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Evaluation of the Hemoglobin Level in Gingival Crevice Fluid and Clinical Periodontal Parameters in Patients with Chronic Periodontitis

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Abstract

Objectives: The present study aimed to evaluate the level of hemoglobin in gingival crevicular fluid (GCF) and its relationship with clinical periodontal parameters in chronic periodontitis.

Methods: This cross-sectional study was conducted in patients with chronic periodontitis. Gingival crevicular fluid was sampled and clinical periodontal parameters PI, PPD, CAL, and BOP were measured. The level of hemoglobin in GCF was measured using a hemoglobin kit. Data were analyzed using Spearman's correlation test and Mann-Whitney's test in Stata 14.1 software.

Results: 315 teeth from patients with chronic periodontitis were evaluated. There was a strong and direct correlation between the amount of GCF hemoglobin and the amount of PPD, CAL and PI, their correlation coefficient was equal to 0.78, 0.88 and 0.82, respectively ($P < 0.001$). The mean hemoglobin GCF in the positive BOP group was 68.84 ± 30.89 and in the negative BOP group was 59.28 ± 8.03, which showed a significant difference in the average hemoglobin between the two groups ($P < 0.001$).

Conclusion: According to the findings of this study, there is a strong correlation between periodontal clinical parameters and the level of GCF hemoglobin, and measuring the level of hemoglobin in the gingival crevice fluid can be an accurate measure and a non-invasive method for investigating periodontal conditions.

Keywords: Chronic periodontitis, hemoglobin, gingival crevicular fluid

Introduction

Chronic periodontitis is an inflammatory disease of the tooth-supporting tissues,[1] which is mainly caused by microbial plaque, but it is a multifactorial disease[2] that causes the release of cytokines and chemokines and ultimately causes periodontal tissue destruction.[3] The methods used to diagnose of periodontal disease should help distinguish between types of periodontal diseases, the degree of destruction of periodontal tissue, and the prognosis of periodontal disease.[4,5] Today, the main method used in the diagnosis of periodontal diseases, in addition to radiographic findings, is examining clinical parameters such as clinical attachment loss (CAL), periodontal probing pocket depth (PPD), plaque index (PI), gingival index (GI) and BOP.[6-8] Gingivitis is checked with bleeding during probing (BOP), which is an important parameter for comparing and evaluating the results of periodontitis treatment. BOP is an important clinical parameter that shows the presence of inflammation in the depth of the pocket, helps in the treatment plan, shows the success or failure of periodontal treatment, and is used to motivate patients to perform oral hygiene at home.[9] Although the examinations of clinical parameters are important diagnostic tools, their definite predictive value is low; they only determine the previous damage to the periodontium and do not show the future state of the periodontal tissue.[10-12] PPD and CAL depend on various factors such as the size of the probe diameter, probing force, and the amount of tissue inflammation. Measuring the depth of the periodontal pocket alone cannot indicate the extent of the disease because soft tissue changes such as gingival resorption affect the estimation of the pocket depth and make the depth of the pocket less than its actual amount.[13] CAL cannot be used as a measure to determine gum health or disease, especially in people who have adhesion destruction, but their gum margin is above the place where cement adheres to tooth enamel.[14] The radiographic examination should be a part of a periodontal evaluation of each patient and should be accompanied by careful examination of CAL, PPD, and BOP. Radiographs often show less bone destruction, and early changes are usually not detected. Comparison of radiographic images at different times is reliable only in recording significant bone surface changes and does not show soft tissue changes.[15] With the advancement of laboratory techniques, GCF components were widely investigated in people with periodontal disease to determine the presence of host response factors, including blood factors such as serum proteins (albumin, globulin, creatine phosphokinase enzyme).[5,16] GCF examination is the most common non-traumatic method, which is used to obtain information about periodontal tissue conditions, including the condition of connective tissue and the degree of bone tissue destruction.[17] According to previous studies, for the accurate diagnosis of periodontal condition, only examining clinical parameters is not enough and biochemical tests are necessary to improve the accuracy of periodontal disease diagnosis.[18,19] Measuring hemoglobin in GCF for early diagnosis of periodontal disease is effective before clinical symptoms appear. Nonvisible pocket bleeding in primary periodontitis occurs even in BOP-negative areas.

Examining the hemoglobin caused by micro bleeding in the gingival groove along with the clinical parameters of PPD, BOP and CAL can be used as an accurate index to diagnose and evaluate the treatment of periodontal disease,[20] so this study aims to investigate the correlation between hemoglobin GCF and Clinical periodontal parameters in patients with chronic periodontitis.

