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Differentially Expressed Genes in Patients with Peri-Implantitis

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ABSTRACT

Peri-implantitis is a biological complication that comprises a range of destructive inflammatory processes causing progressive loss of peri-implant bone and connective tissues, leading to morbidity and eventual implant failure. There is no gold standard treatment currently for this particular problem faced in the branch of dental implantology. Due to the absence of predictable and effective therapeutic interventions for the treatment of peri-implantitis, scientific evidence concerning the host response profile around dental implants could be of significant importance. In the future a wider preventive and/or therapeutic window for this aspect in peri-implant lesions exists, indicating efficient use of biomarkers that provide quantifiable measures of response to peri-implant therapy. The aim of this study was to compare messenger RNA (mRNA) expression profiles between peri-implantitis and healthy controls. Differentially expressed genes (DEGs) in 6 peri-implantitis patients and 6 healthy individuals were analysed using mRNAs gene expression microarrays. 20792 common DEGs between healthy individuals and peri-implantitis patients groups were identified. Results indicated that peri-implantitis exhibit significantly different mRNAs expression profiles. Therefore, these findings highlight potential molecular targets for peri-implantitis therapy development.

Keywords: Differentially expressed genes; Healthy individuals; mRNA; Peri-implantitis;

INTRODUCTION

The use of dental implants as a first line of therapy to rehabilitate the loss of teeth has increased in the last 30 years [1,2]. However, despite the high survival rates of around 90-95% [3], the implant complications are frequently encountered and are garnering increasing focus. Peri-implantitis is a biological complication that comprises a range of destructive inflammatory processes causing progressive loss of peri-implant bone and connective tissues, leading to morbidity and eventual implant failure for which there is no current gold standard treatment [4, 5]. This condition usually develops without any obvious symptoms such as pain [6] so that patients often fail to notice the development of the disease. Due to difficulties in early detection of peri-implantitis, implant failures are steadily increasing, with clinical studies reporting approximately 3-5% implant loss [7]. Several studies proposed that the process of bone loss is influenced by a number of factors, some of which are understood while others are still unclear and share several genetic and environmental causes [8–10]. Hence it is very important to find a fresh approach for diagnosing peri-implantitis and also to better understand the possible biomarkers capable of contributing to the diagnosis of peri-implant diseases. Investigations into molecular pathophysiology are important to provide novel options for effective treatment against peri-implantitis.

The pathological mechanisms underlying peri-implantitis have been reported by a number of studies. Rakic et al reported increased concentration levels of osteoprotegerin (OPG), sclerostin, nuclear factor-κB (NF-B) and soluble RANK ligand

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(sRANKL) in patients with peri-implantitis [11]. The levels of MMP-1, -2, -8, and -9, are also increased in diseased peri-implant tissues when compared to healthy tissues [12]. Another research presented that MMP-8 and PGE-2, regulated by IL-1 to be possible genetic markers for unsuccessful implants based on their role in regulating the extracellular matrix (ECM) which may promote bone healing within defects and enhance osseointegration [13]. Furthermore, peroxisome proliferator-activated receptor γ (PPARγ) that can inhibit inflammation and promote osteoblast function is downregulated [14]. Becker et al reported the upregulation of serglycin (SRGN) gene [15]. It has been suggested that this gene may inhibit bone mineralization in vitro. There is a down regulation of bone matrix molecule, which includes collagen, type IX, α 1 (COL9A1), bone gamma-carboxyglutamate (Gla) protein (BGLAP) and secreted phosphoprotein 1 (SPP1) [16] . Previous research experiences helped us to do the study [17–24] .

However, there is no definitely identified genetic polymorphism and other associated mechanisms that explains biological complications in dental implants. In addition, studies that consider the association between genetic predisposition and implant failure have a weak point due to lack of data reporting the expression of different genes in the pathology.

Therefore, further research should focus on elucidating other potential mechanisms and investigate target genes for the treatment of peri-implantitis. Thus the aim of this study was to compare and analyse the expression of various genes in gingival tissue of healthy individuals and patients with peri-implant disease.

MATERIAL AND METHODS:

Microarray data

RNA sequencing data from gingival tissues of patients with peri-implantitis (n=6), and healthy individuals (n=6) were downloaded from National Centre for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) Data Sets (www.ncbi.nlm.nih.gov/gds) under accession no. GSE106090. Differentially expressed genes (DEGs) in peri-implantitis patients and healthy individuals were analysed using long non-coding RNAs (lncRNAs) and mRNAs gene expression microarrays.

