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ANALYSIS OF WAVE1 GENE EXPRESSION IN PERIODONTITIS

MASTER'S THESIS

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ETHICS CONTRACT

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ÖZET

PERİODONTİTİSTE WAVE1 GEN EKSPRESYONUNUN ANALİZİ

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Periodontitis, periodonsiyumun bozulması ile karakterize multifaktöriyel inflamatuar otoimmün bir hastalıktır. Konağın inflamatuar yanıtı ile periodontopatik bakteriler tarafından üretilen supra- ve sub-gingival biyofilmler arasındaki etkileşimin bir sonucudur. Genetik, mikrobiyal, çevresel ve immünolojik faktörlerin yanı sıra ırk, cinsiyet ve yaş da dahil olmak üzere etkileşimli çoklu risk faktörlerine bağli bir hastaliktir. Bugüne kadar, periodontitis ile doğrudan ilgili spesifik genler tanımlanmamıştır. WASP-ailesi verprolin homolog protein 1 (WAVE1), çoğunlukla nöronal hücrelerde eksprese edilen temel aktin-hücre iskeleti yeniden düzenleme proteinlerinden biridir. Tümör büyümesini, invazyonunu ve metastazını düzenlemek için önemli bir moleküldür. ARP 2/3 kompleksinin aktivasyonu ile aktin polimerizasyonunun kontrolünde WAVE1 proteini, hücre göçünde gözlenen aktin bazlı membran çıkıntılarının oluşumunda görev almaktadır. Bu, periodontitis ve WAVE1 arasındaki ilişkiyi değerlendiren ilk çalışmadır. Bu tez çalışması, WAVE1 geninin periodontitis dokularındaki mRNA ekspresyonunu analiz etmek için 18 periodontitis hastası (10 erkek ve 8 kadın) alındı. Ortalama yaşları 53.1 (34-65) idi. Her hastanın periodontitis ve sağlıklı dokularındaki gen ekspresyon düzeyini değerlendirmek için Kantitatif gerçek zamanlı polimeraz zincir reaksiyonu (qPZR) yöntemi kullanıldı. Analiz edilen veriler, sağlıklı kontrol dokuları ile karşılaştırıldığında WAVE1 geninin periodontitis dokusunda ekspresyonunun arttığı görüldü, bu artışın istatistiksel olarak anlamlı olduğu bulundu ($p = 0.0442$). Bu çalışmanın sınırlılıklarından biri örneklem büyüklüğünün küçük olmasıdır. Sonuç olarak, WAVE1 periodontitisin gelişmesinde ve ilerlemesinde önemli bir role sahiptir.

Anahtar Kelimeler: Ekspresyon; Gerçek zamanlı PZR; Periodontitis; WASF1; WAVE1.

ABSTRACT

ANALYSIS OF WAVE1 GENE EXPRESSION IN PERIODONTITIS

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Periodontitis is a multifactorial inflammatory autoimmune disorder characterized by disruption of the periodontium. It is a result of the interaction between host inflammatory response and supra- and sub-gingival biofilms produced by periodontopathic bacteria. The condition depends on multiple interacting risk factors, including: genetic, microbial, environmental, and immunological factors, as well as race, gender, and age. To date, specific genes directly involved in periodontitis have not been described. WASP verprolin-homologous protein 1 (WAVE1) is one of the essential actin-cytoskeleton rearrangement proteins that is primarily expressed in neuronal cells. It is a very crucial molecule in the regulation of tumor growth, invasion and metastasis. The role of WAVE1 protein in the control of actin polymerization by activation of the ARP 2/3 complex seems to be important in the formation of actin-based membrane protrusions that are observed in cell migration. The current study is the first to investigate the relationship between periodontitis and WAVE1. In this study, 18 periodontitis patients (10 male and 8 female) were recruited in order to analyze the mRNA expression of WAVE1 gene in periodontitis tissues. They had a mean age of 53.1 years (34-65). The quantitative real time PCR (qPCR) method was used to evaluate the gene expression level in periodontitis and healthy tissues of each patient. The analyzed data showed that WAVE1 was overexpressed in periodontitis tissues when compared to healthy control tissues and the difference was statistically significant ($p = 0.0442$). One of the limitations of this study was that it had a small sample size. In conclusion, WAVE1 does have a significant role in the development and progression of periodontitis.

Keywords: Expression; Periodontitis; Real-Time PCR; WASF1; WAVE1.

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LIST OF ABBREVIATIONS

Abi1	Abl-interacting protein 1
ACTB	Beta-actin
ARP	Actin related protein
CAL	Clinical attachment loss
cDNA	Complementary DNA
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease-2019
CRIB	CDC42/Rac-interactive binding
EVH1	Ena-VASP homology 1
F-actin	Filamentous-actin
FcγR	Fc gamma receptors
G-actin	Globular-actin
GBD	GTPase-binding domain
GTPases	Guanosine triphosphatases
IL	Interleukin
JMY	Junction-mediating and regulatory protein
MMP	Matrix metalloproteinase
NPF	Nucleation-promoting factors
N-WASP	Neural-WASP
PCR	polymerase chain reaction
qPCR	Quantitative/real time PCR
RANK	Receptor activator of nuclear factor kB
RANK-L	RANK ligand
RT-PCR	Reverse transcriptase PCR
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SCAR	Suppressor of cyclic AMP repressor
SES	Socio-economic status
SHD	SCAR homology domain

TLR	Toll-like receptor
TNF-α	Tumor necrosis factor-alpha
US	United States
VCA	Verprolin-homology-connecting acidic
VDR	Vitamin D receptor
WAS	Wiskott Aldrich Syndrome
WASP	Wiskott-Aldrich syndrome protein
WASH	WASP and SCAR homologue
WASF1	WASP Family Member 1
WAVE	WASP verprolin-homologous protein
WHAMM	WASP homologue associated with actin, membranes, and microtubules
WH	WASP homology
WHD	WAVE homology domain
WIP	WASP-interacting protein
WRC	WAVE regulatory complex

1. INTRODUCTION

Periodontitis is a common chronic multifactorial inflammatory disorder associated with bacterial dysbiosis, and it is characterized by disruption of the tissues that support the teeth, including the alveolar bone, gingiva, and periodontal ligament; collectively denoted as the periodontium (Hajishengallis, 2015). The disease is a result of the interaction between host inflammatory response and supragingival and subgingival biofilms produced by oral bacterial pathogens (Oppermann et al, 2012). Periodontitis affects 42% of adults in the United States and has a global prevalence of 11% by affecting more than 743 million people, making it the 6th most prevalent human disease on the planet (Eke et al., 2018; Tonetti et al., 2017). The condition frequently leads to gum infection and withdrawal, destruction of periodontium that supports the teeth, and even tooth loss, hence significantly affects the quality of life in these patients (Grønkjær et al., 2018; Borges et al., 2013).

Even though the primary cause of periodontitis is the accumulation of some periodontopathic bacteria, including; *Porphyromonas gingivalsis, Treponema denticola* and *Tannerella forsythia* (Parvaneh et al., 2021), other secondary risk factors play a major role in the development of the disease, including; age, gender, oral hygiene habit, obesity, stress, smoking, diabetes, furcation involvement, frequency of supportive periodontal care, depth of residual pocket, and genetics (Chrysanthakopoulos, 2020; Alhassani & Al-Zahrani, 2020).

Many cellular activities require regulated polymerization and depolymerization of actin, including; adhesion, phagocytosis, and cellular movement (Park et al., 2010). Cellular movement is essential for many normal biological processes, such as activation of the immune system, tissue repair and regeneration. However, abnormally activated cell migration is involved in many diseases (Derry et al., 1994). Regulation of the actin cell skeleton is vital for intracellular activities, particularly for inflammatory cell migration, and the formation of cytotoxic T cell apparatus and immune synapses. Inadequate or abnormal expression of proteins involved in regulating the actin cell skeleton , such as Wiskott-Aldrich syndrome proteins (WASPs) and WASP verprolin-homologous proteins

(WAVEs), increase immunodeficiency and/or autoimmune/inflammatory diseases (Lafouresse et al., 2013).

The WAVE1 is one of the essential actin-cytoskeleton rearrangement proteins that is encoded by WASP Family Member 1 (WASF1) gene (Frugtniet et al., 2015). The protein is mostly expressed in neuronal cells, and also in other tissues in low levels. It is a very crucial molecule in the regulation of tumor growth, invasion and metastasis (Zhang et al., 2016). The role of WAVE1 protein in the regulation of actin polymerization by activating the ARP 2/3 complex seems to be important in the formation of actin-based membrane protrusions that is observed in cell migration. Hence, WAVE1's activity in cell migration has received attention in recent years (Tang et al., 2020).

