For early intervention and dental problems prevention.

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## CASE REPORT

# Inestabilidad cefálica y derrame pericárdico

*Cephalic instability and pericardial effusion*

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## Resumen

Varón de 68 años, con colitis ulcerosa, que ingresa por inestabilidad objetivándose derrame pericárdico severo. Estudio de miocarditis y tratamiento de soporte con evolución tórpida hasta la muerte.

**Palabras clave:** Miocarditis, derrame pericárdico.

## Abstract

A 68-year-old man with ulcerative colitis, who was admitted at the hospital due to instability and showing a severe pericardial effusion. Study of myocarditis and supportive treatment with torpid evolution leading to death.

**Keywords:** Myocarditis, pericardial effusion.

## Presentación del caso

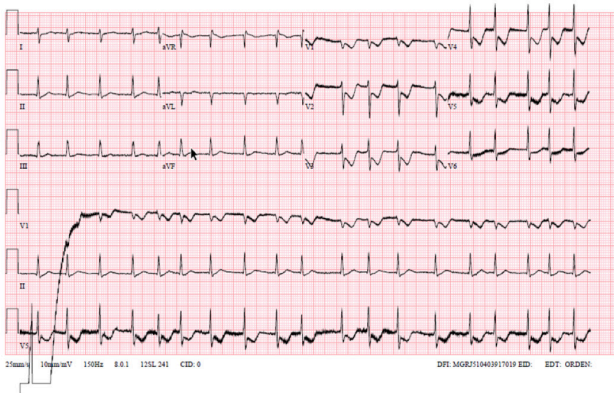
Se trata de un varón de 68 años, sin hábitos tóxicos, con antecedentes de colitis ulcerosa de larga evolución en remisión mantenida con mesalazina. Acudió a Urgencias por segunda vez en un mes por inestabilidad incapacitante de 3 días de evolución. A su llegada se encontraba con tensión arterial de 70/40 mmHg, afebril. El ECG mostró una fibrilación auricular con RVM de 150 lpm, con lesión subendocárdica de V1-V3 (**Figura 1**). El ecocardiograma reflejó un derrame pericárdico severo, por lo que, dada la inestabilidad hemodinámica, se drenó mediante pericardiocentesis guiada por ecografía.

Se realizó un ecocardiograma de control, con mínima persistencia del derrame, con hipocinesia inferobasal, inferolateral medial y basal, y fracción de eyección preservada. Analíticamente destacaba insuficiencia renal aguda y elevación marcada de troponina I (15951,4 ng/L), por lo que se inició doble antiagregación. El

cateterismo cardíaco no mostró lesiones coronarias significativas. Se intentó cardioversión eléctrica sin éxito. Todas las serologías -VHH6, VIH, C.burnetti, C.psittaci, C.pneumoniae, M.pneumoniae, VHC, Erythrovirus B19, adenovirus, enterovirus y VEB- y los cultivos –incluyendo hongos y micobacterias– fueron inicialmente negativos. El análisis del líquido pericárdico mostró un aumento de la celularidad a expensas de linfocitos (230 células -91% MN-, 570 hematíes/mm<sup>3</sup>, glucosa 112 mg/dl, proteínas 3.7 g/dl). No se pudo realizar resonancia magnética cardíaca por inestabilidad clínica.

El paciente persistía hipotenso, iniciándose perfusión de dobutamina, pero por inestabilidad hemodinámica persistente se trasladó a la UCI. A los tres días presentó fiebre, con aislamiento de S.epidermidis linezolid resistente en muestras respiratorias. A pesar de tratamiento antibiótico dirigido y soporte ventilatorio el paciente empeoró rápidamente, siendo finalmente *exitus*.

Figura 1.



## Presentación del caso Discusión del caso clínico

Teniendo en cuenta el cuadro clínico en su conjunto, la primera sospecha clínica sería miocarditis. Según los criterios diagnósticos establecidos por la European Society of Cardiology en 2013<sup>1</sup> (Tabla I), el paciente presentaría 2 de las presentaciones clínicas –arritmias y shock cardiogénico– junto con 3 criterios diagnósticos -alteraciones electrocardiográficas, elevación de troponinas y anomalías funcionales/estructurales en la ecografía. Además, presentaría como característica auxiliar una enfermedad autoinmune extracardíaca (colitis ulcerosa).

