BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY
Volume 21, Issue 1

DOI: 10.58240/1829006X-2025.1-11

RESEARCH ARTICLE

EVALUATION OF EXPRESSION OF SALIVARY ICTP BIOMARKER IN PERIODONTITIS PATIENTS – AN OBSERVATIONAL STUDY

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Received: *Oct 15, 2024;* ***Accepted:*** *Nov 15, 2024;* ***Published:*** *Jan 10, 2025*

Abstract

Objectives: Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is released during the breakdown of Type I collagen which is the primary event which happens in periodontitis. In this study, we primary intention is to find if ICTP biomarker can be used in the diagnosis of periodontitis.

Materials and methods: 30 patients were included. On clinical examination, they were divided into 3 groups 10 healthy, 10 chronic periodontitis patients and 10 chronic periodontitis with diabetes patients. Unstimulated saliva was collected from these patients. Presence of salivary ICTP was assessed using ELISA and qPCR.

Results: The highest ICTP levels were seen in Group 3 which is diabetes with periodontitis, followed by group 2 and group 1. A statistically significant difference was observed when ICTP levels were compared when Group 1 was compared with Group 3 and similarly with Group 2.

Conclusion: Significant difference in the salivary ICTP levels between the gingivitis and periodontitis groups proved that ICTP can be used as a tool for the diagnosis of periodontitis.

Keywords: *ICTP, Periodontitis, Biomarkers, Diagnostic tool, Diabetes.*

INTRODUCTION

Periodontitis is a chronic inflammatory disease that destroys the soft tissue and hard tissue that supports your teeth.[1] It is a progressive condition and is one of the leading causes of tooth loss. Periodontitis is caused by bacteria found in plaque that deposits on the teeth. The primary symptoms of periodontitis include swollen, red, or bleeding gums, bad breath, receding gums, mobile teeth, and eventually loss of tooth which are caused by the various types of microbes present in the periodontal tissues especially the gingival sulcus leading to pocket formation[2] Risk factors for developing periodontitis include poor oral hygiene, smoking, genetic predisposition, certain systemic diseases (such as diabetes), hormonal changes (such as during pregnancy), certain

medications, and a compromised immune system. But these risk factors only tend to enhance the detrimental effects caused by the bacterial strain present within the affected tissues.[3] In the early stages, periodontitis may not cause any discomfort, which is why regular dental check-ups are crucial to catch and treat the condition early.[4] But to detect the disease at its earliest stage, reliable diagnostic methods are still in the process of development. One of them is through the use of various biomarkers.[5] In the oral cavity, saliva and GCF act as a pool of potential biomarkers that could be useful in the prediction of periodontitis.[6]

Numerous studies have been done on the potential for saliva to serve as a hub of biomarkers for detecting the existence, activity, and progression of periodontal disease. Due to saliva's proximity to periodontal tissues, its accessibility to be collected non-invasively, and its potential for site-specific examination, saliva has been widely studied in both healthy and diseased states. For early diagnosis of the disease, to predict the prognosis, and to evaluatethe effects of a particular therapy method, biomarkers are helpful.[7] There have been previous studies on the biomarkers for periodontal disease for a while now.[8] However, the validity of these tests is still in doubt. At least 90 possible biomarkers of various types have been examined during the breakdown of periodontal tissue.

Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP), which is oneof the new biomarkers, is a 12 to 20 kD fragment belonging to type I collagen of the bonethat gets released after processing with trypsin and bacterial collagenase, which, according to Risteli et al., can be positively correlated with the status of periodontal disease.[9] ICTP is a peptide fragment that is released by the breakdown of type I collagen. It is found in the body fluids, and its levels can be measured to provide information about bone resorption, which is the process of breaking down old bone tissue.[10] In clinical settings, ICTP is often used in conjunction with other biomarkers of bone metabolism to evaluate bone turnover and monitorthe effectiveness of treatment for conditions such as osteoporosis, bone metastases, and other bone disorders.[11] By measuring ICTP levels, healthcare professionals can assess it's relationship with bone metabolism, which can help in diagnosing and monitoring bone diseases and evaluating the response to therapy.[12] This study aims to find the association between ICTP with healthy as well as periodontal disease with and without diabetes.

