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Review Article

Periodontal Disease and miRNAs: Biological Mechanisms and Clinical Correlations

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Abstract

MiRNAs have recently emerged as new biomarkers, of high significance and importance, unfolding a new potential in research. These RNAs are capable of inhibiting mRNA translation, or facilitate its degradation. In the present review, we discuss the basic miRNA biological mechanisms of action on bone tissue cells, in periodontal tissues, as well as the impact of miRNAs on the correlation between periodontal disease and other chronic inflammatory diseases. Periodontitis is a chronic, inflammatory disease of the supportive tissues of the teeth. The damage of the periodontal tissues, is a result of the metabolism of the biofilm. At the same time, there is an activation of the host's immune system, through an inflammatory (non-specific) response and subsequently through an immune (specific) response. This combination, of the products of the biofilm's metabolism, as well as those of the host's immune response, is considered one of the main causes of periodontal tissue damage and alveolar bone loss. MiRNA regulation, in periodontal tissue plays an important role in disease progression. Differences in the miRNA expression levels, affects the aforementioned inflammatory mechanisms and their interaction with periodontitis.

Keywords: Biology, Clinical characteristics, miRNAs, Periodontal disease

Introduction

Periodontal Disease

Periodontal disease is a chronic, inflammatory disease of the periodontium affecting the supportive tissues of the teeth, which consist of the gingiva, the alveolar bone, the tooth cementum and the periodontal ligament. Periodontal disease results in the loss of those tissues. The main pathogenetic mechanism of the disease, necessarily requires the existence of a pathologic biofilm, which mainly consists of gram(-) anaerobic bacteria, such as *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Bacteroides forsythus and Eikenella corrodens*. The existence of such a biofilm is a result of the homeostatic disorder amongst the microbes that normally reside into the oral cavity, specifically on teeth surfaces, as well as beneath the soft tissues, subgingival, below the gum line. However, the existence of this biofilm alone, is not enough for the disease to start, as it is a result of the interaction of the biofilm with the host's immune system that is activated, as a response to the rough microbial environment. The tissue damage is a result of the toxic products of biofilm's metabolism, as well as of the products of the activated immune system

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(inflammatory response/non-specific and immune/specific response)[1]. After the activation of the host's immune system response, there is a secretion of cytokines and Reactive Oxygen Species (ROS), i.e. oxidative stress, as well as other enzymes such as Matrix Metalloproteinases (MMPs), which cause periodontal destruction[2].

However, other factors, endogenous and environmental, can also affect the disease's progress. Patient-specific factors are systemic diseases, such as diabetes mellitus, HIV syndrome, cardiovascular disease and others, such as tobacco use[3], preterm low birthweight and non-alcoholic fatty liver disease[4,5]. Also, site-specific (anatomical) factors, such as teeth crowding or morphological characteristics can play a role in the progress of periodontal disease PD[2].

miRNAs

MiRNAs are small, (18-22 nucleotides) single-stranded molecules of ribonucleic acid (RNA), which are regarded as main regulators of gene expression[6]. They participate in various cellular processes and metabolic pathways, such as cellular differentiation, proliferation and apoptosis[7]. A small part of the genome codes for the miRNAs (~3%), however the miRNAs regulate about 30% of those genes coding for a protein[8]. miRNA biogenesis, takes place inside the cell nucleus. Genes which code for miRNAs, are transcribed by RNA Polymerase II. The post-transcription molecule which occurs, consists of introns, which are non-coding genomic regions. Specifically, a molecule of approximately 400 nucleotides is produced, as the original product of transcription, the premature miRNA (pri – miRNA). This molecule is subjected to further process, inside the nucleus, by RNA Dorsha, a splicing enzyme. In this way, it forms a loop shape, consisting of approximately 70 nucleotides and resulting in pre – miRNA. In this form, the pre – miRNA is exported from the nucleus, through a membrane protein, exportin – 5. Outside of the nucleus and in the cytoplasm, RNA Dicer, a second splicing enzyme acts, which forms the mature miRNA as a final product. After the splicing action of Dicer, the loop from the pre–miRNA molecule is receding and that results in the formation of a double-stranded molecule, of approximately 22 nucleotides, in which the one strand is the mature miRNA and is attached to its complementary strand, miRNA* or passenger strand. Finally, specific binding proteins, Argonautes (AGO) are bound with the one of the two strands (the subsequent mature strand) and the complementary strand is receding. This complex of AGO proteins bound to the mature miRNA strand, forms RISC complex (RNA induced silencing complex), which is now the active molecule and regulates gene expression. MIRs regulate gene expression in three different ways: a) Inhibiting mRNA translation by binding to the 3' untranslated region of mRNA, b) promoting mRNA decomposition by binding to 3' untranslated region of mRNA and c) promote changes in DNA methylation patterns[9].

miRNAs	Change in expression
miR-15a	Upregulated
miR-21a	Upregulated
miR-26a	Upregulated
miR-29b	Upregulated
miR-126a	Upregulated
miR-125a	Upregulated
miR-146a	Upregulated
miR-146b	Upregulated
miR-148a	Upregulated
miR-181b	Upregulated
miR-223	Upregulated
let-7e	Upregulated
let-7f	Upregulated
let-7j	Upregulated
let-7k	Upregulated
miR-17	Downregulated
miR-24	Downregulated
miR-30	Downregulated
miR-92a	Downregulated
miR-451	Downregulated

Table 1. Changes in miRNA expression profile in gingival tissues of periodontitis animals compared to control animals (reproduced under the Creative Commons Attribution 4.0 International License from Luan et al. (2018)[10]).

Many chronic and acute diseases are related to aberrant miRNA expression levels, which affect gene expression and subsequently many cellular functions, resulting in a disease phenotype[10]. The levels of miRs are detected in different tissues, in biological fluids such as saliva, gingival crevicular fluid and plasma[11,12].