Currently, there are no related research in the biomedical literature regarding WAVE1 gene expression and periodontitis. Hence there exist a gap of information in this area of research. Genetic analysis may help identify genes that are involved in the pathogenesis of periodontitis. WAVE1-mediated actin cell skeletal system reorganizations may play a central role in regulating autophagy and inflammation activities that occur in response to both chemical and bacterial stimuli seen in periodontitis. In this respect, the aim of the current study is to investigate the role of WAVE1 in the inflammatory process and gum withdrawal due to periodontitis.

2. LITERATURE REVIEW

2.1. General information on periodontitis

2.1.1. Definition

Periodontitis is a common chronic multifactorial inflammatory disorder associated with bacterial dysbiosis, and it is characterized by disruption of the tissues that support the teeth, including the alveolar bone, gingiva, and periodontal ligament; collectively denoted as the periodontium (Figure 2.1) (Hajishengallis, 2015). Periodontitis is a result of the interaction between the host inflammatory response and subgingival biofilms produced by oral bacterial pathogens (Oppermann et al., 2012). The disease has been known since ancient times, and it has especially become prevalent when the oral microbiota make-up of humans went through a distinct change in composition after animal and plant domestication in Neolithic societies (Hajishengallis, 2015).

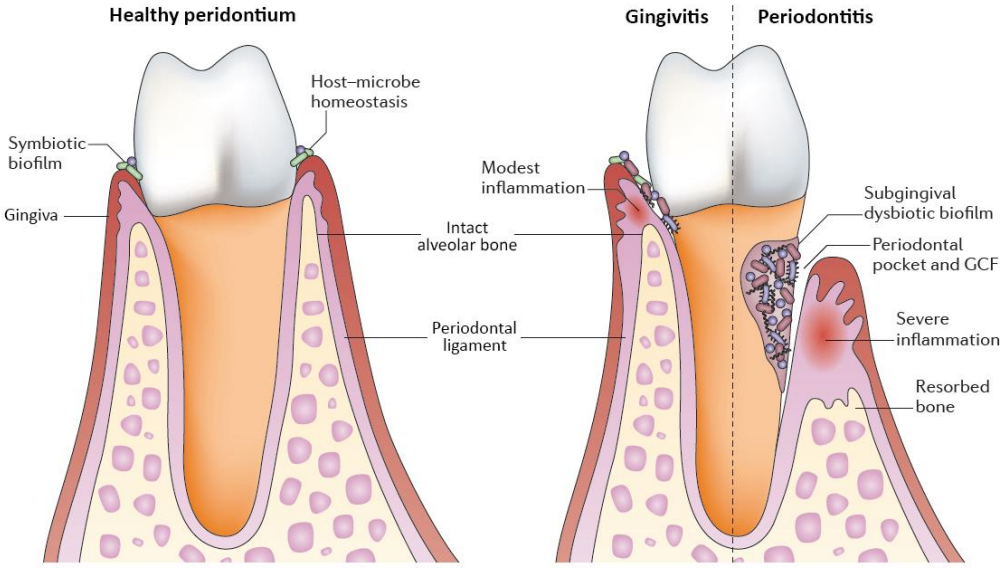

Figure 2.1. Periodontal health and the structure of the periodontium (Hajishengallis, 2015).

Periodontitis is usually associated with gingival inflammation, deepening of the teeth, loss of connective tissue, bleeding upon probing, and alveolar bone loss (Papapanou

et al., 2018; Page & Eke, 2007). The condition frequently leads to gum infection and withdrawal, destruction of the periodontium that supports the teeth, and even tooth loss, hence significantly affects the quality of life in these patients (Figure 2.2) (Grønkjær et al., 2018; Borges et al., 2013). Gum withdrawal due to periodontitis is a highly undesirable disease of the gum that has a very high incidence (Cobb, 2017). Gum withdrawal exposes the periodontium to the oral environment and can be irreversible if left untreated (Pini Prato, 2000).

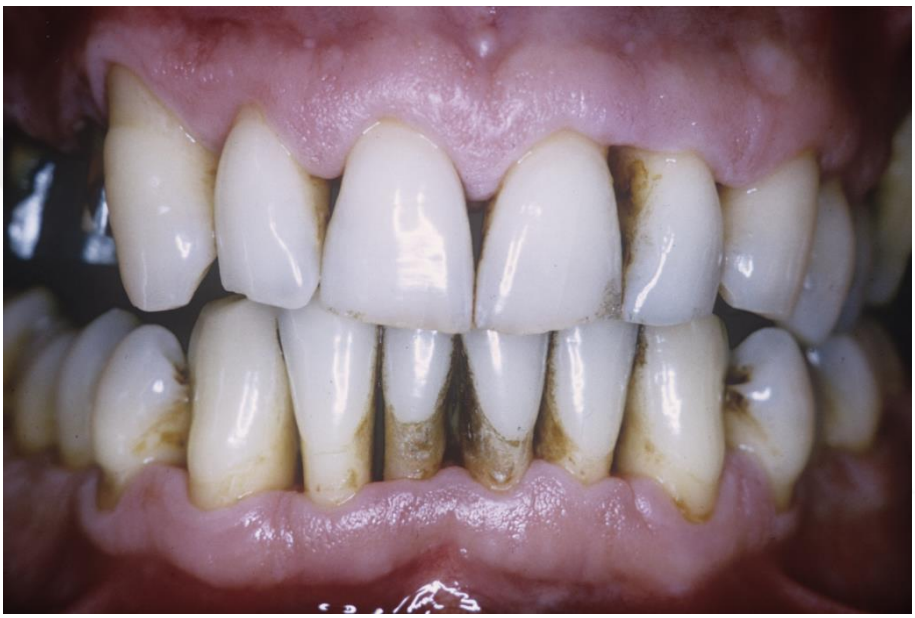

Figure 2.2. A patient with severe periodontitis (Dr. W. GREEN/CNRI/SCIENCE PHOTO LIBRARY).

2.1.2. Classification

The classification of periodontitis subtypes is crucial for making clinical decisions. According to 1999's classification of periodontitis, cases were classified based on clinical attachment loss (CAL) as chronic/aggressive, generalized/localized, and mild/moderate/severe periodontitis (Armitage, 1999). However, in 2018, a new classification system for periodontitis was developed, in which it addressed unresolved problems of the 1999's classification system. Based on recent information, the new system put forward 3 major categories of periodontitis: a) periodontitis (which included both major subtypes of "chronic and aggressive periodontitis" in the previous

classification), b) necrotizing periodontitis, and c) periodontitis as a manifestation of systemic disease (Tonetti et al., 2018; Papapanou et al., 2018).

The new system uses multi-aspect classification of periodontitis by addressing the condition's progression, extent, and severity through applying staging and grading evaluation. In addition, past history of the patient and treatment complexity were also considered (Tonetti et al., 2018). In the new 2018 classification, periodontitis patients are staged between I-IV based on bone loss, CAL, and the number of lost teeth to determine disease severity. More factors are used to further modify the severity staging of the disease, such as furcation involvement, probing-pocket depths, and type of bone loss. Furthermore, the extent of the condition is classified as generalized/localized. Finally, patients are graded A, B, or C based on disease progression, which is affected by smoking, the presence of diabetes mellitus, and bone loss/age index (Graetz et al., 2018).

2.1.3. Diagnosis

For the diagnosis of periodontitis, the medical history of the patient should be obtained to identify the risk factors affecting the patient. Additionally, a thorough periodontal evaluation should be conducted, including; periodontal probing depth, biofilm index, gingival recession, presence of bleeding on probing, occlusal trauma, tooth mobility, furcation involvement, and mucogingival deformity. In addition, a radiographic examination should also be performed to reveal the level of vertical and horizontal alveolar bone loss (Kwon et al., 2021). Periodontitis patients are reported to be associated with raised levels of C reactive protein, interleukin (IL)-6, and markers of oxidative stress in the blood (Chapple, 2014). A number of improvements have been made in understanding the agents involved in the development and progression of periodontitis, but it is seen that a single marker does not fully meet all the criteria for the evaluation of periodontitis, but future research is likely to lead to the production of these marker packages (Ram et al., 2015).

2.1.4. Therapy

Treating biofilm-associated oral infections is a great challenge as the primary etiology for the initiation of gingivitis (which is an inflammation of the gums; if left untreated, it can progress into periodontitis) and periodontitis is bacterial biofilm.

Approaches to prevent or treat these two conditions highly focus on the removal of these biofilms, which can be located subgingivally or suprabgingivally (Donlan & Costerton, 2002). Eliminating the risk factors of periodontitis is crucial in the prevention of the disease. Meanwhile, non-surgical supportive management is used as an initial step, and in severe cases, surgical resection is performed (Kwon et al., 2021). Surgical or non-surgical, successful results can only be achieved if good oral hygiene is performed and maintained by the patient. Hence, toothbrushing to remove/prevent dental plaque is an essential and effective strategy to prevent or treat pocket infections. Yet, mechanical removal of bacterial biofilms should not be the only therapeutic approach for these cases, and substances with antibacterial characteristics should also be used against those bacterial agents that are involved in disease progression (Arweiler et al., 2018).

