Determining the Relationship between Gingival Crevicular Fluid Zinc Levels and Gingivitis, and Gingival Crevicular Fluid Zinc Levels and the Growth of *Streptococcus Mutans* Colonies in Children

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Abstract

Zinc (Zn) is a mineral contained in the enzyme, alkaline phosphatase, present in gingival crevicular fluid. It is believed that a relationship exists between gingival crevicular fluid volume and gingivitis. Gingivitis in children is caused by the accumulation of plaque and bacteria. Plaque is a layer on the surface of teeth containing bacteria. *Streptococcus mutans* is most commonly found on plaque and colonizes on teeth surfaces. This research was conducted to determine the relationship between Zn levels in gingival crevicular fluid, and gingivitis and the growth of *S. mutans* colonies in children. The subjects were 30 children, aged 12–14 years. Zn levels were measured using the atomic absorption spectrophotometry method. A weak but positive correlation was established between Zn levels in gingival crevicular fluid and gingivitis ($p < 0.050$). This finding was not statistically significant. Higher Zn levels in gingival crevicular fluid were equated with increased gingivitis in children. By contrast, a negative and moderate correlation, which did not reach statistical significance, was established between Zn levels and the growth of *S. mutans* colonies in children ($p = < 0.050$). Higher gingival crevicular fluid Zn levels were equated with decreased colony growth *of S. mutans* in children.

Clinical article (J Int Dent Med Res 2018; 11(1): pp. 224-227)

Keywords: Zinc levels, Zn levels, gingival crevicular fluid, gingivitis, Streptococcus mutans, S. mutans, children.

Received date: 20 September 2017 **Accept date:** 25 October 2017

Introduction

Periodontal inflammation is common in children and adolescents. Inflammation affects the gingival tissue in many cases. Gingivitis, an associated disease, is characterized by gingival inflammation without bone loss and without loss of attachment to the connective tissue.[1] The incidence of gingivitis reaches its peak in early puberty. Gingival inflammation varies according to age and is strongly associated with a transitional period of approximately 5–6 years featuring mixed dentition and occurring until puberty. This period is characterized by irregular teeth alignment and hormonal changes. Gingivitis is found in 73% of children aged 6-11 years and in 50-99%% of adolescents.[2] Gingivitis is caused by the accumulation of plaque and bacteria.[1] Plaque is a bacteria-containing layer on the tooth surface. The oral cavity is inhabited by more than 300 species of bacteria, and teeth are usually predominantly colonized by Gram-positive cocci.[3,4] Streptococcus mutans is found soft tissues, saliva, and tongue, more so than on the teeth.[5] Periodontal inflammation can be detected by examining gingival crevicular fluid. It is well known that the volume of gingival crevicular fluid increases during inflammation. This fluid can be found in the gingival sulcus. It contains enzymes, inflammatory mediators and products, and tissue-breakdown products.[6]

Zinc (Zn) is a mineral contained in the enzyme, alkaline phosphatase (ALP), present in gingival crevicular fluid. The number of these enzymes increases with disease progression and Zn levels in the enzyme activate ALP activity.[7,8] It has been stated in previous studies that an increase in the volume of gingival crevicular fluid is an indicator of gingival inflammation. However, it is not yet known if Zn levels influence the growth of *S. mutans* colonies or cause gingivitis

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in children. Thus, this study was conducted to assess the relationship between gingival crevicular fluid Zn levels, and gingivitis and the colony growth of *S. mutans* in this population.

Materials and methods

This was an observational laboratory study in which a cross-sectional, experimental design was employed. It was approved by the Ethical Committee, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. Gingival crevicular fluid and plaque from subjects who met the inclusion criteria were added to samples. The inclusion criteria were children aged 12–14 years with gingivitis in one or more regions and dental caries. The exclusion criteria were subjects who were menstruating and those consuming systemic drugs at the time of the study. All of the subjects, 30 children in total, were sourced from a junior high school in Jakarta, Indonesia. Gingival crevicular fluid and plaque samples were obtained from 10h00–11h00 and gingival index measurements were taken. The fluid was derived by inserting a paper point to a 1 mm depth into the sulcus of teeth numbers 19 or 30 for 30 seconds. Thereafter, it was transferred to an Eppendorf tube containing 1 ml of phosphate-buffered saline (PBS).

