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Dr. Atul Singh
Senior Resident, Department of Dentistry, Dr Yaswant Singh Parmar Government Medical College, Nahan, Himachal Pradesh, India

Dr. R Muthunagai
Reader, Department of Oral and Maxillofacial Surgery, Seema Dental College, Rishikesh, Uttarakhand, India

Dr. Mudit Agarwal
Reader, Department of Oral and Maxillofacial Surgery, Seema Dental College, Rishikesh, Uttarakhand, India

Dr. Arun Prasad
Private practice, GS Dental Care, Saram, Puducherry, India

Assessment of levels of calprotectin levels in cervicular fluid from implant sites with peri implant pathogenesis

Dr. Atul Singh, Dr. R Muthunagai, Dr. Mudit Agarwal and Dr. Arun Prasad

Abstract
Introduction: Peri-implant crevicular fluid (PICF) contains calprotectin, which are markers for inflammation and bone resorption, respectively. The aims of this study were to assessment of levels of calprotectinlevelsincervicular fluid from implant sites with peri implant pathogenesis.in PICF from implant sites with peri-implant diseases and to evaluate the usefulness of calprotectin diagnostic markers for peri-implant diseases.
Methods: 90 patients with dental implants participated in this study. PICF samples were collected from peri-implant disease sites (n=50) and non-diseased (healthy) sites (n=40) after clinical indicators including probing depth (PD), bleeding on probing (BOP), gingival index (GI), and bone loss (BL) rate were investigated. Calprotectin amounts in PICF were measured using their respective ELISA kits and then compared between diseased and healthy samples. The relationship between PICF calprotectinand levels and clinical indicator levels was investigated.
Results: Calprotectin levels in PICF were significantly higher from peri-implant disease sites than from healthy sites.
Conclusions: Calprotectin levelsin PICF have potential as biomarkers for the diagnosis of peri-implant diseases.

Keywords: calprotectin, peri-implant crevicular fluid, peri-implant diseases

Introduction

Dental implant treatment is a successful, widespread and predictable treatment for tooth loss over the past 20 years however, an increasing number of implant failures caused by peri-implant diseases still take part in every day clinical dental practice [1]. Two forms of peri-implant inflammation have been identified in the literature: peri-implant mucositis and peri-implantitis. The American Academy of Periodontology (AAP) [1] stated that from a clinical standpoint, signs that determine the presence of peri-implant mucositis include bleeding on probing (BOP) and/or suppuration, which are usually associated with probing depths (PDs) ≥ 4 mm and no evidence of radiographic loss of bone beyond bone remodelling. Peri-implantitis is a progressive, irreversible disease of the bone and soft tissues around osseointegrated dental implants under masticatory function that is accompanied by bone resorption, reduced osseointegration, deep pocket formation and suppuration [2]. Despite divergences in the definition of peri-implantitis and the differential diagnosis of peri-implant diseases, studies have estimated that peri-implantitis affects approximately 10% of implants and 20% of patients [3]. According to a recent systematic review peri-implant mucositis and peri-implantitis have a prevalence ranging from 19 to 65% and from 1 to 47%, respectively. On the other hand, another systematic review reported mean prevalence for peri-implant mucositis and peri-implantitis as 43% and 22%, respectively [4]. The combination of clinical and radiographic parameters, such as PD, BOP, suppuration, mobility and marginal bone loss, are the commonly used parameters for the diagnosis of peri-implantitis [5].

Calprotectin is an inflammation-related protein that is produced in leukocytes, macrophages/monocytes, and epithelial cells, and its level increases in several inflammatory diseases including ulcerative colitis, rheumatoid arthritis, and cystic fibrosis [6, 7]. Calprotectin was previously detected in GCF, and its level was significantly higher in GCF from periodontal disease sites than in that from healthy non-diseased sites [8, 9]. Furthermore, GCF

Corresponding Author:
Dr. Atul Singh
Senior Resident, Department of Dentistry, Dr Yaswant Singh Parmar Government Medical College, Nahan, Himachal Pradesh, India

calprotectin levels correlated with clinical indicator levels, such as PD, GI, and BOP [9, 10], and was shown to predict periodontal disease activity [11]. These findings indicate that calprotectin is a useful inflammatory biomarker for periodontal diseases. Calprotectin was also detected in PICF, but its levels in PICF samples from healthy and peri-implant disease sites were not compared [12].