2.2. Periodontitis epidemiology

Periodontitis is a common non-transmissible disease that often begins during childhood or adolescence. However, it usually stays silent for years until it appears later in adulthood and the years beyond (Slots, 2017). Data from the National Health and Nutrition Examination Survey between 2009-2014 showed that 42% of United States (US) adults who were 30 years old or older, had periodontitis, and 7.8% of them were associated with the severe form of the condition (Eke et al., 2018). It was also reported that amongst the US population, 59.8% of those aged 65+ years, 46% of those aged 45-64 years, and 29.5% of adults aged 30-44 years had some form of periodontitis (Figure 2.3) (Eke et al., 2020). Approximately half of the adults in the United Kingdom and 60% of those over 65 years are affected by the condition (Chapple, 2014). The global prevalence of periodontitis is thought to be around 11% of the world's population, affecting more than 743 million people, making it the 6th most prevalent human disease on the planet (Figure 2.4) (Tonetti et al., 2017). The condition is a global economic burden on the healthcare sector. In 2008, it was estimated that in the United Kingdom alone, periodontitis related costs were more than 3.75 billion US dollars (Chapple, 2014). According to a study, 43% of periodontitis patients aged between 35-44 years are associated with more than 3 mm of CAL; this proportion significantly increases in patients older than 65 years (91%) (Ilhan et al., 2017).

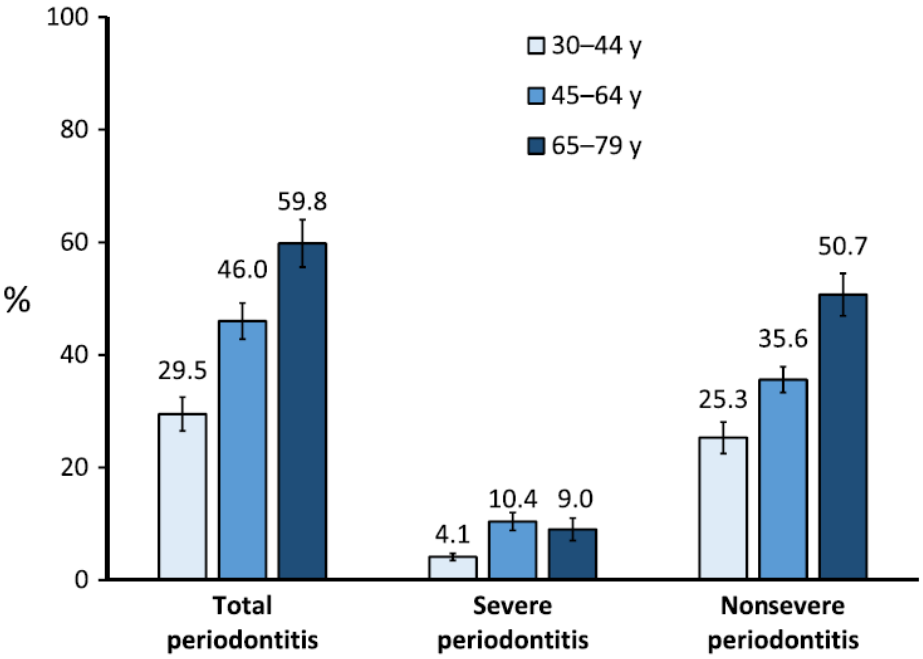

Figure 2.3. Prevalence periodontitis based on severity and age (Eke et al., 2020).

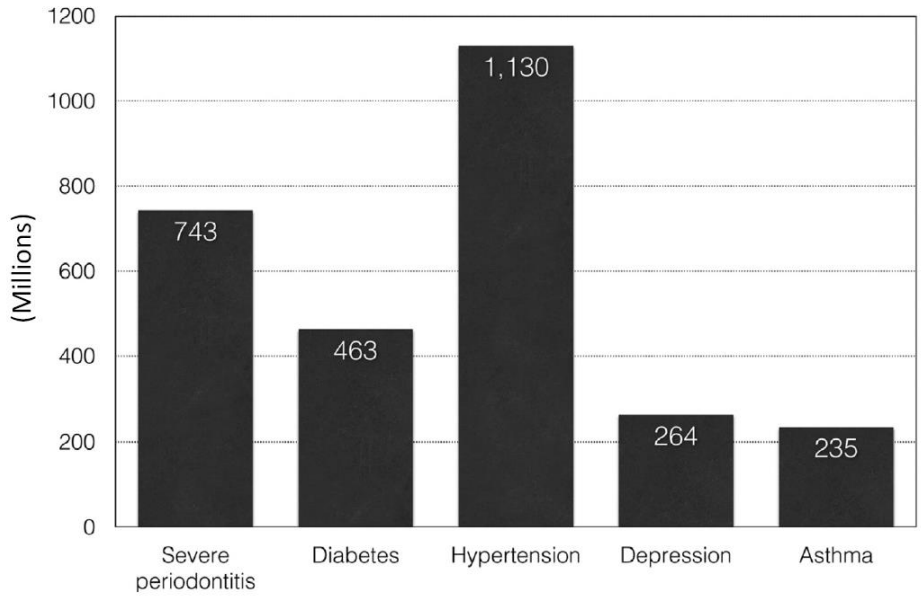

Figure 2.4. Severe periodontitis prevalence in comparison to other comorbidities (Kwon et al, 2021).

2.3. Periodontitis associated systemic diseases

Periodontitis does not only lead to tooth loss, it has also been reported to be potentially linked with a wide range of systemic conditions, such as cardiovascular diseases, respiratory problems, rheumatoid arthritis, fatal pneumonia in hemodialysis patients, adverse pregnancy outcomes, diabetes, chronic kidney disease, metabolic syndrome, and even cancer (Figure 2.5) (Parvaneh et al., 2021; Fischer et al., 2020; Hajishengallis, 2015). Systemic diseases due to periodontitis can be the result of multiple mechanisms, including: a) bacterial spread from the mouth has the potential to induce tissue damage in a variety of organs, b) a rise in systemic inflammatory load that may increase the likelihood of atheromatous plaque development, c) an autoimmune response that might be caused by oral bacterial epitopes (Corbella et al., 2018).

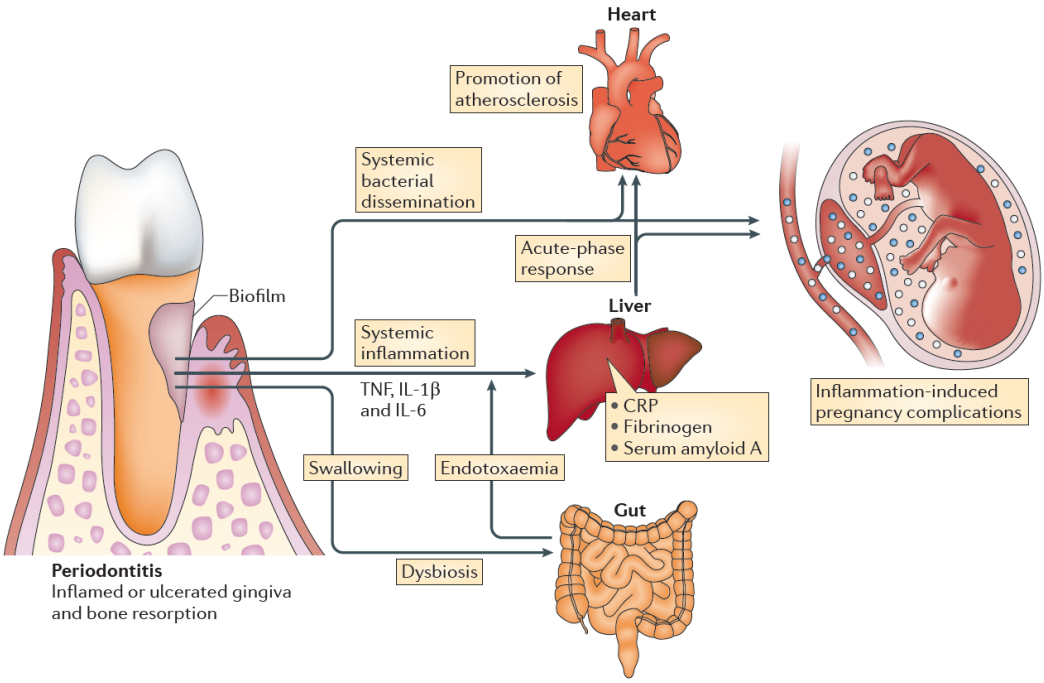

Figure 2.5. Linking periodontitis to other systemic diseases (Hajishengallis, 2015).