Scope of the Present Work

In the present work we reviewed the literature, in order to establish the relation between periodontal disease and miRNAs. Specifically, we concentrated on the biological mechanisms of miRNA action with relation to the periodontal tissues and how this interaction affects the development and progression of periodontal disease and/or bone loss. Also, we presented some of the most important miRNAs, which play a potential role as diagnostic and prognostic biomarkers for PD. Finally, the present work reviewed the possible therapeutic potential, of exosome-related miRNAs in periodontitis.

mRNAs	miRNAs in Diseased tissues	Functions
miRNA-548	Upregulation	Upregulation of IL-8 within the periodontal pocket
miRNA-31	Upregulation	Regulates the expression of ICAM-1, which controls the migration of leukocytes from the bloodstream to the tissues
miRNA-17	Upregulation	Regulates the expression of E- Selectin, which controls the migration of leukocytes from the bloodstream to the tissues
miRNA-146	Upregulation	Negatively regulates the TLR signalling pathway
miRNA-146a	Upregulation	Negatively regulates TLR signalling; reduced expression of NF-κB, TNF-α, IL-1α and IL-6, which induce osteoclastogenesis
miRNA-146b	Upregulation	Negatively regulates TLR signalling
miRNA-155	Downregulation	Regulates TLR release in inflamed tissues
miRNA-200	Upregulation	Reduces the release of IL-6, IL-8, IFRD1 and NF-κB
miRNA-200c	Upregulation	Regulatory effect on TLR4-mediated signalling in macrophages
miRNA-21	Upregulation	Decreases NF-_κB activation
miRNA-let-7	Upregulation	Inhibits TLR4
miRNA-203	Downregulation	Promotes neo-angiogenesis and regulates innate immunity
miRNA-223	Upregulation	Plays a role in alveolar bone loss

Table 2. Overview of miRNAs associated with periodontal disease (reproduced under the Creative Commons Attribution 4.0 International License from Santonocito et al. (2021)[8]).

miRNAs and Exosomes

Until recently, it was considered that miRNAs only act inside the cell in which they are produced. However, miRNAs have been also detected out of cells, having the ability of being stable in extracellular fluids, because of their packing[13,14] connected with lipids and proteins or encapsulated in specific cellular particles, the exosomes. These are small, lipidic particles (soap bubble structure), of 40-130 nm, which are used by cells in order to secrete products of intracellular origin, into the extracellular space[15]. The exosomic miRNAs circulate throughout the body and transfer information among the cells, having the ability to be retained by other cells and promote changes in gene expression. Therefore, exosomes act as an intracellular as well as an extracellular communication mechanism, among the cells. They are detected into plasma, serum or saliva. It has been proven that exosomes play a specific part in inflammation, immune diseases, blood diseases and neoplasmatic diseases of the oral cavity, among others, as well as *Sjögren* Syndrome, Systemic Lupus Erythematosus (SLE), squamous cell carcinoma and periodontitis. For these reasons, exosomes captured the researchers' interest, as they can have clinical application in tissue regeneration, targeted therapy and biomarker research[16,17]. Many studies have investigated the role of exosomes in bone regeneration of the destroyed periodontium and have shown an improvement in periodontal condition, defined by the activation of Wnt/ β-catenin pathway, which induces vascular formation[18]. It has been also shown that exosome therapy improves significantly the regeneration of bone and of periodontal tissues in general. Relevant to this, it has been observed a dose-depending effect. Thus, according to Wang et al. (2019), exosomes may have pro-inflammatory and anti-inflammatory actions, according to the transferred content[19]. However, in order to improve the application of the exosomes for therapeutic reasons, future studies need to focus on the origin of the exosomes[8].

Periodontal Disease and miRNAs

As aforementioned, miRNAs are associated with a plethora of acute or chronic diseases including periodontal disease. Research studies first focused on the collection of samples from gingival tissue, for the isolation of miRNAs. However, over the last years, the isolation of miRNAs from saliva and other biological fluids such as gingival crevicular fluid (GCF), became more preferable[8]. Several reasons exist why biofluids are preferred as miRNA source, which include: a) Sample collection (saliva, gingival crevicular fluid) is more tolerable by patients, since it is minimally invasive and without the risk of bleeding, b) high miRNA stability[20,21] and c) miRNA identification with conventional methods is more feasible[20,21]. Several studies have investigated the potential use of miRNAs as biomarkers, for the diagnosis of periodontal disease[8,22]. Furthermore, miRNAs can also be used as therapeutical biomarkers, by acting on target-genes and affecting many signaling pathways[8,22]. Specific

miRNA expression with respect to periodontal disease is summarized in Table 1 and Table 2.

Homeostasis of Mineralized Bone Tissue and miRNA Regulation

The progenitor cells of the periodontium, have the potential to differentiate in either alveolar bone cells (osteoclasts/osteoblasts), in periodontal ligament connective tissue cells, or in cementoblasts[23,26]. This procedure is regulated by many molecules, such as Runx2 factor, bone morphogenetic proteins (BMPs), as well as many signaling pathways such as Notch. . In these complicated and overlapping pathways, miRNAs are considered to play an important, regulating role, with their expression levels to be either increased or decreased. A research study by Luan et al. (2018) showed that miRNA-31, miRNA-34a and miRNA-34c expression was decreased during the differentiation of the progenitor cells of periodontal tissues, induced by osteogenetic conditions[10]. These miRNA molecules affect osteoblastogenesis, through Runx2 signaling pathway. The process of osteoblastic differentiation begins with the co-ordinated action of multiple signaling pathways and molecules, including BMPs, Wnt and Notch. Among those, BMP pathway plays a major role in osteoblastic differentiation[9]. Studies have shown that decreased expression levels of miRNA-100, improves osteogenesis in human mesenchymal stem cells. The target molecule of miRNA-100 is BMPR2[23,24]. On the contrary, it is found that the overexpression of miR-100, reduces BMPR2 gene expression, leading to the inhibition of Runx2 and osteogenetic differentiation. Subsequently, the reduced expression of miRNA-100 in this study[10], promotes osteogenesis, as the higher levels of the molecule act as a negative regulator of mineralization. Another miRNA which has a regulatory role in mineralization is miRNA-195, whose expression is in low levels during osteogenetic induction of periodontal ligament cells (PDLs). Other studies have shown that miRNA-195 reduces the expression of specific molecules, acting as an antagonist of the BMP pathway in bone tissue cells[25,26]. Therefore, negative regulation in progenitor cells, is an example of an inhibiting miRNA whose expression levels are decreased, in order to induce mineralization. MiRNAs induce osteogenesis by negatively regulating mineralization inhibitors[10].

Wnt pathway is another important pathway for the development, progress and homeostasis of periodontal tissues[24,26-33]. Many of the miRNAs enhance Wnt signaling pathway, by targeting its inhibitors and by inducing osteoblastogenesis[30,32,34,35]. Such miRNAs are miRNA-27, miRNA-29, miRNA-199a, whose expression levels were also low during osteogenetic differentiation of progenitor PDLs. These molecules control the differentiation of mesenchymal cells through the formation of positive feedback mechanisms and the activation of Wnt/β-catenin pathway[10].

