Salivary hemoglobin: A biomarker in periodontitis

Jembulingam Sabarathinam1*, N. P. Muralidharan1, J. Selvaraj2

ABSTRACT

Aim: The aim of the current study is to estimate the levels of salivary hemoglobin and examine whether the cutoff values from these tests could be used as a periodontal disease screening tool. Methods: The salivary hemoglobin was estimated for 45 patients using cyanmethemoglobin method. 100 μL of saliva was added to 5000 μL of Drabkin’s solution and incubated for 20 min, and the optical density was measured using colorimeter under 540 nm wavelength. The optical density of standard, test, and blank was obtained, and the results were calculated and tabulated. Results: In the current study, the mean hemoglobin value in the periodontitis group which was 3.1 μg/dl and the gingivitis group which was 0.71 μg/dl was significantly very high when compared to control group which was 0.1 μg/dl. Conclusion: Salivary hemoglobin levels can be used as a noninvasive, economic tool for periodontal screening in mass population and a viable alternative for predicting prognosis and treatment options.

KEY WORDS: Gingivitis, Periodontitis, Salivary hemoglobin

INTRODUCTION

Periodontitis is one of the most destructive and predominant oral diseases among middle-aged and elderly people, which leads to loss of teeth.1 It is a chronic irreversible inflammatory disease of the periodontium and is characterized by loss of periodontal ligaments and surrounding alveolar bone which is inferred by the signs of loss of attachment of the gingiva and bleeding on probing. Profuse bleeding on probing is an absolute possible symptom of periodontitis.2 Early screening of periodontal diseases plays a vital role to prevent wide spread lesion and to improve the oral health.

Traditionally, periodontal disease screening includes probing of the periodontal pockets of representative teeth in the oral cavity by qualified dental professionals. However, probing the pockets increases the risk of inducing iatrogenic bacteremia which would facilitate the destruction of the fundamental biological tissues of the periodontium.3

Conventionally, the Community Periodontal Index (CPI) which was developed by the WHO is used as a screening tool, but with advances in the techniques and clinical knowledge, many biomarkers have emerged which would help early detection and prevention of progression of the disease. CPI requires well-trained examiners which increases the cost of evaluation and inadequate knowledge and skill would lead to bacteremia which increases the risk for periodontitis patients.

Many salivary biomarkers have been used to correlate with periodontal disease conditions. These biomarkers such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine, and blood urea nitrogen were used for clinical and screening purposes.4,5 The most commonly used biomarker was salivary lactate dehydrogenase which has sensitivity and specificity for periodontal diseases which is <0.71.6

An association between salivary levels of hemoglobin, gingival inflammation, and lactate dehydrogenase has been reported in previous studies.6 In addition, there is a significant relationship between lactate dehydrogenase and probing depth which increases the accuracy of determining the clinical condition. The salivary test developed also helps to overcome the major screening problems as it is noninvasive and reliable.7,8

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Although several biomarkers are available, still their association with periodontal diseases and pocket depth is unclear. Therefore, the objective of the current study is to estimate the levels of salivary hemoglobin and examine whether the cutoff values from these tests could be used as a periodontal disease screening tool.

**METHODS**

**Study Population**

The study population was selected from patients coming to the outpatient department to Saveetha Dental College, Chennai. Patients above 20 years of age and below 60 years of age were selected for the study.

**Ethical Consideration**

The research protocol for this study was submitted to the Scientific Review Board of Saveetha Dental College and Hospitals, and the necessary permission was obtained to conduct this study, and written informed consent was obtained from patients before proceeding with the study.

**Inclusion Criteria**

1. Patients without systemic diseases
2. Patients between 20–60 years of age
3. New patients who have reported with complaints of periodontitis.

**Exclusion Criteria**

1. Patients with smoking and alcohol consumption habits
2. Patients undergoing treatment for periodontitis
3. Patients who had more than 12 missing teeth.

**Clinical Examination and Sampling**

The patients selected for the study were subjected to clinical examination, and the CPI was recorded using a CPI probe and CPI scores were given. 5 ml of stimulated saliva was collected in sterile disposable containers. The saliva was collected 2 h after eating and drinking. Stimulated saliva was collected by asking the patient to chew on paraffin wax for 5 min. The collected saliva was stored at 4°C until hemoglobin estimation was done.

**Sample Size**

A total of 45 patients were selected from the outpatient department coming to Saveetha Dental College and were grouped into control group, gingivitis group, and periodontitis group.

