'Deoxypyridinoline'- an important biomarker in gingival crevicular fluid and serum for alveolar bone loss in patients who have periodontitis: A clinical study

1. Dr. Dasari Vanditha
Senior Lecturer, Department of Periodontics, Sri Venkateswara Dental College, Ariyur Pondicherry.

2. Dr. Pragyan Swain
Post Graduate student, Department of Periodontics, Chhattisgarh Dental College & Research Institute, Rajnandgaon, Chhattisgarh

3. Dr. Saswati Mohanty
Post Graduate student, Department of Periodontics, Rungta College of Dental Sciences and Research, Bhilai, Chhattisgarh

4. Dr. Babita Pawar
Professor and Hod, Department of Periodontics, Chhattisgarh Dental College & Research Institute, Rajnandgaon, Chhattisgarh

5. Dr. Aishwarya Virendra Satpute
Post Graduate Student, Department of Periodontics, New Horizon Dental College & Research Institute, Bilaspur, Chhattisgarh

6. Dr. kirti karda,
Senior Lecturer, Department of Periodontics, Triveni Institute of Dental Sciences Hospital and Research Centre, Bilaspur,chhattisgarh.

7. Dr Ravindra Soni
Senior Lecturer, Department of Periodontics, New Horizon Dental College & Research Institute, Bilaspur, Chhattisgarh
(corresponding author)

Abstract

Background:

Several components of gingival crevicular fluid (GCF) give a pointer toward disease status and represent the course as well as the prediction of periodontal disease. Potential biomarkers such as deoxypyridinoline (DPD), a metallophosphoesterase, would correctly determine the presence of osteoclast-mediated bone turnover activity. Additionally, this metallophosphoesterase seems to hold great promise as a predictive marker to determine bone destruction and active phases in the progression of the disease.

Aim:

It has been claimed that the purpose of the current study is to evaluate the biologic plausibility for the levels of DPD as a biomarker in patients who suffer from chronic periodontitis.

Both the Materials and the Methods:

Participants in the current cross-sectional study were recruited from the outpatient department of Periodontics and included 15 patients who were periodontally healthy and 15 patients who had chronic periodontitis. Patients were matched for age and gender. The enzyme-linked immunosorbent assay kit was used to conduct the analysis on the GCF and blood samples that were taken from each of the patients in order to determine the DPD. Clinical data such as clinical attachment loss (CAL), probing pocket depth (PPD), modified gingival index, bleeding index, and plaque index were recorded. Other clinical measures were bleeding index and plaque index.

Results:

When compared to a group of periodontally healthy individuals, the GCF DPD levels of patients suffering from chronic periodontitis were considerably greater. There were no significant

eISSN 1303-5150

connections detected between GCF and serum DPD levels and the progression of illness severity, age, gender, or rise in PPD or CAL in either of the two groups.
Conclusion:
Increased levels of GCF DPD in chronic periodontitis patients can be used as an indicator of continued periodontal damage, notwithstanding the limitations of this study.
Deoxypyridinoline levels, gingival crevicular fluid, and periodontitis are some of the keywords that may be found in this article.

DOI Number: 10.14704/nq.2022.20.11.NQ66118 **NeuroQuantology 2022; 20(11): 1248-1257**

INTRODUCTION

Periodontitis is a disease of the tooth-supporting tissues that is characterised by persistent inflammation. It is caused by germs that are found in dental plaque. Periodontitis is a complicated aetiology that involves a biofilm, behavioural variables, environmental factors, and the inherited characteristics of the individuals who have it. Although it has been established that microbes in the biofilm play a role in the initiation of periodontal disease, the host's immuno-inflammatory response to the onslaught of microbes plays a pivotal role in the progression of periodontal disease. [1] If the condition is not treated, it will continue with progressive bone destruction, which will eventually lead to tooth mobility and tooth loss .[2]

