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## Use of IL-1 $\beta$ , IL-6, TNF- $\alpha$ and MMP-8 biomarkers to distinguish peri-implant diseases: A systematic review and meta-analysis

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## ABSTRACT

**Objective:** To investigate the use of peri-implant crevicular fluid (PICF) interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and matrix metalloproteinase-8 (MMP-8) biomarkers in distinguishing between healthy implants (H), peri-implant mucositis (MU) and peri-implantitis (PI).

**Material and Methods:** Electronic using three databases (Pubmed, EMBASE and Cochrane) and manual searches were conducted for articles published up to March 2018 by two independent calibrated reviewers. Meta-analyses using a random-effects model were conducted for each of the cytokines; IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , to analyze standardized mean difference (SMD) between H and MU, MU and PI, H and PI with their associated 95% confidence intervals (CI).—Qualitative assessment of MMP-8 was provided consequent to the lack of studies that provide valid data for a meta-analysis.

**Results:** Nineteen articles were included in this review. IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , levels were significantly higher in MU than H groups (SMD: 1.94; 95% CI: 0.87, 3.35;  $p < 0.001$ , SMD: 1.17; 95% CI: 0.16, 3.19;  $p = 0.031$  and SMD: 3.91; 95% CI: 1.13, 6.70;  $p = 0.006$ , respectively). Similar results were obtained with PI compared to H sites (SMD: 2.21, 95% CI: 1.32, 3.11;  $p < 0.001$ , SMD: 1.72; 95% CI: 0.56, 2.87;  $p = 0.004$  and SMD: 3.78; 95% CI: 1.67, 5.89;  $p < 0.001$ ,

respectively). IL-6 was statistically higher in PI than MU sites (SMD = 1.46; 95% CI: 0.36, 2.55; p=0.009); while IL-1 $\beta$  increase was not significant. Despite absence of meta-analysis, MMP-8 show to be a promising biomarker in detection of PI in literature.

**Conclusion:** Within the limitations of this study, pro-inflammatory cytokines in PICF, such as IL-1 $\beta$  and IL-6, can be used as adjunct tools to clinical parameters to differentiate H from MU and PI.

## INTRODCUTION

“Peri-implant diseases” collectively describe plaque – associated pathological conditions that develop inflammatory lesions in tissues around implants (1). A continuum exists between health and peri-implant diseases, categorized into peri-implant mucositis (MU) and peri-implantitis (PI). MU is the precursor to PI, with around 43- 48% prevalence rate among implants (2;3;4). PI is distinguished by progressive loss of supporting bone beyond initial biological bone remodeling; when compared to MU (5), and has a prevalence rate of 12% to 43% (1; 6).

Clinical and radiographic evaluations are the most used methods in diagnosing MU and PI. Although easily applicable, clinical parameters alone do not assess the risk rate, onset, activity, and progression of peri-implant destructive changes (7; 8; 9; 10). Biomarkers are host response molecules that have been investigated in determining disease and its severity in conjunction with clinical assessment (11; 12). Pro-inflammatory cytokines are some of the most investigated

biomarkers in peri-implant diseases, as they play an important role in cascading inflammatory responses that are cellular and vascular (13). The presence of Polymorphonuclear cells (PMN) and abundance of fibroblast matrix metalloproteinase -8 (MMP-8) had also been shown to be pertinent to the early detection of PI, which usually follows the release of those cytokines (14; 15). The destruction of connective tissue is a significant determinant of the progression of peri-implant lesions that is essentially driven by MMP-8. This collagenase is known to be the major MMP detected in periodontitis and is thought to be a potential biomarker of PI.

IL-1 $\beta$ , TNF-  $\alpha$ , IL-6 and MMP-8 have been investigated in conjunction with bleeding on probing, gingival index, and probing depths, to prevent and profoundly comprehend pathogenesis of peri-implant diseases (16). Their concentrations also vary markedly in normal biologic, pathogenic conditions as well as after therapeutic interventions (17). Peri-implant crevicular fluid (PICF), similar to gingival crevicular fluid around teeth, can be an indicator of inflammatory exudates, through which those biomarkers could be collected. PICF is easily accessible, non-invasive and sequentially determinable; therefore, if variation in cytokine and MMP-8 levels matches severity and classification of diseases in reference to health, routine biomarkers testing could become a personalized diagnostic tool in clinical practice (18; 11).