Another signaling pathway is Notch. MiRNAs members of miRNA-34 family, are inhibitors of Notch pathway and subdue osteoblastic differentiation[10]. It has been shown that miRNA-34 insufficiency increases bone formation, affecting osteoblast proliferation.

Osteogenesis regulation by miRNAs under inflammatory conditions, in periodontal disease

Inflammatory conditions, which take place in periodontal disease can cause loss of the alveolar bone and disruption of the connective tissue[27,34-39]. In these conditions, miRNA-138 has been found increased and this fact results in the inhibition of basic genes, associated with mineralization, such as osteocalcin (OC) gene, Runx2 and collagen I type gene. Apparently, the inhibitor of miRNA-138 can be used as a therapeutic factor for the bone loss prevention, associated with advanced periodontitis[38,39]. Another molecule that has been studied is miRNA-17 and its effect on osteogenic differentiation of progenitor PDL cells, making clear that inflammatory environment suspends osteoblastic activity, as well as the differentiation of these cells. Inflammatory cytokines inhibit miRNA-17 and promote Smurf1 expression, which is a direct target of miRNA-17 in periodontium progenitor cells. Furthermore they provoke the attenuation of specific factors associated with osteoblastogenesis, mediated by Smurf1[37,40,41]. MiRNA-146 also affects the differentiation of PDL, in an inflammatory environment. An increase of its expression, during osteoblastic differentiation of PDL cells, is observed[36,42]. This overexpression leads to reduced activity of NF-κB, as well as increased profile expression of genes, which regulate osteoblastic differentiation. The regulation of NF-κB activity blocks miRNA-146 function in osteogenesis, indicating that this molecule promotes PDL cell differentiation, via negative regulation of NF-κB signaling pathway[36,42]. MiRNA-150 and miRNA-151 also contribute to the inhibitory effect of inflammation, in osteoblastic differentiation of pre-osteoblasts and in undifferentiated mesenchymal cells *in vitro*[10].

Osteoclastogenesis regulation by miRNAs under inflammatory conditions, in periodontal disease

Inflammatory conditions which are dominant in periodontal disease, apart from inhibiting osteoblastic differentiation and activation , they also have an effect on osteoclastic activity, by enhancing it and eventually leading to bone resorption[35,36]. Osteoclastic activity is induced by signaling cascades, which are activated by lipopolysacharides (LPS) of bacterial cell walls, as well as by pre-inflammatory cytokines, such as IL-1β, IL-6, TNF-α These cytokines activate RANK molecule on osteoclast cell surface and subsequently, its nuclear molecule-target, NF-κB. They also promote the differentiation of pre-osteoclasts in mature, multinuclear osteoclasts[38,40-46]. Afterwards, mature osteoclasts are attached to bone tissue surface, by forming their ruffled border. After that process, they start to locally create an acidic environment, by secreting acids, which at first, demineralize the inorganic phase of bone tissue, by motivating

exchangeable, non-crystalline Ca^{+2} from the inorganic salts of phosphoric calcium, ($CaHPO_4$). This procedure facilitates the subsequent dissolution of the organic matrix of bone tissue, mediated by Kathepsin K, which consists of collagen type I and other non-collagen organic proteins[43,46-48]. The miRNAs expressed in periodontal tissues , have an impact on inflammatory activation of osteoclasts and on alveolar bone resorption[10]. They affect and induce the catabolic pathway in bone tissue homeostasis, by either inhibiting osteoclastic differentiation and function, or, or by promoting osteoclastogenesis. Some of these miRNAs are miRNA-17, miRNA-15a, miRNA-34a,c, miRNA-146a, miRNA-155, miRNA-223, miRNA-125a,b, miRNA-15b, miRNA-17, miRNA-100, miRNA-132, and miRNA-199a. Specifically, miRNA-146a, miRNA-223, miRNA-34a, miRNA-125a, and miRNA-503, inhibit osteoclast differentiation and function, by regulating various molecules of signaling pathways of osteoclastic differentiation[45,47-52]. The expression of some of these miRNAs in periodontal tissues was found reduced, so this fact promoted osteoclast differentiation. Subsequently, some of these molecules, such as miRNA-146a and miRNA-125a, in increased expression levels, they could block osteoclastic differentiation and bone resorption and in this way they could act as therapeutic molecules and improve periodontal condition. Another category of miRs inhibits osteoclastic differentiation and their expression levels were increased, in order to cause negative feedback loops, associated with osteoclastogenesis. Among thsese miRs are miRNA-26a and miRNA-155, which probably form an interior feedback mechanism of bone remodeling regulation. Subsequently, these two molecules or their agonists, due to their ability to inhibit osteoclastogenesis, they may be used in the future for bone loss therapy[10]. In addition, miRNA-21 is a possible mediator in the association of periodontal disease and estrogen insufficiency, which leads to increased osteoclastic activity, by attaching to FASL (FAS-Ligand) molecule[51,53] in female periodontic patients, during menopause[50,54].

The Correlation of Periodontal and Cardiovascular Diseases: miRNA-146a in Chronic Periodontitis Patients With and Without Coronary Heart Disease

One of the most common diseases with severe mortality rate in the population worldwide, is Coronary Heart Disease (CHD). Research studies over the years, have shown that there are genetic factors which influence the progress of diseases such as CHD, as well as chronic periodontitis (CP). However, this research field is not yet fully discovered. One of the most important tools for further exploration of this field, is considered to be micro-RNAs[52,55]. Some of the processes which cause plaque formation and eventually CHD, are inflammation, apoptosis and angiogenesis. However, those processes are also regulated by miRNAs[53,56].

One of the miRNAs that has a significant role in innate and adaptive immune response of the periodontal tissues, after bacterial infection, is miRNA-146a. It is located on chromosome 5q33.3 and generally it participates in many major processes such as inflammatory cascades[54,57]. This molecule is expressed after stimulation by cytokines and the Toll-like receptors ligands[55,58]. MiRNA-146a has been detected in high levels, in aortic and femoral plaque of the cardiac tissue, in cardiac patients[2,56]. Also, miRNA-146a has been found to play a role in CHD, as well as CP individually, but it is not yet clear if it constitutes a specific and sensitive biomarker that associates CHD with chronic periodontitis. Yagnik et al.(2019) in their study in a South Indian population, they have made an attempt to identify, as well as quantify the miRNA-146a levels, in subgingival plaque samples of CP patients with and without CHD and compare them with healthy controls[52].

