eISSN 2255-0569

ORIGINAL

Effect of zinc on SIRT1 and PGC-1 alpha gene expression among ulcerative colitis patients

Efecto del zinc en la expresión de los genes SIRT1 y PGC-1 alfa en pacientes con colitis ulcerosa

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Received: 17 - IX - 2021
Accepted: 26 - IX - 2021

doi: 10.3306/AJHS.2021.36.04.161

Summary

Zinc has so many beneficial effects as a supplement, particularly for the treatment of diseases and prevention of the occurrence of several disorders through inflammatory reactions, especially in gastrointestinal cases. The present study was done to assess the Zn effect on Silent Information Regulator 1 (SIRT1) and Peroxisome proliferator activated receptor γ coactivator-1α (PGC-1α) gene expression among ulcerative colitis (UC) patients. Fifty patients with mild-to-moderate active UC were included and divided into two groups of treatment (25 patients received Zn (35 mg Zn gluconate/day for 40 days) and control (25 patients received placebo similar to the Zn capsules in shape and color for 40 days). The expression rates of SIRT1 and PGC-1α were examined in the patients using the Real-Time PCR. The mean age of included patients was 37.2±10.6 years. The male to female ratio was 23/27. Totally, the distribution of smoking and alcohol among patients were 55% and 31%, respectively. Pan UC (40%) and lift-sided (40%) had the higher distribution. The mean expression of the PGC1-α gene was increased amongst the UC patients treated with Zn supplement (P <0.05). The mean expression of the SIRT1 gene was increased amongst the UC patients treated with Zn supplement (P <0.05). However, in the control group, no any changes have been recorded for both genes. It seems that Zn caused significant decrease in the inflammatory response of the colon by significant increase in the expression of the SIRT1 and PGC1-α genes.

Keywords: Ulcerative colitis, PGC1-α, SIRT1, Gene expression, Zinc supplement.

Resumen

El zinc tiene muchos efectos beneficiosos como suplemento, en particular para el tratamiento de enfermedades y la prevención de la aparición de varios trastornos por reacciones inflamatorias, especialmente en casos gastrointestinales. El presente estudio se llevó a cabo para evaluar el efecto del Zn sobre el regulador silencioso de la información 1 (SIRT1) y el receptor activado por el proliferador de peroxisomas γ coactivador-1α (PGC-1α) en pacientes con colitis ulcerosa (CU). Se incluyeron 50 pacientes con CU activa de leve a moderada y se dividieron en dos grupos de tratamiento (25 pacientes recibieron Zn (35 mg de gluconato de Zn/día durante 40 días) y control (25 pacientes recibieron placebo similar a las cápsulas de Zn en forma y color durante 40 días). Se examinaron los índices de expresión de SIRT1 y PGC-1α en los pacientes utilizando la PCR en tiempo real. La edad media de los pacientes incluidos fue de 37,2±10,6 años. La proporción entre hombres y mujeres fue de 23/27. En total, la distribución del tabaquismo y el alcohol entre los pacientes fue del 55% y el 31%, respectivamente. La distribución más alta fue la de la CU de tipo pan (40%) y la de tipo lifting (40%). La expresión media del gen PGC1-α aumentó entre los pacientes con CU tratados con suplementos de Zn (P <0,05). La expresión media del gen SIRT1 aumentó entre los pacientes con CU tratados con suplemento de Zn (P <0,05). Sin embargo, en el grupo de control no se registraron cambios en ambos genes. Parece que el Zn provocó una disminución significativa de la respuesta inflamatoria del colon mediante un aumento significativo de la expresión de los genes SIRT1 y PGC1-α.

Palabras clave: Colitis ulcerosa, PGC1-α, SIRT1, expresión génica, suplemento de Zinc.