A published systematic review and meta-analysis, including articles up to 2013, investigated TNF-  $\alpha$  and IL-1 $\beta$  in PICF, showing robust levels in disease compared to health, but no

significant difference between MU and PI (19). Nonetheless, the extent of inflammation markedly increases from MU to PI, as shown by the majority of clinical studies. Additionally, IL-6 is one of the most investigated pro-inflammatory cytokines between healthy and diseased peri-implant tissues (20; 21; 22; 23; 24; 25). MMP-8 was very useful to monitor the rapid destruction of connective tissue that marks the progression of MU and PI (26; 18).

Hence, this study aimed at 1) investigating the potential use of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and MMP-8, as biomarkers of implants health, peri-implant mucositis and peri-implantitis conditions in PICF; 2) attempting to develop a recognizable pattern of cytokines and MMP-8 release; and 3) identifying factors that may influence results of previous studies to minimize discrepancies in future investigations.

## **MATERIAL AND METHODS**

This systematic review and meta-analysis was performed and written following the 27-item PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement (Moher et al. 2009). The PRISMA checklist is attached to the appendix of this article (Supplemental Checklist 1).

### **Focus Question**

The focus question was developed considering the Population, Intervention, Comparison, and Outcome (PICO) elements (Stone 2002) and as follows: Could cytokine and MMP-8 levels in the PICF be used to distinguish between health (H), MU, and PI?

**P:** Systemically healthy subjects who received dental implants.

**I:** IL-1 $\beta$ , IL-6, TNF- $\alpha$  and MMP-8 biomarkers can be used to differentiate between H, MU, and PI.

**C:** Investigating the presence or absence of a difference in the cytokine and MMP-8 levels between implants with H, MU, and PI.

**O:** There is a difference in the level of IL-1 $\beta$ , IL-6, TNF-  $\alpha$  and MMP-8 between 1) H versus MU; 2) H versus PI; and 3) MU versus PI.

### **Search Strategy**

Electronic and manual literature searches were conducted by two reviewers (IG, ZC) independently, using PubMed/MEDLINE, EMBASE and Cochrane Library up to March 2018 without language restriction. For the PubMed library, the search terms were as follows:  
((((((((dental implant[MeSH Terms]) OR dental implantation[MeSH Terms]) OR implant[Title/Abstract]) OR peri-implant crevicular fluid[Title/Abstract]) OR PICF[Title/Abstract]) OR peri-implant sulcus fluid[Title/Abstract]) OR PSF[Title/Abstract]) AND (((((cytokines[MeSH Terms]) OR biomarkers[MeSH Terms]) OR

interlukin[Title/Abstract]) OR IL[Title/Abstract]) OR tumor necrosis factor[Title/Abstract]) OR TNF[Title/Abstract] OR MMP-8[Title/Abstract]). For the EMBASE, the search strategy was: ('tooth implantation'/de OR 'tooth implant'/exp OR 'dental implant':ti,ab,kw) AND ('biological marker'/de OR 'cytokine'/exp OR 'biomarker':ti,ab,kw OR 'interlukin':ti,ab,kw OR 'tumor necrosis factor':ti,ab,kw). For the Cochrane Library, (cytokine OR biomarker) AND implant was applied in Title, Abstract, and Keywords in Trials.

Additionally, to complete the survey, a manual search of periodontics/implantology-related journals from March 1995 to March 2018, including *Journal of Clinical Periodontology*, *Clinical Oral Implants Research*, *Journal of Periodontology*, *Clinical Implant Dentistry and Related Research*, *European Journal of Oral Implantology*, *International Journal of Oral and Maxillofacial Implants*, *Implant Dentistry*, *Journal of Oral Implantology*, *International Journal of Oral and Maxillofacial Surgery*, *Journal of Oral and Maxillofacial Surgery* and *International Journal of Periodontics and Restorative Dentistry*. The related reviews (27; 19; 9) and references of selected studies were further scanned for potentially relevant articles.

According to the selection criteria, titles and abstracts of the search results were screened, and then the full-text articles screen was conducted. The level of agreement between the reviewers (IG, ZC) for potentially relevant articles was calculated using  $k$  statistics. A consent on final decision was reached by discussion with the senior author (HLW).

### **Data Extraction and Selection Criteria**

Data from the eligible studies were extracted by two reviewers (IG, JZ) independently. The inter-reviewer disagreement was resolved by discussion as well as consultation with senior author (HLW). Corresponding authors were contacted in cases of missing or unclear data.

