Saliva based diagnostic approach for caries and periodontitis: A pilot study of Cytokine Interleukin -1 β levels.

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Abstract:
Background*: Salivary diagnostics plays an important role in the early detection and prevention of many of the oral and systemic diseases in a rapid and noninvasive way. Dental caries and periodontitis are the most common infections in the oral cavity of humans. There has to be a quick and easy alternate for the detection of these diseases for early diagnosis and treatment. Salivary diagnostic methods provide simple, easy and reliable method for detecting changes in biological system.*
Materials and Methods*: 30 subjects were recruited into 3 groups(Caries, Periodontitis and Normal group). The Caries status was evaluated by Dentition status and treatment needs by WHO and Periodontal status by CPI index (WHO). Saliva samples were collected and analysed for salivary IL-1β levels using ELISA test .*
Results*: Salivary IL-1β did not show any statistical significance for age, gender and geographical distribution. The mean value of the analyte was more in periodontitis (134.6 ± 97.51 pg/ml) > caries (126.6± 91.2 pg/ml)> normal individuals(117.6 ± 66.71 pg/ml). There was no statistically significant difference between the disease groups and the normals (F value - 0.097, p>.05). The individuals with salivary IL-1β levels above the cut off value of 92.6 pg/mL calculated from ROC analysis may be considered as high risk and require strict follow up.*
Conclusion*: The emerging new trend of liquid biopsy can be used in the identification of biomarkers in various diseases including cancer.*
Key Word*: Saliva, Dental caries,periodontitis,Salivary IL-1β, biomarker,point-of-care diagnostics*

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I. Introduction

Dental caries is one of the most common bacterial infections in humans[1,2]. Periodontitis is another highly prevalent disease of the oral cavity[3,4]. The pathogenesis of these diseases suggest underlying tissue destruction and modulation of host defences by bacterial and host products , which stimulate the host inflammatory process[3]. The clinical parameters used in the detection of these diseases requires significant amount of tissue damage for the change to be detected and documented[5]. So there has to be a quick and easy alternate for the detection of these diseases for early diagnosis and treatment. Salivary diagnostic methods provide simple, easy and reliable method for detecting changes in biological system[6].

Diagnosis with salivary analytes have been increasing in the past decades[6]. It provides a noninvasive and simple method with high rate of accuracy for the detection of oral diseases, oral cancer, drug abuse,forensic odontology, etc[7,8]. Interleukin 1 β is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis[2,3,5]. Apart from immunologic functions IL- 1 β plays vital role in carcinogenesis[6]. Therapies targeting interleukin 1 β are being used in all fields of medicine[9]. The present study evaluates salivary cytokine, IL-1 β, as a quick and easy method for individualized point-of-care diagnostics in caries and periodontitis

II. Material And Methods

II.1 Study Subjects

Subjects were enrolled in the study which was approved by the ethical committee of Government Dental College, Thiruvananthapuram. Informed consent was obtained from each participants between the age group 18-58 years. Willing individuals were included in the study. Individuals with chronic inflammatory diseases(such as arthritis, psoriasis, inflammatory bowel syndrome, granulomatous diseases, sjogrens syndrome), oral inflammatory conditions (apthous,lichen planus, leukoplakia), diabetes mellitus, hypertension, substance abuse,organ transplant, cancer therapy , pregnancy or lactation–were excluded from the study.

II.2 Clinical evaluation

Demographic data was collected from the subjects. All subjects received a full mouth examination. The Caries status of subjects was evaluated by Dentition status and treatment needs by WHO. Periodontal status of individuals was assessed by CPI index (WHO). A *CPITN* probe was used to assess the periodontal status. All clinical findings were recorded in data collection work sheet. Based on caries and periodontal status , 30 subjects were recruited into 3 groups : Caries, Periodontitis and Normal (10 subjects in each group).