Introduction

Ulcerative colitis (UC) is a disease mainly characterized by chronic inflammation and an increased production of pro inflammatory cytokines and reactive oxygen species, particularly in the intestine. UC is consider chronic inflammatory bowel disease (IBD) with higher incidence rate amongst 30-40 years old people[1]. It has a high annual distribution globally, particularly 24.3 per 100,000 cases in Europe and 6.3 per 100,000 cases in Asia[2]. The main clinical signs of the UC are diarrhea, abdominal pain, bowel movements, local inflammation, and rectal bleeding[3]. Epidemiological surveys revealed that many factors play important roles in the UC pathogenesis[4].

Previous report showed that the UC occurrence is frequently associated with zinc (Zn) deficiency[5]. Zn supplementation can be beneficial to UC treatment, owing to its anti-inflammatory and antioxidant properties[6]. Zn deficiency is common in patients with IBD, throughout both active and remission phases, with a prevalence ranging from 15% to 40%[7]. Studies on animal models and translational studies proved that decreased serum Zn concentrations may improve inflammation through numerous pathophysiological mechanism, including disruption of epithelial barrier, altered mucosal immunity, and increased proinflammatory cytokines[8].

Silent Information Regulator 1 (SIRT1) is NAD(+)-dependent histone deacetylases involved in the antioxidant defense regulation, cell apoptosis and inflammation[9]. SIRT1 activation will cause several developments in the UC and IBD[10-13]. SIRT1 is an important factor in the activation of Peroxisome proliferator activated receptor γ coactivator-1α (PGC-1α)[14]. PGC-1α controls numerous biological activities, such as oxidation, mitochondrial biogenesis and inflammation[15]. PGC-1α activation caused several decreased in the occurrence of UC among models[16,17]. In IBD models, increase in the PGC-1α expression caused significant decrease in the occurrence of IBD through antioxidant activities[16,17].

However, the main roles of the Zn supplement on the regulation of and SIRT1 and PGC-1α genes is not determined yet. Thus, this survey was aimed to assess the effects of Zn supplement on SIRT1 and PGC-1αgenes expression in patients with UC.

Materials and methods

Inclusion and exclusion criteria

The current study was conducted on 50 patients with mild-to-moderate active UC who were referred to medical clinics. Patients ≥20 years of age with a confirmed histological and endoscopic diagnosis of UC, body mass index (BMI) 20-30 kg/m^2 were selected. In the survey active mild to moderate UC was determined by a Simple Clinical Colitis Activity Index (SCCAI) score of ≥5 and <12.

Exclusion criteria were use of anti–tumor necrosis factor agents and omega-3, use of non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, use of multivitamin-mineral, polyphenolic and antioxidant supplements and also changing the type or dose of medication over the past month. In addition patients with other diseases (cancer, renal, liver or cardiovascular disease and diabetes mellitus), as well as pregnancy or lactation women were excluded. Additionally, patients with the signs of the Corona Virus Diseases 2019 (COVID-19) were excluded from the study.

Interferences

Patients were randomly divided into two groups according to permuted block randomization design generated via www.Randomization.com website. Patients were given Zn (35 mg Zn gluconate/day for 40 days) or placebo for 40 days (25 patients in each group). Placebo capsules contained rice flour in size and color similar to Zn capsules, also packaged like to Zn boxes. The randomization list and numbered packing of intervention were performed by a person not involved in the study. The intervention packs were placed in the laboratory and given to the patients by the secretary according to the code numbers. Patients and all study personnel, were blinded to treatment assignment throughout the study.

RNA extraction and cDNA synthesis

Buffy coat samples of blood white cells were separated by centrifugation (Shimadzu, Japan). RNA was extracted using RNX-plus Sinacolon Kit (Cinnagen Co, Iran) according to the protocol of the producing company. The RNA concentrations were determined by Nanodrop device (NanoDrop, Wilmington, USA). All RNA samples had a 260:280 absorbance ratio between 1.9 and 2.1. Then, cDNA was synthesized using SinaClon first strand cDNA synthesis kit (Cinnagen Co, Iran) according to the protocol of the producing company. Extracted cDNA samples were subjected to quantification by NanoDrop device (NanoDrop, Thermo Scientific, Waltham, USA), qualification (2% agarose gel) and purity checking (A260/A280). The cDNA was stored at -80°C until subsequent analysis.