Identification of up/down regulated DEGs

Upregulated and downregulated DEGs in the GSE106090 dataset was identified using GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE106090). GEO2R is an interactive web tool and an R-based web application for comparing two groups of datasets in the GEO database, which we used to compare healthy individuals and patients with peri-implantitis groups. Top 3 upregulated and downregulated DEGs in the GSE106090 dataset were extracted. We set $p < 0.05$ and fold change $(FC)| > 2$ as the cut-off criteria.

RESULTS:

We selected the GSE156993 gene expression microarray dataset with peri-implantitis in the GEO database and investigated molecular function, PPI, and molecular pathways using differentially expressed genes (DEGs) to identify clinical biomarker candidates for peri-implantitis diagnosis. A total of 12 gingival tissue samples, including 6 samples from patients with peri-implantitis, and 6 samples from healthy controls were analysed. Differentially expressed genes (DEGs) analysis showed in patients with peri-implantitis compared to healthy controls.

20792 common DEGs in GSE106090 between healthy individuals and peri-implantitis patients groups were identified using GEO2R. The volcano plot and mean difference plot of DEGs are shown in Figure 1 and Figure 2. Specifically, BMP-5, IRAK2, ACVR1B are the top 3 DEGs that are significantly upregulated and DRD5, MMP7 and SOSTDC1 are the top 3 DEGs that were significantly downregulated (Table 1).

The results of functional enrichment analysis on mRNAs in GSE106090 showed that that the cyclooxygenase pathway is the most prominent signal among the up-regulated mRNAs and that hemidesmosome assembly represents the most significant biological process among the down-regulated mRNA when comparing between peri-implantitis with healthy

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patients. Recent studies also revealed that these pathways are associated with inflammation. Therefore, these findings help to study the roles of DEGs and relevant signalling pathways in peri-implantitis.

DISCUSSION:

Peri-implantitis is a destructive inflammatory disease that affects the tissues surrounding dental implants and has been demonstrated to be a crucial element in implant failure [25]. Evidence that periimplantitis tends to highly occur in subsets of individuals may indicate that specific characteristics of the host, such as genetic factors, can jeopardise the osseointegration process [26]. This study aimed to compare and analyse the expression of various genes in gingival tissue of healthy individuals and peri-implant disease. The results showed a statistically significant correlation between the peri implantitis and healthy individuals. There were around 20792 differentially expressed genes identified in patients with peri-implantitis compared with healthy controls. The BMP-5, IRAK2, and ACVR1B genes were upregulated in patients with peri-implantitis compared with healthy controls

The present study reported an upregulation of BMP-5 gene which encodes a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Liu et al, found that BMP-5 gene transcripts were raised at 10-fold in peri-implantitis. BMP-5 is known to increase RANKL/OPG ratio, thus possibly contributing to higher osteoclast-mediated bone resorption noted in peri-implantitis [27]. IRAK2 encodes the interleukin-1 receptor-associated kinase 2, one of two putative serine/threonine kinases that become associated with the interleukin-1 receptor (IL1R) upon stimulation, thereby triggering intracellular signalling cascades leading to transcriptional up-regulation and mRNA stabilisation. The results of the present study is similar to Laine et al, who reported that carriers of IL-1RN genotypes were at higher risk for peri-implantitis with an OR of 3, taking into account smoking status, gender and age of the patients. The carriage rate of IL-1RN allele 2 was 33% in healthy control and peri-implantitis patients carried more often IL-1RN allele 2 (56.5%) [28]. Activin receptor type-1B is a protein that is encoded by the ACVR1B/ ALK-4 gene that acts as a transducer of activin or activin-like ligands (e.g., inhibin) signals [29]. This study shows an upregulation of this gene. However there are not many studies on the association of ACVR1B and peri implantitis.

The presence of titanium in the implants can alter the immune response both in the absence and presence of bacterial antigenic challenge by acting as a secondary stimulus. There are various experimental studies proving that titanium particles exacerbate pro-inflammatory cytokine release in response to bacterial lipopolysaccharide [30]. There was a significant upregulation of the cyclooxygenase pathway in peri-implantitis when compared to healthy sites, consistent with the paradigm of its heightened activity in peri-implantitis.

DA receptors are found to suppress osteoclastic activity and enhance proliferation and mineralization [31]. DRD5 receptors are down regulated in patients with peri implantitis. Other studies have reported the elevation of different molecular forms of MMP-8 and MMP-7 in PISF collected from peri implant disease sites [32]. This study has also been presented with polymorphism of DRD5 receptors. SOSTDC1 gene is a member of the sclerostin family and encodes an N-glycosylated, secreted protein with a C-terminal, cystine, knot-like domain. This protein functions as a bone morphogenetic protein (BMP) antagonist. Specifically, it directly associates with BMPs, prohibiting them from binding their receptors, thereby regulating BMP signalling during cellular proliferation, differentiation, and programmed cell death [33].