2.3.1. Cardiovascular Diseases

Many studies point out that periodontitis can be linked with cardiovascular diseases, regardless of other causative factors, including obesity and smoking (Oppermann et al., 2015). In addition, it has been postulated that controlling and managing periodontitis

lowers systemic inflammation and has positive effects on atherosclerosis and its progression (Hajishengallis, 2015). In a study by Parvaneh et al., it was shown that endothelial dysfunction is induced by periodontitis in mice (Parvaneh et al., 2021). This is in line with most studies conducted on the relationship between periodontitis disease and cardiovascular conditions in human patients.

2.3.2. Respiratory Diseases

Aspiration pneumonia and chronic obstructive pulmonary disease are two respiratory diseases reportedly associated with periodontitis.

2.3.2.1. Aspiration pneumonia.

Bacterial biofilms produced in gingivitis or periodontitis are considered a reservoir for pulmonary infections. From lung abscesses and aspiration pneumonia, oral anaerobic bacteria are regarded as frequent isolates. Periodontitis is identified as a risk factor for mortality in aspiration pneumonia, especially amongst the elderly population (Hajishengallis, 2015).

2.3.2.2. Chronic obstructive pulmonary disease (COPD).

Polybacterial infection, including Pseudomonas aeruginosa, can lead to the worsening of COPD and raise mortality and morbidity associated with the disease. It has been reported that in tracheal aspirates of acute COPD patients, *Porphyromonas gingivalis* is frequently detected alongside *Pseudomonas aeruginosa*, which can further promote *P. aeruginosa*'s pathogenicity (Hajishengallis, 2015).

2.3.3. Rheumatoid Arthritis

Rheumatoid arthritis is an autoimmune condition of the joints. An association between periodontitis and rheumatoid arthritis disease has been indicated by previous studies (Hoare et al., 2019; Hajishengallis, 2015). According to a study, patients with detectable *P. gingivalis* in dental biofilms were associated with a raised level of anti-citrullinated protein antibodies, which correlates with the severity of rheumatoid arthritis (Mikuls et al., 2014).

2.3.4. Adverse Pregnancy Outcomes

Clinical studies on periodontitis suggest that the disease is linked with a raised risk of unfavorable outcomes of pregnancy, such as pre-term birth, low weight at birth, stillbirth and miscarriage. It has been postulated that oral bacteria can spread systematically reach the fetal circulation through the placenta, and/or acute phase response caused by inflammatory mediators in the circulation, which are produced in the periodontium, negatively impact the fetus and/or placenta (Oppermann et al., 2015; Hajishengallis, 2015).

2.3.5. Diabetes Mellitus

Despite diabetes being known as a risk factor for periodontitis, it has been suggested that periodontitis and diabetes have a bidirectional association (Taylor, 2001). Diabetic patients who have periodontitis may have poor glycemic control, and the control and management of periodontitis can improve glycemic control in diabetic patients (Oppermann et al., 2015).

2.3.6. Cancer

Periodontitis patients have been found to be linked with a higher risk of some forms of cancer, both locally and generally (Corbella et al., 2018). More specifically, a link has been well established between periodontitis and orodigestive cancers, such as pancreatic, oral, esophageal, gastric, and colonic cancers. Cancers of the breast, prostate, and bladder have also been reported to be linked to periodontitis (Hoare et al., 2019). Another study reported that there is a positive correlation between periodontitis and all kinds of cancer (Fitzpatrick et al., 2010). However, additional research are needed to further evaluate this correlation.

Multiple mechanisms have been put forward to explain cancer development due to periodontitis. One theory postulates the role of several viruses (Epstein-Barr virus and Human Papilloma virus) that may reside in the periodontal pocket in causing oral cancer via multiple oncogenes activations. *P. gingivalis*, which is a key player in periodontitis, has also been suggested to be involved in cancer initiation by preventing cell apoptosis after invading the epithelium, as these bacteria can be found in gingival carcinomas and also in relation to other distant cancers (Corbella et al., 2018). It has also been proposed that inflammatory reactions due to periodontitis can cause reactive oxygen release and

other metabolites, which can in turn promote the development of cancer. In addition, cell stimulating signals in the inflammatory pathway can provide an ideal environment for cellular transformation (Coussens & Werb, 2002; Mantovani & Pierotti, 2008). Furthermore, periodontal bacteria can cause nuclear factor kappa B-mediated responses, activate oncogenic pathways, promote cell survival, increase cell migration and invasion, reduce proapoptotic protein expression, and enhance metastasis (Hoare et al., 2019).

2.4. Periodontitis etiology

Even though the main etiology of periodontitis is the accumulation of some dental opportunistic bacteria (also known as periodontopathic bacteria) such as; *Tannerella forsythia, Porphyromonas gingivalis,* and *Treponema denticola* (also referred to as red-complex bacteria) (Parvaneh et al., 2021), other secondary risk factors might play a major role in the development of the disease, including; socio-economic status, age, gender, race/ethnicity, oral hygiene habits, obesity, sleep deficiency, stress, smoking, diabetes, osteopenia/osteoporosis, furcation involvement, frequency of supportive periodontal care, depth of residual pocket, and genetics (Chrysanthakopoulos, 2020; Alhassani & Al-Zahrani, 2020). The majority of people have a symbiotic relationship with their oral microbiota (De Vries et al., 2017). However, these risk factors can disrupt this symbiosis and directly raise the possibility of developing periodontitis, or in their absence, lower the likelihood of acquiring the disease (Borrell & Papapanou, 2005). Bacterial infection due to the bacterial pathogen leads to a host immune response of the teeth-supporting tissue. Meanwhile, the inflammatory response of the tissue is highly affected by genetic and environmental factors which differ from one person to another (Chrysanthakopoulos, 2020).

2.4.1. Modifiable Risk Factors

2.4.1.1. Cigarette smoking

Smoking is one of the most critical secondary etiologies for periodontitis, and it is highly considered to be associated with the development and severity of the disease (Van Dyke & Dave, 2005). The smoking population has a much higher prevalence of periodontopathic bacteria compared to non-smokers (Chigasaki et al., 2018). Smoking is

thought to have a negative effect on immune cells, such as neutrophils. Smokers are also more prone to have alveolar bone and periodontal attachment loss (Kwon et al, 2021) (Van Dyke & Dave, 2005). In addition, it has been reported that smokers with periodontitis have a poorer response to treatment and prognosis when compared to non-smokers (Borrell & Papapanou, 2005). Hence, patients and physicians should be aware that for the successful treatment of periodontitis, smoking has to be ceased (Kwon et al, 2021).

2.4.1.2. Diabetes mellitus

Despite diabetes being an uncurable disease, it is still considered a modifiable risk factor for periodontitis as it can be controlled (Van Dyke & Dave, 2005). Previous studies suggest that cases with uncontrolled diabetes (both type I or II) are at a much higher risk of developing periodontitis when compared to the healthy population. Hence, diabetic patients are associated with a higher prevalence, severity, and extent of periodontitis (Borrell & Papapanou, 2005). It has been hypothesized that the underlying mechanism of this association is that uncontrolled diabetes leads to hyper-responsive macrophages and impaired neutrophils that produce pro-inflammatory cytokines. In addition, altered connection tissue metabolism in diabetic patients speeds up the reabsorption process in periodontitis (Kwon et al, 2021).

2.4.1.3. Obesity

Recent studies point out a possible link between periodontitis and obesity. A study on Japanese adults observed a considerable increase in the risk of periodontitis in cases with a high body mass index and body fat (Saito et al., 2001). It has been proposed that this association could be due to the abnormal lipid metabolism, hyperinflammatory condition, and insulin resistance found in obese people, all of which can together promote and induce periodontal connective tissue breakdown (Borrell & Papapanou, 2005).

2.4.1.4. Psychological factors

It has been demonstrated that the development alveolar bone loss and clinical attachment loss, found in periodontitis, have a higher likelihood in people under psychological stress (Van Dyke & Dave, 2005). The exact mechanism by which psychosocial stress induces periodontitis is not known; however, it has been proposed

that people under stress are more likely to smoke or have a poor oral and dental hygiene, which can in turn increase the likelihood of periodontitis development (Borrell & Papapanou, 2005). Even though studies in this regard is conflicting, a study conducted in the USA showed that individuals with financial crisis or those with poor stress coping behavior were associated with a higher risk for periodontitis (Genco et al., 1999).