Gene expression

Real-Time Polymerase Chain Reaction (Real-Time PCR) was carried out to determine the levels of SIRT1 and PGC-1α genes expression in extracted cDNA samples. **Table I** describes list of primers used in the real-time PCR reaction.

Table I: Primers used in the real-time PCR reaction[18].

Target gene	Oligonucleotide primers (5'-3')
PGC-1α	F: GTCAACATTCAAAGCAGCAGAGAG R: GACACATAATCATTACCTACTGGAAGC
SIRT1	F: TAGTAGGCGGCTTGATGGTAATC R: GGTTCTTCTAAACTTGGACTCTGG

Real-Time PCR thermocycler (Rotorgene 6000, Corbett research, Mortlake, London, UK) was used to assess the expression rates of the SIRT1 and PGC-1α genes in examined groups using SYBR Green PCR master mix (Applied Biosystems), according to the manufacturer's instructions. The real-time PCR reaction were performed by 5 µL of SinaSYBR Blue HS-qPCR Mix (2x) (Sinaclone. Iran), 1 µL of extracted cDNA, 0.25 µL of each 10 µM forward and reverse primers, and 3.5 µL sterile distilled water, making a total volume of 10 µL. Forty cycles of 95ºC for 15 sec, 60ºC for 30 sec, and 72ºC for 20 sec were run after the denaturation of DNA at 95ºC for 5 min. The melting curve analysis was conducted from 55ºC to 99ºC with 0.2 sec interval. The data were analyzed according to the delta–delta Ct (ΔΔCT) method and were normalized to SIRT1 and PGC-1α expression in each sample.

Statistical analysis

Data were analyzed using the SPSS statistical software. Chi-square and fisher exact two tailed tests. Quantitative data were analyzed using the ANOVA test. *P*-value was considered to be statistically significant when 0.05.

Results

Study population

Table II shows the demographic characters of an included population. According to data, the mean age of included patients was 37.2±10.6 years. The male to female ratio was 23/27. Totally, the distribution of smoking and alcohol among patients were 55% and 31%, respectively. Pan UC (40%) and lift-sided (40%) had the higher distribution.

Table II: Demographic characters of an included population.

Characters	Frequency (%)
Mean age (SD)	37.2 (10.6)
sex (M/F)	23/27
Mean Weight (SD)	70.7 (13.4)
Smoking (%)	55
Alcohol (%)	31
Extension of disease Pan UC (%)	40
Left-sided (%)	40
Extensive (%)	5
Rectosigmoid (%)	15

Gene expression

Figures 1 and **2** show the PGC1-α and SIRT1 gene expression amongst the patients of the present survey. The mean expression of the PGC1-α gene was increased amongst the UC patients treated with Zn supplement (*P* <0.05). However, in the control group, no any changes have been recorded for this gene.

The mean expression of the SIRT1 gene was increased amongst the UC patients treated with Zn supplement (P <0.05). However, in the control group, no any changes have been recorded for this gene.

Figure 1: Effect of Zn on PGC1-α gene expression.