There are a variety of host-immuno inflammatory factors, either induced or inherent, that work together to coordinate the pathophysiology of periodontal disease. In a healthy individual with normal physiological circumstances, the ratio of bone creation to bone resorption is kept in a healthy equilibrium. Several inflammatory metabolic illnesses, such as osteoporosis, periodontal disease, and rheumatoid arthritis, are instances of a disrupted balance between bone production and bone resorption. [3] Bone turnover is associated with a variety of osseous metabolic and osteolytic diseases. Ninety percent of the organic matrix that makes up bone is composed of type I collagen. Pyridinoline cross-links are a kind of collagen degradation product that belongs to the family of C, N-telopeptides, which also includes pyridinoline and deoxypyridinoline (DPD). DPD is one among the products described above, and it is most commonly found in bones. It is produced when the side chains of one lysine and two hydroxylysine molecules react with each other. The cross-link molecules that result from posttranslational alteration of collagen molecules are unable to be reused throughout the process of collagen synthesis; as a result, they are unique to the process of bone resorption. These molecules are released into the blood as a consequence of osteoclastic bone resorption as well as the destruction of the collagen matrix. Because it is produced during the process of collagen maturation or biosynthesis, DPD is regarded to be a bone marker that is particular to resorption. [4,5,6] This is because DPD only emerges as a resorption product of the mature matrix.

Even while regular diagnostic procedures are simple and inexpensive, such parameters are of limited help in the early diagnosis, and they constitute an indicator of previous destruction. This is the case despite the fact that such parameters are useful. Because of this, there is a need for an innovative diagnostic test that can correctly determine the current status of disease activity, predict sites that are vulnerable for the possibility of breakdown, and evaluate the response to periodontal interventions. [4] This is where biomarkers assume a significant diagnostic function, monitoring the outcomes of the discovery in life sciences. [5] .[7]

eISSN 1303-5150

In the search for a sensitive and accurate periodontal disease marker, the majority of the molecules in oral fluids, such as saliva, gingival crevicular fluid (GCF), and serum or plasma molecules in the circulation, have been explored up to this point. Saliva is one example. Both gingival crevicular fluid (GCF) and saliva are examples of non-invasive oral fluids that are easily acquired, cause the patient minimum or no discomfort, and include chemicals that are both systemically sourced and locally generated [7,8].

The levels of GCF DPD are expected to be more accurate markers of disease activity, and their quantification can provide helpful information for the diagnosis of active periodontal disease as well as an estimate of the course of the illness. The primary objective of the current research is to investigate the biological plausibility of the levels of DPD in healthy individuals and those with chronic periodontitis.

MATERIALS AND METHODS

Study design

After receiving authorization from the institutional ethics committee, researchers recruited participants in the age range of 30–60 years old from the outpatient department of periodontics and carried out the study between January and July of 2018. Following the completion of the clinical and radiographic examinations of the people, they were split into two separate groups. Every patient who was enrolled for the research was given information on the procedures, and their signed informed consent was acquired. All of the patients who were recruited underwent extensive periodontal treatment, in addition to having a comprehensive clinical evaluation that included the recording of all pertinent data.

Criteria for acceptance and rejection of applicants

Group A (the healthy group) consisted of 15 persons who were systemically healthy and had clinically healthy periodontium with a PPD of less than 3 mm. Group B (the chronic periodontitis group) consisted of 15 individuals who were in good overall health and had a total of at least 14 teeth. They were diagnosed with moderate-to-severe periodontitis and had at least one site with a PPD of less than 6 millimetres and a CAL of less than 3 millimetres in each quadrant. Additionally, there was radiographic evidence of bone loss in this group. Patients with any known systemic diseases (hypertension and diabetes) that can alter the course of periodontal disease, history of any recent infections, smokers, patients with alcohol intake, pregnancy and lactation, patients on any analgesics and antibiotics within the past three months, patients with prior history of periodontal therapy within six months of the study, and patients with aggressive periodontitis were not included in the study. Patients with aggressive periodontitis were those who had periodontitis that was severe enough to require