The samples were adjusted using 1–2 ml of nitric acid (HNO_3) (60%) until homogeneity was achieved. The Zn levels were then measured using the atomic absorption spectrophotometry (AAS) method. The plaque samples were also obtained via the paper point technique and added to an Eppendorf tube. An *S. mutans* colony count was carried out (colony forming units/ml). Analysis of any correlation between gingival crevicular fluid and *S. mutans* colony growth was performed using Pearson's correlation coefficient. Spearman's rank correlation coefficient was employed to assess any correlation between Zn levels and gingivitis prevalence, and between the growth of *S. mutans* colonies and gingivitis. Statistical significance was set at a *p*-value of < 0.050.

Results

The samples were obtained from children aged 12–14 years at a Jakarta junior high school. Thirty research subjects met the inclusion criteria. Zn levels in gingival crevicular fluid were determined and the *S. mutans* colony count was measured at the Oral Biology Laboratory, Dentistry Faculty, Universitas Indonesia. Spearman's rank correlation coefficient was used to determine the significance of a relationship between the Zn levels and the Gingival Index. A positive, weak, and insignificant relationship was established (r = 0.130, p = 0.493) (Table 1). The relationship between Zn levels and the growth of *S. mutans* colonies in children was found to be negative, moderate, and insignificant (r = -0.410, p = 0.829) (Table 2). The Spearman rank correlation coefficient results are shown in Table 3. A moderately strong, positive, and significant relationship was demonstrated between the Gingival Index and the growth of *S. mutant* colonies in children (r = 0.389, p = 0.034).

	Mean±SD	*r*	*p*-value
Zinc levels in gingival crevicular fluid (ppm)	1.436±0.832	0.130	0.493
Gingival Index	1.031±0.620		

Table 1. The relationship between zinc levels in gingival crevicular fluid and the Gingival Index.

	Mean±SD	*r*	*p*-value
Zinc levels in gingival crevicular fluid (ppm)	1.436±0.832		
The growth of *S. mutans* colonies in children (CFUs/ml × 10^3)	9.081±5.154	-0.410	0.829

Table 2. The relationship between zinc levels in gingival crevicular fluid and the growth of *S. mutans* colonies in children. CFUs: colony-forming units.

	Mean±SD	*r*	*p*-value
Gingival Index	1.031±0.620		
The growth of *S. mutans* colonies in children (CFUs/ml × 10^3)	9.081±5.154	0.389	0.034

Table 3. The relationship between the Gingival Index and the growth of *S. mutans* colonies.

Discussion

The study objective was to determine the relationship between the Zn levels in gingival crevicular fluid and gingivitis, and between the Zn levels in gingival crevicular fluid and the growth of *S. mutans* colonies in children. This was based on the high prevalence of gingival inflammation in children for which early prevention is essential. Samples were collected from a school in Jakarta, and then transported to the Oral Biology Laboratory, Dentistry Faculty, Universitas Indonesia. The location was selected based on

consideration being given to schools and laboratory locations that were easily accessible. The subjects were children aged 12–14 years. This age range was chosen according to the theory that the incidence of gingivitis reaches its peak at puberty. The prevalence of gingival inflammation has been reported to be 73% in children 6-11 years old and 50-99% in adolescents.[2] The inclusion criteria were subjects who were not menstruating at the time of the sample collection process and those without dental caries on their posterior teeth. ALP plasma concentration is affected by the menstrual cycle in women. Most ALP plasma activity occurs during the proliferative phase, reaching a peak during ovulation and decreasing until the start of menstruation.[6] In addition, subjects were excluded if they were taking systemic medication at the time that the study was conducted as this would have affected the normal oral flora.

