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A New Screening Method For Periodontitis; Substitute For The Conventional Invasive Method.

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Abstract
Background: *Conventional periodontal screening methods including Community Periodontal Index which are used for the detection of periodontal diseases are time consuming and not cost effective even though they are precise and effective. The current study is aimed at testing the accuracy of periodontal screening using salivary biomarkers (Ferritin and Lactate dehydrogenase) in assessing periodontal diseases and compare it to routine periodontal examination.*
Materials and Methods: *Study population consisted of 60 subjects in which both conventional and biochemical screening methods are carried out. Inclusion criteria were 60 adults aged over 20 years with at least 20 teeth remaining. Full mouth clinical periodontal parameters including pocket depth, bleeding on probing and CPI were recorded. Salivary levels of LDH and Ferritin were recorded by using multimode analyser.*
Results: *Study showed a significant increase in levels of salivary LDH and ferritin proportional to periodontal diseases.*
Conclusion: *The salivary ferritin and LDH levels can be used as an adjunct to community periodontal index values. Thus, salivary tests may be a less invasive and viable adjunct to the Community Periodontal Index for periodontal screening.*
Key words: *Periodontitis, Screening, Community Periodontal Index, Saliva test*

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I. Background

Periodontal disease is widespread inflammatory condition ranging from gingivitis to severe periodontal breakdown which is closely associated with specific immune responses. It is considered as one of the major causes for tooth loss and its related function [1]. In the presence of these problems, early detection and prevention of periodontal disease prove to be crucial.

Periodontitis is generally diagnosed based on clinical criteria, such as probing depth, bleeding on probing, clinical attachment loss and alveolar bone loss on radiograph. The Community Periodontal Index (CPI) which was originally developed by the WHO in the year 2005 to measure community oral health is commonly used for periodontal screening[2]. It detects gingival bleeding and subgingival calculus and measures PD with probe and it is used for epidemiologic studies and routine periodontitis patient screening. Furthermore, the measure requires a large amount of inspection time, effort, and examiner skills. Also, it has another disadvantage that it doesn't represent current disease activity. Conventional disease diagnosis techniques lack the capacity to identify high susceptibility patients who are at risk for future breakdown.

A periodontal diagnostic tool provides pertinent information for differential diagnosis, localization of disease and severity of inflammation. These diagnosis in turn serve as a basis for planning treatment and provide a mean of assessing the effectiveness of periodontal therapy. Hence, instead of routine periodontal examinations, other diagnostic methods can be used that would provide very rapid estimates of periodontal health of the patients. Therefore, by using advanced diagnostic techniques and knowledge a new method should be developed which can act as an adjunct to the current diagnostic techniques. Saliva is an important resource of biomarkers that provides clinical information relevant to oral and systemic health. Thus, saliva is used as a surrogate variable to monitor the active status of the periodontal diseases and prognosis after treatment. An association between salivary levels of Ferritin and gingival inflammation has been reported [3]. In gingival inflammatory conditions as BOP increases, the amount of ferritin in saliva also increases which can further add the ease of periodontal disease diagnosis([3]. In addition, LDH activity in saliva may constitute a specific indicator of oral mucosal lesions with tissue breakdown, including periodontal disease [1], [4]–[8]. Therefore,

analyzing the level of LDH in saliva will help in diagnosing periodontal diseases in which the clinical attachment is already been lost.[1]

The aim of this study is to evaluate and compare salivary Ferritin and LDH levels with CPI values in patients with chronic generalized gingivitis and periodontitis and to determine whether this new method can be used as an adjunct to conventional screening method or not.

II. Materials And Methods

Study population

The study population was selected from patients who came to OPD in Department of Periodontology, Government Dental college and hospital Ahmedabad. A detailed history was taken by the clinician in every patient. Patients older than 20 years who had more than 20 teeth remaining were included. Other inclusion criterias were chronic generalised periodontitis patients with pocket depth ≥ 4 mm which involves more than 30% no. of teeth and chronic generalised gingivitis patients with bleeding on probing and ≤ 3 mm pocket depth with no clinical attachment loss (CAL). Those who were missing any index teeth from the sextant classified by the CPI or who had life style related diseases like diabetes, kidney and liver diseases, and history of blood transfusion were excluded. Also, patients with habit of using tobacco products were not included in this study. Smoking status, current medication and presence of lifestyle related diseases were checked by clinicians.