Today; there is large variation for the threshold of diagnosis for peri-implantitis, which may explain the wide range of percentages reported for its prevalence. Researchers and clinicians are always looking for adjunctive measures to aid in proper disease diagnosis, and the measurement of levels of enzymes possible tool, and have gathered a lot of interest. Therefore, the purpose of this article was to review the current understanding of the enzymes associated with peri-implant diseases and how their level changes took part in the pathogenesis of the disease.

Material and Methods
Patients who received dental implants from 3 to 10 years ago, had healthy or diseased implants with peri-implant diseases, and visited at for the maintenance of dental implants and treatment was recruited for the present clinical study. Total 50 patients (15 males and 35 females; aged 66.7±5.5 years) gave written informed consent after receiving an explanation of this study (Table 1). Participants with healthy and diseased dental implants did not have any systemic inflammatory diseases or a history of antibiotic therapy within 3 months. PD, BOP, and gingival index (GI) were examined as clinical indicators after the collection of PICF. GI scores were evaluated according to modifications of the standard of Löe and Silness. Diseased sites with peri-implant diseases were defined as periodontal sites with PD≥3 mm, BOP negative or positive, and GI score ≥1. Healthy implant sites were defined as sites with PD <3 mm, BOP negative, and GI score=0.

Table 1: Characteristics of participants and examining sites

Participants		
Number of participants	90	
Gender (male/female	35/55	
Age (years	66.7±5.5	
Examining site	Healthy	Diseased
Number of PICF sample	40	50
PD (mm	2.22±0.46	4.90±1.16
Gingival index	0.0±0.0	1.3±0.5
BOP-positive rate (%	0.0±0.0	38.0±13.1
Bone loss rate (%)	18.±8.8	39.6±17.0

Collection of PICF samples
PICF sampling and sample preparation PICF samples were collected from peri-implant sites using sterile paper strips. Briefly, PICF sampling sites were isolated with cotton rolls, supra-gingival plaque was removed, and sites were then very gently air-dried. Periopaper (Oraflow Inc., NY, USA) was gently inserted into a peri-implant crevice and held for 30 s. The volume of PICF was measured using a Periotron 8000 (Harco Electronics, Winnipeg, MB, Canada). Paper strips containing blood and pus were not used in the present study.

PICF in the paper strip was extracted in 100 μl of phosphate-buffered saline (pH=7.4) containing 0.2 μMphenylmethylsulfonyl fluoride by centrifugation and used in ELISA for calprotectin.

