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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[1]. 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 7th century BC. In the 18th century, a Pennsylvanian physician[2] 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[3].

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[4]. *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[5]. The area around the dental plaque has a low oxidation potential promoting the growth of facultative anaerobes. Newman[6] 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[7] 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 ad 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 microaerophically (5% oxygen, 85% nitrogen, and 10% CO_2) 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)[8-14]. Suspected colonies were then identified using Gram stain, motility, colony morphology, and biochemical tests such as urease, oxidase, and catalase tests[15]. 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[16]. 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[17,18]. Purity (A260/A280) and concentration of extracted DNA were then plaids (NanoDrop, Thermo Scientific, Waltham, MA, USA), and the DNA quality was scrutinized by electrophoresis[19-22]. PCR was accompanied using a PCR thermal cycler (Eppendorf Co., Hamburg, Germany) rendering to the described procedure[23-25]. *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[26-28]. 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[29-33].

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[34-36].

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 (%) Canine teeth (%) Premolar teeth (%) Molar teeth (%)	 25 (10.0) 30 (12.0) 82 (32.8) 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 *H. pylori* (%)
Dental plaque position	Incisor teeth	25	3 (12.0)
	Canine teeth	30	4 (13.3)
	Premolar teeth	82	13 (15.8)
	Molar teeth	113	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[38-40]. In this regard, *H. pylori* have become an essential public health issue in the last century[41]. 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*[42]. 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[43]. 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[44,45]. Chitsazi et al. (2006)[46] 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)[47] mentioned that *H. pylori* was detected in 18.3% of oral samples and 88.3% of gastric biopsies. Saudia authors[48] 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[49]. High distribution of *H. pylori* in dental plaque has been reported in surveys conducted in Mexico[50], Iran[51], Japan[52], and Morocco[53]. An Iranian survey[54] 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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