La miocarditis se trata de una enfermedad inflamatoria del músculo cardíaco, de etiología variada y con una incidencia no bien definida, fundamentalmente por la gran variabilidad en la presentación clínica que posee: enfermedad subclínica, dolor torácico, insuficiencia

cardíaca, derrame pericárdico, arritmias, muerte súbita, etc. Las causas se engloban en dos subgrupos, infecciosas (virus, bacterias, hongos y parásitos y protozoos) y no infecciosas (autoinmunitarias, por hipersensibilidad, por toxinas y por patógenos físicos).

Una vez se tiene la sospecha clínica, el diagnóstico definitivo lo otorgaría la biopsia endomiocárdica, una técnica que realizada por manos expertas es de bajo riesgo<sup>2</sup>. Aún así, durante los últimos años se han potenciado las pruebas de imagen cardíaca como método diagnóstico, sobre todo la resonancia magnética cardíaca. De esta manera se han establecido los criterios de Lake Louise (Tabla II) que, si se cumplen 2 o más, consigue una especificidad del 96% y una sensibilidad del 76%.

Pese a la importancia de obtener el diagnóstico, finalmente el tratamiento básico de toda miocarditis es el de soporte de la insuficiencia cardíaca.

Tabla II: Criterios en RM cardíaca diagnósticos de miocarditis (criterios de consenso de Lake Louise).

Ante la sospecha clínica de miocarditis, los hallazgos en la RM cardíaca son compatibles con inflamación miocárdica si están presentes al menos 2 de los siguientes criterios:

1. Aumento de la señal focal o difusa en las secuencias potenciadas en T2.
2. Realce precoz con Gd (midiendo el realce global relativo del miocardio).
3. Al menos un foco de realce tardío focal no isquémico.

Un estudio de RM cardíaca es compatible con daño del miocito y/o cicatriz por inflamación miocárdica si el tercer criterio está presente.

Si el estudio inicial con RM es normal, se recomienda repetir RM entre la primera y la segunda semana si la sospecha clínica es muy alta y el comienzo de la clínica ha sido muy reciente.

- La presencia de disfunción del ventrículo izquierdo o derrame pericárdico son datos adicionales que favorecen la presencia de miocarditis.

Tabla I: Criterios diagnósticos de miocarditis de la Sociedad Europea de Cardiología.

Diagnostic criteria for clinically suspected myocarditis	
Clinical presentations <sup>a</sup>	
Acute chest pain, pericarditic, or pseudo-ischaemic	
New-onset (days up to 3 months) or worsening of: dyspnoea at rest or exercise, and/or fatigue, with or without left and/or right heart failure signs	
Subacute/chronic (>3 months) or worsening of: dyspnoea at rest or exercise, and/or fatigue, with or without left and/or right heart failure signs	
Palpitation, and/or unexplained arrhythmia symptoms and/or syncope, and/or aborted sudden cardiac death	
Unexplained cardiogenic shock	
Diagnostic criteria	
I. ECG/Holter/stress test features	
Newly abnormal 12 lead ECG and/or Holter and/or stress testing, any of the following: I to III degree atrioventricular block, or bundle branch block, ST/T wave change (ST elevation or non ST elevation, T wave inversion), sinus arrest, ventricular tachycardia or fibrillation and asystole, atrial fibrillation, reduced R wave height, intraventricular conduction delay (widened QRS complex), abnormal Q waves, low voltage, frequent premature beats, supraventricular tachycardia	
II. Myocardialcytolysis markers	
Elevated TnI/Tnl	
III. Functional and structural abnormalities on cardiac imaging (echo/angio/CMR)	
New, otherwise unexplained LV and/or RV structure and function abnormality (including incidental finding in apparently asymptomatic subjects): regional wall motion or global systolic or diastolic function abnormality, with or without ventricular dilatation, with or without increased wall thickness, with or without pericardial effusion, with or without endocavitary thrombi	
IV. Tissue characterization by CMR	
Oedema and/or LGE of classical myocarditic pattern (see text)	
Clinically suspected myocarditis if ≥1 clinical presentation and ≥1 diagnostic criteria from different categories, in the absence of: (1) angiographically detectable coronary artery disease (coronary stenosis ≥ 50%), (2) known pre-existing cardiovascular disease or extra-cardiac causes that could explain the syndrome (e.g. valve disease, congenital heart disease, hyperthyroidism, etc.) (see text). Suspicion is higher with higher number of fulfilled criteria.	
<sup>a</sup> If the patient is asymptomatic >2 diagnostic criteria should be met.	