Protein determination by ELISA
Calprotectin in PICF samples was determined using Calprotectin Human ELISA kit® (Hycult Biotech, PB Uden, the Netherlands) according to the instruction manual. Briefly, the extracted PICF solution was diluted to 100-200-fold using dilution buffer provided in the kit. The diluted PICF solution was added to wells coated with an antibody of human calprotectin and incubated at room temperature for 1 h. After washing the wells, a biotinylated anti-calprotectin antibody was added and incubated at room temperature for 1 h. An immune complex in the wells was reacted with a streptavidin-peroxidase conjugate for 1 h and further incubated with 3,3′,5,5′-tetramethylbenzidine (TMB) for 15 min in the dark. After stopping the reaction using a stop solution, the absorbance of the reacting solution in wells was determined using a microplate reader at 450 nm. The concentrations of calprotectinwere expressed as nanograms per microliter of PICF
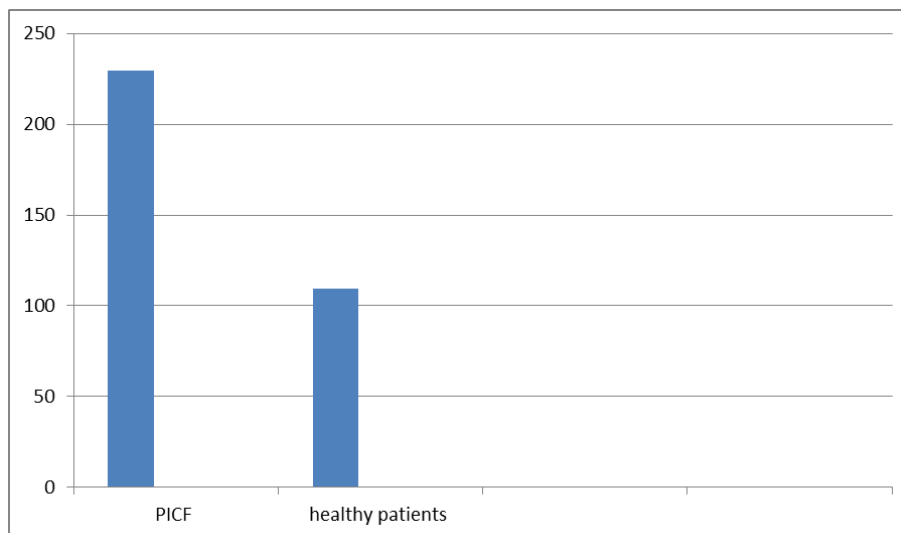
Statistical analysis
Differences in PD, GI, and calprotectin levels, between healthy and diseased groups were statistically analyzed by the Mann-Whitney U test. Differences in the BOP-positive rate between healthy and diseased groups were statistically evaluated using Fisher's exact test. Difference in calprotectin amounts among the GI score 0, 1, and 2 groups were analyzed by the Mann-Whitney U test. Data were analyzed using statistical analysis software (SPSS version 20, IBM, Chicago, IL, USA). P values less than 0.05 were considered to indicate significance.
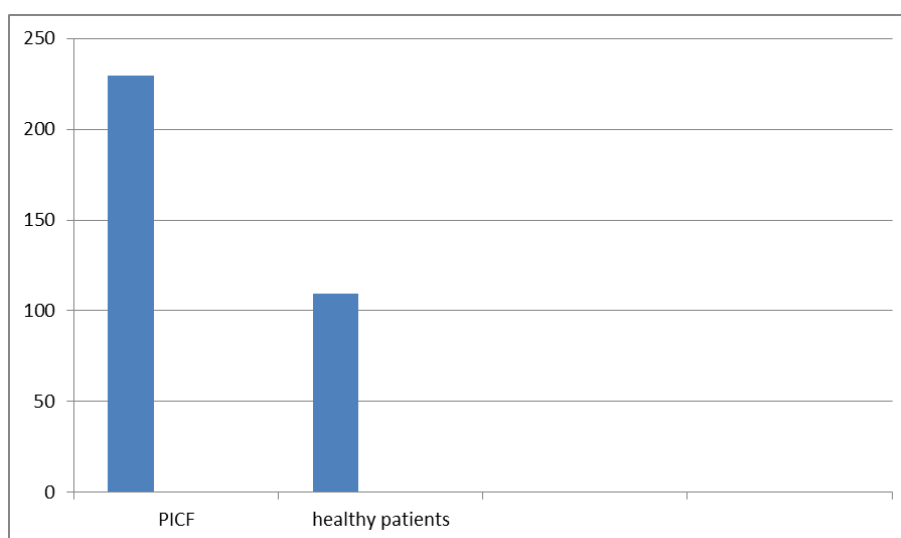
Results
Characteristics of PICF samples and sites of PICF collection 40 of PICF samples were collected from healthy peri-implant sites and 50 samples from diseased sites (Table 1). The mean PD in diseased sites was 4.90 mm, which was significantly deeper than that of healthy sites (2.22 mm). The mean GI score of diseased sites was 1.3 which was significantly higher than that of healthy sites. A significant difference was observed in the BOP-positive rate between diseased and healthy sites (diseased=38.0 vs healthy=0.0; $P<0.01$). Furthermore, the mean BL rate of peri-implant disease sites was 39.6%, which was approximately 2.2-fold that of healthy sites (18.0%).

