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Salivary hydroxyproline levels and gingivitis in 6–7 years children: a case-control study

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Abstract

Aim: Gingivitis is the most prevalent periodontal infection in children and adolescents. Various salivary markers have become potentially important in the early diagnosis of oral diseases, and some salivary amino acids play a significant role in predicting periodontal status. Therefore, this study investigated the relationship between the concentration of hydroxyproline in unstimulated saliva and the occurrence of gingivitis in 6–7 years children.

Methods: This case-control study was conducted on children aged 6–7 years in elementary schools in Birjand from November 2022 to June 2023. A boy's school was randomly selected based on the list of primary schools in Birjand city. Then, among the students in the first grade of elementary school and after the initial examinations, they were assigned to study groups according to the presence of inflammation in the gums and consent to participate in the study. Data analysis was done using SPSS-19 and statistical tests such as chi-square and Mann-Whitney *U* tests were employed for data analysis at a significance level of 5%.

Results: The unhealthy group had poorer dental health compared to the healthy group ($P = 0.013$) and there were no differences in the dental care attendance status of participants between the two groups ($P = 0.288$). The mean of salivary hydroxyproline levels in the unhealthy group was 0.197 ± 0.289 mg/L and in the healthy group was 0.079 ± 0.006 mg/L. This difference was statistically significant between 2 study groups ($P = 0.001$).

Conclusions: It can be concluded that the level of hydroxyproline in unstimulated saliva samples can be used as a biomarker in the diagnosis of gingivitis. However, to confirm the results of this study, further studies with a larger sample size comparing different severities of periodontitis such as mild, moderate, and severe forms are needed.

Keywords

Salivary, hydroxyproline, children, gingivitis

Introduction

Gingivitis is the most prevalent periodontal infection in children and adolescents [1], so today the problem of gingivitis has been identified with a significant long-term social and medical impact on individuals, groups, populations, and social levels in the world, especially in childhood [2]. Gingivitis or gum inflammation is caused by the accumulation of microbial plaque (bacteria) on the tooth surface [3]. It is characterized by halitosis and painless gum bleeding, either spontaneously or during brushing [3]. The gum is one of the components of the periodontal tissue and its diseases can lead to the destruction of the supporting structures of the teeth and alveolar bone [4]. Most of the gum is made up of connective tissue that contains high levels of collagen [5]. In normal healthy gums, collagen makes up around 60% of the total proteins [6]. During the development of gingivitis and periodontitis, significant changes occur in the collagen and non-collagenous proteins of the gums [7], so that 70% of the connective epithelium collagen is lost in the early stages of inflammation [8]. Collagen is composed of different amino acids, the most important of which are glycine, proline, hydroxylysine, and hydroxyproline [5]. Collagen stability is enhanced by hydroxyproline and proline [9, 10].

On the other hand, due to recent advanced technology, various salivary markers have become potentially important in the early diagnosis of oral diseases, and some salivary amino acids play a significant role in predicting periodontal status, including plaque-induced gingivitis [2]. Saliva is an available biological fluid and its biomarkers provide necessary information about oral health. [11]. Factors such as saliva composition and saliva flow rate are very important and necessary for diagnostic, experimental and clinical protocols [12]. Saliva can be extracted from the mucosal surfaces and gingival crevices as well as from the dental surfaces of the oral cavity [11]. Unstimulated saliva is a popular method for examining saliva's composition. Unstimulated saliva refers to saliva that is passively collected by pouring saliva into the sample container [13].

Several studies have examined the predictive power of hydroxyproline in saliva in the diagnosis of periodontal diseases [14, 15]. These studies have mostly investigated the concentration of hydroxyproline in gingival crevicular fluid (GCF) in adults, for which sampling requires clinical intervention. A few studies conducted in children have measured the amount of hydroxyproline in other body fluids such as urine [16]. In children, oral cavity examination is mostly limited to hard tissue assessment, and soft tissues like gums are given less attention. The short lifespan of primary dentition may have led to a lack of attention to gum health in children. Furthermore, the progression of untreated gingivitis can be accompanied by more serious types of periodontal disease in children. In addition, most children are afraid of receiving dental services and care [17], and traditional diagnostic methods for these diseases include clinical observation, patient history, and measurements such as probe depth assessment and radiography, which are uncomfortable and prone to measurement errors [18]. Therefore, providing simple, convenient diagnostic services without the intervention of health workers in this age group is of particular importance. Therefore, this study investigated the relationship between the concentration of hydroxyproline in unstimulated saliva and the occurrence of gingivitis in 6 to 7-year-old children.

