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PROGRESS IN THE STUDY OF GINGIVAL CREVICULAR FLUID BIOMARKERS FOR THE DIAGNOSIS OF PERI-IMPLANT DISEASE

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ABSTRACT

A recent study of patients undergoing implant surgery showed a significant increase in the prevalence of peri-implant disease, with peri-implant mucositis and peri-implantitis predominating. Peri-implant disease mostly appears after several years of implant use. Currently, the diagnosis of peri-implant disease relies on peri-implant soft tissue exploration and imaging, but abnormalities in these indicators only occur when tissue destruction has already occurred and cannot predict whether an implant has the potential to develop peri-implant disease. Therefore, the early diagnosis of peri-implant disease remains challenging and early diagnosis is important to achieve a good prognosis. Gingival crevicular fluid is a biological exudate present in the gingival crevicular, which is mainly derived from serum and contains metabolic components of bacteria and host cells. Gingival crevicular fluid is considered to be an important diagnostic material and its biomarkers can be used as reaction indicators to determine the development of the disease. In this paper, we describe the progress and effectiveness of research on the use of gingival crevicular fluid biomarkers for the prediction and diagnosis of peri-implant disease, intending to provide a reference for subsequent studies.

Keywords: Peri-implant disease; gingival crevicular fluid; peri-implant crevicular fluid; biomarkers

INTRODUCTION

With the continuous development of implant technology and the increasing number of patients receiving dental implant treatment modalities, the incidence of some diseases associated with implants is increasing year after year. A large cross-sectional study of patients undergoing implant surgery showed a 22% prevalence of peri-implantitis and a 43% prevalence of peri-implant mucositis(Derks and Tomasi 2015). Studies have shown that peri-implantitis (PI), which often occurs after several years of implant use (Renvert, Persson et al. 2018), is a pathological disease that occurs in the peri-implant tissue and is characterized by peri-implant soft tissue inflammation and progressive loss of peri-implant bone tissue (Schwarz, Derks et al. 2018), defined by imaging criteria as bone loss ≥ 3 mm (Berglundh, Armitage et al. 2018). In contrast, peri-implant mucositis (PIM) is mainly an inflammatory lesion of the peri-implant soft tissues without loss of bone tissue and is a reversible disease caused by disruption of the host-microbe balance at the implant-mucosal interface (Heitz-Mayfield and Salvi 2018). Currently, the traditional clinical methods used for the diagnosis of

peri-implant diseases (PID), such as peri-implant soft tissue exploration and imaging, have their limitations, as these abnormalities only occur when tissue destruction has already occurred and cannot determine whether the current implant is at risk of developing peri-implant disease or recurring after treatment(Chen, Cai et al. 2019, Esberg, Isehed et al. 2019). As of now, there are no models to predict the PID process. Despite the lack of predictive models, biomarkers may offer some potential (Alassy, Parachuru et al. 2019).

In recent years, a large number of biomarkers are detected in saliva, blood, urine, and other body fluids. Biomarkers are objective indicators of biological processes in normal or pathological states(Roydeva and Reinders 2021), and there are some potential differences between different diseases and different stages of the same disease. It has been shown that the relationship between pre-angiography biomarkers and contrast-associated acute kidney injury can be predicted by analyzing the incidence of adverse renal complications(Parikh, Liu et al. 2020). A study of the microflora in saliva of pancreatic cancer patients confirms the imbalance of oral flora in pancreatic cancer patients, with periodontal chondrocytes and Neisseria mucosa as potential biomarkers for early diagnosis of pancreatic cancer(Sun, Zhao et al. 2020).