For this systematic review, eligible studies met the following inclusion criteria: 1) original cross-sectional and longitudinal prospective clinical studies with the collection of pro-inflammatory cytokines in PICF from individuals with PI or MU; 2) studies analyzed protein expression by enzyme-linked immunosorbent assay (ELISA) or flow cytometry using a cytometric bead array system. The exclusion criteria comprised of: 1) animal, in vitro studies, case reports, and reviews; 2) studies with quantification of pro-inflammatory cytokines in tissue biopsies or saliva; 3) only analysis of osteogenic markers; 4) unreported exact numbers of cytokine levels; 5) fluid collection during early osseointegration; 6) unclear peri-implant disease criteria; and 7) unreported anti-inflammatory and antibiotic medication in inclusion criteria.

### **Risk of Bias Assessment**

The criteria used to assess the quality of selected studies is the NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies. This constituted of 14 questions and provided guidelines for evaluating the research question, study population, exposure, outcomes, follow-up

rate and statistical analyses. Consequently, studies are rated good and fair. This was completed by two examiners (IG, ZC) independently. The possibility of publication bias (see Appendix Fig. 1) was assessed with Egger linear plots for continuous-data elements. A significant publication bias is found if  $P < 0.05$  (28)

### **Statistical Analysis**

Stata 14.0 (StataCorp LP, College Station, TX, USA) was used to conduct all of the statistical analyses. The standardized mean difference (SMD) between two groups was analyzed with random effects model to compare the IL-1 $\beta$ , IL-6 and TNF- $\alpha$  levels between H and MU, H and PI, as well as MU and PI. SMD was used rather than the weighted mean difference (WMD) since the measurement units for each biomarker varied between studies; i.e. in accordance with Cochrane guidelines (29). Heterogeneity was estimated by the Q statistic (significant at  $P < 0.1$ ) and quantified with the  $I^2$  test. The value of  $I^2 \geq 75\%$  suggests high or “considerable/substantial” heterogeneity. Moderate heterogeneity is deduced from results showing 30% to 60% and 0% to 40% may not be important (30). Galbraith plots analyses were conducted to investigate the potential source of heterogeneity among studies (31).

## **RESULTS**

### **Study selection**

The literature selection process is illustrated by a PRISMA flowchart (Figure 1). Initial screening yielded a total of 291 records, 140 articles via PubMed, 89 via EMBASE and 62 via Cochrane

Library. Additionally, 32 records were found by hand-searching. After duplicates discarded, 152 records remained. The review of the titles and abstracts resulted in 42 articles selected for full-text screening, 21 of them were then excluded with reasons listed in Table 1. Finally, 19 eligible articles (26; 32; 33; 34; 35; 36; 37; 15 ; 38; 25; 24; 39; 23; 22; 40; 11 ; 2; 41; 42) were included in this systematic review and quantitative synthesis. The details of the included studies are reported in Table 2. The *k* value for inter-reviewer agreement of potentially relevant articles was 0.84 (title/ abstract screening) and 0.89 (full-text screening), indicating a consistent agreement between the two reviewers. A consent on final decision was reached by discussion with another reviewer (HLW).

### **Description of the Studies**

The most studied cytokine was IL-1 $\beta$  (n = 14), followed by IL-6 (n = 8) and TNF- $\alpha$  (n = 6). Other cytokines, IL-4, IL-8, IL-10, IL-12, and IL-17, were also linked to peri-implant diseases. MMP-8 was investigated in 12 studies, of which 8 were excluded.

All included studies were either cross-sectional or longitudinal experimental ones. Regarding the age of patients, the most common range was between 30 and 60 years. Meyer in 2017, showed the highest age group of patients, with a mean age of 77. Among the 19 included studies, five used ELISA R&D System; a type of sandwich immunoassay where two highly specific antibodies are used to detect a target analyte (35; 25; 39; 23; 22). Eight used other ELISA kits

(32; 26; 36; 15; 38; 11; 2; 42), 2 CBA assays (33; 34), 3 multiplexes (37; 25; 40) and 1 Luminex kit (41).