Clinical evaluation and observation

During the clinical examination, the following parameters were evaluated: the plaque index (PI),[9] the modified gingival index (MGI),[10] the Saxton bleeding index,[11] the probing pocket depth (PPD)[12] on each tooth from the gingival margin to the bottom of the sulcus/pocket, and clinical attachment loss (CAL), which was measured as the distance between a fixed point on the crown, such as the cementoenamel junction, and the base Full mouth intraoral periapical radiographs and an orthopantomogram were used for the radiographic assessment of the teeth, which

eISSN 1303-5150

was done to confirm the bone loss. In order to prevent GCF contamination with the blood that is associated with probing at inflamed areas, patients were reminded to return for their future sessions.

Site selection as well as the collection of gingival crevicular fluid

The patients were instructed to sit in an upright position in the dentist chair, and the area that had the greatest probing depth was then chosen, separated with cotton rolls, and allowed to air-dry. Plaque that was supragingival was scraped away with a curette without making contact with the marginal gingiva in order to prevent the microcapillary pipette from being clogged or contaminated. The gingival crevice fluid (GCF) was collected using a calibrated 1–5 l volumetric optimised black color-coded microcapillary pipette (Sigma Aldrich, USA [Catalog No. p0549]). This was done by inserting the tip of the pipette at the entrance of an unstimulated gingival crevice for 5–20 minutes. The amount of GCF that was collected was 3 l, which is a normal volume. [5,13]

Because it was difficult to collect sufficient GCF from periodontally healthy individuals, samples were pooled from different locations in order to get the minimum needed amount (3 l) from each patient. This allowed adequate GCF to be obtained. The collection of gingival crevicular fluid (GCF) was shown to be easier in instances of chronic periodontitis since the needed quantity could be taken from the region with the greatest PPD. The samples that were found to be contaminated with blood or saliva, as well as those that included air bubbles, were thrown out, and the remaining samples were frozen at a temperature of 80 degrees Celsius until the time of the analysis.

A collection of blood

After disinfecting the skin above the antecubital fossa, three millilitres of blood were drawn from a vein using a syringe with a capacity of five millilitres and a needle of twenty-gauge. After collecting the blood sample, it was left to coagulate at room temperature for around half an hour.

Serum preparation

After that, the specimen was centrifuged at a speed of 3000 rpm for ten minutes in order to separate the serum part. As a result, the component of the serum that had been isolated was placed in a plastic vial and kept at a temperature of 80 degrees Celsius until the time of the assay .[8]

Test principle

When measuring the amount of human DPD present in samples, the kit carried out an enzyme-linked immunosorbent test (ELISA) in the form of a double-antibody sandwich.

For the statistical study, we made use of SPSS version 20.0, which was developed by IBM (a firm based in the United States). The Shapiro–Wilk test was performed to determine whether or not the data followed a normal distribution, and it was discovered that the data did. Because of this, the student's t test was utilised for the purposes of comparison and analysis. The Pearson's correlation coefficient test was utilised to investigate the degree to which HDPD concentrations in blood and GCF were correlated with age, MGI, PI, BI, PPD, and CAL. The data were given with the mean value together with the standard deviation.

eISSN 1303-5150

RESULTS

The demographics, clinical parameters, serum, and GCF DPD levels are summarized in Table 1. Table 1 Intergroup comparison of demographic data, clinical parameters, and Deoxypyridinoline concentration in gingival crevicular fluid and serum

	Group, mean±SD		***P***
	Control	**Test**	
Age (years)	41.1±6.54	40.7±5.8	0.853 (NS)
Sex (%)			
Female	8 (46.7)	7 (53.3)	
Male	7 (53.3)	8 (46.7)	
Overall PPD (mm)	2.03±0.4	6.75±0.41	≤0.001 (S)
Overall CAL (mm)	0%	7.0±0.38	≤0.001 (S)
MGI	0.68±0.16	2.41±0.52	≤0.001 (S)
PI	0.62±0.2	1.76±0.35	≤0.001 (S)
BI	0.5±0.2	1.42±0.31	≤0.001 (S)
Serum DPD (nmol/L)	0.37±0.42	0.52±0.42	0.234 (NS)
GCF DPD (nmol/L)	4.71±1.84	11.5±3.73	≤0.001 (S)