Correlation Between Periodontal Disease and Acute Coronary Syndrome (ACS): Dysregulation of miRNA-146a by periodontal pathogens

According to the Global Burden of Disease Study[59,60] periodontitis is one of the most widespread diseases, specifically the 6th most (11.2%) common disease worldwide. Bacteremia, endotoxemia as well as systemic low-grade inflammation, are some of its main effects, conditions which are also linked with systemic diseases such as diabetes mellitus and coronary heart disease[57,61], as it has been previously mentioned.

Acute Coronary Syndrome (ACS) is a pathological condition that is caused by chronic inflammation, a biological process that affects all stages of atheroma formation and that also causes rupture of the fibrous cap around the plaque[58,62]. Subsequently it is the diversion of the balance between progress and inhibition of inflammation that provokes the risk of developing ACS. The host's susceptibility is affected by the immune response to the biofilm formation and by immune-inflammatory factors. The chronic inflammatory conditions, consisting of cytokines, oxidative stress and epigenetic factors, are considered to result from the exaggerated and disproportionate inflammatory response and also to play a significant role in the disease. However, the effect of inflammation on the Acute Coronary Syndrome is a field which needs further investigation[2,55]. P. *gingivalis* is a pathogen that has been involved in many systemic diseases, therefore its role has been intensely researched[63,64]. It has also been found that P. *gingivalis* affiliates with an amount of virulent factors such as fimbriae, serine phosphatases (SerB), cysteine proteases and endotoxins, such as lipopolysaccharides (LPS). LPS-Toll-like Receptor activation leads to translocation of factor NF-κB in the nucleus and eventually to the transcription induction of inflammation-related genes[60,65]. MiRNA-146a is considered to have a significant role in the regulation of the innate immune response to inflammation[61,66].

Many inflammatory components such as cytokines and pathogen products like LPS in macrophages and dendritic cells, can induce the expression of miRNA-146a. Specifically, NF-κB factor induces the transcription of this molecule, as a response, after the innate immune signaling activation. The induction of miRNA-146a causes a downregulation of the production of proteins such as TRAF6 and IRAK1, involved in the inflammatory responses, as well as the pro-inflammatory cytokines such as TNF-α and IL-1β, affecting the NF-κB signaling pathway, resulting eventually in the inhibition of NF-κB activation, by a negative feedback loop[62]. Subsequently, miRNA-146a is considered to regulate inflammation negatively[55]. Furthermore, miRNA-146a has been involved in the pathogenesis of many other inflammatory diseases such as diabetes mellitus, atherosclerosis, cancer and chronic periodontitis. Specifically, in chronic periodontitis it has been found highly expressed[62], whereas as far as atherosclerosis is concerned, its over-expression may lead to its prevention and treatment[64]. For this reason, miRNA-146a may be a novel therapeutic target for ACS[65]. Nevertheless, the role of miRNA-146a as a link between CP and ACS is still unclear. The study of Gita et al. (2019), aimed to assess the relative miRNA-146a expression levels in ACS patients with and without CP, as well as clarify the role of miR-146a in the regulation of the immune-inflammatory response, in CP patients with ACS[66]. This study concluded that there was a statistically significant increase in the levels of miRNA-146a, TNF-α, IL-6 and IL-1β in ACS patients with CP ($p<0.01$). Eventually, it established the linking role of miRNA-146a in chronic periodontitis and the acute coronary syndrome. MiRNA-146a can also be regarded as a potential serum biomarker of the inflammatory conditions related to CP and ACS, in a background of co-morbidities such as smoking and diabetes[66].

Correlation Between Periodontal Disease And Diabetes Mellitus: Evaluation of miRNA-223, miRNA-203 and miRNA-200b Expression Levels in Periodontitis Patients With and Without Diabetes Mellitus (Type 2)

Diabetes mellitus type 2 is another metabolic disease, which is associated with inflammatory response, angiogenesis as well as tissue repair[67]. It is considered one of the main causes of mortality in the adult population and it has been found that its relationship with periodontitis is bidirectional[68]. The relationship of diabetes mellitus with periodontitis is a medical field that has been intensely researched. Many studies have been developed in order to elucidate details of these two diseases and the effect the one has on the other. The study by Elazazy et al. (2020) aimed to evaluate the relation of some miRNA molecules, specifically miR-223, miR-203 and miR-200b expression levels in serum and gingival crevicular fluid (GCF), in chronic periodontitis patients, with and without diabetes mellitus of type 2)[69]. This study was also the first to link the miRNA expression levels, detected in the GCF and the serum, with clinical parameters in periodontal patients as well as with TNF-α and IL-10 levels. TNF-α factor was highly expressed in patients group compared with the control group[70,71], whereas IL-10, which is an anti-inflammatory cytokine, was found decreased in patient groups compared with the control group[72]. Subsequently, in patient groups, the high TNF-α levels reflect the presence of systemic inflammation[73].

As far as the miRNA-223 is concerned, it was found that it was overexpressed in GCF as well as serum, in CP patients group with and without diabetes type 2, compared with the control group. MiR-223 can be used in the differential diagnosis of periodontitis patients with type 2 diabetes mellitus and periodontitis patients alone[69,74,75]. MiR-223 is a very important regulator of innate immune system and normally, it plays a role in tissue homeostasis and differentiation of many immune cells. Moreover, it affects activation pathways in granulopoiesis, hematopoiesis and lipopolysaccharide (LPS) exposure[76]. MiRNA-223 also participates in bone metabolism. As shown by many studies, it is associated with osteoblast differentiation control and combined with miR-214 and miR-338, it inhibits osteogenesis by inducing osteoblast apoptosis and at the same time, it induces osteoclast differentiation by acting to nuclear factor 1-A (NF1-A). Therefore, the final result is the activation of osteoclast activity and the inhibition of osteoblast activity, leading to the divertion of bone metabolism balance, towards bone absorption. Eventually, its increased levels in CP, affect alveolar bone loss[77]. However, miRNA-223 is found dysregulated in type 2 diabetes, as a result of the action of the advanced glycation end products, a process that eventually affects osteoblast and endothelial cell apoptosis[77].