Clinical examination

Routine oral examination and CPI were carried out by dentist in all subjects. Each tooth was examined using the six-point method, and the number of remaining teeth, presence or absence of calculus, bleeding on probing and pocket depth were recorded. Each patient was diagnosed CPI probe according to CPI criteria from code 0 to code. The (CDC) – American Academy of Periodontology case definition for periodontitis was used [(21)]

. Briefly, patients with periodontal pockets in two or more interproximal sites with clinical attachment level of ≤3 mm, in two or more interproximal sites with a PD≥4mm, or in one site with PD ≥5mm were diagnosed as having periodontitis. Clinical attachment loss were only measured when a periodontal pocket≥ 3mm was found at an interproximal site.

Measuring salivary Ferritin and LDH

Unstimulated salivary samples were collected during day time, atleast 2 hr after eating, drinking or toothbrushing. All collected salivary samples are kept under 4ºC and a volume of 100 µL immediately transferred into working solutions which were made according to manufacturer's instructions. After incubation under 37ºC it was kept under a multimode analyser.

III. Statistical Analysis

Descriptive statistics, including means and standard deviations, were calculated to describe the data according to participant's clinical parameters and LDH and ferritin enzymatic levels, among the selected subjects. The unpaired t test was applied to assess the association of clinical parameters (BOP, PD) with biochemical parameters (LDH , Ferritin). The correlation between the groups were analysed using Pearson correlation test.

IV. Results

The study population consisted of 60 participants including 28 males and 32 females. The mean age of participants was 34±3years in the control group and 45±2 in study group. When it comes to the biochemical parameters it was observed that salivary ferritin and LDH values were higher in periodontitis patients when compared to that of gingivitis patients.

Table 1 Demographic data

Descriptive analysis of bleeding on probing (BOP), pocket depth (PD), and salivary levels of Ferritin and Lactatedehydrogenase (LDH) by Community Periodontal Index (CPI) score

CPI scores	0(n=4)	1(n=6)	2(n=5)	3(n=7)	4(n=8)	P
BOP(%)	0.45 ± 0.23	1.002 ± 0.001	1.02 ± 0.03	2.28 ± 0.08	2.77±0.21	<0.001
PD (mm)	1.92± 0.95	2.61 ±0.18	3.10 ± 0.07	3.37 ±0.36	4.12± 0.19	<0.001
Ferritin (µg/ml)	5.42 ± 0.33+	7.23± 0.69	9.56 ± 0.45	19.78± 1.55	24.03±24.03	<0.001
LDH (IU/L)	187 ± 4.76	199.5 ± 5.43	232.8 ± 20.60	343.29 ± 20.36	438.6± 30.34	<0.001

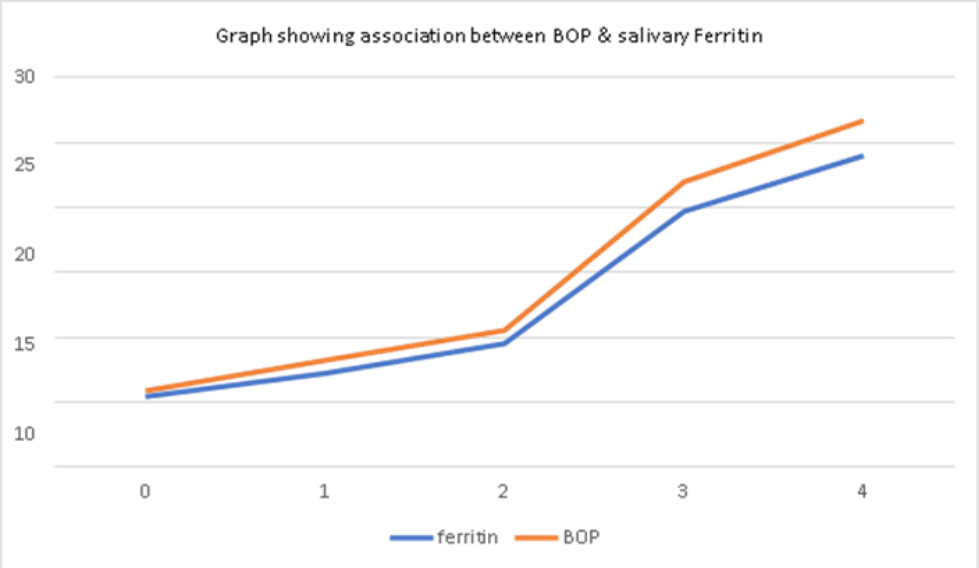
Table 2 Descriptive analysis of BOP, PD, ferritin and LDH in participants with and without periodontitis.