The patients who were periodontally healthy had a mean age of 41.113 6.54 years, while the patients who had chronic periodontitis had a mean age of 41.2 7.84 years. In the A Group (the control), there were eight men (53.3%), and there were seven females (46.7%). In the B Group, there were seven males (46.7%), and there were eight females (53.3%). (case). There was no statistically significant difference found between the A Group and the B Group's mean ages.

The comparison of clinical indicators between the different groups, including the mean MGI, Saxton BI, PI, PPD, and

Deoxypyridinoline concentration

[Graph 2] found that DPD concentrations were measurable in the serum and GCF levels of all patients, regardless of whether they were in Group A or Group B. [Graph 3] shows that the maximum levels of DPD in the serum of patients in Group A were 1.05 nmol/L, whereas the highest levels of DPD in the serum of patients in Group B were 1.128 nmol/L. The mean serum concentration of DPD in Group A was 0.37 0.42 nmol/L, while the mean serum concentration of DPD in Group B was 0.52 0.42 nmol/L. An intergroup comparison of the mean serum DPD levels of Group A and Group B reveals that there is no statistical difference between the two groups [Table 2]. While the highest DPD levels in GCF of healthy persons was 6.03 nmol/L and 18.36 nmol/L in the B Group, the mean DPD GCF concentration in Group A patients was 4.71 1.84 nmol/L and 11.5 3.73 nmol/L in Group B. The DPD GCF concentration in healthy individuals was 6.03 nmol/L. There is not a statistically significant difference seen when GCF DPD levels from Group A and Group B are compared with one another across groups as shown in [Table 2].

eISSN 1303-5150

Table 2

Details of Deoxypyridinoline concentration in Group A and B

Group	Mean±SD	Minimum-Maximum
A		
Serum (nmol/L)	0.37±0.42	0.022-1.05
GCF (nmol/L)	4.71±1.84	1.25-6.03
B		
Serum (nmol/L)	0.52±0.42	0.52-1.128
GCF (nmol/L)	11.5±3.73	7.5-18.36

Correlation between serum and gingival crevicular fluid deoxypyridinoline levels and clinical parameters

According to the results of a Pearson correlation analysis, the concentrations of serum and GCF DPD, as well as age and gender, did not have a statistically significant relationship between Group A and Group B [Table 3]. In Group B, the PPD, CAL, MGI, BI, and PI concentrations with serum DPD and GCF DPD concentration were not found to have an association that was statistically significant [Table 4]. This was determined by using the Pearson correlation analysis.

Table 3

Correlations between serum and gingival crevicular fluid, deoxypyridinoline concentration with age and gender in the Groups A and B using Pearson correlation

With age		
Group Age	**Serum DPD**	**GCF DPD**
A		
Correlation coefficient	−0.208	0.106
P	0.45 (NS)	0.70 (NS)
B		
Correlation coefficient	0.311	0.654
P	0.26 (NS)	0.008 (NS)

Table 4

Correlation between serum and gingival crevicular fluid deoxypyridinoline concentration with clinical parameters of Group B using Pearson correlation

	Mean		MGI	PI	BI
	PPD	CAL			
Serum DPD					
Correlation coefficient	0.159	0.082	−0.246	−0.236	0.054
P	0.57 (NS)	0.77 (NS)	0.38 (NS)	0.85 (NS)	0.4 (NS)
GCF DPD					
Correlation coefficient	0.086	0.058 (NS)	−0.076	−0.236	−0.063
P	0.76	0.84 (NS)	0.79	0.38 (NS)	0.82 (NS)