MATERIALS & METHODS STUDY DESIGN

The study was approved by the institutional human ethical approval committee (IHEC/SDC/PERIO - 2103/22/197). 75 patients who were included in this study were segregated into three groups. 25 patients were in the periodontally healthy group (C, n = 25), 25 in the chronic periodontitis group (P, n = 25), and 25 in periodontitis with diabetes (P+D, n = 25). All participants who were recruited were outpatient of the Department of Periodontology, Saveetha Dental College & Hospital, Chennai. The patients were made to read, understand and sign the consent forms before taking part in the study.

Inclusion criteria

Patients in the age group 25-50 years who do not present with any systemic diseases other than diabetes were included in the study. Patients with infectious disease such as hepatitis or HIV, who are under medication which influences the periodontium, pregnant or lactating women and finally patients who underwent periodontal therapy in the last 6 months.[13] At least 20 teeth should be present in both healthy and chronic periodontitis patients. Periodontally healthy patients should have a pocket depth (PPD) of less than 3 mm and less than 10% bleeding on probing. Whereas periodontitis patients should have PPD of more than 5 mm with attachment loss and more than 30% bleeding on probing. On clinical examination, probing pocket depth (PPD) and clinical attachment level (CAL) were measured. All possible clinical measurements were made with the help of Williams probe after measuring in all 6 sites around each tooth.[14]

Sample collection

Every patient had their whole, unstimulated saliva taken by the drool method in the morning (9 to 10 am), prior to a clinical evaluation, into a sterile vial (5 mL) (Figure 1). After asking patients to expectorate and properly rinse their mouths with distilled water for 30 seconds, saliva was collected using the passive drool method. One hour prior to the experiment, the

participants were instructed to remain calm and refrain from eating, drinking, chewing gum, and brushing their teeth in order to exclude any potential outside influences that might have an impact on the volume of saliva secreted. If the patient ate or drank one hour before saliva collection, or if there was insufficient saliva, the sample would be discarded. The salivary sample vials were labelled for identification and sealed. The sealed, labelled vials containing the salivary samples were sent to the Saveetha Dental College's Clinical Biochemistry Laboratory for storage (at 80 °C) for further clinical biochemistry examination by a senior member with experience. To determine whether ICTP was present in the obtained salivary samples, antibody sandwich ELISA (Enzyme-Linked Immunosorbent Assay) was carried out using ELISA kit.[15]

Figure 1. *Saliva sample stored in a sterile 5 ml container*

Principle of the assay

This assay follows two site sandwich ELISA to quantitatively assess the presence of ICTP in human saliva. Antibody specific to ICTP are pre coated on the titre plates. Samples along with HRP conjugated ICTP antibody are added to the titre plate. Then the unbound HRP conjugate reagent is washed off, after which a substrate solution is added. The intensity of thecolour appears in accordance with the amount of ICTP present in the sample. Once the color stops developing, the intensity of the colour is measured.[16]

Procedure

After centrifuging the samples at 1000 rpm for about 20 minutes, the sediments were removed and send for assay (Figure *2*). The samples can also be stored at a temperature of - 20° or -80°C. Do not freeze the sample repeatedly to avoid damage. Follow the instructions properly to prepare the reagents, working protocols and samples. With no solution, prepare a blank well. Add 50 µL of sample to each well and also 50 µL of horse radish peroxidase conjugate to each well. Mix them well and place them in the incubator for 60 minutes at37°C. Aspirate the contents from each well and wash thrice repeatedly. They are washed witha wash buffer (1X) with the help of proper armamentarium. To achieve good outcome, the cleansing step has to be done properly. The excess water is removed by blotting with clean paper. Add 50 µL of each sample and mix well. Place them for incubation at 37°C. Keepthem away from direct light to prevent temperature changes. Later add 50 µL stop solution toeach well. The wells which contain highest concentrations of the standard turns into blue colour. Check for the optical density at 450 nm or if possible, at 540 or 570 nm within 5 minutes of colour change to achieve higher accuracy.[16]