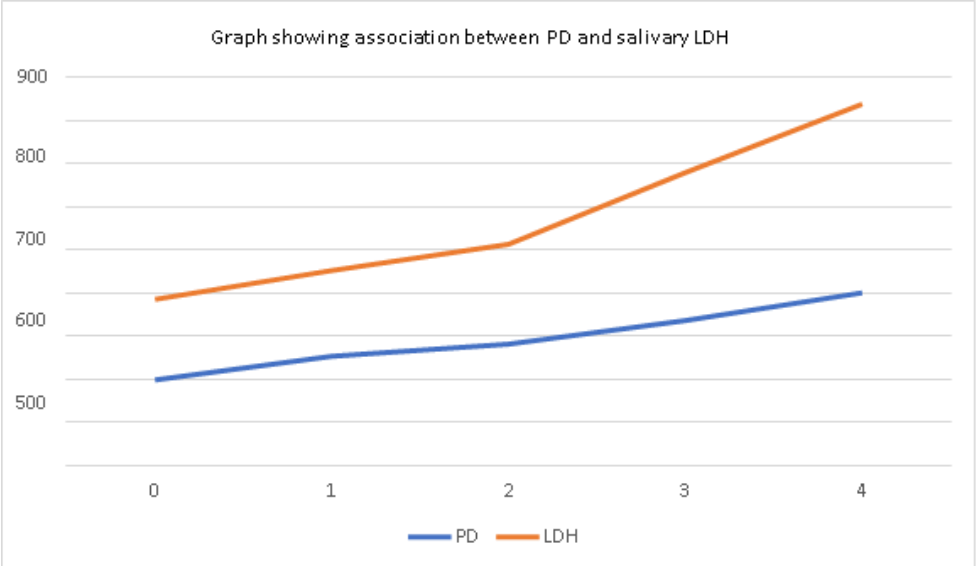
	-(n= 15) without periodontitis	+(n=15) with periodontitis	**P**
BOP(%)	1.01±0.02	2.57±0.31	S
PD (mm)	2.58±0.49	3.83±0.42	S
Ferritin(µg/ml)	7.66±1.62	21.74±2.57	S
LDH (IU/L)	206.20±22.98	405.60±50.46	S
Unpaired t test, p <0.01 – statistically significant(S)			

higher in participants with periodontitis than without periodontitis.

Table 3 Pearson correlation of Ferritin and LDH in relation to BOP and PD

		Ferritin {Pearson correlation (p-value)}	LDH {Pearson correlation (p-value)}
Periodontitis +	BOP	0.607(0.023)*	0.581(0.023)*
	PD	0.715(0.003)*	0.907(0.001)*
Periodontitis -	BOP	0.445(0.585)	-0.153(0.585)
	PD	0.832(0.001)*	-0.128(0.650)
Pearson corelation test; p< 0.01 (statistically significant)(*)			





V. Discussion

The use of biomarkers as a diagnostic tool during routine dental examinations could be helpful in early diagnosis of periodontal disease. Additionally, screening periodontal disease in large populations by the means of biomarkers could be done less invasively than conventional methods of periodontal diagnosis. Therefore, during recent decades salivomics gain its popularity in detecting biomarkers for diagnosis of periodontal disease and has attracted attention.

Periodontal disease is a chronic microbial and inflammatory process characterised by the presence of sulcular pathogenic bacteria, impaired host immune response, and destruction of the connective tissue attachment.

Periodontal diseases can be detected by conventional methods but it has several limitations like accumulation of evidence regarding odontogenic bacteraemia has also been reported[23]. During dental procedures, and even during tooth brushing or mastication, oral bacteria and their components can easily disseminate into the systemic circulation. According to a systematic review by the American Academy of Orthopaedic Surgeons and the American Dental Association, the prevalence of bacteraemia by mastication is less than 5 %, and by pocket probing is more than 30 %. In this respect, saliva tests are more advantageous than the CPI for mass screening[1]

In practice, biomarkers include tools and technologies that can aid in understanding the prediction, cause, diagnosis, progression, regression, or outcome of treatment of disease. Periodontal disease biomarkers are substances that could be produced during the host's defensive responses against bacterial invasion, reflect inflammation, or be released from cell death as a result of tissue destruction due to periodontal disease[15,17]. In the affected tissues, biochemical signalling involving three biological phases (Inflammation, connective tissue degradation and alveolar bone turnover) contributes to the clinical morbidity observed. Circulating molecules in these biological phases have been detected at elevated levels in the gingival crevicular fluid and whole saliva of patients who have periodontal disease making them putative biomarkers for the disease. Among these, salivary biomarkers act as a better tool for diagnosis due to its special characteristics The composition of saliva varies in every individual depending on their disease severity. If there are changes in its composition, there may be significant alterations in deterioration of the health of the host. These revelations have formed the basis of the field of saliva diagnosis and, therefore, have triggered research that culminated in the identification of saliva- based biomarkers for disorders ranging from cancer to infectious diseases.