With gender			
Group	**Gender, mean±SD**		***P***
	Female	**Male**	

eISSN 1303-5150

A			
Serum DPD	0.47±0.46	0.28±0.38	0.17 (NS)
GCF DPD	5.44±1.61	4.07±1.9	0.16 (NS)
B			
Serum DPD	0.54±0.41	0.49±0.47	0.17 (NS)
GCF DPD	12.04±4.83	11.8±2.74	0.91 (NS)

DISCUSSION

Periodontal disease is a persistent microbial infection that causes inflammation, has an effect on the immune system, and ultimately leads to the breakdown of alveolar bone. The body of evidence supporting the interaction between the immune system and the skeletal system has been bolstered. Immune response cytokines including tumour necrosis factor-alpha (TNF-a), interleukins (ILs), and interferon inhibit the activity and development of osteoclasts, which ultimately leads to bone resorption [5,14,15].

The current study is thought to be the first of its type to examine the concentrations of DPD in blood and GCF in periodontal disease and health, as well as to assess the influence of periodontal damage on DPD levels. This is to the best of our knowledge, but we cannot be certain of this.

The current investigation discovered that the mean concentration of DPD levels had a substantially higher value in Group B (11.5 nmol/L) compared to Group A (4.21 nmol/L). This difference was statistically significant. These findings are consistent with the value that was reported by Dharmayanti, according to which the group suffering from periodontitis exhibited considerably greater levels of GCF DPD than the healthy group. Other investigations, such as I-carboxy telopeptide pyridinoline (ICTP), osteopontin, and osteocalcin, all assessed various bone markers, and their findings are in agreement with the findings of the current research. [16,17]

Initial steps in the progression of chronic periodontitis are triggered by the presence of lipopolysaccharides. At active periodontal destruction sites, activated macrophages and monocytes respond by releasing cytokines such as tumour necrosis factor alpha (TNF), interleukin-1 (IL-1), and receptor activator of nuclear factor-kappa B ligand. These cytokines are responsible for mediating osteoclastogenesis and bone breakdown. Bone-specific indicators such as ICTP and DPD are released into the surrounding environment and transferred to the sulcus or pocket by GCF. This process, which acts as a possible biomarker for periodontal disease, is called resorption. Therefore, local DPD levels were reported to be raised in the gingival crevicular fluid of individuals with periodontitis [16,18,19].

There was not a statistically significant difference identified between the two groups. The mean serum concentration of DPD in Group A was 0.37 nmol/L, whereas the mean serum concentration in Group B was 0.52 nmol/L. This suggests that the GCF is a localised site-specific potent inflammatory exudate fluid that seeps out into the sulcus or periodontal pockets and that it is a more reliable method to detect the DPD levels in comparison to serum, which is a diluted fluid that is present throughout the body. In addition, the GCF seeps out into the sulcus or periodontal pockets.

eISSN 1303-5150

During the course of this research project, DPD was also discovered in the GCF and serum of healthy participants. Because bone is a dynamic tissue that is always being remodelled and experiences bone turnover during the course of a person's lifetime, physiological bone metabolism may be the cause of the problem .[20]

In the current investigation, neither age nor gender exhibited a statistically significant link with levels of DPD in either of the two groups. It is possible that the individuals who participated in the research all had generally good health and did not show any signs of bone or collagen-related inflammatory alterations elsewhere, both of which would have had an effect on DPD levels. This is the most likely explanation. Another possible explanation for the absence of a correlation between illness and blood levels is the very small sample size, which consisted of only thirty individuals. It is possible that the impact of chronic periodontitis on DPD levels can be better understood with the help of additional research using bigger sample sizes.

In the current investigation, a rise in disease severity as defined by PPD and CAL did not correlate with the DPD levels both in GCF and serum in the chronic periodontitis group. This was shown to be the case in the group that had periodontitis for a longer length of time. In contrast to these findings, Dharmayanti Augustin[17] (2012) revealed that the levels of GCF DPD strongly linked with the severity of periodontal disease. This contradicts the findings presented here. However, it was also proposed earlier (1984)[21] that disease activity is not a continuous process but rather consists of phases of active disease state and a remission state. It has been generally accepted that an increase in pocket depth and a concomitant increase in the total amount of biomarker in the disease. [13] However, it was also proposed that disease activity is not a continuous process. In the event that a sample were to have been taken from a location that was in a state of remission but otherwise would have been active earlier, the levels would not have been greater. It is sufficient to say that in the current study, the DPD levels did not correlate with the increase in PPD/CAL, and this aspect requires further investigation with more samples of patients having varying degrees of active and inactive sites. In spite of the contradictory opinions, it is sufficient to say that the present study found that.