II.3 Saliva Samples

Salivary samples were collected between 9 and 12 am. Patients were asked to refrain from eating, drinking or oral hygiene measures at least one hour prior to saliva collection. Subjects were asked to pool saliva and expectorate 2 ml of saliva into collecting vials. Samples were stored at -70 degree Celsius at Rajiv Gandhi Center for biotechnology, Thiruvananthapuram for further analysis. Concentration of salivary IL 1 β levels were assessed by commercial chemiluminescent enzyme linked immunoassay kit (Biolegend). Absorbency at 450nm was measured and the corresponding OD values were noted.

II.4 Statistical analysis

Descriptive statistics were calculated for the demographic data and salivary analyte. Data are expressed in its frequency and percentage as well as mean and standard deviation. To elucidate the associations and comparisons between different parameters, Chi square ($\chi 2$) test was used as nonparametric test. Analysis of variance (One Way ANOVA) were performed as parametric test to compare 3 groups .Confidence interval was calculated at 95% confidence level. Receiver operated characteristic (ROC) analysis was carried out to conclude upon the cut off value for IL1B for normal population.

III. Result

III.1 Demographic data

Age: Samples were obtained from the age group 18- 58 years. Most subjects were in 28-38 age group (12/30 ie, 40%). Salivary IL 1 β levels were not significant in different age groups (Table 1).
Gender : There were more male subjects in the study (17/30 ie, 56.67%)(Table 1). More than half of the study subjects were males. No significant variation in levels of salivary IL 1 β levels could be noted between males and females.
Geographical area: There were more subjects from the rural areas accounting to 53% (Table 1). IL 1 β levels in subjects coming from urban, semiurban and rural areas did not show any significant correlation.

	Caries	Periodontitis	Healthy
Age group	29-38(40%)	29-38(40%)	29-38(40%)
Males	40%	70%	60%
Rural area	40%	70%	50%
Table 1: Age, gender and geographical distribution of the 3 groups			

III.2.Disease conditions

Caries: The mean DMFT score in the study population was 5.1. A minimal difference in the mean value of salivary IL 1 β levels in caries group(126.6± 91.2 pg/ml) was noted when compared to the normals (mean value : 117.6 ± 66.71 pg/ml) but was not statistically significant(p>0.05)

Periodontitis: Mean CPI score was in the study subjects was 1.26. The levels of salivary IL 1 β was higher in the periodontitis group (mean value 134.6 ± 97.51 pg/ml) when compared to healthy(117.6± 66.71 pg/ml) subjects.(p>0.05)

95% CI value of 126.05± 29.4 pg/mL was obtained for salivary IL1 β for the population . ROC analysis gave a F value was .097. A cut off value of 82.6 pg/mL for salivary IL1 β for normal population with sensitivity .90 and specificity of .63.

IV. Discussion

Saliva is a mixture of different components and their levels may change or remain constant with age. The study by Nasser *et al*, on the age related changes in salivary biomarkers showed no statistically significant difference in salivary biomarker levels according to age [10]. The elderly group showed significantly lower salivary flow rate and Ca levels than the young group[10]. In our study, there was no significant difference of IL 1 β levels in different age. This indicates that age may not have any influence on salivary biomarker

concentration. In the current study, the number of people coming for treatment was more in 28-38 age group which is in accordance with earlier studies[5].

No significant variation in levels of salivary IL 1 β levels could be noted between males and females. Takai *et al* also noted no gender specific differences in the resting salivary biomarker parameters[11]. However, inter-individual variability should be accounted[12]. IL 1 β levels in subjects coming from urban, semiurban and rural areas did not show any significant correlation in our study. The geographical area in which a person lives does not influence the biomarker concentration. No studies could be found related to this topic; apart from a study conducted in malarial areas which was a related to the disease transmission[13].