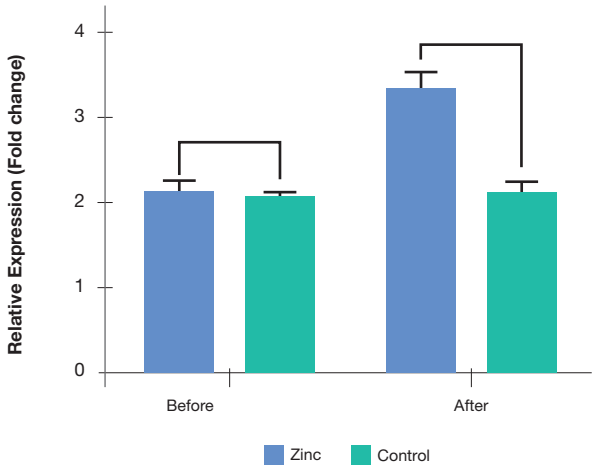
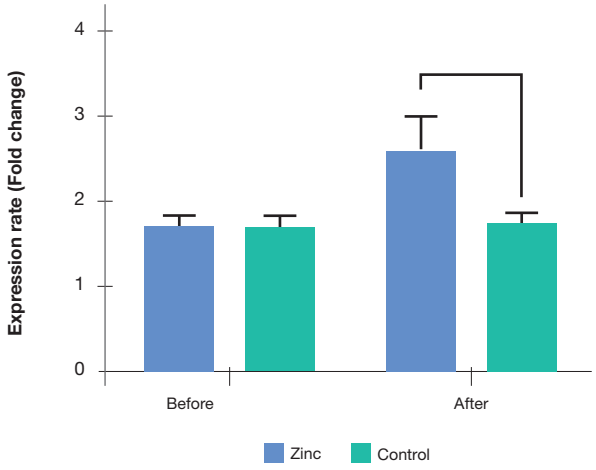


Figure 2: Effect of Zn on SIRT1 gene expression.



Discussion

Diet plays a role in the pathogenesis of gastrointestinal diseases, particularly UC and IBD. Dietary Zn may influence risk of disease through effects on autophagy, innate and adaptive immune response and maintenance of the intestinal barrier[19]. In cell culture experiments and colitis animal models, Zn administration improves intestinal barrier function and reduces expression of pro-inflammatory cytokines[20,21].

According to our knowledge, this is the first clinical trial that investigated the effects of Zn supplementation on expression of genes involved in the inflammatory response in UC patients. The study findings revealed that the expression of both SIRT1 and PGC1-α genes were significantly increased after Zn supplementation. However, in the control group no significant changes was occurred in the expression of examined genes. This finding may show the Zn therapeutic effect in UC patients through the up-regulate expression of SIRT1 and PGC1-α genes.

Zn deficiency is associated with more severe colitis and a larger inflammatory burden[22]. In human researches, Zn administration reduces intestinal permeability in Crohn's disease[23] and is effective in numerous diarrheal diseases treatment. Zn also is a co-factor for numerous enzymes involved in maintenance of intestinal integrity and regulates autophagy and bacterial clearance in macrophages[24].

In a similar survey, Khazdouz et al. (2020)[25] reported that Selenium supplement caused some changes in the SIRT1 and PGC1-α genes in UC patients. They reported that the SIRT1 gene expression in the Se group was significantly increased compared to the placebo ($p < 0.001$). An increase in the expression of the PGC-1α gene in the Se group was not statistically significant. It seems that Se supplementation caused a significant decrease in the inflammatory response of the colon by a significant increase in the expression of the SIRT1 gene. Researches established that SIRT1 can regulation of intestinal inflammation and tissue homeostasis in UC model[26-28]. Since, down-regulation the expression of SIRT1 upraise the pro-inflammatory cytokines concentrations, that are involved in UC pathogenesis, whereas, activation of SIRT1 caused significant reduction in the symptoms of the disease in IBD[29].

Conclusion

This is the first report of the effect of Zn supplements on the SIRT1 and PGC-1α gene expression in UC patients. It seems that the Zn caused significant decrease in the inflammatory response from the over expression of the SIRT1 and PGC-1α genes. However, further surveys are required to found the exact role of the Se in the controlling of UC through expression of the SIRT1 and PGC-1α genes.

Conflict of interests

The authors have no conflict of interest.