MiR-203 levels, on the contrary, were found lower in all patient groups, in serum and GCF. One of the functions of miR-203, among others, is that it plays a major role in the regulation of wound-specific cell functions, having also a negative effect on the network of cytokines associated with wound healing[10]. It is considered that the lower levels of miR-203 in periodontal tissues, increase angiogenesis, whereas in healthy tissues the normal expression levels act on the vascular endothelial growth factor alpha (VEGF-a) and inhibit angiogenesis[78]. Subsequently, miR-203 lower expression levels in patients and also the negative correlation with TNF-α (higher levels of TNF-α), promotes reduced healing process and thus, it defines the irreversible tissue damage of periodontal disease. It is considered that miR-203, when in normal levels, may have a protective role in CP, as it reduces TNF-α levels and may also have the possibility to be used as a therapeutic molecule in healing promotion. Finally, it is considered as a biomarker for differential diagnosis of CP patients with type 2 diabetes and CP patients without type 2 diabetes, and also for distinguishing type 2 diabetes patients with periodontitis

from diabetes patients without periodontitis[69,79,80].

MiRNA-200b, another miR molecule, was also found overexpressed in serum as well as in GCF of both patient groups. Specifically, this study showed that miR-200b is positively correlated with TNF-α and negatively correlated with anti-inflammatory IL-10 cytokine, in the CP - diabetes type 2 patient group. It is referred that miRNA-200b is induced by cytokines in inflammatory conditions and therefore it may play a role in any inflammatory disease[76]. Moreover, the higher levels of miR-200b were considered to play a role in the increased apoptosis rate of pancreatic β-cells[77], showing that miR-200b is more involved in the pathogenesis of diabetes. MiR-200b also acts on VEGF and promotes an increase in angiogenesis and vascular permeability. Subsequently, these findings explain the fact that this molecule is overexpressed in inflammatory conditions[78].

The expression levels of miRNA-223, miRNA-202 and miRNA-200b in CP patients with and without diabetes mellitus type 2, suggest that they constitute very promising serum biomarkers which can elucidate the molecular mechanisms as well as the pathophysiology of CP.

In conclusion, the expression levels of miRNA-223, miRNA-202 and miRNA-200b in CP patients with and without diabetes mellitus type 2, suggest that these molecules constitute some very promising serum biomarkers which can elucidate the molecular mechanisms as well as the pathophysiology of CP.

Periodontal and Adipose Tissue Correlation: Anti-Inflammatory Impact of miRNA-146a, Induced in Adipose and Periodontal Tissues

Obesity is considered as a multifactorial chronic disease, which is defined by genetic as well as environmental factors[81]. Obesity is also considered to amplify the risk for other chronic conditions with high mortality rate, such as coronary heart disease (CHD) and cancer. It affects the systemic metabolism and participates in inflammatory responses, as it can cause low-grade inflammatory conditions. Furthermore, research studies on humans and animals suggest that obesity is associated with periodontal disease[82-84]. Specifically, obesity has been found to enhance periodontal inflammation[85]. TNF-α expression occurs as an inflammatory response, which is evoked by adipose tissue and is considered to enhance systemic low-grade inflammation, which in turn, acts as a compounding factor for inflammation and specifically, for other inflammatory diseases and eventually, periodontal disease. Many miRNAs have been found highly expressed in the inflamed gingiva of obese patients, compared to healthy ones[86]. White adipose tissue, under inflammatory conditions, express high levels of miRNA-146a. On the contrary, the transfection of miRNA-146a in adipocytes, causes downregulation of the expression of the inflammatory cytokines[87]. As far as periodontal disease is concerned, it was observed that miRNA-146a expression levels were positively correlated with clinical periodontal parameters such as periodontal pocket depth (PPD)[88]. In the study of Sanada et al,(2020) the results demonstrated that miRNA-146a transfection in adipocytes, gingival fibroblasts and macrophages, suppresses inflammatory gene expression. Macrophages were found to play a major role in miR-146a induction[89]. Specifically, IRAK1 and TRAF6 are target molecules for miRNA-146a, as well as for its homologue miRNA-146b[55]. It has been found that miRNA-146a downregulates the expression of IRAK1 and TRAF6, molecules that mediate TNF-α signals. Specifically, IRAK1 phosphorylation and eventually activation, induces its binding with TRAF6 and this process activates the signaling cascade of MAP kinase (JNK, p38 MAPK), as well as NF-κB signaling pathway[90]. Subsequently, the transfection of miRNA-146a into macrophages, caused IRAK1 and TRAF6 downregulation and promoted a decreased intracellular TNF-α expression and also low levels of phosphorylated JNK MAP kinase. In this way, miRNA-146a plays a major role in inflammatory response regulation. Furthermore, overexpression of miRNA-146a is found to inhibit foam cell formation and cytokine production as well[64]. In conclusion, as it has been observed in this study, with further injection of miRNA-146a, it has occurred a downregulation of inflammatory response in adipose and gingival tissue, in vivo. For that reason, this process mediated by miRNA-146a may have an impact on long-term immune regulation, in diseases with an inflammatory base, such as obesity and periodontal disease[89].

miRNAs as Biomarkers and Therapeutic Molecules

In the aforementioned studies, only some of the miRNAs associated with periodontal disease, were presented. These molecules constitute major expression regulators of a large amount of genes, because of their ability to bind, without the necessity of a high complementarity, with mRNAs[19,27-29,31,32]. The result is that miRNAs not only have a diagnostic value as biomarkers, but also have the potential to be used as therapeutic markers for various diseases, such as periodontal disease. Single miRNA molecules act on target-genes and affect many signaling pathways. Combination of miRNAs can regulate many molecules of a significant signaling pathway. Their role as diagnostic and therapeutic markers as well, is still to be elucidated, as this research field is still under development and constantly new information and data are coming to light.

Conclusions

Until today, the most common biomarkers for periodontitis, that can be isolated from the oral cavity, are separated in the following 3 categories: a) The host's enzymes and inhibitors, b) inflammation mediators and immune response modulators of the host and c) tissue

degeneration products.

MiRNAs constitute a novel category of biochemical markers, of high sensitivity and specificity. It has been shown that these molecules affect the disease's progress, by taking part in the process of bone formation, as well as in the bone resorption process. They have an impact on many signaling pathways, with final aim the regulation of genes associated with the differentiation of precursor periodontal ligament cells (PDLs), in osteoblasts and osteoclasts. This is the exact ability that makes miRNAs also therapeutic markers which can possibly modify the disease's process. It has also been shown that miRNAs take part in other inflammatory diseases, such as coronary heart disease (CHD), acute coronary syndrome (ACS), diabetes mellitus type 2 and obesity, associated with periodontal disease. However, more research needs to be done in this field, in order to clarify the action mechanisms of the miRNAs and also reveal more details about the link of periodontitis with these chronic, inflammatory diseases.