### **Meta-analyses of IL-1 $\beta$ , IL-6 and TNF- $\alpha$**

For IL-1 $\beta$ , there were 5 articles (35; 25; 24; 23; 22) comparing its level between H and MU (Figure 2a). In one article (23), there were two subgroups of MU (i.e. early and advanced MU), so we included both arms in meta-analysis. MU group showed significantly higher IL-1 $\beta$  level than H group (SMD: 1.94; 95% CI: 0.87, 3.35;  $p < 0.001$ ). The heterogeneity between studies was significant ( $I^2 = 92.1\%$ ,  $p < 0.001$ ). The Galbraith plot (Figure 2b) showed that the considerable heterogeneity was generated by 1 study (25). With this study removed, the heterogeneity decreased effectively ( $I^2 = 9.4\%$ ,  $p = 0.353$ ), and the result remained significant (SMD: 1.21, 95% CI: 0.89, 1.53;  $p < 0.001$ ).

Four studies (35; 37; 25; 40) compared IL-1 $\beta$  release level between implants with MU and PI (Figure 2c). The result demonstrated that the IL-1 $\beta$  in PI site was similar to that in MU site (SMD: 1.52, 95% CI: -0.03, 3.07;  $p = 0.055$ ), with significant heterogeneity among these studies ( $I^2 = 91.5\%$ ,  $P < 0.001$ ). The Galbraith plot (Figure 2d) also showed that the considerable heterogeneity was generated by 1 study (25). The heterogeneity decreased effectively ( $I^2 = 0.0\%$ ,  $P = 0.734$ ) upon removal of that study, and the result became significant (SMD: 0.60, 95% CI: 0.12, 1.08;  $p = 0.015$ ).

Nine studies (32; 34; 35; 38; 25; 39; 11; 2; 41) reported the difference in IL-1 $\beta$  between H and PI (Figure 2e). Meta-analysis of these data showed IL- $\beta$  release was much higher in PI compared to H sites (SMD: 2.21, 95% CI: 1.32, 3.11;  $p < 0.001$ ). However, high degree of heterogeneity was noted ( $I^2 = 92.6\%$ ,  $P < 0.001$ ). The Galbraith plot (Figure 2f) demonstrated that the heterogeneity came from 3 studies (32; 35; 25). After excluding the data of these studies, the homogeneity test showed moderate heterogeneity among the remaining 12 studies ( $I^2 = 43.6\%$ ,  $P = 0.115$ ), and results showed that the SMD was 1.06 (95% CI: 0.71 to 1.42;  $p < 0.001$ ).

### **Meta-analysis of IL-6 and TNF- $\alpha$**

When comparing H and MU, 4 studies (33; 25; 24; 42) reported levels of IL-6 and 3 studies (25; 24; 23) reported the levels of TNF- $\alpha$ . Statistical differences were found in both cytokines (Figure 3a and 4a), which indicated that in MU sites, the release of IL-6 and TNF- $\alpha$  was increased (SMD: 1.17; 95% CI: 0.16, 3.19;  $p = 0.031$  and SMD: 3.91; 95% CI: 1.13, 6.70;  $p = 0.006$ , respectively). Regarding MU versus PI, there were 3 studies on IL-6 (25; 37; 42) (Figure 3b). PI group showed significantly higher levels of IL-6 than that in MU group (SMD = 1.46; 95% CI: 0.36, 2.55;  $p = 0.009$ ). For PI versus H, IL-6 was investigated in 5 studies (34; 25; 2; 41; 42) (Figure 3c) and TNF- $\alpha$  were reported in 4 studies (34; 36; 25; 23; 41) (Figure 4b). Results showed that PI group has higher releasing level in both IL-6 (SMD: 1.72; 95% CI: 0.56, 2.87;  $p = 0.004$ ) and TNF- $\alpha$  (SMD: 3.78; 95% CI: 1.67, 5.89;  $p < 0.001$ ). Meta-analyses revealed high

heterogeneity between these studies, with  $I^2$  ranged from 80.3% to 98.1%. The Galbraith plot (Appendix figure 1) did not show any specific papers contributing to heterogeneity.

### **MMP-8 Results:**

Only 5 articles were included in this review investigating MMP-8 in healthy conditions vs. peri-implant diseases (32; 26; 15; 22; 11). In Salvi et al. 2012, MMP-8 increased significantly between H and MU. The 3 other articles compared H to PI (26; 15; 11). Mean values and SD of MMP-8 levels were only provided in two of the three articles (26; 11); thus, meta-analysis could not be performed. Nonetheless, MMP-8 was found in elevated amounts in PICF collected from a total of 85 implants; that were diagnosed with peri-implant diseases. Fifteen implants had MU and 70 had PI. Arakawa et al. only found MMP-8 in PICF. The sensitivity of technique and depth of PICF samples were marked both high and significant (15). Further, a combination of microbiological profiling and MMP-8 found to have increased the accuracy of results previously obtained (11).