2.4.1.5. Sleep deficiency

Sleeping is a normal physiological activity with regulatory and restorative effects. Sleep deficiency is considered as a sleep duration of less than 6-7 hours (da Silva et al, 2016). It has been reported that more than 30% of the adults in the USA sleep less than 7 hours (Alhassani & Al-Zahrani, 2020). Sleep deficiency has been found to lead to immune system function impairment, and alter endocrine and metabolic activities in the body, which are indicators of obesity and aging (Besedovsky et al., 2012). Previous studies regarding periodontitis and sleep deficiency have been contradictory. In the latest study by Alhassani et al., it was reported that periodontitis was much more prevalent (36%) in sleep deprived individuals in comparison to the control group with a normal sleeping pattern (Alhassani & Al-Zahrani, 2020).

2.4.1.6. Socio-economic status

The role of socio-economic status (SES), such as education and income, has not been properly studied independently from other risk factors. However, SES can have an influence on the ability to have access to proper education regarding dental and oral care, hence, indirectly increasing the risk of developing periodontitis (Borrell & Papapanou, 2005).

2.4.2. Non-Modifiable Risk Factors

2.4.2.1. Age

Even though the relationship is not straightforward, aging has been linked with a higher incidence of periodontitis. It has been suggested that both the severity and prevalence of the disease increase with aging. However, considering periodontitis as a disease of the elderly is not quite correct, as it has been proposed that the higher incidence in the elderly is due to prolonged exposure to the true risk factors and accumulated

destruction of the periodontal tissues (Van Dyke & Dave, 2005; Borrell & Papapanou, 2005).

2.4.2.2. Gender

Differences between males and females in susceptibility to developing periodontitis is yet to be demonstrated. However, studies have shown that males usually have poorer oral health when compared to women. Hence, it is possible that an association might exist that is yet to be addressed (Borrell & Papapanou, 2005).

2.4.2.3. Race/Ethnicity

Despite the fact that there are differences in the incidence of periodontitis between different regions, when age and oral hygiene are taken into consideration, no persistent differences between racial/ethnic groups have been identified (Borrell & Papapanou, 2005).

2.4.2.4. Osteopenia/Osteoporosis

According to some studies, systemic loss of bone density found in osteoporosis, in combination with some other factors, can make a host more susceptible to periodontitis (Wactawski-Wende, 2001). Even though most studies in this regard point out the possible association between alveolar bone loss and osteoporosis, the relationship between osteoporosis and CAL is not well established (Van Dyke & Dave, 2005). However, it has been reported that loss of bone mineral density is significantly associated with CAL in the elderly population (Yoshihara et al., 2004).

2.4.2.5. Host response and genetic factors

Even though periodontopathic bacteria are the main etiologic factor for the initiation and progression of periodontitis, it is well established that most of the periodontal tissue destruction observed in periodontitis is due to the host's own impaired immune response and not the bacteria themselves (Kaarthikeyan & Meenakshi, 2019). This dysregulation in the host's immune response can be strongly linked to genetics. A recent study by Nibali et al. in 1996 suggested that up to 1/3 of periodontitis is due to genetic factors, and more severe forms of the disease are associated with a higher heritability (Nibali et al., 2019). Research on identical twins has shown that host factors

account for 50% of periodontitis susceptibility (Van Dyke & Dave, 2005). Another study by Monteiro et al. showed that periodontitis-affected parents have an effect on the subgingival colonization of their children; however, it was not exactly pointed out whether this was due to genetic factors or not (Monteiro et al., 2021).

2.4.2.6. Other syndromes

Multiple conditions that result in neutrophil function deficiency have been reported to be related to periodontitis, such as leukocyte adhesion deficiency, Chediak-Higashi syndrome, cyclic neutropenia, agranulocytosis, Down syndrome, lazy leukocyte syndrome, and Papillon Lefevre syndrome (Van Dyke & Dave, 2005).

2.5. Pathogenesis of periodontitis

Generally, in a healthy state, the oral environment contains a balanced symbiotic microbial community that is mostly composed of facultative bacteria, including bacteria belonging to the Streptococci and Actinomyces genera. However, a drastic alteration in the composition of these microbial communities can convert this symbiosis of the oral environment into a dysbiotic state (Hajishengallis, 2015). It is suggested that infection in the subgingival area due to periodontopathic bacteria (*P. gingivalsis, T. denticola, and T. forsythia*), which are usually found in subgingival crevices and deep periodontal pockets of periodontitis cases, leads to inflammation in the gingival and periodontal tissues (Socransky et al., 1998). It is estimated that the inflammatory immune response of the host contributes to 80% of the risk of developing periodontitis (Meyle & Chapple, 2015). Despite *P. gingivalis* being a key bacterial player in the initiation of periodontitis, it has been found that *P. gingivalis* cannot cause periodontitis alone in germ-free subjects (Hajishengallis et al., 2011). Hence, *P. gingivalis* acts through inducing dysbiosis in normal oral microbial communities, causing imbalance and allowing other pathogenic bacteria to flourish. In addition, *P. gingivalis* impairs and manipulates the host immune response, preventing proper immune actions while also inducing inflammation at the site of periodontitis to receive more nutrients via increased blood flow (Figure 2.6) (Hajishengallis, 2015).

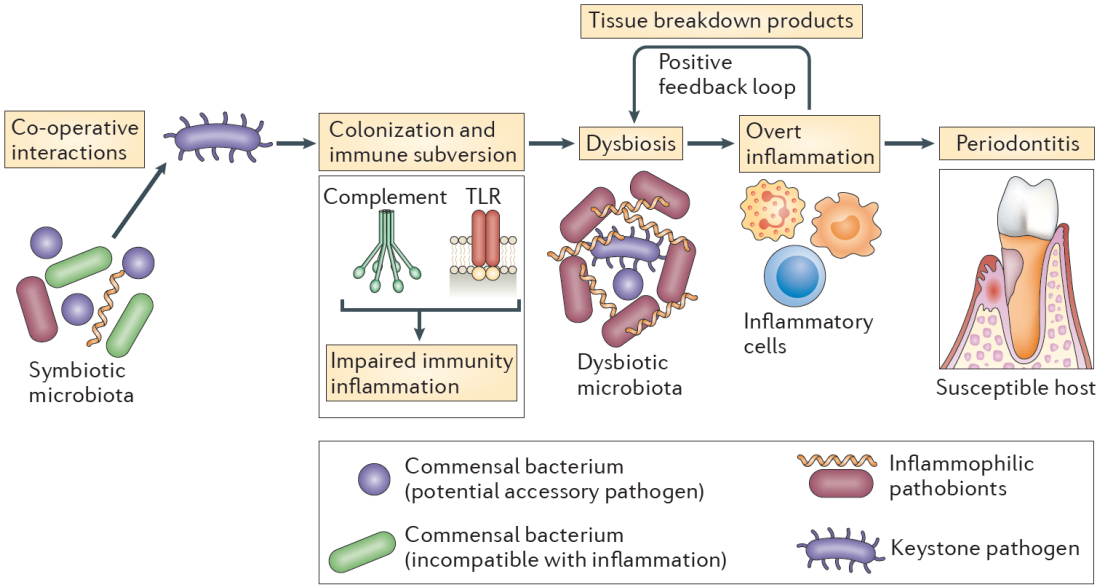

Figure 2.6. Microbial dysbiosis in periodontitis (Hajishengallis, 2015).

Regarding the pathogenesis of periodontitis, it has been reported that repeated infection with periodontopathic pathogens can disrupt host immune and inflammatory responses and lead to local tissue destruction of alveolar bone, connective attachment, and periodontal ligament, and can even lead to tooth loss (Cekici et al., 2014). Toxic products produced by these periodontopathic bacteria, such as lipopolysaccharide, in addition to other virulence factors, activate the first line of immune response and attract granulocytes (polymorphonuclear cells), neutrophils, macrophages, and natural killer cells and overwhelm them in the process. These bacteria, in addition to the host's own immune/inflammatory response, will disrupt the immune surveillance in the local tissue environment and induce macrophages and other immune cells to produce and secrete various inflammatory signals, such as IL-1, IL-2, IL-6, multiple other proinflammatory cytokines, and tumor necrosis factor-alpha (TNF-α) (Chrysanthakopoulos, 2020). These signaling molecules will in turn stimulate neutrophils, macrophages, junctional epithelial cells, and fibroblasts to produce secondary inflammatory mediators, which will further amplify the inflammatory signaling pathways and induce fibroblasts to produce matrix metalloproteinases (MMPs) that cause the destruction of collagen fibers in the periodontal tissue (Kwon et al., 2021). Meanwhile, the pro-inflammatory cytokines that are produced by T CD4+ lymphocytes will stimulate receptor activator of nuclear factor kappa B

(RANK) and its ligand (RANK-L), which is found on osteoblasts and T helper cells, to be further expressed. The expressed RANK-L molecule will bind to the RANK, which is found on osteoclast precursor cells (osteoblasts), and lead to the formation and maturation of osteoclasts, which will in turn mediate the destruction of alveolar bone (see Figure 2.7) (Darveau, 2020; Rosier et al., 2014).