Authors' contributions

AIA: Drafted the manuscript, reviewed literature. GIL: Proof-edited the manuscript, reviewed the manuscript and gave final permission for publication. All authors read and approved the manuscript.

References

1. Dereka XE, Markopoulou CE, Vrotsos IA. Role of growth factors on periodontal repair. Growth factors (Chur, Switzerland) 2006;24(4):260-7.
2. Meyle J, Chapple I. Molecular aspects of the pathogenesis of periodontitis. Periodontology 2000 2015;69(1):7-17.
3. Kodovazenitis G, Pitsavos C, Papadimitriou L, Deliargyris EN, Vrotsos I, Stefanadis C, et al. Periodontal disease is associated with higher levels of C-reactive protein in non-diabetic, non-smoking acute myocardial infarction patients. Journal of dentistry 2011;39(12):849-54.
4. Basha S, Shivalinga Swamy H, Noor Mohamed R. Maternal Periodontitis as a Possible Risk Factor for Preterm Birth and Low Birth Weight--A Prospective Study. Oral health & preventive dentistry 2015;13(6):537-44.
5. Iwasaki T, Hirose A, Azuma T, Ohashi T, Watanabe K, Obora A, et al. Correlation between ultrasound-diagnosed non-alcoholic fatty liver and periodontal condition in a cross-sectional study in Japan. Scientific reports 2018;8(1):7496.
6. Hobert O. Gene regulation by transcription factors and microRNAs. Science 2008;319(5871):1785-6.
7. Stefani G, Slack FJ. Small non-coding RNAs in animal development. Nature reviews Molecular cell biology 2008;9(3):219-30.
8. Santonocito S, Polizzi A, Palazzo G, Isola G. The Emerging Role of microRNA in Periodontitis: Pathophysiology, Clinical Potential and Future Molecular Perspectives. International journal of molecular sciences 2021;22(11).
9. Foessl I, Kotzbeck P, Obermayer-Pietsch B. miRNAs as novel biomarkers for bone related diseases. J Lab Precis Med 2019;4(2):2.
10. Luan X, Zhou X, Naqvi A, Francis M, Foyle D, Nares S, et al. MicroRNAs and immunity in periodontal health and disease. International journal of oral scienc. 2018;10(3):24.
11. Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids--the mix of hormones and biomarkers. Nature reviews Clinical oncology. 2011;8(8):467-77.
12. Turchinovich A, Cho WC. The origin, function and diagnostic potential of extracellular microRNA in human body fluids. Frontiers in genetics 2014;5:30.
13. Cheng L, Sharples RA, Scicluna BJ, Hill AF. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood. Journal of extracellular vesicles 2014;3.
14. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proceedings of the National Academy of Sciences of the United States of America 2008;105(30):10513-8.
15. Montecalvo A, Larregina AT, Shufesky WJ, Stolz DB, Sullivan ML, Karlsson JM, et al. Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. Blood 2012;119(3):756-66.
16. Barile L, Vassalli G. Exosomes: Therapy delivery tools and biomarkers of diseases. Pharmacology & Therapeutics 2017;174:63-78.
17. Cheng Q, Shi X, Han M, Smbatyan G, Lenz HJ, Zhang Y. Reprogramming Exosomes as Nanoscale Controllers of Cellular Immunity. Journal of the American Chemical Society 2018;140(48):16413-7.
18. Qin Y, Sun R, Wu C, Wang L, Zhang C. Exosome: A Novel Approach to Stimulate Bone Regeneration through Regulation of Osteogenesis and Angiogenesis. International journal of molecular sciences 2016;17(5).
19. Wang T, Nasser MI, Shen J, Qu S, He Q, Zhao M. Functions of Exosomes in the Triangular Relationship between the Tumor, Inflammation, and Immunity in the Tumor Microenvironment. Journal of immunology research 2019;2019:4197829.
20. García-Giménez JL, Sanchis-Gomar F, Lippi G, Mena S, Ivars D, Gomez-Cabrera MC, et al. Epigenetic biomarkers: A new perspective in laboratory diagnostics. Clinica chimica acta; international journal of clinical chemistry 2012;413(19-20):1576-82.
21. Kim S-H, Lee S-Y, Lee Y-M, Lee Y-K. MicroRNAs as biomarkers for dental diseases. Singapore Dental Journal 2015;36:18-22.
22. Santonocito S, Ronsivalle V, Fichera G, Indelicato F. Psychological impact and patient perception of occlusion and orthodontic treatment in periodontitis patients. Mediterranean Journal of Clinical Psychology 2020;8(3):1-16.
23. Zeng Y, Qu X, Li H, Huang S, Wang S, Xu Q, et al. MicroRNA-100 regulates osteogenic differentiation of human adipose-derived mesenchymal stem cells by targeting BMPR2. FEBS letters 2012;586(16):2375-81.
24. Lim WH, Liu B, Mah SJ, Yin X, Helms JA. Alveolar bone turnover and periodontal ligament width are controlled by Wnt. Journal of periodontology 2015;86(2):319-26.
25. Grünhagen J, Bhushan R, Degenkolbe E, Jäger M, Knaus P, Mundlos S, et al. MiR-497-195 cluster microRNAs regulate osteoblast differentiation by targeting BMP signaling. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2015;30(5):796-808.
26. Yin X, Li J, Salmon B, Huang L, Lim WH, Liu B, et al. Wnt Signaling and Its Contribution to Craniofacial Tissue Homeostasis. Journal of dental research 2015;94(11):1487-94.
27. Darveau RP. Periodontitis: a polymicrobial disruption of host homeostasis. Nature reviews Microbiology 2010;8(7):481-90.
28. Diekwisch TG. Our periodontal tissue: a masterpiece of evolution. Journal of clinical periodontology 2016;43(4):320-2.