### **Quality and Risk Assessment**

According to NIH Quality Assessment tool, risk assessment of bias was conducted. A study reporting less than 4 (No/NR) was defined with low risk. Any higher scores than 4 increased the bias risk and were concluded to be fair. Ten studies were regarded “good” and 9 were “fair”. These were also shown in Table 3.

### **Publication Bias**

The publication bias was presented by Egger test in appendix Figure 2. For IL-1 $\beta$ , there was no evidence of publication bias, according to Egger's test, in the comparison of H to MU sites (p=0.159), MU and PI (p=0.08). However, publication bias was found in the comparison between H and PI (p=0.013). Studies measuring IL-6 demonstrated no publication bias in all three comparisons (p=0.234, 0.641, 0.08 respectively). Egger's test, combined with funnel plots, showed that for TNF- $\alpha$ , H versus MU (p=0.067), as well as H versus PI groups (p=0.082) failed to prove the presence of publication bias.

### **DISCUSSION**

Peri-implant soft tissues demonstrate a strong inflammatory response to plaque accumulation; influencing the occurrence and development of peri-implant diseases; MU and PI (22; 43;11). Lipopolysaccharide in plaque directly stimulates macrophages, PMNs and fibroblasts to produce proinflammatory cytokines and MMPs. These elicit an inflammatory response that overlaps bacterial action, inducing degenerative pathways of tissues; namely, an irreversible, rapid connective tissue destruction and an alteration of osteoclast and osteoblasts' activities (37; 12; 17; 44). Cytokines and MMPs are released in varying detectable amounts between different diseases and health conditions around implants; therefore, are recently investigated in the course

of peri-implant diseases. In fact, IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and MMP-8 are the most investigated biomarkers in literature (9; 27; 43).

Inflammation continues to occur simultaneously with changes in clinical parameters around diseased implants, such as bleeding on probing; which indicates profuse bleeding and an increased amount of exudate around the implant. Accordingly, concentrations of the selected biomarkers from 19 studies showed an enhanced host response of PICF between health, MU, and PI. Their increased levels in PICF could facilitate an early diagnosis of peri-implant disease and prediction of its onset (45;17). MMP-8 increased levels were previously associated with early implant failures (46;47). One suggested reason is polymorphism in the promoter region of MMP-8. In this meta-analysis, early implant failure was supported by a marked increase in proinflammatory cytokines IL-1  $\beta$ , IL-6 and TNF- $\alpha$  expressions between H and MU.

While the molecular mechanism is not fully comprehensible, it is illustrated that in the inflammatory stage of wound healing, IL-1 $\beta$  and TNF- $\alpha$  are chiefly responsible for prolonging plasminogen pathway of clot lysis and propagating collagenase degradative pathway (48). Some evidence suggests that IL-1 $\beta$  acts synergistically with TNF- $\alpha$  to initiate and propagate inflammation (50). This is demonstrated by the robust levels of the 3 investigated cytokines in PI when compared to H, suggesting that they are indicative of the absence or presence of disease. Our data agree with Faot meta-analysis (19); especially for IL-1  $\beta$  and TNF- $\alpha$ . A correlation

between pro-inflammatory cytokines and MMPs would suggest that they may cooperatively promote inflammation and tissue degradation in peri-implant diseases (40; 48). IL-1 $\beta$  and TNF- $\alpha$  induce the synthesis and secretion of MMP-8; which in turn, cleaves the triple helix of collagen and collectively degrade the extracellular matrix. The biomarkers' association to one another seem to be an added benefit supporting the choice of four in this review.