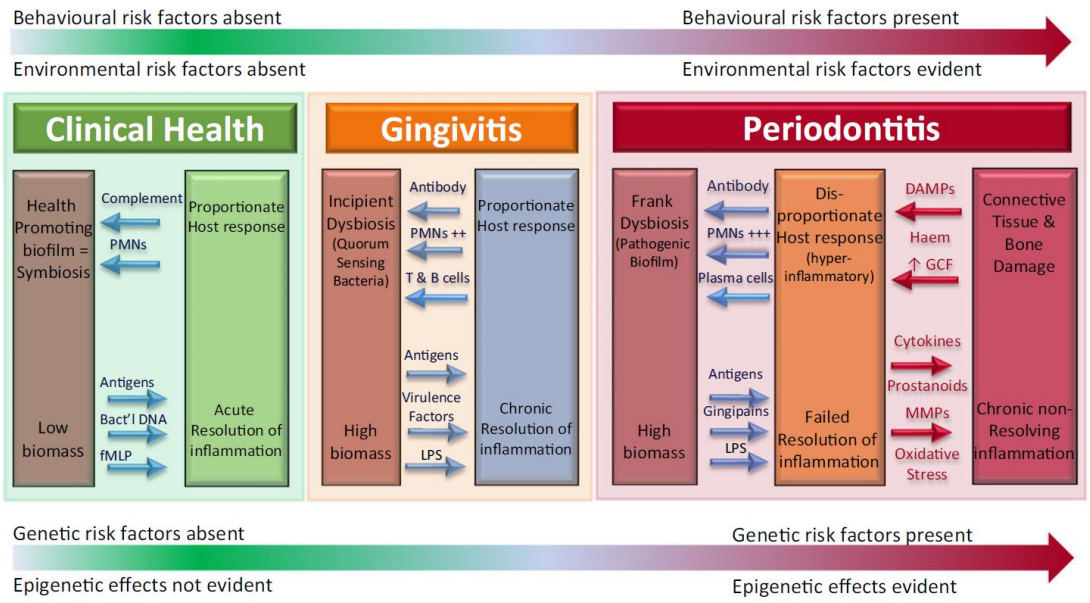

Figure 2.7. The new model of periodontitis pathogenesis (Meyle & Chapple, 2015).

2.6. Gene polymorphisms in periodontitis

Evidence has been building up regarding the role of single-nucleotide polymorphisms in the development of periodontitis. These gene polymorphisms can either alter the protein structure or change its expression level, which may in turn dysregulate the adaptive and innate immunity of the host (Laine et al., 2010). Many studies have been published in this regard to evaluate the significance of more than 65 gene polymorphisms in relation to periodontitis, including tumor necrosis factor, IL-1 gene cluster, IL-2, IL-4, IL-6, IL-10, transforming growth factor-b 1, CD14 receptor, Fc gamma receptors (FcγR), N-acetyltransferase 2, vitamin D receptor, and MMPs (Loos & Van Dyke, 2020) (Borrell & Papapanou, 2005). In addition, in new studies,

polymorphisms in long non-coding RNA genes have also been reported to be linked to periodontitis (Sayad et al., 2020). However, a direct association has not been established between periodontitis and all the studied genes.

2.6.1. Candidate Gene Polymorphisms Associated with Periodontitis

Most emerging evidence supports the involvement of some gene polymorphisms more than others in the pathogenesis and progression of periodontitis, such as IL-1, IL-6, IL-10, FcγR, CD14, and VDR (Laine et al., 2010; Laine et al., 2012; da Silva et al., 2017).

2.6.1.1. IL-1 Gene cluster

IL-1 is a pro-inflammatory mediator that is produced by dendritic cells, macrophages, and monocytes. Increased levels of IL-1α and IL-1β, and IL-1 receptor antagonist have been observed in periodontitis tissues. The genes for these proteins are located on chromosome 2q13–q21 (Laine et al., 2010; Laine et al., 2012).

2.6.1.2. IL-6 Gene

IL-6 is produced by various cell types, the level of section of IL-6 is based on the type of stimuli and the cell being stimulated. It is reported that IL-6 gene polymorphisms can affect the circulating IL-6 serum levels, and these polymorphisms have the potential to make the individuals carrying them more susceptible to periodontitis (Laine et al., 2010; Laine et al., 2012).

2.6.1.3. IL-10 Gene

IL-10 is an anti-inflammatory cytokine that is produced and secreted by monocytes, T cells, and macrophages. It downregulates the proinflammatory immune responses (IL-1 and TNF-α) produced by macrophages and monocytes. However, certain IL-10 gene polymorphisms can make hosts more susceptible to periodontitis. Its gene is located on chromosome 1q31-q32 (Laine et al., 2010; Laine et al., 2012).

2.6.1.4. Fc gamma receptors (FcγR) Gene

FcγR are members of the immunoglobulin superfamily, they are the most significant Fc receptors for initiating phagocytosis of marked microorganisms. FcR acts as a link between the humoral and cellular parts of immunity. In the periodontal tissues,

FcγRs are found on a wide range of immune cells and are highly considered to have a role in periodontitis pathogenesis. The genes of FcγRs are on chromosome 1q21 (Laine et al., 2010; Laine et al., 2012).

2.6.1.5. CD14 Gene

CD14 is a protein found in humans that is primarily produced by macrophages as part of the innate immune system. It aids in the detection of microorganisms in the body. Its gene is located on chromosome 5q21–q23. It has been reported that CD14 -260 polymorphisms can increase the susceptibility of developing periodontitis (Laine et al., 2010; Laine et al., 2012).

2.6.1.6. Vitamin D receptor (VDR) Gene

Vitamin D has a crucial role in the metabolism of bone. As reabsorption of alveolar bone is a primary feature of periodontitis, it has been proposed that gene polymorphisms of bone metabolism mediators, such as VDR, can make hosts more susceptible to periodontitis. It has also been observed that VDR and Vitamin D also have a role in phagocytosis and differentiation of monocytes. The gene encoding for VDR is on chromosome 12q12–q14 (Laine et al., 2010; Laine et al., 2012).

2.7. Expression of autophagy and apoptosis related genes in periodontitis

2.7.1. Autophagy Gene Expression and Periodontitis

Autophagy is a well-known natural cellular degradation activity that removes dysfunctional or unwanted cell components to allow intracellular recycling and tissue homeostasis maintenance. The mechanism of this cellular activity includes the engulfment of cytoplasmic material by phagophores to form autophagosomes. Lysosomes will later bind to these autophagosomes and release degradative enzymes to breakdown their content (Mizushima et al., 2010). It has been reported that autophagic response deficiency leads to increased inflammation and infection susceptibility. In a study by Ebersole and associates, a significant difference was noted in the expression of autophagy-related genes in the tissues of periodontitis patients when compared to healthy

patients. The differences were reflected in the mTOR complex, ATG12 conjugation/LC3 lipidation, PI3K complex, ULK1/ATG1 complex, ATG9 complex, and lysosome fusion/vesicle degradation (LF/VD) activities within the broader autophagic pathway. The genes most greatly altered in gingival tissues of naturally occurring periodontitis were identified in the LF/VD and ATG12 pathways (Ebersole et al., 2020). Another study reported that changes in the expression of genes related to autophagy are observed in the later progression of periodontitis (Ebersole et al., 2021).

2.7.2. Expression Of Apoptosis-Related Genes and Periodontitis

Programmed cell death, also known as apoptosis, is an important natural phenomenon in the maintenance of growth and homeostasis of tissue, which is also a key player in host immune and inflammatory responses (Kim et al., 2006). Apoptosis dysregulation has been shown to be involved in multiple human conditions, such as neoplasia, autoimmune and degenerative diseases, and acquired immunodeficiency syndrome. It has been postulated that apoptosis might also play a significant role in the development or progression of periodontitis (Kim et al., 2006; Gholami et al., 2020). Recent studies have revealed that in periodontal tissues, apoptosis-related factors, including DNA fragmentation, have been increased in correlation with increased probing depth (Gholami et al., 2021). Another study also found a significant alteration in the expression of apoptosis genes with the initiation of periodontitis (Ebersole et al., 2021). In 2021, a study by Gholami et al. showed different levels of expression of apoptosome-related genes (WDR33, AGO2, HUR1, and CPSF7) in periodontitis tissues, and considered AGO2 as a potential contributor to the development of the disease (Gholami et al., 2021).