1. Biological Role Of Gingival Crevicular Fluid And Collection Method

1.1 Biological effects

Gingival crevicular fluid (GCF) is a biological exudate present in the gingival crevicular,mainly derived from serum. Because it contains host cell products, immunoglobulins, tissue destruction products, plasma-derived molecules, and subgingival microbial products, GCF is considered to be an important diagnostic material(Esberg, Isehed et al. 2019). Peri-implant crevicular fluid (PICF) has also been reported to contain biomarkers that can be used to diagnose and predict disease, and effective treatment options can be selected based on these specific biomarkers(Alassy, Parachuru et al. 2019). A study comparing the secretion of gingival crevicular fluid at healthy and diseased sites found that the secretion of both GCF and PICF was higher in the presence of disease than at healthy sites and increased with the degree of inflammation at the lesion(Bevilacqua, Biasi et al. 2016). Higher levels of pro-inflammatory cytokines and matrix metalloproteinases were detected in the PICF of implants with inflammation compared to healthy implants(Duarte, Serrão et al. 2016, Dursun and Tözüm 2016), including IL-1β, IL-6, TNF-α, vascular endothelial growth factor, matrix metalloproteinases, and cathepsin, were detected in the PICF of implants with inflammation(Dursun and Tözüm 2016). Akram Zet al.(Akram, Abduljabbar et al. 2016)demonstrated that detection of inflammatory biomarkers in GCF or PICF can be effective in determining the progression of periodontal and peri-implant disease.

1.2 Collection method

Currently, there are three main methods of collecting gingival crevicular fluid: the gingival crevicular irrigation method, the micropipette method, and the filter paper strip method. The quality of gingival crevicular fluid obtained by different collection methods varies. Depending on the experimental design and the purpose of the study, the most suitable sampling method can be used.

The gingival crevicular irrigation method involves inserting a thin "syringe needle" into the bottom of the gingival pocket and a "collection needle" at the gingival margin during the sampling process, and

the operator manually injects the isotonic solution into the gingival pocket and continuously aspirates the gingival crevicular irrigation solution through the collection needle(Salonen and Paunio 1991). Bleeding is an inevitable disadvantage of this method, not only that, it is difficult to accurately quantify the gingival crevicular fluid during the operation, and each sample is time-consuming, so the method has some limitations. At present, this method is not commonly used.

A sample of gingival crevicular fluid can also be obtained by placing a micropipette over a dried and moistened sulcus inlet (Gupta, Mohindra et al. 2021). The advantage is that it does not have to be inserted into the gingival pocket, but instead collects the gingival crevicular fluid outside the gingival crevicular. Pradeep A Ret al.(Pradeep, Raghavendra et al. 2011)concluded that this method not only ensures non-invasiveness to the patient but also allows obtaining undiluted samples while avoiding non-specific attachment of analytes to the filter paper fibers.

The same method of obtaining gingival crevicular fluid is possible using filter paper strips. A special filter paper strip is inserted into the gingival crevicular at the proposed sampling site until slight resistance is encountered, left for 1 minute, then removed and stored in an Eppendorf tube(Elazazy, Amr et al. 2021). Although this method is susceptible to blood or saliva contamination in practice and often requires multiple sampling, it is currently the method of choice because it is simpler to perform and can be quantified.

2. Research application of gingival crevicular fluid biomarkers in the diagnosis of oral diseases

In some cases of oral inflammation, such as gingivitis, periodontitis, and dental caries, the amount and composition of gingival crevicular secretion are significantly changed(Bostanci and Belibasakis 2018). Several studies have shown that interleukins and tumor necrosis factors are reliable biomarkers for the diagnosis of chronic periodontitis and that the concentrations of IL-1β and TNF-α correlate significantly with the progression of chronic periodontitis(da Silva, Pessoa et al. 2021, Isler, Soysal et al. 2021). In addition, MMP-8 and MMP-9 are also reliable indicators for diagnosing the presence of periodontitis(Kim, Kim et al. 2020, Sorsa, Alassiri et al. 2020). Studies analyzing the relationship between gingival crevicular fluid biomarkers and oral diseases are still ongoing, but there is no consensus on biomarkers with diagnostic significance for oral diseases such as periodontitis and caries.

2.1 Periodontal diseases

Gingival crevicular fluid contains a variety of biomarkers associated with the early diagnosis and prognosis of periodontal disease. Although some of the biomarkers associated with periodontal disease have been identified, there are still a large number of studies showing that other biomarkers are also closely related to periodontal disease. Several recent reports have shown that bone-related biomarkers such as bone bridging proteins(Ahmad, Arshad et al. 2020), neutrophil elastase(Aral, Ölçer et al. 2020) in the gingival crevicular fluid can also effectively reflect the activity status of periodontal disease. Increased levels of bone bridge protein positively correlate with active periodontal disease(Ahmad, Arshad et al. 2020). In contrast, the level of neutrophil elastase in the gingival crevicular fluid was significantly higher in patients with periodontal disease compared to patients without periodontal disease(Aral, Ölçer et al. 2020).