Materials and methods

Study design and study population

This case-control study was conducted on children aged 6–7 years in elementary schools in Birjand from November 2022 to June 2023.

Inclusion and exclusion criteria

All 6 to 7-year-old children living in Birjand city with gingivitis, whose tooth number 6 had erupted, were included in the study if they did not suffer from oral and dental systemic diseases (dental plaque, tooth decay, need for nerve extractions) and the consent of the parents. Also, children who did not cooperate with sampling and children with a gingival index (GI) of 3 were excluded from the study.

Sample size and sampling method

According to the results obtained from the study of Kejriwal et al. [19] and considering 99% accuracy and 90% power, the sample size was calculated for each group of 16 people using the mean difference formula.

A boy's school was randomly selected based on the list of primary schools in Birjand city. Then, among the students in the first grade of elementary school and after the initial examinations, according to the presence of gingivitis and consent to participate in the study, they were assigned to study groups (16 cases and 16 controls).

Study implementation

One day before saliva collection, the study objectives and methods and child preparation conditions for saliva collection were explained to the participants and their parents. The next day, after obtaining informed consent from the parents, gum health, absence of dental plaque, and tooth brushing/non-brushing were checked. Gum health and the absence of dental plaque were checked through observation and clinical examination. We assessed gum by observing visual characteristics such as color and edema, using the Williams probe for bleeding after probing, and evaluating gingival inflammation based on the GI. Then, saliva samples were collected.

We classified GI based on Löe and Silness [20]:

GI 0: healthy gums.

GI 1: mild discolouration and oedematous gingiva. No bleeding on probing.

GI 2: red, oedematous, and shiny gingiva. There is bleeding on probing.

GI 3: red, oedematous, and ulcerated gingiva. There is spontaneous bleeding.

In this study, we considered children with a GI of 0 healthy and a GI of 1 to 3 unhealthy.

Saliva collection

Unstimulated saliva samples were collected using the direct method. Samples were collected at least 2 hours after meals and at least 1 hour after brushing (between 10:00 AM and 12:00 PM) to minimize diurnal variations in saliva flow and composition. After sitting upright in a chair and resting for 5 min before saliva collection, children were instructed to swallow all the saliva and then pour the saliva through a glass funnel into a graduated saliva collection container. As a result, 5 mL of saliva, which was collected by continuous movements of the tongue to the mucosa and teeth, was collected in a 15 mL falcon tube.

After saliva collection, the samples were stored at 4°C and immediately subjected to biochemical analysis. Hydroxyproline diagnostic kit (Kiazist, KHPA96) and ELISA method were used to analyze the samples. The color change analysis was done with an ELISA reader [spectrophotometer (Uvikon 923, NorthStar Scientific, Bedfordshire, UK)].

Data analysis

Data analysis was done using SPSS-19 (SPSS Inc, Chicago, IL, United States) software. Descriptive statistics (mean and standard deviation) were used to describe the measured variables. Chi-square and Mann-Whitney *U* tests were employed for data analysis at a significance level of 5%.

Results

Demographic information

In this study, a total of 32 boys aged 6–7 years were enrolled, with 16 having gum inflammation (case group) and 16 considered healthy (control group). The average age of the participants in the case group and the control group was 7.09 ± 0.55 and 7.09 ± 0.20 years, respectively.

Dental health and dental care attendance

According to the chi-square test result, there is a significant statistical difference in dental health between the two groups, indicating poorer dental health in the unhealthy group (P = 0.013). There were no differences in the dental care attendance status of participants between the two groups (P = 0.288) (Table 1).

Table 1. Dental health and dental care attendance comparison

Group index		Unhealthy		Healthy		*P*-value
		No.	Percent (%)	No.	Percent (%)	
Dental health	Poor	7	21.9	1	3.1	0.013
	Average	9	28.1	11	34.4	
	Good	0	0.0	4	12.5	
Dental care attendance	Yes	7	21.9	10	31.3	0.288
	No	9	28.1	6	18.8	

Salivary hydroxyproline levels

The mean of salivary hydroxyproline levels in the unhealthy group was 0.197 ± 0.289 mg/L and in the healthy group was 0.079 ± 0.006 mg/L. Based on the Mann-Whitney U test results, this difference was statistically significant between the 2 study groups and the level of salivary hydroxyproline in the unhealthy group was significantly higher than in the healthy group (P = 0.001) (Table 2).