The third possible explanation for the lack of a correlation in this study is that the sample size was rather small, the illness development was not standardised, and the sample was collected from a single site. Furthermore, it can be explained based on the fact that GCF collection in periodontitis sites may not yield comparable volumes of GCF as a result of the difference in the rate of GCF production and flow based on the inflammatory status of the periodontium. This difference can be explained based on the fact that GCF collection in healthy sites may yield comparable volumes of GCF. In addition, there was no effort made to analyse DPD levels both before and after the therapy in order to ascertain whether or not a reduction in PPD and CAL would ultimately result in a reduction in DPD levels. [13,21]

It is important to keep in mind the findings of Loos and Tjoa, who examined almost one hundred different components as indicators of GCF in terms of the potential value they may bring to the process of diagnosing a case of periodontitis. They argued that the identification of a single diagnostic marker for all forms of periodontal disease appears to be misleading. [22] Although there are some advantages of these marker systems over

eISSN 1303-5150

conventional diagnosis, only a few of them may be capable of detecting or predicting periodontal disease activity. However, there are certain limitations to consider, such as selecting the best relevant biomarker and determining whether or not it is cost-effective .[23]

Estimating the levels of GCF DPD might be beneficial in recognising the early stages of periodontal tissue degradation in individuals who do not have clinically evident attachment loss. This is because it is difficult to diagnose early periodontitis in people who do not have this condition .[24]

CONCLUSION

The results of this investigation led the researchers to the following conclusions:

1. When compared to the periodontally healthy group, the GCF DPD levels in patients with chronic periodontitis were considerably greater.

2. In the chronic periodontitis group, an increase in the periodontal disease severity, as measured by PPD and CAL, did not demonstrate a significant connection with the GCF and serum DPD levels.

When the findings of the current study and those of earlier studies are combined, one could reach the conclusion that additional studies, both interventional and cross-sectional, with larger sample sizes are required to evaluate the GCF DPD levels in chronic periodontitis and gauge it as a potential biomarker for alveolar bone loss. This conclusion could be reached by combining the findings of the current study with those of earlier studies.

REFERENCES

1. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: Current concepts. *J Periodontol.* 1992;63:322–31. [PubMed] [Google Scholar]
2. Genco RJ. Host responses in periodontal diseases: Current concepts. *J Periodontol.* 1992;63:338–55. [PubMed] [Google Scholar]
3. Darveau RP. Periodontitis: A polymicrobial disruption of host homeostasis. *Nat Rev Microbiol.* 2010;8:481–90. [PubMed] [Google Scholar]
4. Taba M, Jr, Kinney J, Kim AS, Giannobile WV. Diagnostic biomarkers for oral and periodontal diseases. *Dent Clin North Am.* 2005;49:551–71, vi. [PMC free article] [PubMed] [Google Scholar]
5. Champagne CM, Buchanan W, Reddy MS, Preisser JS, Beck JD, Offenbacher S. Potential for gingival crevice fluid measures as predictors of risk for periodontal diseases. *Periodontol 2000.* 2003;31:167–80. [PubMed] [Google Scholar]
6. Ram VS, Parthiban US, Sudhakar U, Mithradas N, Prabhakar R. Bone biomarkers in periodontal disease. A Review article. *J Clin and Diag Res.* 2015;9:7–10. [PMC free article] [PubMed] [Google Scholar]
7. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clin Pharmacol Ther.* 2001;69:89–95. [PubMed] [Google Scholar]
8. Pussinen PJ, Paju S, Mäntylä P, Sorsa T. Serum microbial- and host-derived markers of periodontal diseases: A review. *Curr Med Chem.* 2007;14:2402–12. [PubMed] [Google Scholar]
9. Silness J, Loe H. Periodontal disease in pregnancy. II Correlation between oral hygiene and periodontal condtion. *Acta Odontol Scand.* 1964;22:121–35. [PubMed] [Google Scholar]
10. Lobene RR, Weatherford T, Ross NM, Lamm RA, Menaker L. A modified gingival

