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Evaluating the role of inflammatory biomarkers as a diagnostic tool in peri-implantitis

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ABSTRACT

Background: Most studies have analyzed the presence of cytokines in Gingival crevicular fluid (GCF) in different periodontal diseases, but very limited studies have been conducted on Peri-implant crevicular fluid (PICF) in peri-implantitis.

Aim: The present study was conceptualized to explore the levels of Interleukin (IL)-1β and Interleukin (IL)-6 as a diagnostic marker in peri-implantitis.

Materials and methods: A total of 40 patients each having at least one dental implant were enrolled in the study. Clinical parameters were recorded around each implant and tooth nearest to it. Gingival crevicular fluid (GCF)/ Peri-implant crevicular fluid (PICF) was collected to evaluate the concentration of IL-1β and IL-6. Conservative treatment was performed in peri-implantitis cases, 3 months after treatment their clinical parameters, IL-1β and IL-6 levels were recorded and compared with their pre-treatment values.

Results: Clinical parameters like Modified Plaque Index (MPI), Modified Bleeding Index (MBI) and Probing Pocket Depth (PPD) were statistically significantly higher in the peri-implantitis group as compared to the healthy implant group and healthy teeth group. IL-1β and IL-6 levels were also statistically significantly higher in the peri-implantitis group in comparison to healthy implants and healthy teeth group.

Conclusion: The study concludes that biomarkers in PICF can be used as a diagnostic tool to supplement the diagnosis of peri-implantitis along with the use of clinical parameters to make an early diagnosis of peri-implantitis possible.

1. Introduction

The ultimate goal of dental therapy is to restore normal function, comfort, esthetics, speech, and overall health of patients which is affected by the loss of teeth. A few decades back, the fabrication of removable and fixed prosthesis was the sole treatment modality for these conditions. However, dental implants have now become an indispensable tool for oral rehabilitation as high survival rates of 82.9 % have been reported even after a follow-up period of 16 years.[1] Implant prosthesis allows normal muscle functions and maintains the dimensions in a manner similar to natural teeth.

However, in the last decade, there has been an increase in the number of reported cases of peri-implant inflammation affecting both soft and hard tissues, ultimately leading to implant failure. Similar to gingivitis and periodontitis affecting the periodontium of natural teeth, peri-implant mucositis and peri-implantitis terms are given for inflammation and destruction of peri-implant soft and hard tissues respectively.[2]

Diagnosis of peri-implantitis still largely relies on conventional diagnostic parameters which mainly include mobility, Bleeding on probing (BOP), PPD, and bone loss. The main drawback of the above clinical diagnostic parameters is their lack of sufficient sensitivity and specificity for early diagnosis of peri-implant tissue destruction. As a result, proper management cannot be initiated until significant supporting bone is lost, ultimately leading to implant failure, which has a drastic impact on patients who have invested time, trust, and money in implant rehabilitation.

Various biomarkers present in GCF and PICF which are released following bone destruction and inflammation, can serve as specific and sensitive parameters for early detection of peri-implantitis, so that

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Table 1
Intergroup comparison of various parameters among the groups.

Parameter	Group I (N = 20)	Group II (N = 20)	Group III (N = 20)	F - value	p-value
Bleeding Indices Values (Mean ± SD)	1.51 ± 0.211	2.23 ± 0.172	1.62 ± 0.116	104.74	**<0.001**
Plaque Indices Values (Mean ± SD)	1.62 ± 0.159	2.35 ± 0.201	1.72 ± 0.110	122.12	**<0.001**
PPD (Mean ± SD)	3.67 ± 0.259	5.14 ± 0.264	3.75 ± 0.124	269.63	**<0.001**
IL-1β (pg/ml) (Mean ± SD)	71.39 ± 15.352	213.2 ± 41.713	85.29 ± 12.065	172.85	**<0.001**
IL-6 (pg/ml) (Mean ± SD)	14.33 ± 2.286	23.15 ± 2.844	15.46 ± 1.514	88.58	**<0.001**

SD- Standard deviation, PPD- Probing pocket depth, IL- Interleukin, pg-picogram, ml-milliliter, value of $p < 0.05$ considered statistically significant.