Considering the absence of effective and predictable therapeutic interventions for the therapy of peri-implantitis, scientific evidence on host response profile around dental implants could be important for providing in the future a wider preventive and/or therapeutic window for this peri-implant lesion, indicating biomarkers that provide quantifiable measure of response to peri-implant therapy.

CONCLUSION:

Within the limitations of the study, it was observed that BMP-5, IRAK2, ACVR1B are the top 3 DEGs that are significantly upregulated and DRD5, MMP7 and SOSTDC1 are the top 3 DEGs that were significantly downregulated in patients with periimplantitis.

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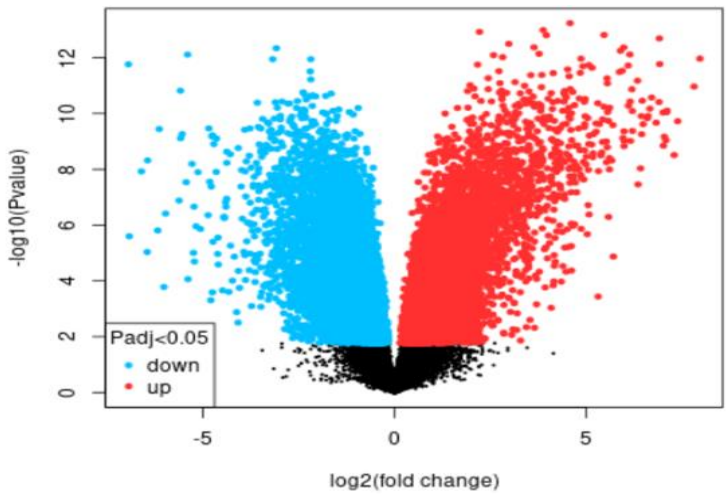

Figure 1 showing Volcano plot of expression difference for expression dataset (GSE156993). Comparison of gene expression in peri-implantitis and healthy individuals.

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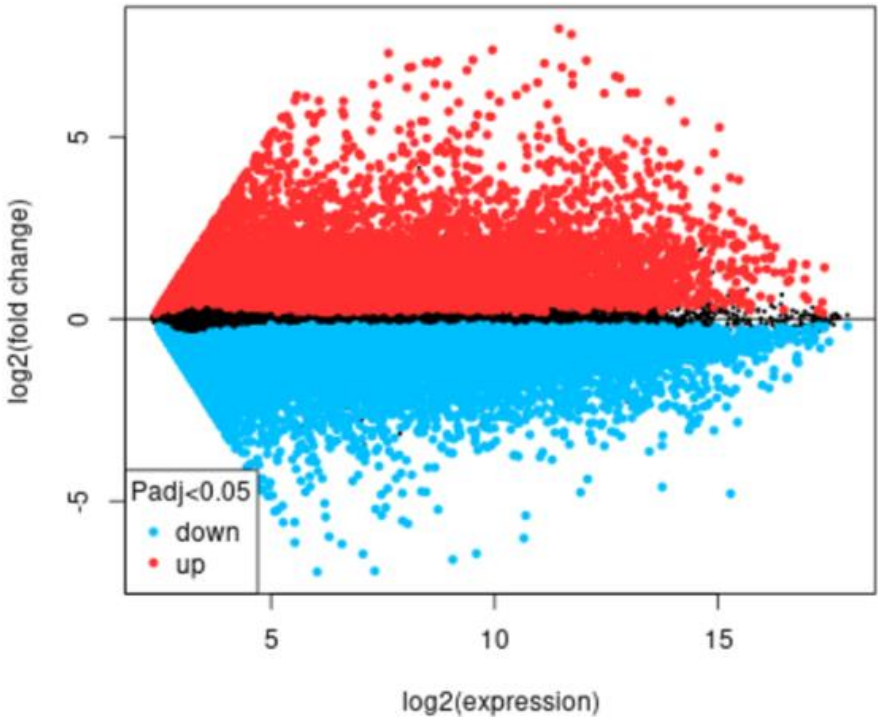

Figure 2. Comparison of gene expression in peri-implantitis and healthy individuals. Mean difference plot of expression difference for expression dataset (GSE156993).

TABLE 1: Top 6 differentially expressed genes in peri implantitis vs healthy individual

Gene (mRNA)	Expression change
BMP-5	Upregulated
IRAK2	Upregulated
ACVR1B	Upregulated
DRD5	Downregulated
MMP7	Downregulated
SOSTDC1	Downregulated