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## Evaluation of calprotectin levels in crevicular fluid from implant sites in patients with peri-implantitis

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### Abstract

**Background:** Peri-implantitis are also chronic inflammatory diseases associated with the accumulation of a pathogenic biofilm on the implant surface. Calprotectin is a calcium- and zinc-binding protein, which for practical purposes can be considered to be neutrophil-specific, although low levels are found in other phagocytic cells. Hence; the present study was conducted for evaluating the calprotectin levels in crevicular fluid from implant sites in patients with peri-implantitis.

**Materials & Methods:** A total of 10 patients with clinical and radiographic confirmed diagnosis of peri-implantitis and 10 healthy controls were enrolled. Complete demographic and clinical details of all the patients were obtained. Peri-implant crevicular fluid (PICF) sampling and sample preparation PICF samples were collected from peri-implant sites using sterile paper strips. Calprotectin in PICF samples was determined using Calprotectin Human ELISA. All the results were recorded in Microsoft excel sheet and were analysed by SPSS software.

**Results:** Mean levels of calprotectin in peri-implantitis patients and in healthy controls was 204.82 ng/ $\mu$ L and 115.36ng/ $\mu$ L respectively. Significant results were obtained while comparing the mean calprotectin levels in between the two study groups.

**Conclusions:** Calprotectin levels in crevicular fluid are significantly altered in patients with peri-implantitis highlighting their role in the pathogenesis of the disease.

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

### Introduction

Peri-implantitis are also chronic inflammatory diseases associated with the accumulation of a pathogenic biofilm on the implant surface. They are characterized by inflammation of the peri implant mucosa and by loss of the implant supporting bone. Calprotectin is a calcium-and zinc-binding protein, which for practical purposes can be considered to be neutrophil-specific, although low levels are found in other phagocytic cells. Calprotectin accounts for approximately 60% of total soluble proteins in the cytosol fraction of neutrophils. Neutrophils are the common effector cells that define acute inflammation in response to a number of factors<sup>[1, 2]</sup>. Once the neutrophil migrates to a site of chemoattraction, the contact sets off a cascade of events leading to a respiratory burst, oxygen radical generation, and disintegration of the neutrophil with the release of its cytosolic granules (and calprotectin), which contain a variety of hydrolytic and proteolytic enzymes. In this way, the neutrophil deals with the chemo attractant but at the same time cause indiscriminate damage to its surroundings<sup>[3, 4]</sup>. Hence; the present study was conducted for evaluating the calprotectin levels in crevicular fluid from implant sites in patients with peri-implantitis.

### Material and methods

The present study was conducted for evaluating the calprotectin levels in crevicular fluid from implant sites in patients with peri-implantitis. A total of 10 patients with clinical and radiographic confirmed diagnosis of peri-implantitis and 10 healthy controls were enrolled. Complete demographic and clinical details of all the patients were obtained. Diseased sites with peri-implant diseases were defined as periodontal sites with probing depth  $\geq 3$  mm, bleeding of probing negative or positive and gingival index score  $\geq 1$ . PICF sampling and sample preparation PICF samples were collected from peri-implant sites using sterile paper

strips. Calprotectin in PICF samples was determined using Calprotectin Human ELISA. All the results were recorded in Microsoft excel sheet and were analysed by SPSS software.

## Results

In the present study, a total of 10 patients with peri-implantitis and 10 healthy controls were enrolled. Mean age of the patients of peri-implantitis group and control group was 29.3 years and 31.8 years respectively. There were 6 males and 4 females in the peri-implantitis group and 7 males and 3 females in the control group. In the present study, mean levels of calprotectin in peri-implantitis patients and in healthy controls was 204.82 ng/ $\mu$ L and 115.36 ng/ $\mu$ L respectively. Significant results were obtained while comparing the mean calprotectin levels in between the two study groups.