Materials and Methods

The present study was commenced upon receiving approval from the Research Ethics Committee of Golestan University of medical sciences (IR.GOUMS.REC.2019.091). This cross-sectional study was conducted in patients with chronic periodontitis referred to the periodontics department of the Dental School of Golestan University of Medical Sciences. The study inclusion criteria included patients with chronic periodontitis according to the American Academy of Periodontology (AAP) classification in 1999,[21] having at least 20 teeth, and being over 30 years old. The criteria for exclusion from the study include the presence of a systemic disease affecting the periodontium tissue, the use of drugs affecting the periodontium, addiction to tobacco, drugs and alcohol, the use of antibiotic drugs in the last 3 months for one week, treatment Periodontal diseases in the last 6 months and pregnancy and breastfeeding.

After obtaining a written consent from the patients, the region was isolated with a cotton roll and dried with mild air. At first, the plaque index (PI) of Sillness & Loe (1964) was measured in the patients.[22] Then, a sample was taken from the gingival crevice fluid using paper probe No. 30. A paper cone number 30 was inserted with gentle pressure inside the periodontal pocket (buccal or palatal) to a depth where resistance was felt and remained in the area for 30 seconds.[23] Later, without getting contaminated with the patient's saliva, the paper cone was slowly taken out of the pocket and quickly transferred to the microtube containing 200 microliters of phosphate buffered saline solution (PBS).[24] In the next step, BOP was checked by the Bay & Ainamo method (1975).[25]

Finally, PPD and CAL were measured at 6 levels: mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual by Williams periodontal probe. The samples were sent to the laboratory to measure the level of hemoglobin. In the laboratory, the level of hemoglobin was calculated by a laboratory expert using a hemoglobin kit (Zist Shimi, Hemoglobin-Total Kit, Iran) by the Cyanmethemoglobin method with Drabkin's reagent according to the instructions of the manufacturer of the kit.[25] 100 microliters of sample content were mixed with 100 microliters of Drabkin's reagent and incubated for 5 minutes. Finally, the optical density of the final solution was measured using an Elisa reader (Awarness, USA) at a wavelength of 540 nm. Data were entered into Stata 14.1 software and analyzed.

Results

This study was conducted on 315 teeth of patients with chronic periodontitis. Using the Kolmogorov Smirnov test, the normality of the data was investigated, and the result was the absence of normality in any of the clinical parameters and hemoglobin. The average hemoglobin of the gingival crevice fluid was 62.58 ± 32.07, CAL was 4.63 ± 1.32, PPD was 4 ± 0.98, and PI was 1.4 ± 0 (Table 1).

84.4 percent of samples were BOP positive and 15.6% were BOP negative. The average hemoglobin in gingival crevice fluid in positive BOP samples was 68.84 ± 30.89 and 28.59 ± 8.03 in negative BOP samples. The average difference of GCF hemoglobin content in these two groups was reported as 40.25 and was statistically significant (P value < 0.001) (Table 2).

Spearman's test was used to check the correlation between hemoglobin GCF and CAL, PPD, and PI due to the absence of normality. The correlation between the hemoglobin content of gingival crevice fluid and PPD was obtained with a correlation coefficient of 0.78 (P < 0.001). The correlation between the hemoglobin content of gingival crevice fluid and CAL was obtained with a correlation coefficient of 0.88 (P < 0.001). The correlation between the hemoglobin content of gingival crevice fluid and PI was obtained with a correlation coefficient of 0.82 (P < 0.001). The results indicate a strong and direct correlation (Table 3).

Discussion

In the past few decades, several studies have been focused on finding an accurate method for the early diagnosis of periodontal diseases. Many of these studies suggest that GCF is a good source of biomolecular samples that can be used to investigate the state of periodontal tissues.[19,20] This study showed that there is a direct relationship between the level of hemoglobin in GCF and periodontal clinical parameters, and in people who had the disease but BOP was negative in them, hemoglobin was detected in GCF, and this indicates that this marker can be used for early diagnosis of periodontal diseases in people who do not have clinical symptoms. Diagnosis of periodontal diseases, in addition to radiographic findings, is the examination of periodontal clinical parameters. Examining BOP is the most important periodontal diagnostic

Table 2. **Comparison of average hemoglobin according to positive and negative BOP**

	BOP+	BOP–
Number of samples	266 (84.4%)	49 (15.6%)
The lowest amount	11.23	8.35
The greatest amount	165.33	45.62
Mean ± Standard deviation	68.84 ± 30.89	28.59 ± 8.03

P-value < 0.001.