2.2 Caries

In terms of caries, it was found that the levels of MMP-1 and MMP-2 in the

gingival crevicular fluid of patients with caries were significantly higher than those of healthy people, and the MMP levels in the gingival crevicular fluid of patients with caries decreased after treatment(Matuszczak, Cwalina et al. 2020), so it was concluded that the MMP concentration in the gingival crevicular fluid could reflect the oral health status of patients with caries. The IL-1β and vascular endothelial growth factor, which are important biomarkers for the diagnosis of periodontal disease, are also important in childhood milk tooth caries. A recent study showed that the levels of these two biomarkers differed significantly before and after treatment of adjacent surface caries, and therefore it was suggested that they could be used as indicators to assess the severity of adjacent surface caries in children's milk teeth(Duruk, Gurbuz et al. 2020).

2.3 Oral squamous cell carcinoma

Currently, the diagnosis of oral squamous cell carcinoma relies primarily on tissue biopsy, but the results of several studies have demonstrated the potential of saliva biomarkers for early diagnosis. According to a Meta-analysis, saliva can be used as a highly sensitive and specific diagnostic tool for OSCC, where MMP-9 and chemokines in adipokines are used as salivary biomarkers to screen and diagnose oral squamous cell carcinoma(Hema Shree, Ramani et al. 2019). The concentrations of IL-1α, IL-6, IL-8, and TNF-α also showed an increasing trend among numerous salivary biomarkers in OSCC patients, with IL-8 being the most important biomarker for early monitoring of OSCC(Babiuch, Kuśnierz-Cabala et al. 2020). This conclusion is also supported in another study where IL-8 showed high sensitivity and specificity for the diagnosis of OSCC(Bugshan and Farooq 2020).

3. Application of gingival crevicular fluid biomarkers in the diagnosis of peri-implant disease

Smoking, plaque, improper type of prosthesis, and uncontrolled moderate/severe periodontitis are known to be risk factors for peri-implant disease(Romandini, Lima et al. 2021), and PI has a more extensive lesion area than periodontitis, with a significantly higher expression of biomarkers in the gingival crevicular fraction than periodontitis(Isler, Soysal et al. 2021). As peri-implant disease is a difficult disease to treat, early detection of peri-implant inflammatory changes before the onset of clinical signs is essential for early diagnosis and prevention of further damage to the lesion. It has been reported that the level of biomarker expression in PICF is valuable in the diagnosis of peri-implant disease and that the increased secretion of PICF and the level of expression of the contained biomarkers reflect the progression of inflammation in the early stages of the disease (Wang, Garaicoa-Pazmino et al. 2016). These findings suggest that PICF may be a source of early diagnostic biomarkers for peri-implant disease.

3.1 Gingival crevicular fluid biomarker detection techniques

Numerous studies have been reported on saliva, GCF, PICF, and other body fluids using traditional assay techniques(Marcello-Machado, Faot et al. 2020, Babady, McMillen et al. 2021, Ramírez-De Los Santos, López-Pulido et al. 2021). Among them, real-time fluorescence quantitative polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) techniques are more commonly used.The RT-PCR technique has the feature of detecting and quantifying trace amounts of gene expression, while the ELISA technique can observe the effect of different concentrations of stimuli on the target protein.

Metabolomics is an emerging technology that has become the technique of choice

for the analysis of metabolites in the body fluids of many patients in recent years. This is because metabolomic techniques are more sensitive and simpler than traditional immunoassays for the detection of relevant indicators(Millet, Khoudour et al. 2021). For example, a recent study identified 12 biomarkers with potential value for the early diagnosis of periodontitis, which provides a tremendous boost to the development of a rapid test to screen for periodontitis in the population, thus enabling early and accurate monitoring of periodontitis(Yi, Shen et al. 2021).