Table 2
Pre to Post Treat Changes in Clinical Parameters in Group – II according to PPD status.

Parameter	Time	Mean	SD	Mean Diff.	t-value	p-value
PPD <5 (N = 10)						
Bleeding indices	Pre.	2.15	0.14	0.11	4.25	**0.005**
	Post.	2.04	0.11			
Plaque indices	Pre.	2.25	0.16	0.24	3.50	**0.013**
	Post.	2.02	0.13			
PPD	Pre.	4.91	0.04	0.12	3.07	**0.022**
	Post.	4.78	0.09			
IL-1β (pg/ml)	Pre.	182.57	10.23	10.83	2.72	**0.034**
	Post.	171.74	9.03			
IL-6 (pg/ml)	Pre.	20.90	1.60	1.75	2.55	**0.044**
	Post.	19.14	1.24			
PPD ≥ 5 (N = 10)						
Bleeding indices	Pre.	2.28	0.18	0.06	2.64	**0.022**
	Post.	2.22	0.16			
Plaque indices	Pre.	2.41	0.20	0.13	2.63	**0.022**
	Post.	2.27	0.13			
PD	Pre.	5.27	0.25	0.15	1.90	0.082
	Post.	5.12	0.09			
IL-1 β (pg/ml)	Pre.	229.69	43.14	11.81	1.94	0.077
	Post.	217.88	33.42			
IL-6 (pg/ml)	Pre.	24.36	2.64	0.70	1.44	0.175
	Post.	23.66	1.98			

SD- Standard deviation, PPD- Probing pocket depth, IL- Interleukin, pg-picogram, ml-milliliter, value of $p < 0.05$ considered statistically significant.

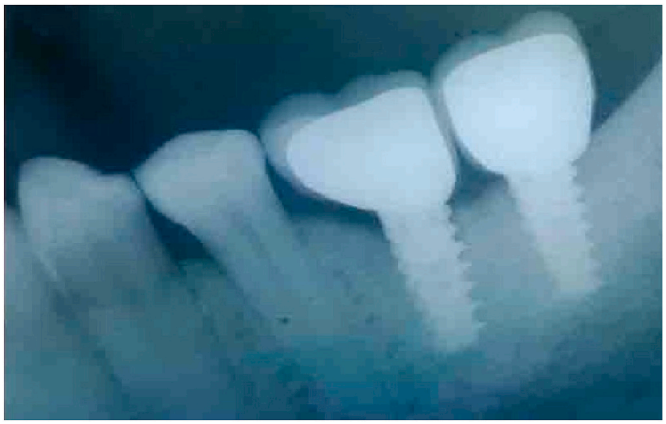

Fig. 1. IOPA of healthy implant case.

proper management can be initiated much before considerable peri-implant tissue destruction has occurred, thus preventing implant failure. Biomarkers can also aid in monitoring the progress of various treatment therapies in peri-implantitis. Among various biomarkers, cytokines have gained more attention in the medical research field. Interleukin (IL) - 1β and Interleukin (IL)-6 are one of the most potent cytokines to be involved in the pathogenesis of inflammation. Effectiveness at low concentrations and the transient nature of production is a characteristic feature of IL-1β and IL-6.

A large number of studies have been conducted in the past to analyze

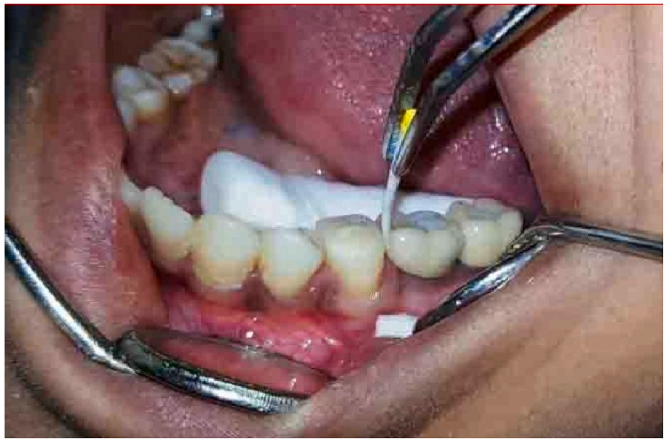

Fig. 2. Pooled PICF collection from healthy implant site.