References

1. Porter RJ, Kalla R, Ho GT. Ulcerative colitis: Recent advances in the understanding of disease pathogenesis. F1000Research. 2020;9.

2. Gajendran M, Loganathan P, Jimenez G, Catinella AP, Ng N, Umapathy C, Ziade N, Hashash JG. A comprehensive review and update on ulcerative colitis. Disease-a-month. 2019 Dec 1;65(12):100851.

3. Kayal M, Shah S. Ulcerative colitis: current and emerging treatment strategies. Journal of clinical medicine. 2020 Jan;9(1):94.

4. Taku K, Britta S, Chen WS, Ferrante M, Shen B, Bernstein CN, et al. Ulcerative colitis (Primer). Nature Reviews: Disease Primers. 2020;6(1).

5. de Moura MS, Soares NR, de Lima Barros SE, de Pinho FA, Silva TM, Bráz DC, et al. Zinc gluconate supplementation impacts the clinical improvement in patients with ulcerative colitis. BioMetals. 2020 Feb;33(1):15-27.

6. Soares NR, de Moura MS, de Pinho FA, Silva TM, de Lima Barros SÉ, de Castro Amorim A, et al. Zinc supplementation reduces inflammation in ulcerative colitis patients by downregulating gene expression of Zn metalloproteins. PharmaNutrition. 2018 Sep 1;6(3):119-24.

7. Ananthakrishnan AN, Khalili H, Song M, Higuchi LM, Richter JM, Chan AT. Zinc intake and risk of Crohn's disease and ulcerative colitis: a prospective cohort study. International journal of epidemiology. 2015 Dec 1;44(6):1995-2005.

8. Gîlcă-Blanariu GE, Diaconescu S, Ciocoiu M, Ştefănescu G. New insights into the role of trace elements in IBD. BioMed research international. 2018 Sep 6;2018.

9. Singh V, Ubaid S. Role of Silent Information Regulator 1 (SIRT1) in Regulating Oxidative Stress and Inflammation. Inflammation. 2020 Oct 1;43(5).

10. Caruso R, Marafini I, Franzè E, Stolfi C, Zorzi F, Monteleone I, et al. Defective expression of SIRT1 contributes to sustain inflammatory pathways in the gut. Mucosal immunology. 2014;7(6):1467-79.

11. Sharma M, Mohapatra J, Wagh A, Patel HM, Pandey D, Kadam S, et al. Involvement of TACE in colon inflammation: A novel mechanism of regulation via SIRT-1 activation. Cytokine. 2014;66(1):30-9.

12. Singh UP, Singh NP, Singh B, Hofseth LJ, Price RL, Nagarkatti M, et al. Resveratrol (trans-3, 5, 4′-trihydroxystilbene) induces silent mating type information regulation-1 and down-regulates nuclear transcription factor-κB activation to abrogate dextran sulfate sodium-induced colitis. Journal of Pharmacology and Experimental Therapeutics. 2010;332(3):829-39.

13. Bansal K, Singh N, Jaggi AS. Dual role of sirtuin 1 in inflammatory bowel disease. Immunopharmacology and Immunotoxicology. 2020(just-accepted):1-22.

14. D'Errico I, Salvatore L, Murzilli S, Sasso GL, Latorre D, Martelli N, et al. Peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC1α) is a metabolic regulator of intestinal epithelial cell fate. Proceedings of the National Academy of Sciences. 2011;108(16):6603-8.

15. Rius-Pérez S, Torres-Cuevas I, Millán I, Ortega ÁL, Pérez S. PGC-1α, inflammation, and oxidative stress: an integrative view in metabolism. Oxidative medicine and cellular longevity. 2020 Oct;2020.

16. Cunningham KE, Vincent G, Sodhi CP, Novak EA, Ranganathan S, Egan CE, et al. Peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC1α) protects against experimental murine colitis. Journal of Biological Chemistry. 2016;291(19):10184-200.