En este caso, debido a la ausencia tanto de la biopsia endomiocárdica como de la resonancia magnética, la prueba analítica que mayor información nos aporta es el análisis del líquido pericárdico. Es un líquido seroso con baja celularidad aunque con predominio linfocitario (hematíes 570/ul, leucocitos 230/ul, linfocitos 91%), con glucosa similar a la que hay en sangre y con proteínas elevadas (3'7 g/dl), pudiéndose orientar como exudado (aunque no disponemos de la LDH).

Por lo tanto, iniciando el diagnóstico diferencial, estas serían las principales causas de miocarditis a tener en cuenta<sup>3-5</sup>:

- Miocarditis viral: se trata de la causa más común, teniendo como patógenos más frecuentes el parvovirus B-19, virus herpes humano 6, adenovirus, enterovirus (*Coxsackie*), *Citomegalovirus*, *Virus Epstein Barr*, VIH, *Virus hepatitis C*, gripe, *Varicela Zóster*... A favor de este diagnóstico tendríamos el exudado de predominio linfocítico. En contra, que las serologías para VIH, VHC, PVB19, adenovirus y CMV fueron negativas.
- Miocarditis bacteriana: los patógenos más frecuentes son la mycobacteria, chlamydia, estreptococo, mycoplasma, legionella, salmonella, rickettsia, corynebacteria, borrelia... En este caso se evidenció crecimiento en esputo de *Estafilococo Epidermidis* en el día 6 de ingreso hospitalario, por lo que se engloba más dentro de las complicaciones habituales de ingreso en Unidad de Cuidados Intensivos que como agente causal de la miocarditis. Por lo demás no hay datos de neutrofilia en el líquido pericárdico, y el resto de cultivos -hemocultivos, urocultivo, coprocultivo y líquido pericárdico- fueron negativos. Sería interesante disponer también de una determinación de ADA en la bioquímica del líquido, ya que las miocarditis tuberculosas pueden presentarse con predominio linfocítico.
- Miocarditis autoinmune: suele estar relacionada con trastornos intestinales inflamatorios, miocarditis de células gigantes, Lupus eritematoso sistémico, Sarcoidosis, síndrome de Sjögren, Síndrome de Churg-Strauss, Granulomatosis de Wegener, Arteritis de Takayasu. A favor de este diagnóstico tendríamos el antecedente de la colitis ulcerosa y la evolución fulminante del cuadro. En contra, que estos cuadros suelen ir acompañados de brotes o agudizaciones de las enfermedades autoinmunes de base, que no se especifican para este caso.
- Miocarditis por hipersensibilidad: suelen tener lugar como respuesta alérgica a ciertos fármacos como los antirreumáticos, penicilina, antidepresivos tricíclicos, clozapina, sulfamidas, cefalosporinas... A favor de esta orientación estaría el tratamiento con mesalazina del paciente, aunque se trata de una medicación iniciada años atrás. En contra, que no se ha evidenciado la eosinofilia típica de estas patologías ni en suero ni en el líquido pericárdico.

Por último, sería interesante tener en cuenta el síndrome MINOCA<sup>6</sup> (*acute Myocardial Infarction with Non-Obstructive Coronary Arteries*) ya que se trata de un caso en el que el paciente presentaba alteraciones de la repolarización en forma de elevación del ST junto con elevación de troponinas, aunque con una coronariografía sin lesiones significativas. Dicho síndrome engloba múltiples causas como el espasmo coronario, la trombosis aguda, la disección coronaria, la miocardiopatía de Takotsubo, la disfunción microvascular y la embolia coronaria, entre otras. Aunque para este caso la clínica no cuadraría ya que el paciente presentó una clínica subaguda sin dolor torácico, y con evolución desfavorable rápidamente progresiva.

Por lo tanto, las principales sospechas diagnósticas serían:

1. Miocarditis viral: al ser la causa más frecuente junto con un exudado de predominio linfocítico.
2. Miocarditis autoinmune: debido al antecedente de colitis ulcerosa.
3. Miocarditis por hipersensibilidad: secundario a la mesalazina, aunque poco probable al tratarse de un tratamiento crónico.