**Table 1:** Comparison of mean calprotectin levels in GCF/PICF

Calprotectin levels	Peri-implantitis patients	Healthy control	p-value
Mean (ng/ $\mu$ L)	204.82	45.76	0.00
SD	115.36	27.88	

## Discussion

Calprotectin is a small calcium-binding protein and is a member of the S100 family of zinc-binding proteins, being a heterodimer of S100A8/A9. It was first discovered in 1980 and was found to contribute ~60% of the protein content of the cytosol in neutrophils. It has also been detected in monocytes and macrophages, albeit at lower concentrations than in neutrophils and may have anti-microbial properties. In the presence of active intestinal inflammation, polymorphonuclear neutrophils migrate to the intestinal mucosa from the circulation. Any disturbance to the mucosal architecture due to the inflammatory process, results in leakage of neutrophils, and hence, calprotectin, into the lumen and its subsequent excretion in feces<sup>[6-9]</sup>. Hence; the present study was conducted for evaluating the calprotectin levels in crevicular fluid from implant sites in patients with peri-implantitis.

In the present study, a total of 10 patients with peri-implantitis and 10 healthy controls were enrolled. Mean age of the patients of peri-implantitis group and control group was 29.3 years and 31.8 years respectively. There were 6 males and 4 females in the peri-implantitis group and 7 males and 3 females in the control group. Sakamoto E *et al.* compared calprotectin and NTx levels in PICF from implant sites with or without peri-implant diseases and to evaluate the usefulness of calprotectin and NTx as diagnostic markers for peri-implant diseases. Thirty-five patients with dental implants participated in this pilot study. PICF samples were collected from peri-implant disease sites (n=40) and non-diseased (healthy) sites (n=34) after clinical indicators including probing depth (PD), bleeding on probing (BOP), gingival index (GI), and bone loss (BL) rate were investigated. Calprotectin and NTx amounts in PICF were measured using their respective ELISA kits and then compared between diseased and healthy samples. The relationship between PICF calprotectin or NTx levels and clinical indicator levels was investigated. A receiver operating characteristic (ROC) curve analysis of calprotectin and NTx was performed to predict peri-implant diseases. Calprotectin and NTx levels in PICF were significantly higher from peri-implant disease sites than from healthy sites. PICF calprotectin amounts correlated with PD, and its levels were significantly higher in the GI-1 and GI-2 groups than in the

GI-0 group. PICF NTx amounts correlated with PD and the BL rate. ROC curves indicated that PICF calprotectin and NTx are useful biomarkers for peri-implant diseases. Calprotectin and NTx in PICF have potential as biomarkers for the diagnosis of peri-implant diseases<sup>[10]</sup>.

In the present study, mean levels of calprotectin in peri-implantitis patients and in healthy controls was 204.82 ng/ $\mu$ L and 115.36 ng/ $\mu$ L respectively. Significant results were obtained while comparing the mean calprotectin levels in between the two study groups. Friedmann A *et al.* recruited 22 patients with 22 implants and their contra-lateral corresponding teeth for longitudinal observation. Clinical parameters probing depth (PD), bleeding on probing (BoP), plaque index (PI), assessment of gingival crevicular fluid (GCF) and peri-implant crevicular fluid (PCF) volumes and periapical radiographs were performed at 2-and 3-year control appointments. Calprotectin (MRP 8/14) and cross-linked N-terminal telopeptide (NTx) levels in both crevicular fluids were determined by ELISA. PD was significantly reduced from years 2 to 3 appointments at implant sites as at teeth sites. At the 3-year appointment in both compartments, fluid volumes were significantly increased, which corresponded well with ascending NTx levels. The total amount of calprotectin decreased non-significantly in both GCF and PCF samples. Periapical radiographs revealed stable bone conditions around implants without significant changes from years 2 to 3 examinations. Clinical peri-implant parameters were considered as stable as the periodontal parameters of their corresponding teeth. A parallel increase in NTx levels in both GCF and PCF at 3-year appointment is not clearly understood; it may reflect an upregulation in the overall bone turnover rate<sup>[11]</sup>.

## Conclusion

From the above results, the authors conclude that calprotectin levels in crevicular fluid are significantly altered in patients with peri-implantitis highlighting their role in the pathogenesis of the disease.

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