eISSN 1303-5150

index for use in clinical trials. *Clin Prev Dent.* 1986;8:3–6. [PubMed] [Google Scholar]
11. Saxton CA, van der Ouderaa FJ. The effect of a dentifrice containing zinc citrate and Triclosan on developing gingivitis. *J Periodontal Res.* 1989;24:75–80. [PubMed] [Google Scholar]
12. Yoneyama T, Okamoto H, Lindhe J, Socransky SS, Haffajee AD. Probing depth, attachment loss and gingival recession. Findings from a clinical examination in Ushiku, Japan. *J Clin Periodontol.* 1988;15:581–91. [PubMed] [Google Scholar]
13. Barros SP, Williams R, Offenbacher S, Morelli T. Gingival crevicular fluid as a source of biomarkers for periodontitis. *Periodontol 2000.* 2016;70:53–64. [PMC free article] [PubMed] [Google Scholar]
14. Takayanagi H. Osteoimmunology: Shared mechanisms and crosstalk between the immune and bone systems. *Nat Rev Immunol.* 2007;7:292–304. [PubMed] [Google Scholar]
15. Persson GR. Site-based versus subject-based periodontal diagnosis. *Periodontol 2000.* 2005;39:145–63. [PubMed] [Google Scholar]
16. Sharma CG, Pradeep AR. Gingival crevicular fluid osteopontin levels in periodontal health and disease. *J Periodontol.* 2006;77:1674–80. [PubMed] [Google Scholar]
17. Dharmayanti AW. Deoxypyridinoline level in gingival crevicular fluid as alveolar bone loss biomarker in periodontal disease. *Dent J.* 2012;45:102–6. [Google Scholar]
18. Talonpoika JT, Hämäläinen MM. Type I collagen carboxyterminal telopeptide in human gingival crevicular fluid in different clinical conditions and after periodontal treatment. *J Clin Periodontol.* 1994;21:320–6. [PubMed] [Google Scholar]
19. Lee AJ, Walsh TF, Hodges SJ, Rawlinson A. Gingival crevicular fluid osteocalcin in adult periodontitis. *J Clin Periodontol.* 1999;26:252–6. [PubMed] [Google Scholar]
20. Shetty S, Kapoor N, Bondu JD, Thomas N, Paul TV. Bone turnover markers: Emerging tool in the management of osteoporosis. *Indian J Endocrinol Metab.* 2016;20:846–52. [PMC free article] [PubMed] [Google Scholar]
21. Socransky SS, Haffajee AD, Goodson JM, Lindhe J. New concepts of destructive periodontal disease. *J Clin Periodontol.* 1984;11:21–32. [PubMed] [Google Scholar]
22. Loos BG, Tjoa S. Host-derived diagnostic markers for periodontitis: Do they exist in gingival crevice fluid? *Periodontol 2000.* 2005;39:53–72. [PubMed] [Google Scholar]
23. Reddy S, Kaul S, Prasad MG, Agnihotri J, Asutkar H, Bhowmik N. Biomarkers in Periodontal Diagnosis: "What The Future Holds. *Int J Clin Dent Sci.* 2011;2:76–83. [Google Scholar]
24. Khiste SV, Ranganath V, Nichani AS, Rajani V. Critical analysis of biomarkers in the current periodontal practice. *J Indian Soc Periodontol.* 2011;15:104–10. [PMC free article] [PubMed] [Google Scholar]

eISSN 1303-5150