## Examen anatómico y patológico postmortem

El examen externo mostró un varón obeso, con abdomen globuloso, sin que se palpasen masas/visceromegalias. En fosa ilíaca derecha, piernas y brazos se observaron lesiones equimóticas. A la apertura, en la cavidad torácica no se observan lesiones. El pulmón derecho pesa 620g y el izquierdo 520g, con congestión general del izquierdo y ambos con aspecto antracótico. Presencia de leve derrame pericárdico de carácter seroso. Corazón de tamaño y peso incrementado (550g). No se observan lesiones en pericardio ni material fibrináceo. Se remite muestra de miocardio al servicio de microbiología para estudio de microorganismos. A la apertura de cavidades cardíacas se observa leve incremento de grosor en ventrículo izquierdo. Placas de ateroma no oclusivas en la salida de la coronaria izquierda, así como también en la aorta. Con respecto a la cavidad abdominal, se observó hematoma en partes blandas en fosa ilíaca derecha, así como también gran hematoma en pared abdominal posterior, con afectación de psoas derecho principalmente (**Imagen 1**). Esófago, estómago e intestino delgado sin lesiones; colon con presencia de aspecto congestivo en sigma y recto, sin otras alteraciones. Hígado de 1930g, de superficie lisa y amarillento al corte. Vesícula, vía biliar, páncreas y suprarrenales sin alteraciones macroscópicas. El bazo pesó 210g, congestivo. Los riñones de 220g y 210g, de superficie irregular con múltiples cicatrices corticales, así como lesión quística de contenido seroso de 4 cm en riñón derecho. Próstata de 20g, sin alteraciones; ambos testículos presentaron hidrocele. Encéfalo de 1410g con hemisferios simétricos, con surcos y circunvoluciones sin alteraciones.



Microscópicamente, se observaron múltiples focos parcheados de distribución pancardíaca con destrucción y necrosis de cardiomiocitos, a expensas de un infiltrado macrófagico y linfocitario con fenotipo T (CD3+, CD4+) y sin inflamación aguda (**Imagen 2**)<sup>7,8</sup>. No se observaron microorganismos con las tinciones para Citomegalovirus, Epstein-Barr, ni hongos mediante PAS/Grocott. Parénquima pulmonar congestivo generalizado, de predominio en lóbulo inferior izquierdo, con focal hemorragia alveolar en algunas áreas (**Imagen 3**). El resto del parénquima pulmonar mostró patrón enfisematoso en lóbulos superiores, antracosis, presencia de émbolos de médula ósea y se observaron tapones mucoides en bronquios principales con escamas córneas y presencia de estructuras fúngicas por probable contaminación de vías superiores/orofarige (**Imagen 4**). Mucosa gastrointestinal autolítica, con áreas congestivas en sigma y recto, sin evidencia de inflamación. Parénquima hepático con arquitectura conservada, marcada esteatosis macrovacuolada en zonas 3 y 2 de Rappaport y congestión sinusoidal. Los riñones presentaron áreas de necrosis tubular y cambios congestivos glomerulares y medulares, con quiste simple en riñón derecho. En el estudio histológico cerebral, se observó mediante la inmunotinción para Tau la presencia

de depósitos puntiformes en amígdala, hipocampo anterior y posterior y circunvoluciones adyacentes, llamados cuerpos argirófilos (**Imagen 5**), que se acompañan de previllos y ovillos, neuronas balonzadas, astrocitos “en arbusto” y “coiled bodies”<sup>9-11</sup>. El resto de órganos no mostró alteraciones relevantes.