29. Diekwisch TG. Novel approaches toward managing the micromanagers: 'non-toxic' but effective. Gene therapy 2016;23(10):697-8.
30. Li Z, Hassan MQ, Jafferji M, Aqeilan RI, Garzon R, Croce CM, et al. Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. The Journal of biological chemistry 2009;284(23):15676-84.
31. Tamura M, Nemoto E. Role of the Wnt signaling molecules in the tooth. The Japanese dental science review 2016;52(4):75-83.
32. Wang T, Xu Z. miR-27 promotes osteoblast differentiation by modulating Wnt signaling. Biochemical and biophysical research communications 2010;402(2):186-9.
33. Lim WH, Liu B, Cheng D, Williams BO, Mah SJ, Helms JA. Wnt signaling regulates homeostasis of the periodontal ligament. Journal of periodontal research 2014;49(6):751-9.
34. Bartold PM, Van Dyke TE. Periodontitis: a host-mediated disruption of microbial homeostasis. Unlearning learned concepts. Periodontology 2000 2013;62(1):203-17.
35. Hienz SA, Paliwal S, Ivanovski S. Mechanisms of Bone Resorption in Periodontitis. Journal of immunology research 2015; 2015:615486.
36. Hung PS, Chen FC, Kuang SH, Kao SY, Lin SC, Chang KW. miR-146a induces differentiation of periodontal ligament cells. Journal of dental research 2010;89(3):252-7.
37. Liu Y, Liu W, Hu C, Xue Z, Wang G, Ding B, et al. MiR-17 modulates osteogenic differentiation through a coherent feed-forward loop in mesenchymal stem cells isolated from periodontal ligaments of patients with periodontitis. Stem cells (Dayton, Ohio) 2011;29(11):1804-16.
38. Wiebe SH, Hafezi M, Sandhu HS, Sims SM, Dixon SJ. Osteoclast activation in inflammatory periodontal diseases. Oral diseases 1996;2(2):167-80.
39. Zhou X, Luan X, Chen Z, Francis M, Gopinathan G, Li W, et al. MicroRNA-138 Inhibits Periodontal Progenitor Differentiation under Inflammatory Conditions. Journal of dental research 2016;95(2):230-7.
40. Boyce BF. Advances in osteoclast biology reveal potential new drug targets and new roles for osteoclasts. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2013;28(4):711-22.
41. Boyce BF, Xiu Y, Li J, Xing L, Yao Z. NF-κB-Mediated Regulation of Osteoclastogenesis. Endocrinology and metabolism (Seoul, Korea) 2015;30(1):35-44.
42. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontology 2000 2014;64(1):57-80.
43. Karsenty G, Wagner EF. Reaching a genetic and molecular understanding of skeletal development. Developmental cell 2002;2(4):389-406.
44. Lee EJ, Song DH, Kim YJ, Choi B, Chung YH, Kim SM, et al. PTX3 stimulates osteoclastogenesis by increasing osteoblast RANKL production. Journal of cellular physiology 2014;229(11):1744-52.
45. Sugatani T, Hruska KA. MicroRNA-223 is a key factor in osteoclast differentiation. Journal of cellular biochemistry 2007;101(4):996-9.
46. Teitelbaum SL. Bone resorption by osteoclasts. Science 2000; 289(5484):1504-8.
47. Chen C, Cheng P, Xie H, Zhou HD, Wu XP, Liao EY, et al. MiR-503 regulates osteoclastogenesis via targeting RANK. Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research 2014;29(2):338-47.
48. Nakasa T, Shibuya H, Nagata Y, Niimoto T, Ochi M. The inhibitory effect of microRNA-146a expression on bone destruction in collagen-induced arthritis. Arthritis and rheumatism 2011;63(6):1582-90.
49. Guo LJ, Liao L, Yang L, Li Y, Jiang TJ. MiR-125a TNF receptor-associated factor 6 to inhibit osteoclastogenesis. Experimental cell research 2014;321(2):142-52.
50. Shapiro LF, Freeman K. The relationship between estrogen, estrogen receptors and periodontal disease in adult women: a review of the literature. The New York state dental journal 2014;80(3):30-4.
51. Sugatani T, Hruska KA. Down-regulation of miR-21 biogenesis by estrogen action contributes to osteoclastic apoptosis. Journal of cellular biochemistry 2013;114(6):1217-22.
52. Yagnik K, Mahendra J, Kurian VM. The Periodontal-Cardiovascular alliance: Evaluation of miRNA-146a in subgingival plaque samples of chronic periodontitis patients with and without coronary heart disease. Journal of investigative and clinical dentistry 2019;10(4):e12442.
53. Papageorgiou N, Tousoulis D, Androulakis E, Siasos G, Briasoulis A, Vogiatzi G, et al. The role of microRNAs in cardiovascular disease. Current medicinal chemistry 2012;19(16):2605-10.
54. Lukiw WJ, Zhao Y, Cui JG. An NF-kappaB-sensitive micro RNA-146a-mediated inflammatory circuit in Alzheimer disease and in stressed human brain cells. The Journal of biological chemistry 2008;283(46):31315-22.
55. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proceedings of the National Academy of Sciences of the United States of America 2006;103(33):12481-6.
56. Malik R, Mushtaque RS, Siddiqui UA, Younus A, Aziz MA, Humayun C, et al. Association Between Coronary Artery Disease and MicroRNA: Literature Review and Clinical Perspective. Cureus 2017;9(4):e1188.
57. Offenbacher S. Periodontal diseases: pathogenesis. Annals of periodontology 1996;1(1):821-78.
58. Libby P, Tabas I, Fredman G, Fisher EA. Inflammation and its resolution as determinants of acute coronary syndromes. Circulation research 2014;114(12):1867-79.
59. Tonetti MS, Jepsen S, Jin L, Otomo-Corgel J. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. Journal of clinical periodontology 2017;44(5):456-62.
60. Zenobia C, Hajishengallis G. Porphyromonas gingivalis virulence factors involved in subversion of leukocytes and microbial dysbiosis. Virulence 2015;6(3):236-43.
61. Nahid MA, Pauley KM, Satoh M, Chan EK. miR-146a is critical for endotoxin-induced tolerance: IMPLICATION IN INNATE IMMUNITY. The Journal of biological chemistry 2009;284(50):34590-9.
62. Park H, Huang X, Lu C, Cairo MS, Zhou X. MicroRNA-146a and microRNA-146b regulate human dendritic cell apoptosis and cytokine production by targeting TRAF6 and IRAK1 proteins. The Journal of biological chemistry 2015;290(5):2831-41.
63. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nature reviews Immunology 2015;15(1):30-44.
64. Yang K, He YS, Wang XQ, Lu L, Chen QJ, Liu J, et al. MiR-146a inhibits oxidized low-density lipoprotein-induced lipid accumulation and inflammatory response via targeting toll-like receptor 4. FEBS letters 2011;585(6):854-60.
65. Guo M, Mao X, Ji Q, Lang M, Li S, Peng Y, et al. miR-146a in PBMCs modulates Th1 function in patients with acute coronary syndrome. Immunology and cell biology 2010;88(5):555-64.
66. Bagavad Gita J, George AV, Pavithra N, Chandrasekaran SC, Latchumanadhas K, Gnanamani A. Dysregulation of miR-146a by periodontal pathogens: A risk for acute coronary syndrome. Journal