Interestingly, each cytokine showed a distinct pattern of shift from MU to PI. IL-1 $\beta$  levels increase, but not significantly between MU and PI. This may suggest that there is a 'peak' response to enhanced IL-1 $\beta$  levels termed substrate saturation; i.e., all receptor sites being fully occupied with IL-1 $\beta$ . Polymorphism in IL-1 $\beta$  gene expression could be another reason for varied responses between different individuals of the same disease category (38; 39). Contrary to IL-1 $\beta$ , IL-6 increases significantly between MU and PI (p=0.009); suggesting that it may play a more important role in the non-linear pattern of bone loss occurring in PI (51). IL-6 links innate to acquired immune responses; in which it induces differentiation of activated B cells in antibody-producing cells as well as naïve CD4+T cells. It is conspicuous in chronic inflammation surrounding implants, leading to osteoclastic activation and peri-implant bone loss (52;53;54). These correspond to the pattern of IL-6 increase in concentration between H and MU; H and PI (33; 55). To our knowledge, this is the first meta-analysis investigating IL-6 in H, MU and PI. IL-6 was found to be more sensitive to severity of inflammation in peri-implant tissues.

Regarding TNF- $\alpha$ , there were insufficient studies to compare its level between MU and PI; therefore, meta-analysis could not be conducted. Further investigations are needed to comprehend its role in inflammation and in the progression of peri-implant diseases. In qualitative assessment of MMP-8, four out of five included articles showed MMP-8 being highly elevated in peri-implant diseases along with enhanced bleeding on probing and gingival index values. Specific and sensitive tests exist that facilitate accurate detection of MMP-8 in PICF (16; 11). MMP-8 shows a lot of potentials to be used in the progression of disease (26;16). In contrast; Abo Youssef found that MMPs were not reliable indicators of implant health.

In this meta-analysis, a strict inclusion criterion featured clear definitions of H, MU, and PI. Unclear definitions were either lacking a specific definition (55) or did not mention bone loss as the distinguishing parameter of MU from PI (23). In the study of Panagakos (1996) (39), MU was comprised of bone loss to a limit of 30%. MU was stringently defined as the absence of bone loss, so this study was excluded from MU. This criterion leads to a better distinction of disease and thus enables better judgment of biomarkers use.

While many methodological features of analysis were similar in the included studies, the functioning time of implants differed. This is a limitation to relating cytokine levels to the onset of peri-implant diseases. Similarly, publication bias was found in the comparison of IL-1 $\beta$  in H to PI. Results show high heterogeneity  $I^2$  varying from 80.3% to 98.1%; which led to the use of

the random effect model. Liu et al. depict the highest heterogeneity in evaluation of IL-1 $\beta$  in all three conditions. One reason could be that cytokine levels were associated with high mobility group box 1 (HMGB1) protein increase as the principal investigated variable (25). In other studies, the main reason for high heterogeneity could be attributed to the nature of studies, i.e., the majority of included studies are cross-sectional. Alternatively, longitudinal peri-implant disease monitoring, combined with a non-treatment phase, could recognize a cluster of host response biomarkers associated with breakdown of tissues (22; 47; 57).

Limited evidence exists to show the number of subjects with a sufficient sample size; i.e., no calculations of statistical power were documented. Among the 19 included studies, only few studies accounted for smoking habits, though smoking has been identified as a risk indicator for peri-implant inflammation (37; 58; 59; 41). Other limitations include but are not limited to no consideration of other potential risk indicators for PI, such as history of periodontitis and tissue phenotype. Subsequently, biomarkers' concentrations could show marked discrepancy around implants with the same diagnosed implant disease (35; 60). Moreover, the type of protein assay used could affect the quality of results based on using different manufacturer products of ELISA and multiplex bead assays. Cytokine multiplex assays were found to be "comparable in sensitivity, accuracy and reproducibility" to ELISA for the same analyte (61). Concentration values followed a similar pattern among ELISA and bead assays but yielded different absolute cytokine concentrations. A trend of varied cytokine levels was expressed in this review (62). In

accordance with Khan's study, a trend is most important to monitor development and progression of disease. Finally, most studies lacked data on sensitivities and specificities to cytokines and time at which PICF samples were acquired; hence the probability of false positive or false negative results could not be calculated.

## **CONCLUSION**

Within the limitations of this study, pro-inflammatory cytokines in PICF, such as IL-1 $\beta$  and IL-6, can be used as adjunct tools to clinical parameters to differentiate H from MU and PI. The results of this review indicate moderate evidence in literature to support the use of biomarkers with peri-implant diseases. A significant increase in IL-6 is shown between MU and PI while IL-1 $\beta$  levels did not increase as remarkably. Future research should focus more on longitudinal monitoring of biomarkers in order to deduce a suitable range in health and disease conditions.

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