Figure 2. *Centrifuging the collected saliva samples to remove the sediments*

STATISTICAL ANALYSIS

Statistical analysis was done with the help of SPSS software version 21 (SPSS Inc., Chicago, IL, USA). The salivary ICTP levels between the 3 groups were compared with One way ANOVA and pair wise inter group comparison using Tukey's HSD post hoc analysis and if the p value was less than 0.05, it was considered statistically significant. The sample size

was calculated using G power analysis. The obtained outcome data was represented in the form of Mean ± SD. With power of study (1− β) = 80% with alpha = 0.05, medium effect size (0.5) with three groups, we obtained a value of 9.5 which is supposed to be the size of the sample in each group, which was rounded off to 10 individuals.[16]

RESULTS

This study included 25 healthy gingiva patients (41.3 ± 4.73), 25 periodontitis patients (39.4 ± 5.2), and 25 periodontitis with diabetes patients (40.3 ± 4.8) (Table 1).

Table 1. *Demographic details*

Parameter	Group 1 (n=25)	Group 2 (n=25)	Group 3 (n=25)
Age (years)	41.3 ± 4.73	39.4 ± 5.2	40.3 ± 4.8
Sex (Male/Female)	14/11	14/11	14/11

Table 2 represents the intergroup comparison of salivary ICTP biomarker levels between the three groups using a one-way ANOVA. The intergroup comparison by ANOVA revealed that the highest ICTP levels were seen in group 3, which is diabetes with periodontitis, followed by groups 2 and 1. A significant difference was observed when ICTP levels were compared when group 1 was compared with group 3 and also with groups 2 & 1.

Table 2. *Intergroup comparison of salivary ICTP levels between three groups*

Variables	Group 1 (Mean ± SD)	Group 2 (Mean ± SD)	Group 3 (Mean ± SD)	p value
Salivary ICTP (ng/ml)	0.34 ± .02	1.29 ± 0.04	2.44± 0.02	.000*
ICTP - Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen **The mean difference is significant at the 0.05 level.*				

Figure 3 shows the graphical representation of salivary ICTP biomarker levels in all the three groups.

In pairwise comparison, in terms of ICTP levels, a statistically significant difference was observed between all three groups as shown in Table 3.

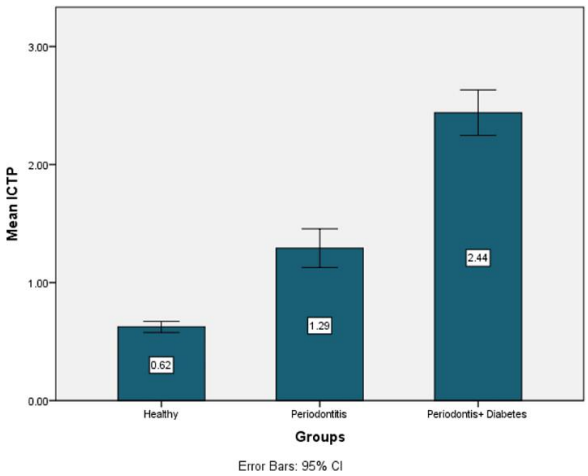

Figure 3. *Graphical representation of the difference in the salivary ICTP levels among the 3 groups*

Table 3. *Pairwise comparison of ICTP levels between 3 groups*

Groups		Mean difference	p value
Group 1	Group 2	-0.6679	0.000*
	Group 3	-1.8156	0.000*
Group 2	Group 1	0.6679	0.000*
	Group 3	-1.1477	0.000*
Group 3	Group 1	1.8156	0.000*
	Group 2	1.1477	0.000*

ICTP - Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen
**The mean difference is significant at the 0.05 level*

Table 4 shows the Pearson's correlation of salivary ICTP levels with periodontal clinical parameters PPD and CAL. ICTP and PPD (r = 0.686) and ICTP and CAL (r=0.659) revealed a strong positive correlation with statistically significant difference.