Cogulu *et al* suggested that dental caries was not correlated with salivary or serum concentrations of IL-1β[2]. But S. mutans level positively correlated with saliva IL-1β concentration and inversely correlated with saliva IL-1ra concentration[2]. S. mutans were found to stimulate production of proinflammatory cytokines. The present study showed a minimal difference in the mean value of salivary IL 1 β levels in caries group(126.6± 91.2 pg/ml) when compared to the normals (mean value : 117.6 ± 66.71 pg/ml) but was not statistically significant.

The levels of salivary IL 1 β was higher in the periodontitis group (mean value 134.6 ± 97.51 pg/ml) when compared to healthy(117.6± 66.71 pg/ml) subjects. Earlier evidence suggests increased interleukin-1 β (IL-1β) concentration in gingival tissue from periodontitis patients[3,14,15]. But no IL- 1 β could be found in normal gingival tissue[14]. This finding may have important consequences relevant to connective tissue destruction and episodes of alveolar bone resorption characteristic of chronic periodontitis.[14] Levels of salivary IL 1β showed reduction after oral prophylaxis suggesting that the biomarker could facilitate screening, diagnosis and management of periodontitis[3,15].

One way ANOVA analysis gave an F value of 0.097 when the caries, periodontitis and normal groups were compared(p> .05). There was no statistically significant difference between the disease groups and the normals . There was wide range of distribution of salivary IL-1β which may be attributed to the small sample size(Graph 1).
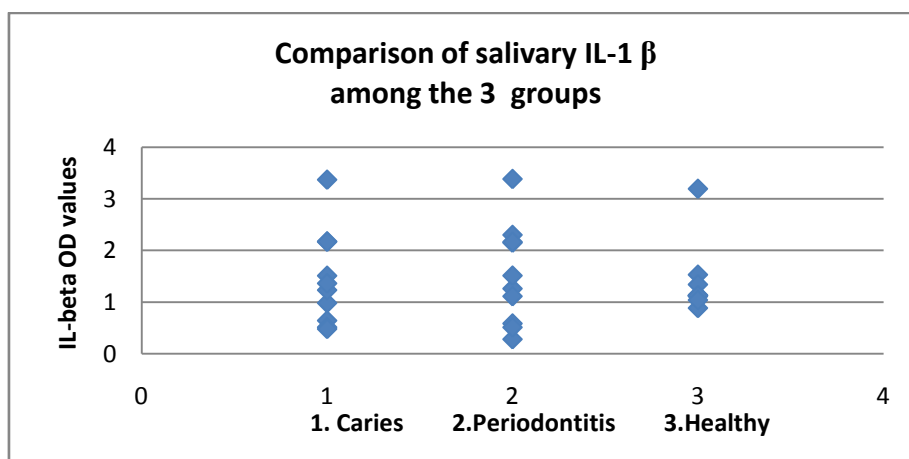
Graph 1: Graph showing the comparison of salivary IL-1 β OD values among caries, periodontitis and healthy group

95% CI value of 126.05± 29.4 pg/mL was obtained. For 95% of the population the salivary IL1β values fall in the range of 96.65- 155.45 pg/mL. The wider range may be due to the small sample size and uneven distribution of the samples. ROC analysis gave a cut off value of 92.6 pg/mL for salivary IL1 β for normal population with sensitivity .90 and specificity of .63. This throws light to the fact that persons with salivary IL1β values above 92.6 pg/mL are at higher risk of oral diseases, like caries and periodontitis and need proper follow up. This may be used as a guideline for further analysis.

The draw backs of the study include
1. Small sample size: The study has to be conducted in larger population.
2. Single biomarker: Combination of different biomarkers will provide better validity for the study.

V. Conclusion
Salivary diagnostics plays an important role in the early detection and prevention of many of the oral and systemic diseases in a rapid and noninvasive way. The individuals with salivary IL-1β levels above the cut off value may be considered at high risk and require strict follow up. The study can be conducted in a larger

sample for better validity. All the more, multiple biomarkers will provide valid information in the diagnosis of risk groups. This study may pave the way for further research in this field. The emerging new trend of liquid biopsy can be used in the identification of biomarkers in various diseases including cancer.

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