2.8. Periodontitis as an inflammatory condition of oxidative stress

Evidence is building up regarding the involvement of reactive oxygen species in forming an oxidatively stressed environment, which underpins the pathogenesis of a wide scope of human inflammatory diseases. Hence, oxidation plays an important role in these diseases. It has been reported that antioxidants, detoxification enzymes, and nuclear factor erythroid 2-related factor 2-associated antioxidant upregulation promote cell protective

effects through reducing inflammation following oxidative tissue damage. Thus, management strategies that raise the level of antioxidants and/or antioxidant activity can be possible options to treat or prevent periodontitis or other conditions caused by oxidative stress (Sczepanik et al., 2020).

2.9. Periodontitis and coronavirus disease 2019 (COVID-19)

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a recently emerged viral pathogen that leads to coronavirus disease-2019 (COVID-19). SARS-CoV-2 was first found in China in late 2019, and it quickly spread into a pandemic. Most cases of COVID-19 lack symptoms or have only mild ones. However, in severe cases, it can even lead to death (Salih et al., 2021). Even though clinical research regarding the relationship between COVID-19 and periodontitis is scarce or lacking, according to the theoretical basis, a bidirectional biological pathway has been suggested between the two diseases (Fabri, 2020). Some studies suggest that due to the direct connection between periodontitis and overall health, there could be a connection between COVID-19 complications and periodontitis as well (Jafer et al., 2020). As periodontitis can result in bacteremia and adverse systemic inflammation, it can influence COVID-19 progression and COVID-19 associated systemic complications, and the dysregulation in immunity caused by COVID-19 can also in turn affect periodontitis progression (Fabri, 2020). According to recent reports, a similarity between COVID-19 and periodontitis was evident when observing changes in the main cytokine profile serum levels (Hajizadeh et al., 2021; Deo et al., 2021). A clinical study by Gupta and colleagues showed a direct link between periodontitis and COVID-19 outcomes (Gupta et al., 2021). In addition, in a case–control study conducted by Marouf et al., a significant connection between periodontitis and a raised risk of COVID-19 complications was observed, such as requiring ventilation, intensive care unit admission, and death. Hence, COVID-19 patients with periodontitis were linked to significantly higher levels of D-dimer, white blood cells, and C-reactive protein in the blood (Marouf et al., 2021).

2.10. Actin cytoskeleton and its regulation

The cytoskeleton is a crucial part of eukaryotic cells that has a role in maintaining cell shape, participates in the regulation of vital dynamic cellular activities, and serves as a necessary scaffolding for the activation/inhibition, and localization of several cytoplasmic signaling molecules. It is especially important for the immune system cells as many of their activities rely on their mobilization, such as immune cell activation, secretory function, cell–cell interaction, phagocytosis, and migration. The cytoskeleton is primarily composed of three different macromolecules, which include intermediate filaments, microtubules, and filamentous-actin (F-actin) that is made up of globular-actin (G-actin) monomers and compromises the actin cytoskeleton (Wickramarachchi et al., 2010).

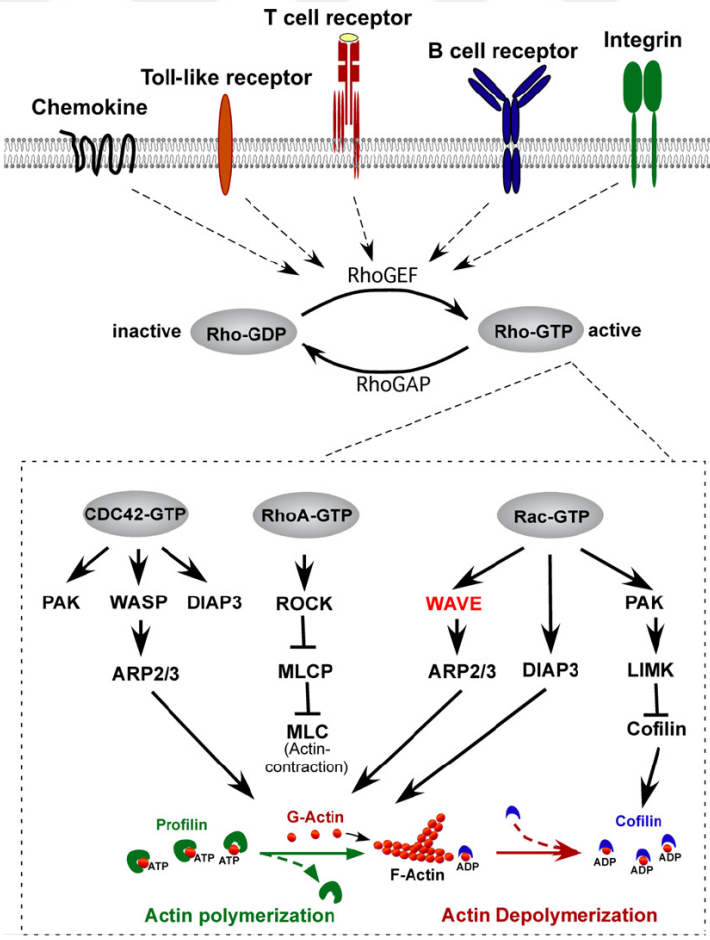

Figure 2.8. Actin polymerization regulation (Park et al., 2010).

Actin is a family of cell skeleton proteins that are expressed in almost all eukaryotic cells. Regulation and reorganization of the actin cytoskeleton are vital for intracellular interactions and many aspects of the immunological/inflammatory response to infections, especially the formation of cytotoxic T cell apparatus and immune synapses, cell polarization, inflammatory cell migration, phagocytosis of foreign bodies, immune cell adhesion to the vascular endothelium, and cellular movement (Lafouresse et al., 2013). Cellular movement is essential for many normal biological processes, including the activation of the immune system, tissue regeneration and repair. However, abnormally activated cell migration is involved in many diseases (Derry et al., 1994). Because of the instability of actin dimers, the polymerization of actin molecules into filaments is difficult (Pollard & Borisy, 2003). Hence, these activities, and many other cellular activities, require regulated, directed, and coordinated polymerization and depolymerization of actin filaments (Park et al., 2010). Cytoskeleton regulating proteins have various roles that act in a time and cell-specific way. They can reduce inflammation both outside and inside the cell and also affect the development of healing in inflammatory autoimmune diseases such as epidermolysis bullosa (Kopecki et al., 2016). Recent studies suggest that inadequate or abnormal expression of proteins involved in regulating the actin cell skeleton, increase immunodeficiency and/or autoimmune/inflammatory diseases (see Figure 2.8) (Lafouresse et al., 2013).

2.10.1. Rho Family of Guanosine Triphosphatases (GTPases)

Various extracellular stimuli lead to changes in the cell skeleton at different locations in the cell via the activation of downstream Rho proteins, such as extracellular matrix interaction, cell–cell adhesions, and signaling molecules (cytokines, hormones, and growth factors) that interact with the cell surface receptors, such as Toll-like receptors (TLRs), cytokine and chemokine receptors, and B and T cell antigen receptors (Sit & Manser, 2011). The core of actin polymerization and depolymerization is initiated by Rho GTPase members (Cdc42, Rho, and Rac) and highly regulated by downstream effecters, such as nucleation-promoting factors (NPF). Amongst the mammalian Rho GTPases family, Cdc42, Rho, and Rac members have been best studied. Only a few members have been shown to be involved in the function and development of acquired and innate immune cells, such as Cdc42, RhoA, RhoH, RhoG, Rac1, and Rac2 (Park et al., 2010). In the presence of stimuli, guanine nucleotide exchange factors can switch Rho proteins

from a GDP-bound inactive state to a GTP-bound active state, hence upregulating the active levels of membrane-bound Rho protein members and leading to a signaling cascade that will result in actin cytoskeletal reorganization. On the other hand, GTPase-activating proteins can switch off Rho family members by promoting the hydrolysis of GTP-bound active Rho proteins to a GDP-bound inactive state (see Figure 2.9) (Sit & Manser, 2011; Park et al., 2010).