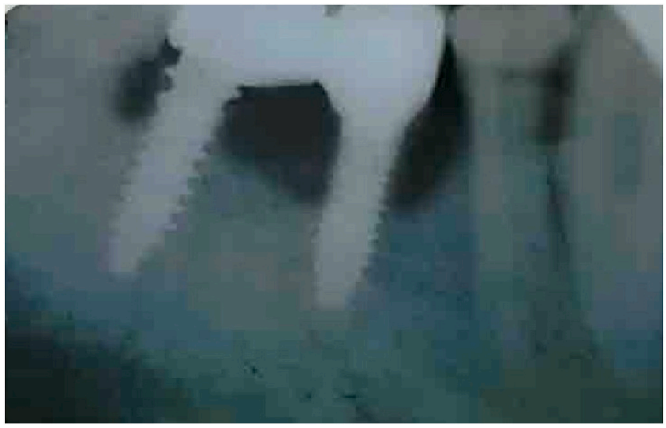

Fig. 3. IOPA of implant suffering from peri-implantitis.

the presence of cytokines specially Interleukins (IL) in GCF in periodontal diseases, which has established their potential role as a diagnostic tool in periodontitis and also to monitor the progress of various treatment modalities performed in periodontitis cases. However, very limited studies have been conducted to analyze PICF around implants with peri-implantitis. Therefore, the present study was designed to compare clinical parameters and biomarker levels in healthy implants, implants with peri-implantitis, and natural healthy tooth nearest to the peri-implantitis site. Additionally, a comparison was made between these parameters in peri-implantitis cases before and after performing conservative therapy at the peri-implantitis site.

1.1. Materials and methodology

The sample size was determined by referencing a study conducted by Song Z et al. (2019).[3] The variations in IL-6 and C-reactive protein (CRP), the inflammatory biomarkers used across the three study groups

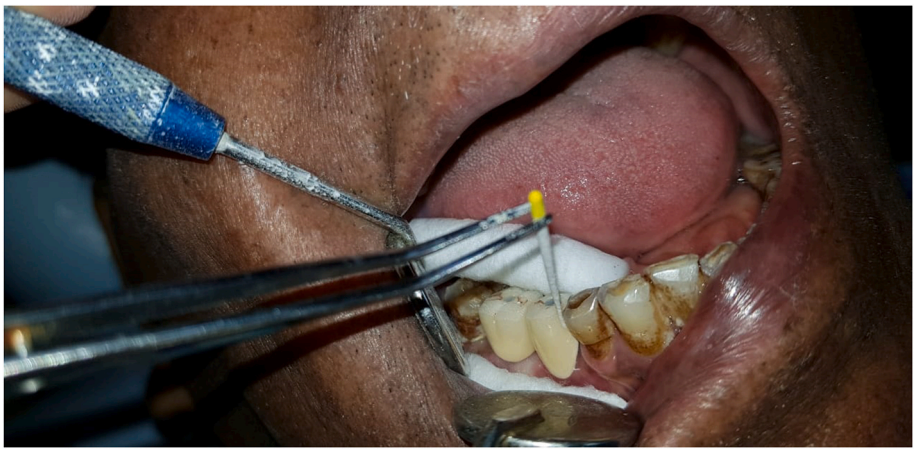

Fig. 4. Pooled PICF collection from peri-implantitis site.

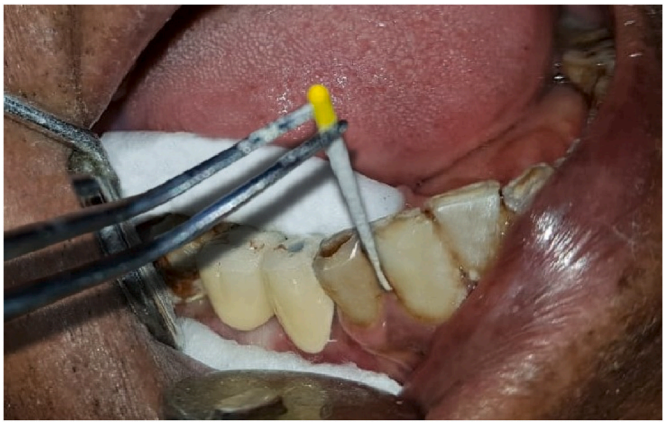

Fig. 5. Pooled GCF collection from healthy teeth nearest to peri-implantitis site.