Comparison of calprotectin levels between diseased and healthy sites Mean calprotectin amounts in PICF samples from diseased and healthy sites were 166.9 and 37. 1 ng per site, respectively and their mean concentrations were 229.30 and 109.2 ng/μl PICF, respectively. Calprotectin amounts and concentrations in the diseased group were significantly higher than those in the healthy group by approximately 4.5-fold and 2.1-fold, respectively (healthy vs diseased; $P<0.01$).

Graph 1: Comparison of mean calprotectin amounts levels between diseased and healthy sites

Graph 2: Comparison of Mean concentration of calprotectin levels between diseased and healthy sites

Discussion

The present study demonstrated that calprotectin amounts and concentrations in PICF samples were significantly higher from diseased sites than from healthy sites, and a positive association was observed between calprotectin levels and clinical indicators such as PD and GI scores. This result for peri-implant diseases was similar to previous findings obtained in diagnostic studies on periodontal diseases [13, 14].

We did not classify peri-implant diseases into peri-implant mucositis and peri-implantitis in this study. Peri-implant mucositis does not show BL, whereas peri-implantitis shows BL of more than 2.5 or 3 mm on intra-oral radiographs [15, 16]. Figuero *et al*. [17]. Introduced plural diagnostic criteria for peri-implant mucositis and peri-implantitis. Rakic *et al*. [18]. Defined peri-implantitis as a PD of more than 5 mm, BOP positive, and BL of at least two threads of implant. Furthermore, Sanz *et al*. [19]. Elevated calprotectin levels in diseased groups might be due to the increased activation of inflammatory cells by bacterial components as well as greater release of intracellular material in gingivitis and periodontitis [20, 21, 22]. As a result, calprotectin could contribute to the host inflammatory immune defense against bacteria in periodontal disease [23, 24, 25, 26]. In an *in vitro* study, epithelial calprotectin was shown to promote resistance to *Porphyromonas gingivalis* invasion and thereby reduce bacterial invasion [23]. A significant difference was noted in calprotectin amounts between GI-0 group and GI-1 or GI-2 group, suggesting that PICF calprotectin indicates initial, weak inflammation in peri-implant diseases because calprotectin is mainly existed in leukocytes that more express at early stage of inflammation and acute inflammation [27, 28]. In contrast, there was a little difference of the median of calprotectin level between the GI-1 and GI-2 groups, but not significant difference, supposing that calprotectin amounts may reach to almost the maximum level at inflammation sites with GI-1 and GI-2

We evaluated BL around dental implants using Schei *et al*.'s method [29]. Which has been used to evaluate BL rate in periodontal diseases. The mean BL rate was significantly higher at peri-implant disease site than at healthy sites without inflammation and deep PD. The ability of some biomarkers including pro-inflammatory cytokines, inflammation-related factors, and proteolytic enzymes to diagnose peri-implant diseases has been examined [30, 31]. Treatments for peri-implant diseases are selected by CIST [32]. In which clinical indicators including PD, BOP, implant mobility, and BL on radiographs are used to diagnose peri-implant diseases. However, these clinical indicators are not considered to be sufficiently accurate or objective for the diagnosis of peri-implant diseases.

Conclusions

Our data show that calprotectin levels in crevicular fluid were

able to predict site-specific periodontal disease activity in the study population. Detection of site-specific activity was related to disease activity on subject basis. The calculated cut-off levels provide a dichotomous basis for prospective evaluation of calprotectin as a diagnostic marker for monitoring periodontal treatment

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