3.2 Research applications of biomarkers for the diagnosis of peri-implant disease

Yang Let al. (Yang, Zhu et al. 2018)found that CyPA, IL-1β, and Emmprin could be used as biomarkers for the early diagnosis of peri-implant disease. CyPA may be an early signal for the appearance of peri-implant inflammation but remains unclear for the detection of the recovery phase of the disease after early intervention. The study by Marcello-Machado R Met al.(Marcello-Machado, Faot et al. 2020) similarly supports this result. Gürlek Ö et al. (Gürlek, Gümüş et al. 2017)compared the composition of biomarkers in the gingival crevicular fluid of healthy implants and peri-implantitis in the same individual and found that IL-1β levels were significantly higher in peri-implant disease than in healthy implants. IL-1β is expressed by a variety of cell types and is a major pathogenic mediator of sterile inflammatory, autoimmune, infectious, and degenerative diseases (Klück, Liu et al. 2021). IL-1β is elevated in peri-implantitis and may play a role in the early detection of peri-implantitis. This is similar to the results of the previously mentioned study on periodontitis, suggesting that peri-implant disease and periodontal disease share a similar pattern in terms of the host response. A recent study showed that CypA can activate the NF-kB pathway and promote IL-1β expression(Li, Luo et al. 2020). And Song et al.(Song, Yang et al. 2015)found that injection of CypA antibody in mice resulted in a significant decrease in IL-1β levels. These findings suggest that CyPA may be an upstream factor of IL-1β, so detecting CyPA can help in the early diagnosis of peri-implantitis.

Another study suggests that increased MMP-8 levels may be associated with peri-implant inflammation and that the presence of periodontitis affects neutrophil activation leading to more MMP-8 release(Teixeira, Lira-Junior et al. 2020). A recent study similarly found significantly higher levels of IL-1β and MMP-8 in implants with peri-implantitis compared to healthy implants(Hentenaar, De Waal et al. 2021). During the initiation and course of the inflammatory response, pro-inflammatory mediators, including MMP-8, are upregulated in lesional tissues and present in the PICF(Sorsa, Tervahartiala et al. 2011).IL-1β and TNF-α act synergistically to initiate and propagate inflammation, while both induce the synthesis and secretion of MMP-8, which together degrade the extracellular matrix. (Ghassib, Chen et al. 2019). Similar to findings in periodontitis, elevated MMP-8 levels may be associated with peri-implant inflammation and may serve as a useful diagnostic aid for peri-implant disease.

In addition to the most studied biomarkers such as interleukin and MMP-8, procalcitonin (PCT) may also be a biomarker with diagnostic value. The results of a recent study on PICF containing biomarkers for peri-implant disease suggest that PCT may play a role in peri-implant inflammation and that its increased levels may indicate the development of peri-implant disease(Algohar and Alqerban 2020). In the development of peri-implantitis, inflammatory cytokines increase due to massive bacterial invasion, and the

production of inflammatory cytokines contributes to active osteoclastogenesis and altered PCT expression.(Severino, Napimoga et al. 2011). It has been shown that PCT is significantly increased in the presence of bacterial infection(Mohan, Srirangarajan et al. 2021). Although high levels of C-reactive protein are associated with periodontal and peri-implant inflammation, PCT is considered to be a better indicator of disease due to its short half-life, and PCT has been shown to have a higher predictive value than CRP in comparison with other biomarkers.

4. Prospect

Although numerous studies have yielded biomarkers with diagnostic significance, the consensus has still not been reached and further clinical studies are needed to elucidate the exact role of these biomarkers in the early diagnosis of peri-implant disease. If the validity and reliability of early diagnosis of biomarkers in PICF are confirmed, the potential for future application of PICF as a diagnostic body fluid will be greatly enhanced. Second, combining portable gingival crevicular fluid testing with biomarkers may be a more effective diagnostic method. Finally, the molecular mechanisms behind the metabolic alterations of each biomarker of PICF should be elucidated, which may require multi-omics analysis such as metabolomics and proteomics to identify the pathways associated with the metabolic alterations and thus become potential targets for the treatment of peri-implant diseases.

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