- Group 1 – Control group – Patients with healthy gingiva with CPI score 0 (n = 15)
- Group 2 – Gingivitis group – Patients with generalized chronic gingivitis with CPI scores 1 and 2 (n = 15)
- Group 3 – Periodontitis group – Patients with generalized chronic periodontitis with CPI scores 3 and 4 (n = 15)

**Biochemical Analysis**

The salivary hemoglobin was estimated using cyanmethemoglobin method using Drabkin’s reagent. The optical density was measured using colorimeter under 540 nm wavelength. The optical density of standard, test, and blank was obtained, and the results were calculated and tabulated.

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\text{Optical density of test} \times \text{dilution factor} = \frac{\text{concentration of standard}}{\text{Optical density of standard}}
\]

**RESULTS**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean value</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Gingivitis group</td>
<td>0.7</td>
<td>0.15</td>
</tr>
<tr>
<td>Periodontitis group</td>
<td>3.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

In this study, 45 patients participated and their salivary levels of hemoglobin were assessed. In patients with CPI score 0 (health gingiva), the mean salivary hemoglobin level was 0.1 + 0.05 μg/dl. In patients with CPI scores 1 and 2 (gingivitis), the mean salivary hemoglobin level was 0.7 + 0.20 μg/dl. In patients with CPI scores 3 and 4 (periodontitis), the mean salivary hemoglobin level was 3.1 + 0.1 μg/dl which was higher than the gingivitis group and control group.

**DISCUSSION**

In our current study, the hemoglobin was estimated using the fecal occult blood present in the saliva which
is not visible clinically, and the mean hemoglobin value in the periodontitis group was 3.1 μg/dl and in the gingivitis group which was 0.71 μg/dl was significantly very high when compared to control group which was 0.1 μg/dl.

Periodontitis is a chronic irreversible inflammatory disease of the periodontium and is characterized by loss of periodontal ligaments and surrounding alveolar bone.[2] This leads to loss of teeth in majority of middle-aged and elderly people. Smoking, alcohol consumption, improper oral hygiene maintenance, improper brushing techniques, and tobacco chewing habits show increased risk periodontitis.[9]

The diagnosis of the periodontal condition is by measuring the loss of periodontal ligament according to the Canadian survey 2007 which is the gold standard for diagnosis.[10] The CPI has also been a consistent tool in the diagnosis of periodontal conditions in mass population and has helped in epidemiological studies.

Several biomarkers for diagnosis of the periodontitis such as tumor necrosis factor-alpha, alanine phosphatase, alkaline phosphatase, matrix metalloproteinase (MMP)-2, MMP-8, and C-reactive protein have to be identified, but their use is limited to research studies because of their increased cost of reagents and need for expertise in the field to obtain accurate results.[11]

Periodontal disease screening techniques must be economical, simple, and user-friendly. In addition to it, the samples must be collected in a noninvasive technique like saliva.[12] Although CPI proves to be the traditional technique to be used for mass periodontal screening, it involves the use of periodontal probes which injures the periodontal tissues and causes pain. Inadequate knowledge of the technique and negligence might cause bacteremia which would increase the risk of the disease.

Hence, this study measured the salivary hemoglobin levels of patients with gingivitis and periodontitis and patients with healthy gingiva, were the salivary hemoglobin levels where tremendously increased in case of periodontitis.

The principle behind the increase in the salivary hemoglobin is the increase in the bleeding of the inflamed gingival tissue which contaminates the blood and this free hemoglobin is measured and used as a biomarker.[13]

There are various factors which influence the results of the study such as the dietary habits of the patient, familial history of periodontal diseases, and history of systemic diseases.

Patient dietary habits could greatly influence the levels of hemoglobin because there are many foods containing hemoglobin such as the fruits and fish, which could be in the saliva which shoes increases levels of salivary hemoglobin.

Patient’s history of familial periodontal diseases which have decreased bleeding and increased bone loss might show decreased hemoglobin levels despite the patients having periodontal condition.

Using salivary hemoglobin levels as a biomarker for the diagnosis of periodontal conditions can give accurate results in simpler, easier, and in an economical way when compared to other biomarkers which pose the risk of high cost of reagents and more invasive way of sample collections.

CONCLUSION

Salivary hemoglobin levels can be used as a noninvasive, economic tool for periodontal screening in mass population and a viable alternative for predicting prognosis and treatment options.

REFERENCES


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