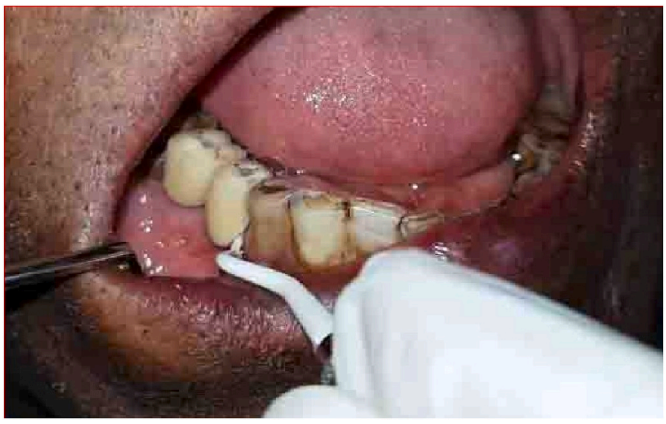

Fig. 6. Performing conservative therapy in peri-implantitis case.

in the above mentioned study, were applied in the following formula:

$$N = \frac{(z\alpha/2 + z\beta/2)\ (\sigma_1{}^2 + \sigma_2{}^2)}{d^2}$$

Where:
$\sigma_{1=}$ SD of IL-6 and CRP value in peri-implant group
$\sigma 2$ = SD of IL-6 and CRP value in healthy group
d = Max ($\sigma_{1,}$ $\sigma 2$) (margin of error)

Type 1 error (α) = 5 %
Type 2 error (β) = 10 %

Taking into account a 95 % confidence level and 90 % study power, the sample size was calculated separately for the CRP and IL-6 groups. The higher value between the two was chosen for the sample size in our study. Therefore, the final sample size for each respective group was determined at 20.

Institutional ethical committee permission (Ref. no. 89th ECM II-B Thesis/P88) and patient consent were taken prior to the commencement of the study. A total of 68 patients who reported to the Out-Patient Department (OPD) of, Department of Periodontology and Department of Prosthodontics, who were having at least one implant-retained prosthesis in function for at least 6 months, were screened for enrollment into the study. Among the patients who were screened, patients suffering from systemic diseases which affect the healing process, patients who were current smoker or smoker over the past year, pregnant and lactating women were excluded from the study.

Of these, 46 patients were selected for the study, out of which 6 patients dropped out of the study due to various reasons. The remaining 40 patients (26 male + 14 female) with an age range from 25 to 56 years were enrolled in the study and three groups were formed. Group I represents the healthy implant group, Group II represents the peri-implantitis group, while Group III comprises healthy teeth nearest to the peri-implantitis site of patients belonging to Group II. The reason for framing Group III was to scrutinize the site-specific action of cytokines in peri-implantitis.

1.2. Inclusion parameters

Healthy Implant group inclusion parameters: (1) Age ≥18 years (2) Modified bleeding Index ≤2.0 (3) Modified plaque Index ≤2.0 (4) Probing depth ≤ 4 mm (5) No sign of exposure of Implant thread in the radiograph.

Peri-implantitis group inclusion parameters: (1) Patient age ≥18 years (2) Modified bleeding Index ≥2.0 (3) Modified plaque Index ≥2.0 (4) Probing depth ≥ 4 mm (5) Exposure of at least 2 Implant Threads in the radiograph.

Healthy teeth group inclusion parameters: (1) Bleeding Index ≤2.0 (2) Plaque Index ≤2.0 (3) Probing depth ≤3 mm.

1.3. Clinical parameters assessed

Modified Plaque Index (MPI),[4] Modified Sulcus Bleeding Index (MBI),[4] Probing Pocket Depth (PPD) were recorded at baseline for Group I and Group II, while Plaque Index (PI),[5] Gingival Index (GI)[6] and PPD were recorded at baseline for Group III, at each surface by using UNC 15 periodontal probe (PCP UNC 15; Hu-Friedy Manufacturing Co., Chicago,

IL, USA)

The same investigator assessed all clinical parameters during various recall visits. Calibration training was conducted over consecutive days, during which 10 volunteers were examined. Examinations were repeated until satisfactory consistency was attained, as indicated by an intra-class correlation coefficient of 0.80.