Table 2. Comparison of mean, SD, median, and quartiles of salivary hydroxyproline levels between two study groups

Salivary hydroxyproline levels (mg/L) group	Mean	SD	Median	1st quartile	3rd quartile	*P*-value
Healthy group	0.079	0.006	0.078	0.076	0.080	0.001
Unhealthy group	0.197	0.289	0.099	0.081	0.109	

Discussion

In this case-control study, which was conducted to evaluate and compare the level of salivary hydroxyproline in healthy 6 to 7-year-old children and those with gingivitis, higher levels of salivary hydroxyproline were observed in the group with gingivitis. The results of this study are in agreement with the results of Akalin et al. (1993) [21], Ravi et al. (2021) [14], Koss et al. (2010) [22], and Koss et al. (2009) [23] are consistent. In the study of Ravi et al. (2021) [14] who compared hydroxyproline levels in gingival tissue and GCF samples of healthy people and people with chronic periodontitis, observed a positive correlation between clinical parameters in both groups with hydroxyproline levels and higher concentrations of hydroxyproline reported in GCF and gingival tissue samples of people with chronic periodontitis [14]. Koss et al. (2010) [22] in their study investigated the quantification of inflammation and tissue destruction biomarkers in the GCF to help diagnose the severity of gingival-periodontal disease in adults with gingival-periodontal disease. In that study, total protein increased in gingivitis, moderate and severe periodontitis, and hydroxyproline increased in moderate and severe periodontitis. Finally, they concluded that the levels of GCF biomarkers specific for inflammation and collagen degradation are associated with clinical diagnosis in patients with gingivitis and chronic periodontitis. Also, Koss et al. (2009) [23] in another study that aimed to identify salivary parameters effective in diagnosing different stages of periodontal disease, after examining adults with mild, moderate, or severe chronic periodontitis, concluded that hydroxyproline significantly attention increased in all groups with periodontal diseases compared to healthy people. The study of Akalin et al. (1993) [21] also observed higher gingival hydroxyproline levels in periodontitis samples compared to the control group.

Therefore, it can be concluded that in addition to GCF samples, the level of hydroxyproline in unstimulated saliva samples can also be used as a biomarker in the diagnosis of gingivitis. Because, periodontitis is the primary cause of tooth loss in today's society [24]. In addition, compared to serum or

other saliva-based methods, unstimulated saliva collection is easily performed without any professional assistance [25] and can reduce children's fear of receiving dental diagnostic services. Also, storage and transportation as well as large sampling make saliva the best research fluid compared to serum or urine [25]. However, to confirm the results of this study, further studies with a larger sample size comparing different severities of periodontitis such as mild, moderate, and severe forms are needed.

On the other hand, considering that the consequences of periodontal diseases observed in adulthood are established in childhood [26, 27], prevention, timely diagnosis and control of gum-related diseases in childhood has a lot of importance [27]. In the present study, the condition of oral and dental health in students with gingivitis was worse than in the non-affected group. So that in the affected group, 21.9% had poor oral hygiene, while this rate was 3.1% in healthy students. In terms of dental care attendance, no difference was observed between the two groups. This result is consistent with the results of other studies [28–30]. Improper oral and dental hygiene and ineffective brushing are known to be the most important factors affecting gum disease in children [30, 31]. Studies conducted in Iran have found that improper oral hygiene (lack of daily brushing), male gender, oral breathing, lower income, family with a higher number of children in the family, mother's employment status, and mother's low education level are effective in reducing gum health [28, 29, 32]. Therefore, it is necessary to implement educational programs in intervention methods for children and their parents.

This study has several limitations. The most important limitation is the small sample size, conducting the study in a specific age group (6 to 7 years) and gender (male). Therefore, generalizing the results to other age and gender groups should be done with caution.

Conclusions

Based on this study's findings, it can be concluded that the level of hydroxyproline in unstimulated saliva samples can be used as a biomarker in the diagnosis of gingivitis. However, to confirm the results of this study, further studies with a larger sample size comparing different severities of periodontitis such as mild, moderate and severe forms in both genders and other age groups are needed.

Abbreviations

GCF: gingival crevicular fluid

GI: gingival index

Declarations

Author contributions

MMM: Conceptualization, Investigation, Writing—review & editing, Supervision. HN: Investigation, Writing—original draft. FO: Conceptualization, Investigation, Writing—original draft. All authors read and approved the submitted version.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Ethical approval

This study was approved by the ethics committee of Birjand University of Medical Sciences with code IR.BUMS.REC.1402.071.

Consent to participate

Parents filled out the informed consent before sampling and clinical exams of their children. In addition, the child's parents were assured of the confidentiality of their information and the non-disclosure of information individually.

Consent to publication

Not applicable.

Availability of data and materials

The data are available based on the eligible request to the corresponding author.

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