of periodontology 2019;90(7):756-65.
67. Santamaria-Jr M, Bagne L, Zaniboni E, Santamaria MP, Jardini MAN, Felonato M, et al. Diabetes mellitus and periodontitis: Inflammatory response in orthodontic tooth movement. Orthodontics & craniofacial research 2020;23(1):27-34.
68. Preshaw PM, Bissett SM. Periodontitis and diabetes. British dental journal 2019;227(7):577-84.
69. Elazazy O, Amr K, Abd El Fattah A, Abouzaid M. Evaluation of serum and gingival crevicular fluid microRNA-223, microRNA-203 and microRNA-200b expression in chronic periodontitis patients with and without diabetes type 2. Archives of oral biology 2021;121:104949.
70. Miranda TS, Heluy SL, Cruz DF, da Silva HDP, Feres M, Figueiredo LC, et al. The ratios of pro-inflammatory to anti-inflammatory cytokines in the serum of chronic periodontitis patients with and without type 2 diabetes and/or smoking habit. Clinical oral investigations 2019;23(2):641-50.
71. Hatem SA, Nsaif ZN, AL-Rubaee EAS. Evaluation of Interleukin IL-1β, TNF-α, and VDB Protein Levels in Serum of Patients with Chronic Periodontitis. Journal of Biochemical Technology 2020;11(1):14-7.
72. Acharya AB, Thakur S, Muddapur MV. Evaluation of serum interleukin-10 levels as a predictor of glycemic alteration in chronic periodontitis and type 2 diabetes mellitus. Journal of Indian Society of Periodontology 2015;19(4):388-92.
73. Acharya AB, Thakur S, Muddapur MV, Kulkarni RD. Cytokine ratios in chronic periodontitis and type 2 diabetes mellitus. Diabetes & metabolic syndrome 2017;11(4):277-8.
74. Liu L, Xiao Z, Ding W, Wen C, Ge C, Xu K, et al. Relationship between microRNA expression and inflammatory factors in patients with both type 2 diabetes mellitus and periodontal disease. American journal of translational research 2022;14(9):6627-37.
75. Pan W, Wang Q, Chen Q. The cytokine network involved in the host immune response to periodontitis. International journal of oral science 2019;11(3):30.
76. Matsui S, Zhou L, Nakayama Y, Mezawa M, Kato A, Suzuki N, et al. MiR-200b attenuates IL-6 production through IKKβ and ZEB1 in human gingival fibroblasts. Inflammation research: official journal of the European Histamine Research Society [et al] 2018;67(11-12):965-73.
77. Belgardt BF, Ahmed K, Spranger M, Latreille M, Denzler R, Kondratiuk N, et al. The microRNA-200 family regulates pancreatic beta cell survival in type 2 diabetes. Nature medicine 2015;21(6):619-27.
78. McArthur K, Feng B, Wu Y, Chen S, Chakrabarti S. MicroRNA-200b regulates vascular endothelial growth factor-mediated alterations in diabetic retinopathy. Diabetes 2011;60(4):1314-23.
79. Ke XF, Fang J, Wu XN, Yu CH. MicroRNA-203 accelerates apoptosis in LPS-stimulated alveolar epithelial cells by targeting PIK3CA. Biochemical and biophysical research communications 2014;450(4):1297-303.
80. Al-Rawi NH, Al-Marzooq F, Al-Nuaimi AS, Hachim MY, Hamoudi R. Salivary microRNA 155, 146a/b and 203: A pilot study for potentially non-invasive diagnostic biomarkers of periodontitis and diabetes mellitus. PloS one 2020;15(8):e0237004.
81. Dennison EM, Syddall HE, Aihie Sayer A, Martin HJ, Cooper C. Lipid profile, obesity and bone mineral density: the Hertfordshire Cohort Study. QJM : monthly journal of the Association of Physicians 2007;100(5):297-303.
82. Genco RJ, Grossi SG, Ho A, Nishimura F, Murayama Y. A proposed model linking inflammation to obesity, diabetes, and periodontal infections. Journal of periodontology 2005;76(11 Suppl):2075-84.
83. Genco RJ, Grossi SG, Ho A, Nishimura F, Murayama Y. A Proposed Model Linking Inflammation to Obesity, Diabetes, and Periodontal Infections. Journal of periodontology 2005;76 Suppl 11S:2075-84.
84. Virto L, Cano P, Jiménez-Ortega V, Fernández-Mateos P, González J, Esquifino AI, et al. Obesity and periodontitis: An experimental study to evaluate periodontal and systemic effects of comorbidity. Journal of periodontology 2018;89(2):176-85.
85. Dursun E, Akalin FA, Genc T, Cinar N, Erel O, Yildiz BO. Oxidative Stress and Periodontal Disease in Obesity. Medicine 2016;95(12):e3136.
86. Kalea AZ, Hoteit R, Suvan J, Lovering RC, Palmen J, Cooper JA, et al. Upregulation of gingival tissue miR-200b in obese periodontitis subjects. Journal of dental research 2015;94(3 Suppl):59s-69s.
87. Roos J, Enlund E, Funcke JB, Tews D, Holzmann K, Debatin KM, et al. miR-146a-mediated suppression of the inflammatory response in human adipocytes. Scientific reports 2016;6:38339.
88. Motedayyen H, Ghotloo S, Saffari M, Sattari M, Amid R. Evaluation of MicroRNA-146a and Its Targets in Gingival Tissues of Patients With Chronic Periodontitis. Journal of periodontology 2015;86(12):1380-5.
89. Sanada T, Sano T, Sotomaru Y, Alshargabi R, Yamawaki Y, Yamashita A, et al. Anti-inflammatory effects of miRNA-146a induced in adipose and periodontal tissues. Biochemistry and biophysics reports 2020;22:100757.
90. Blasius AL, Beutler B. Intracellular toll-like receptors. Immunity 2010;32(3):305-15.