1.4. Biochemical parameters assessed

1. IL-1β
2. IL-6

1.5. Clinical procedures

At baseline, the selected site (mesial to teeth/implant) was dried with a gentle stream of compressed air. Absorbent cotton rolls were used to maintain isolation during the PICF/GCF collection. 3–4 Paper points (Sure-Endo, size #20, 6 % taper) were gently placed in the pocket till slight resistance was felt. They were held in site for 30 s. Paper points soaked in blood or saliva were excluded. After pooled PICF/GCF collection, paper points were transferred in a sterilized micro-centrifuge tube (Eppendorf tube) containing 500 μl of phosphate buffer saline (PBS) at 4 °C. Then these tubes were immediately transported to the Department of Biochemistry where they were stored at −80 °C until analysis to minimize biomarker reactivity loss. On the day of analysis, Eppendorf tubes containing sample solution were taken out from at −80 °C, paper points were removed and sample solutions were centrifuged at 10000 rpm for 15 min at 4 °C. Prepared PICF/GCF supernatant samples were evaluated for different biochemical parameters using the ELISA method.

Now for the group comprising patients suffering from peri-implantitis i.e. group II, conservative therapy using a plastic tip curette (Hu-Friedy Manufacturing Co., Chicago, IL, USA) was performed at the peri-implantitis site. Along with that oral hygiene instructions which include rinses with chlorhexidine gluconate mouthwash (0.2 % w/v) for two weeks was given. This conservative therapy procedure was again performed after 1 month. Following conservative therapy, recording of clinical parameters and sample collection for immunological analysis was again performed after 3 months post conservative therapy in group II patients.

The gingival crevicular fluid was analyzed for IL-1β and IL-6 using commercially available ELISA kits (USCN Business Co., Ltd, Hubei, China). All ELISA procedures were carried out according to the manufacturer's instructions. The ELISA plates were then assessed spectrophotometrically at an optical density of 450 nm. Results were reported as the total amount of IL-1β and IL-6 (pg± SD) per 30 s. This was expressed as pg/sample.

1.6. Statistical tools employed

Data are presented as means and Standard Deviation (SD). One-way ANOVA was used for the comparison of Bleeding indices, Plaque indices, PPD, IL-1β, and IL-6 levels in three groups of patients. Tukey HSD test was used to perform paired comparisons. Data were analyzed using SPSS version 20.0 (IBM Co., Armonk, NY, USA). p-value less than 0.05 was considered statistically significant.

2. Results

The patients were evenly distributed between males and females and the mean age of selected patients for group I was 38.00 ± 9.60 years, while the mean age of selected patients for group II was 39.30 ± 8.35 years and the same patients were selected for group III.

On comparing the mean values of clinical parameters like bleeding indices, plaque indices, and PPD between the three groups, it was found that the mean values of all three were maximum in group II and minimum in group I. A highly significant difference was found in mean plaque indices values among the three groups ($p < 0.001$) (Table 1).

On comparing the mean of IL-1β and IL-6 values between the three groups, it was found that the IL-1β and IL-6 were maximum in group II and minimum in group I. A highly significant difference was found in mean IL-1β and IL-6 values among the three groups ($p < 0.001$) (Table 1).

Among the cases with PPD <5, reduction in pretreatment IL-1β and IL-6 mean values was found to be significant ($p < 0.05$). While in cases with PPD ≥5, reduction in pretreatment IL-1β and IL-6 mean values were found to be non-significant ($p > 0.05$) (Table 2).

3. Discussion

In the recent era, implant placement has become a revolutionizing treatment modality for the rehabilitation of missing teeth. However, in the last decade, there has been an exponential increase in the incidence of implant-related complications which ultimately lead to the failure of implants. The most common implant-related complications include inflammation of the peri-implant soft and hard tissues, referred to as peri-implant mucositis and peri-implantitis, which are analogous to gingivitis and periodontitis, respectively.