17. Lai H, Nie T, Zhang Y, Chen Y, Tao J, Lin T, et al. Selenium Deficiency-Induced Damage and Altered Expression of Mitochondrial Biogenesis Markers in the Kidneys of Mice. Biological Trace Element Research. 2020:1-12.

18. Heshmati J, Golab F, Morvaridzadeh M, Potter E, Akbari-Fakhrabadi M, Farsi F, et al. The effects of curcumin supplementation on oxidative stress, Sirtuin-1 and peroxisome proliferator activated receptor γ coactivator 1α gene expression in polycystic ovarian syndrome (PCOS) patients: A randomized placebo-controlled clinical trial. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2020;14(2):77-82.

19. Jarosz M, Olbert M, Wyszogrodzka G, Młyniec K, Librowski T. Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF-κB signaling. Inflammopharmacology. 2017 Feb;25(1):11-24.

20. Hu C, Song J, Li Y, Luan Z, Zhu K. Diosmectite–zinc oxide composite improves intestinal barrier function, modulates expression of pro-inflammatory cytokines and tight junction protein in early weaned pigs. British journal of nutrition. 2013 Aug;110(4):681-8.

21. Hu CH, Song ZH, Xiao K, Song J, Jiao LF, Ke YL. Zinc oxide influences intestinal integrity, the expressions of genes associated with inflammation and TLR4-myeloid differentiation factor 88 signaling pathways in weanling pigs. Innate immunity. 2014 Jul;20(5):478-86.

22. Siva S, Rubin DT, Gulotta G, Wroblewski K, Pekow J. Zinc deficiency is associated with poor clinical outcomes in patients with inflammatory bowel disease. Inflammatory bowel diseases. 2017 Jan 1;23(1):152-7.

23. Ananthakrishnan AN, Khalili H, Song M, Higuchi LM, Richter JM, Chan AT. Zinc intake and risk of Crohn's disease and ulcerative colitis: a prospective cohort study. International journal of epidemiology. 2015 Dec 1;44(6):1995-2005.

24. Hassan A, Sada KK, Ketheeswaran S, Dubey AK, Bhat MS. Role of zinc in mucosal health and disease: a review of physiological, biochemical, and molecular processes. Cureus. 2020 May;12(5).

25. Khazdouz M, Daryani NE, Alborzi F, Jazayeri MH, Farsi F, Hasani M, Heshmati J, Shidfar F. Effect of Selenium Supplementation on Expression of SIRT1 and PGC-1α Genes in Ulcerative Colitis Patients: a Double Blind Randomized Clinical Trial. Clinical nutrition research. 2020 Oct;9(4):284.

26. Sharma M, Mohapatra J, Wagh A, Patel HM, Pandey D, Kadam S, et al. Involvement of TACE in colon inflammation: A novel mechanism of regulation via SIRT-1 activation. Cytokine. 2014;66(1):30-9.

27. Singh UP, Singh NP, Singh B, Hofseth LJ, Price RL, Nagarkatti M, et al. Resveratrol (trans-3, 5, 4′-trihydroxystilbene) induces silent mating type information regulation-1 and down-regulates nuclear transcription factor-κB activation to abrogate dextran sulfate sodium-induced colitis. Journal of Pharmacology and Experimental Therapeutics. 2010;332(3):829-39.

28. Sasso GL, Ryu D, Mouchiroud L, Fernando SC, Anderson CL, Katsyuba E, et al. Loss of Sirt1 function improves intestinal anti-bacterial defense and protects from colitis-induced colorectal cancer. PloS one. 2014;9(7):1-10.

29. Caruso R, Marafini I, Franzè E, Stolfi C, Zorzi F, Monteleone I, Caprioli F, Colantoni A, Sarra M, Sedda S, Biancone L. Defective expression of SIRT1 contributes to sustain inflammatory pathways in the gut. Mucosal immunology. 2014 Nov;7(6):1467-79.