Imagen 1

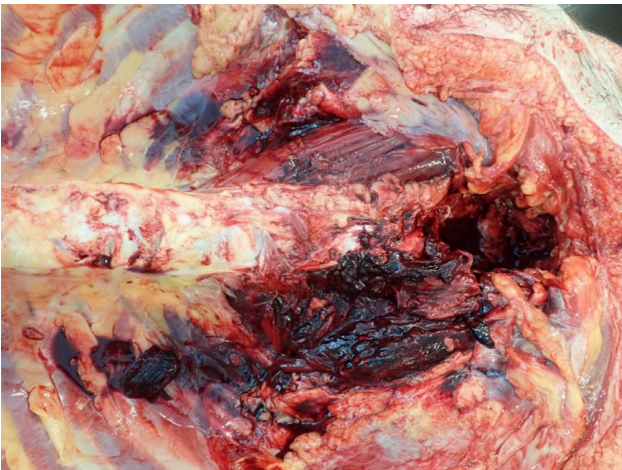


Imagen 2

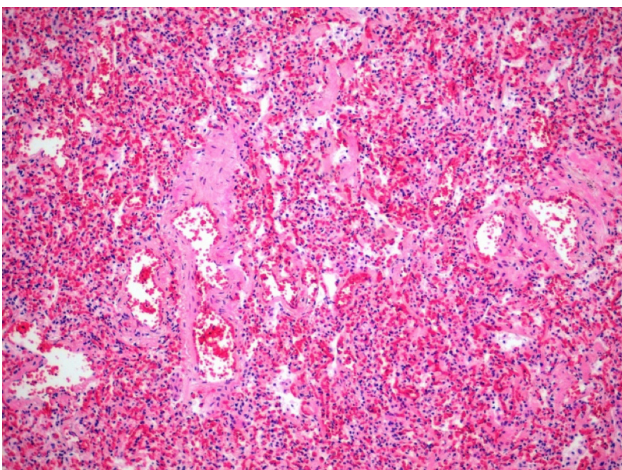


Imagen 3

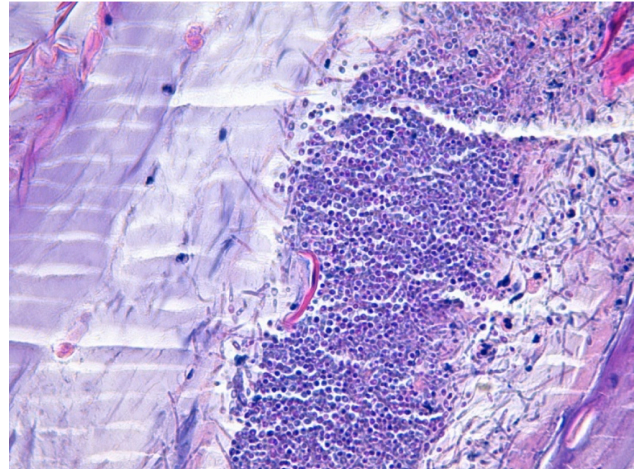


Imagen 4

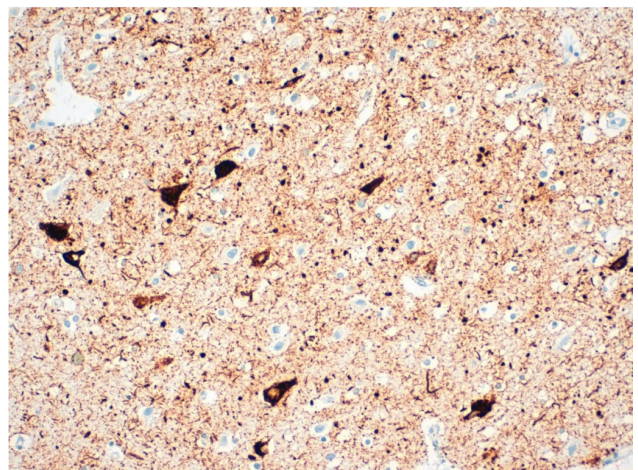
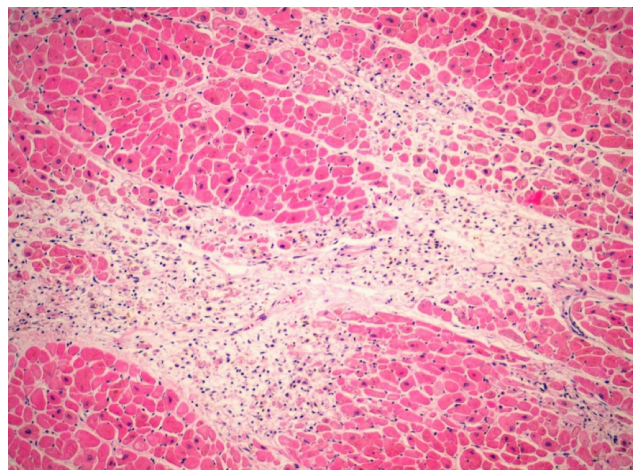


Imagen 5



Los diagnósticos realizados fueron: Miocarditis evolucionada idiopática, con cultivos negativos para bacterias en la biopsia miocárdica; signos de disfunción multiorgánica (con congestión hepatoesplénica y necrosis tubular aguda incipiente); arterioesclerosis generalizada (ateromatosis coronaria y aórtica e hipertrofia del ventrículo izquierdo); enfermedad de los granos argirófilos, estadio III de Saito. Como otros diagnósticos se identificó enfisema pulmonar, émbolos de médula ósea pulmonares (en relación con maniobras de RCP),

esteatosis hepática, neoplasia intraepitelial pancreática (PanIN) de bajo grado y quiste renal simple de 4 cm.

Se estableció como probable causa etiológica de la miocarditis un posible origen vírico, si bien no pudo ser demostrado microbiológicamente. Se descartó una miocarditis asociada a mesalazina por tratarse de una reacción de hipersensibilidad y no haberse observado eosinófilos, así como también causas bacterianas y fúngicas por la ausencia de infiltrado neutrofilico.

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