The pathogenesis of inflammation of peri-implant tissues is similar to those seen in natural teeth during gingivitis and periodontitis. Shortly after implant placement, the exposed titanium surface becomes covered by salivary glycoprotein along with microbial colonization, which consists mainly of gram-negative bacteria and motile rods.[7,8] Due to the release of chemotactic peptides and damage of epithelial cells by bacterial population, leucocytes (mainly neutrophils) get accumulated in the peri-implant pocket. These neutrophils phagocytose the bacteria which leads to the elimination of bacteria from the pocket. However, in cases of progressive microbial plaque accumulation, neutrophils and the epithelial barrier fail to contain the infection, resulting in inflammation of the peri-implant tissues, clinically diagnosed as peri-implant mucositis. Spreading of this inflammation from marginal gingiva into supporting tissues around the peri-implant region resulting in bone destruction is clinically termed peri-implantitis.[9]

The interaction between periodontopathogenic bacteria and a dysregulated host immune response to the antigen leads to the intense production of proinflammatory mediators, such as IL-1β, IL-6, and matrix metalloproteinases (MMP), alongside a reduced production of anti-inflammatory mediators like IL-10, Transforming growth factor β1.[10] Seymour et al. proposed that a patient's susceptibility to disease progression is determined by a shift in the lymphocyte population in the inflammatory infiltrate, from predominantly T-cells in mucositis to an increased proportion of B-cells in peri-implantitis.[11]

Among various biomarkers, cytokines are the inflammatory mediators that have grabbed most of the attention in the field of medical research due to their vital role in the initiation and progression of several autoimmune, infectious, and inflammatory diseases. Cytokines are soluble proteins secreted by the cells of adaptive and innate immunity. They bind to specific receptors on target cells and initiate intracellular signaling cascades which via altered gene regulation results in phenotypic changes in cells. This soluble mediator of immune reaction is present in saliva, GCF/PICF in the oral cavity, and is produced by the physiological interaction of gingival epithelium and local leukocytes with dental plaque and oral microorganisms.[12,13]

To date, diagnosis of peri-implantitis is primarily based on a combination of clinical and radiographic parameters like PPD, BOP, suppuration, mobility, and bone loss. The major disadvantage of these parameters is that they lack the sensitivity or specificity enough to distinguish disease onset, activity, and risk rate. Early detection of peri-implant tissue destruction, as well as its monitoring, is extremely important for implant survival, but the use of these clinical and radiographic parameters lacks this ability.[14]

Various biomarkers present in PICF which are released following

bone destruction and inflammation can serve as specific and sensitive parameters for early and prompt detection of peri-implantitis so that proper management can be initiated much before considerable peri-implant tissue destruction has occurred.

Many studies have been conducted in the past to analyze the presence of cytokines in GCF in periodontal diseases, but very limited studies have been conducted to analyze PICF around implants with peri-implantitis. The present study was designed to compare clinical parameters and biochemical parameters in healthy implants, implants with peri-implantitis, and healthy tooth nearest to the implant suffering from peri-implantitis so that perspectives of new diagnostic strategies can be looked into. Among biochemical parameters, IL-1β, and IL-6 were used as a diagnostic tool in this study since their role in inflammation is established as discussed previously. The levels of IL-1β and IL-6 in GCF/PICF were compared among healthy implants, implants with peri-implantitis, and healthy teeth nearest to the peri-implantitis site, and their correlation with clinical parameters was also taken into consideration.

Plaque has been demonstrated as the primary etiologic factor in peri-implant tissue destruction as observed with the natural dentition.[15] Assessment of plaque accumulation is an effective parameter for monitoring peri-implant health and it was the only index observed that positively correlated with the histological changes.[16] While comparing group-wise, a significant difference was found between group I & group II ($p < 0.001$) and between groups II & III ($p < 0.001$). The difference between groups I & III was not found to be significant ($p = 0.095$). Similar results were obtained by Al-Askar M et al. (2018),[17] with significantly higher ($p < 0.001$) plaque indices values in patients with peri-implantitis than in those without peri-implantitis.