Table 3. **Correlation coefficient of hemoglobin with PI, PPD and CAL**

	PI	CAL	PPD
Hemoglobin	0.82	0.88	0.78
P-value	<0.001	<0.001	<0.001

Table 1. **Average GCF hemoglobin, CAL, PI and PPD**

	Hemoglobin	PI	CAL	PPD
The lowest amount	8.35	0	2	2
The greatest amount	165.33	3	7	6
Mean ± Standard deviation	62.58 ± 32.07	1.40 ± 0.71	4.63 ± 1.32	4.00 ± 0.98

parameter,[4] but its diagnostic value alone is low and cannot to determine the future state of periodontal tissue.[5] According to the report of Lang and his colleagues, if the BOP is negative four times a year, in 98% of the examined areas, the destruction of adhesion is less than 2 mm, but in a few areas, the destruction of adhesion still occurs.[19] Investigating the depth of the pocket probing and destruction of adhesion cannot show the real histological changes in the tissue.[5] Examining the samples taken from the periodontal pocket is important in diagnosing and treating periodontitis.[16] One of the most common non-traumatic methods for examining the condition of periodontal tissue, including the condition of connective tissue and the degree of destruction of hard bone tissue, is the analysis of gingival crevice fluid.[7,16-20] GCF consists of serum, products resulting from host tissue destruction, and subgingival biofilm derivatives, which have diagnostic value. Hemoglobin indicates the bleeding reaction and can be detected in the GCF of the diseased areas.[11,19,20]

In this study, which was conducted on 315 teeth of patients with chronic periodontitis, 15.6% of the teeth were BOP negative, while hemoglobin was discovered in their pockets. In the study of Ito et al. (2020), clinical parameters such as PI, PPD, CAL, BOP, GCF, and biochemical parameters such as hemoglobin and proteins were investigated in 76 patients during the periodontal supportive treatment phase. In this study, it was seen that all clinical and biochemical parameters were significantly higher in diseased areas than in healthy areas,[20] which is in line with the results of our study. In the study of Ito et al. (2014), the relationship between the activity of GCF enzymes and periodontal clinical parameters, especially BOP, was investigated. Their study divided patients based on BOP and PPD, and clinical and biochemical parameters were measured in all groups. According to their findings, 14 areas out of 29 areas that needed periodontal treatment according to the definition were BOP negative. This result clarifies that a combination of clinical and biochemical parameters is helpful for the accurate and reliable diagnosis of periodontal.[26] In our study, the relationship between GCF hemoglobin content and periodontal clinical parameters showed that with the increase of GCF hemoglobin, periodontal clinical parameters have clear changes towards worsening and causing periodontitis disease. In our study, 68% of BOP-negative areas indicated periodontal health had hemoglobin, resulting from micro bleeding in the gingival sulcus. The findings of this study are in line with the study of Ito (2016) and indicate that hemoglobin GCF can be an accurate biomarker for diagnosing and predicting periodontal health and disease. In the same study, a significant correlation was observed between all measured periodontal clinical parameters (PI, GCF, PPD, CAL, BOP) with GCF hemoglobin content, which was consistent with the results of our study because in our study, all the measured clinical parameters (PI, PPD, CAL, BOP) were correlated with the hemoglobin GCF content.[19] Sabarathinam et al. (2019) investigated the relationship between salivary hemoglobin level and periodontal health status in 45 patients. According to their findings, the amount of salivary hemoglobin in patients with chronic periodontitis and gingivitis was significantly higher than in periodontally healthy people. In this study, healthy tissue, gingivitis and periodontitis were defined according to CPI (Community periodontal index). According to the findings of their study, salivary hemoglobin level can be used as a non-invasive and cost-effective tool to screen for periodontal diseases, determine prognosis, and make decisions about treatment options in the population. The result is in line with the result of our study, which was on the level of hemoglobin GCF.[27]

Conclusion

This study investigated periodontal clinical parameters with hemoglobin GCF content in patients with chronic periodontitis. Correlation between GCF hemoglobin content and periodontal clinical parameters was observed. Therefore, according to the findings of this study, the content of hemoglobin GCF can be used to screen for periodontal diseases and treat them.

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Conflicts of Interest

The authors have no conflict of interests to declare that are relevant to the content of this article.

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