This was an observational, cross-sectional, experimental study. A cross-sectional design was chosen because it allows the research subjects to be observed together simultaneously. This design is practical, simple, economical, time saving, and the results can be rapidly obtained. A limitation of this design is that a large number of subjects is needed.[9] Gingival crevicular fluid samples were used in the current research as increased volumes of this fluid are indicative of gingival inflammation. Gingival crevicular fluid is usually obtained using paper points that are inserted into the gingival sulcus to determine Zn levels.[6] Zn is a mineral contained in the enzyme, ALP, present in gingival crevicular fluid.[10] Zn plays a role in epithelial cell differentiation and growth, and in the epithelialization process. It is an important mineral that influences growth, maturation, and the immune system.[11]

Sample collection usually involves obtaining plaque swabs, again using paper points, from the buccal surface of the maxillary posterior teeth. Bacteria from dental plaque is known to start the infiltration of inflammatory cells in the gingival crevicular fluid and an increase in ALP concentration is observed 1-2 weeks without oral hygiene practice.[6,8] The posterior teeth tend to accumulate a large amount of plaque because they are harder to reach during cleaning.[12] *S. mutans* is most commonly found in supragingival plaque.[13] It has been shown in previous studies that a greater number of *S. mutans* colonies are found in molars, compared to that found in the anterior teeth.[14] *S. mutans* obtained from samples is cultured without differentiating serotypes. TYS20B agar, containing 20% sucrose concentration to kill other bacteria, is the medium of choice used to culture it.[15]

The Zn levels are quantified by AAS. Electromagnetic wave radiation absorption by atoms is used with this method. The energy level difference between the ground and excitation states is specific to every element. Thus, the absorbed light wavelength for every element is also specific.[16,17] AAS is used because this method is quick, selective, sensitive, and has high accuracy.[17]

The results from the correlation tests for this research were analyzed to determine the relationship between the Zn levels in the gingival crevicular fluid and gingivitis, and gingival crevicular fluid Zn levels and the growth of *S. mutans* colonies in children. A positive, weak, and insignificant relationship was also shown between the Zn levels and Gingival Index (r = 0.130, p = 0.493) (Table 1). It was demonstrated that higher Zn levels in the gingival crevicular fluid corresponded with a higher the incidence of gingivitis. This finding is similar to that of others in the literature that reported an increase in gingival crevicular fluid quantity and flow in relation to the progression of plaque formation.[8] The Zn levels in ALP also activate ALP. ALP is an enzyme that has a role in bone formation activity, which is produced by osteoblasts. It is used as an indicator of periodontal disease activity.[11]

Correlation tests to determine the relationship between the Zn levels in gingival crevicular fluid and the growth of *S. mutans* colonies were performed. The relationship between Zn levels and the growth of *S. mutans* colonies in children was shown to be negative, moderate, and insignificant (r = -0.410, p = 0.829). Higher Zn levels in the gingival crevicular fluid correlated with less *S. mutants* colonies. It is known that gingival sulcus is a suitable environment for the growth of complex microbial flora and can cause periodontal disease. *S. mutans* is a Gram-positive cocci bacteria that is found in gingival sulcus. This organism may produce enzymes, endotoxins, exotoxins, and cell wall-specific antigens. ALP is an enzyme in gingival crevicular fluid that contains Zn.[8] Our findings were similar to those of previous studies.

Zn is known to inhibit S.mutans growth in pelicles by inhibiting certain metabolic enzymes and interfere the substrate transport and oxidation process.[18,19] Zn deficiency is also known to be associated with increased susceptibility of infections. Higher levels of zinc causes an activation of various immune cells.[19]

A moderately strong, positive, and significant relationship between the Gingival Index and the growth of *S. mutans* colonies in children was established (r = 0.389, p = 0.034). Further research, using a population with a wider age range and with more samples, is needed, in addition to further exploration of the correlation between gingivitis and the growth of *S. mutans* colonies in children.

Conclusion

A weak and insignificant correlation was established in the current study between Zn levels in gingival crevicular fluid and gingivitis. Higher Zn levels in gingival crevicular fluid equated with a higher incidence of gingivitis in children. A negative and moderate correlation, that did not reach statistical significance, was found between Zn levels and the growth of *S. mutans* colonies in children. Accordingly, higher Zn levels in gingival crevicular fluid corresponded with reduced *S. mutans* colony growth in children.

Declaration of Interest

The authors report no conflict of interest.

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