Smithloff and Fritz assessed that BOP occurred concurrently with other signs of implant failure, such as increased PPD and radiographic bone loss. They also pointed out that BOP and radiographic changes were the most valid indicators of peri-implant breakdown.[18] In the bi-comparison of the groups, a significant difference was found between groups I & II ($p < 0.001$) and between groups II &III ($p < 0.001$), but the difference between groups I & III was not found to be significant ($p = 0.105$). These results are in conjunction with the findings of the study conducted by Rakic et al. (2013).[19]

Probing measurements aid in the accuracy and variability of comprehensive peri-implant assessment.[20] The principal resistance to probe tip penetration in clinically healthy peri-implant tissue is the connective tissue fibers attached to the implant, while in inflamed tissue the probe tip penetrates up to the bone level.[21] On doing group-wise bi-comparison, it was observed that a highly significant difference was found between groups I & II ($p < 0.001$) and between groups II & III ($p < 0.001$) but the difference between groups I & III was not found to be significant ($p = 0.508$). These findings are supported by results obtained by Wang et al. (2015).[22]

PICF/GCF was collected at baseline from all three groups using absorbent paper points. In group II patients it was recollected after 3 months interval period following conservative therapy. All the samples were then stored at −80 °C. After collecting all samples, levels of IL-1β and IL-6 were detected by ELISA.

On comparing IL-1β values between various group pairs, a significant difference was found between groups I & II ($p < 0.001$) and between groups II &III ($p < 0.001$) but the difference between groups I & III was not found to be significant ($p = 0.232$). These results are on a similar node to the results observed by Ata-Ali et al. (2015)[23] and Milinkovic et al. (2021)[24] in which statistically significant ($p < 0.001$) higher IL-1β values were observed at the peri-implantitis site as compared to the healthy peri-implant tissue site. Conversely, Melo et al. (2012)[25] stated that no statistically significant difference ($p > 0.05$) occurs between IL-1β levels of peri-implantitis sites and healthy implant sites.

On doing bi-comparison among the groups with respect to IL-6 values, a highly significant difference was found between groups I & II ($p < 0.001$) and between groups II &III ($p < 0.001$) but the difference between groups I & III was not found to be significant ($p = 0.263$). The results are in conjunction with the results of a study conducted by Severino et al. (2016)[26] and Hentenaar et al. (2021)[27] in which higher levels of IL-6 were found in peri-implantitis sites as compared to the healthy implant site with statistically significant difference ($p < 0.05$). In contrast to this, a study conducted by Melo et al. (2012) shows no statistically significant difference ($p > 0.05$) occurred between IL-6 levels of peri-implantitis site and healthy implant site.

An interesting observation in our study was the comparison of post-treatment changes in clinical parameters in group II patients, categorized according to their pre-treatment PPD status. Patients with pre-treatment PPD ≥5 mm showed a less significant reduction in both clinical and immunological parameters compared to those with pre-treatment PPD ≤5 mm. This finding highlights the importance of early diagnosis of peri-implantitis, as an early intervention with minimally invasive procedures may help control disease progression and prevent implant failure. To further explore this perspective, larger studies with longer follow-up periods and involving different treatment modalities are needed.

However, our study has some limitations, with the most significant being the small sample size and the short follow-up period. Additionally, future studies employing micropipettes and Periotron for crevicular fluid collection could improve precision, reduce contamination, and enable more detailed quantitative analysis. Therefore, further research incorporating more refined methods for crevicular fluid collection, larger sample sizes, and longer follow-up periods is essential to more thoroughly assess the role of biomarkers in the early detection, monitoring of treatment progress, and evaluation of prognosis in peri-implantitis.

4. Conclusion

The results of our study strongly indicate that cytokines present in PICF can be used to supplement the diagnosis of peri-implantitis along with the use of clinical parameters to make an early diagnosis of peri-implantitis and to make it more sensitive and specific. A statistically significant difference in cytokine levels between the peri-implantitis site and the natural tooth nearest to it highlights the site-specific action of cytokines in peri-implant tissue destruction. Reduction in cytokine levels, post-conservative therapy strengthens the fact that along with using biomarkers as a diagnostic tool in peri-implantitis, they can also be utilized for monitoring the progress of management therapy in peri-implantitis cases and for determining their prognosis.

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Declaration of Competing Interest

Nil (see Figs. 1–6)

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