Ferritin is an iron containing protein that is present in the blood and commonly extracellular fluids as well. In periodontal diseases, increased bleeding on probing can also increase the amount of salivary ferritin levels which can be used as a useful diagnostic tool for detection of periodontal diseases [9,14,15]. Ferritin levels are also increased in transfusion iron overload, severe liver disease, hemophagocytic syndromes, renal failure, sepsis, severe inflammation and other severe illnesses[14,15]. The raise in the levels of salivary ferritin in subjects with iron deficiency anemia can be attributed to the iron-dependent enzymatic function of saliva. A study was conducted by Lokesh Sundaran and Vidya Ratnavedu to assess the levels of salivary Ferritin in iron deficiency anemia subjects and healthy subjects. It showed that, salivary ferritin can become a biomarker that helps in the diagnosis of iron deficiency anemia also[9]. Another study on salivary ferritin changes in patients with COVID -19 was conducted by Lorena Franco in which Ferritin levels were higher in COVID-19 patients in serum and saliva, and the highest values were found in those patients presenting severe symptomatology. In conclusion, ferritin in saliva is the valid marker for diseases like COVID 19, iron deficiency anemia and pernicious anemia [10]

LDH is a ubiquitous enzyme present almost in every cell of human body. When cell viability reduced leakiness of the plasma membrane increases and therefore LDH enzyme is released into the extracellular space. The inflammatory and immune processes that develop in the periodontal tissues in response to the long-term presence of subgingival biofilm are protective by intent but result in considerable tissue damage. Majority of the tissue damage in periodontitis derives from the excessive and dysregulated production of a variety of inflammatory mediators such as LDH. Thus, LDH concentration in saliva as an expression of cellular necrosis, could be a specific indicator for clinical attachment loss. Furthermore, a possible correlation may exist between the levels of salivary LDH and the aggressiveness of oral OSCC lesion, as the mitotic rate of more aggressive lesion is higher and thus the salivary LDH is also expected to increase[4]–[7], [11]–[13] Thus, along with the periodontal diseases if the salivary LDH levels are much higher in such patients we can also screen the oral cancerous lesion activity[4]–[7], [11]–[13]. An increase in LDH levels can be seen in liver diseases, megaloblastic anemia, heart attack, cancers and infectious diseases. Stress induced epinephrine can enhance the levels of LDH.(22) Therefore, all these conditions can be a confounding factor while analysing the salivary biomarkers.

The results of this study indicated that LDH salivary level was significantly higher in subjects with periodontitis. Therefore, in the test group that included periodontitis patients, a higher salivary LDH level was shown significant difference ($p<0.01$)(table 2) . A descriptive analysis of clinical markers and salivary levels of LDH and ferritin were calculated against CPI criteria. Dose-response relations were observed for all markers.

All differences were statistically significant based on pearson correlation test. Next, a descriptive analysis of clinical markers and salivary levels of Ferritn and LDH were calculated against with and without periodontitis (Table 2). Ferritn and LDH levels were markedly higher in patients with periodontitis than in those without and these differences were also statistically significant according to the Pearson Correlation test. Then to compare the accuracy of the study, a graph is plotted on BOP and PD versus Ferritin and LDH respectively (Graph I &II). Graph plotted that, as the clinical parameters increases biochemical parameters also raised up .

A similar study was conducted by Yoshiaki Nomura1 on salivary Hb and LDH levels, sensitivity and specificity for Hb levels were 0.75 and 0.76 respectively and .722 and 0.711 respectively, for LDH levels. Combining these two tests, when samples tested positive for both Hb and LDH, the positive predictive value was 91.7%. They concluded that measuring Hb and LDH levels in saliva is a less invasive method than the CPI method.

Therefore, this saliva tests may be a viable alternative to the CPI method for periodontal screening [1]. Another study by Lin-Na Guo (2018) also supported present study result, in which there was positive correlation between salivary ferritin levels and patient's periodontal status.[23]

VI. Limitations of study

Even though the present study can be used as an adjunct for mass periodontal screening, it is not a site-specific method for diagnosing the disease. Also, in terms of cost the reagents and availability of biochemical laboratory set up may be inconvenient under certain circumstances. But despite all these limitations, this adjunct method can be used for diagnosing periodontal diseases for better understanding of the underlying disease severity by using chemical mediators and proteins which are involved in it. Thus, both the clinical and subclinical disease activities of periodontitis become easily accessible for determining the prognosis. Along with that other undiagnosed diseases like oral cancer and anemia can also be detected by test like this.

VII. Conclusion

Our study results show that as the value of clinical parameters like probing depth and bleeding on probing increases both LDH and ferritin levels in saliva also increases in patients with periodontal diseases. Thus, saliva tests may be a viable adjunct to the CPI for routine periodontal screening.

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