Table 4*. Correlation of ICTP with PPD and CAL*

Correlation Variable	Correlation Coefficient (r)	p value
PPD vs ICTP	0.686**	.000*
CAL vs ICTP	0.659**	.000*

***Correlation is significant at the 0.01 level (2- tailed)*
PPD - Probing Pocket Depth
CAL - Clinical Attachment Loss
ICTP - Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen

DISCUSSION

The role of saliva in diagnosing the oral and systemic health of an individual has alreadybeen studied in many researchers so far. Salivary biomarkers play a major role in assessing periodontal health and have a close association with systemic diseases.[17] Various genetic as well as microbiological studies have been carried out so far to establish a connection between the microbial environment and the clinical status of the disease.[18] In recent times,the researchers are hopeful about the potential development of periodontal vaccines, which might help to prevent periodontal disease at an earlier stage or even prevent it from even occurring.[19] Saliva is mostly preferred, since it's cost-effective and the method of collection is comparatively easier than GCF. Kc S et al., implied that for the verification of markers specifically in each stage of disease progression, research of the variations in biomarker expression for cases at different stages like gingivitis and periodontitis may be relevant.[20]

Based on the results of this study, ICTP biomarker can be used as a potential diagnostic tool for the prediction of periodontitis. The salivary levels of ICTP were the highest in patients with both diabetes and periodontitis, followed by those with only periodontitis, and the least in healthy patients. ICTP

reflected the periodontal status of the individuals. But also, their increase in diabetic patients, urges us to study the association between ICTP and diabetes. According to Zhang Y et al., since there was no bone loss in the gingivitis group or the healthy group, ICTP was not shown to be substantially different between the two groups. On comparing, the disease group to the healthy groups, there was a marked increase in ICTP levels due to apparent alveolar bone loss in the periodontitis group.[21] Similarly, Mishra D et al., concluded that on examination of ICTP levels from healthy samples and gradually moving on to the diseased sample, a significant rise was noticed. This indicated that ICTPwas more positively associated with active disease sites than with healthy or inactive sites.[22] Gursoy et al.,[23] and Kinney et al.,[24] gave a similar assessment of the levels ofICTP when compared between healthy and periodontally compromised patients.

Giannobile et al., demonstrated in his study that the presence and absence of pyridinoline cross links represent the status of bone loss and that this data can be used as an indicator to identify active forms of gingival and periodontal disease.[25] Palys et al., found that there was a significant increase in the levels of ICTP with increased levels of periodontal pathogens, which are mainly responsible for periodontal destruction.[26] Quesada JG et al., confirmed that the levels of ICTP marker was higher in the active sites of disease than in the inactive sites when seen in patients after periodontal therapy.[27] Payne et al., also revealed the importance of ICTP in the detection of bone loss or bone metabolism in patients.[28] However according to Lappin DF et al., his study failed to see any positive relationship or correlation between ICTP with diabetic periodontitis patients and those without diabetes. There was no significant difference.[29] Another study by Gursoy et al., revealed a similar result of elevated salivary ICTP levels were found in periodontitis subjects, however, without statistical significance.[30] Based on the results of the mentioned studies and current study, majority of the studies revealed that ICTP is a reliable biomarker for the prediction of periodontitis. However, its association with diabetic patients is still debatable since not much evidence has been found.

CONCLUSIONS

We have evaluated the use of the ICTP biomarker as a potential diagnostic marker to differentiate between healthy and periodontitis patients as well as diabetic periodontitispatients. The results of the study revealed that there is a significant difference between the ICTP levels of the periodontally healthy and diseased groups. This concludes that checking for ICTP biomarker levels would enhance the probability of early detection of periodontal disease in clinical practice. But, however, the expense of the molecular biologic analysis, like checking biomarker levels, would be much higher than the clinical and radiographic examination. Hence, the rate of acceptance of patients to undergo these examinations to predict the probability of the development of periodontitis, even before being symptomatic, would be unlikely.

DECLARATIONS

Conflicts of interest and financial disclosures

The author declares that he has no conflict percent and there was no external source of funding for the research in question.

Ethical approval

The study was approved by the Institutional Ethics Committee and was conducted in accordance with the Declaration of the World Medical Association.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Source of funding

The work was not funded.

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