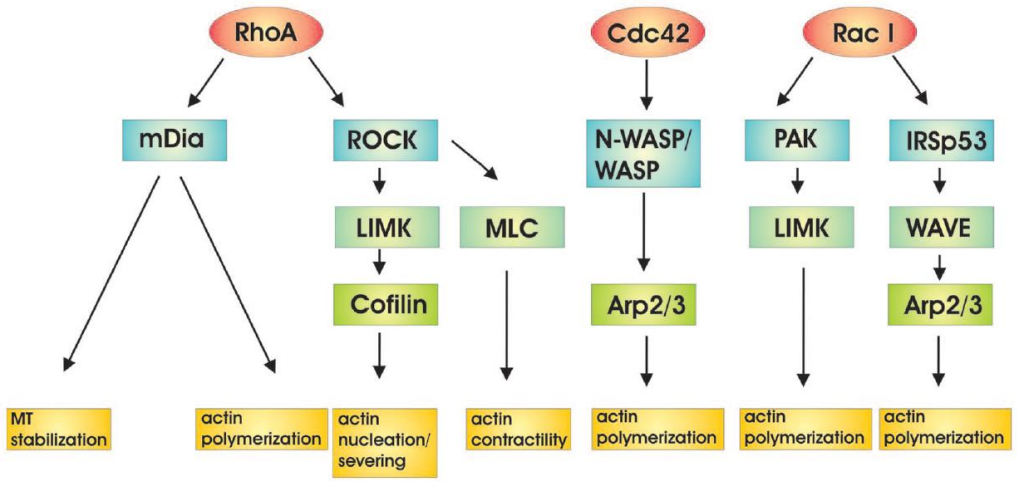

Figure 2.9. Rho family of GTPases downstream effector targets (Spiering & Hodgson, 2011).

2.10.2. Actin Related Protein (ARP) 2/3 Complex

The ARP2/3 is a protein complex composed of seven transmembrane proteins; five novel proteins (ARPC1-ARPC5) and two actin-related proteins (Arp2 and Arp3) (Goley & Welch, 2006). Even though a few actin nucleators exist in human cells that initiate monomeric G-actin polymerization into F-actin filaments (formin, spire, cordon-bleu, ARP2/3 complex, and leiomodin), branched-chain F-actin is only nucleated by the ARP2/3 complex, which adds actin at a 70-degree angle to pre-existing actin filaments. Hence, any abnormality in this complex can have a devastating effect on cell survival and function (Moulding et al., 2013). The ARP2/3 complex is indirectly activated via GTPases through NPFs, such as WASP and WAVE family proteins (WASP family through Cdc42 and WAVE family through Rac) (see Figure 2.10) (Wickramarachchi et al., 2010).

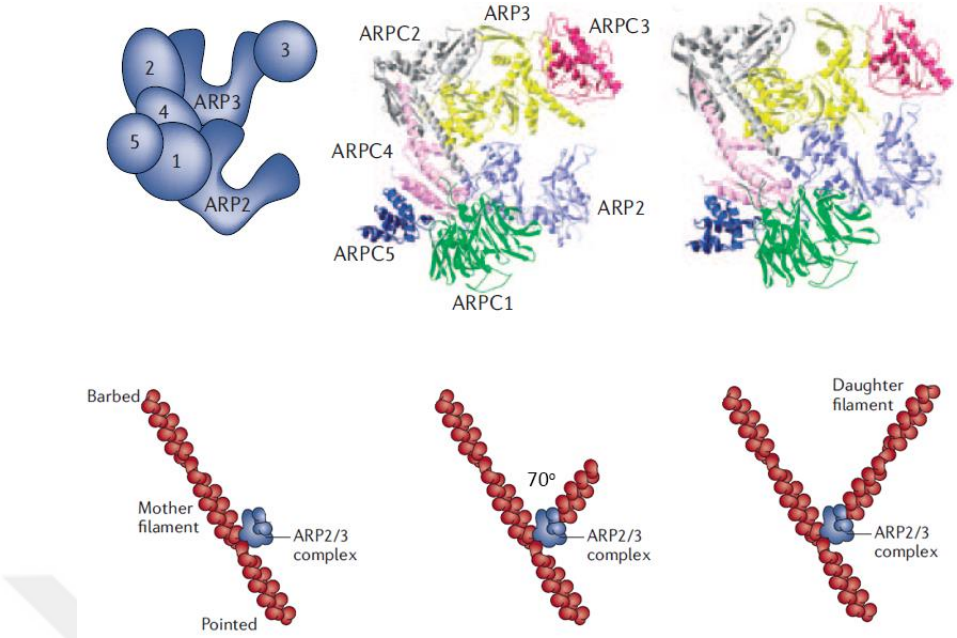

Figure 2.10. The ARP2/3 complex structure and function (Goley & Welch, 2006).

2.11. Wiskott Aldrich Syndrome (WAS) family of proteins

2.11.1. Discovery of WAS Family of Proteins

WAS is an X-linked primary immunodeficiency syndrome that results from mutations in the gene encoding for WAS protein (WASP), characterized by eczema, micro-thrombocytopenia, higher frequency of autoimmune diseases, and recurrent bacterial, fungal, and viral infections (Ngoenkam et al., 2021; De Meester et al., 2010). WASP was the first protein of the WASP family, which was identified in 1994 while searching for the genetic abnormality responsible for WAS (Badour et al., 2003). It belongs to the actin NPF family and is an important regulator of actin in hematopoietic cells (Ngoenkam et al., 2021). Neural (N-) WASP is another WASP family member which was later found from a proteomic search for mammalian proteins which are most abundant in the brain (Kurisu & Takenawa, 2009). In 1996, a new sub-family of the WASP family of proteins was discovered called the WASP verprolin-homologous proteins (WAVEs), which consists of WAVE1/SCAR1 (suppressor of cyclic AMP repressor 1), WAVE2, and WAVE3 proteins (Moazzam et al., 2015). Hence, it is currently agreed that mammals have five WASP genes belonging to two subfamilies: the

WASP sub-family (WASP and N-WASP) and the WAVE sub-family (WAVE1/SCAR1, WAVE2, and WAVE3) (Kurisu & Takenawa, 2009). In recent years, other proteins related to the WASP family of proteins have been discovered, including WASP and SCAR homologue (WASH), junction-mediating and regulatory protein (JMY), and WASP homologue associated with actin, membranes, and microtubules (WHAMM). However, the role of these new members has not been completely understood yet (Mughees et al., 2021).

2.11.2. WASP and WAVE Family Proteins

The WASP and WAVE family proteins comprise a pentameric complex consisting of two sub-families; the WASP family and the WAVE family. WASP family are found in many multicellular organisms and are responsible for reorganization of actin filaments into other shapes and forms, they are subdivided into two other subtypes; WASP which was the first protein of its kind to be found and neural (N)-WASP which is a brain molecule that is also expressed in various tissues (Svitkina, 2018). Meanwhile WAVE is a multi-subunit protein sub-divided into WAVE1-WAVE3, which regulate the polymerization of branched actin networks at the leading edge (Lane et al., 2014).

The WASP-WAVE family proteins are potent NPFs that form a crucial link between the actin cell skeleton and GTPases. WASP-WAVE family proteins have a crucial role in regulating and controlling rapid actin polymerization through translating morphogenic extracellular signals via Rho GTPases into cytoskeletal changes as they are downstream effectors of the Rho GTPases. This translation can be achieved via the activation of ARP 2/3 complex by WASP-WAVE proteins via inducing conformational change and bringing ARP 2 and 3 subunits into close proximities, which will act as a seed in promoting F-actin branching in a 70-degree angle manner (Weeks et al., 2016). These protein families also play a major role in other regulatory cellular activities such as maintaining cell shape, contractility and adhesion, cell motility, and also in vesicle traffic and microbial infection (García et al., 2012).

In recent times, the members of the WASP family of proteins have been revealed to have multiple crucial functions, especially in autophagy. WASH deficiency has been reported to lead to impaired lysosomal digestion and autophagosome formation. WHAMM and WAVE proteins have been found to be important in autophagosome

formation and endo/exocytosis. WASP is reported to be involved in adaptive and innate immunity via controlling and regulating actin cytoskeleton-dependent cellular activities, such as cell signaling, immune synapse formation, cytokine release, and migration (Rivers & Thrasher, 2017). WAVE1 protein has been reported to be responsible in the formation of dorsal ruffles, WAVE2 in the formation of lamellipodia, and WAVE3 reportedly plays a role in cell differentiation (Mughees et al., 2021).

2.11.3. Structure of the WASP-WAVE Family

The structural properties of WASP-WAVE family members vary. However, they all share two homologous regions; a central segment that is rich in proline units and a carboxy-terminal module composed of three characteristic domains called the Verprolin-homology-connecting acidic (VCA) domain, which consists of verprolin homology (WASP homology 2 (WH2)) domain, cofilin homology (Central) domain, and an acidic region, that is responsible for ARP2/3 complex activation. The V domain is a single sub-unit actin binding site, and the CA domain binds the ARP2/3 complex. N-WASP has 2 WH2 domains; hence, it exhibits a much higher nucleation rate when compared to the other members (Lane et al., 2014). Despite their homology, WASP-WAVE family members each have a different and distinct amino-terminal domain that enables them to respond to different types of upstream signals and have different modes of regulation to control different cellular processes (Kim et al., 2000). The amino-terminal domain of WASP sub-family proteins is characterized by having a WASP homology 1 (WH1; also called Ena-VASP homology 1 (EVH1)) domain which acts like a protein-protein interaction domain and promotes binding with WASP-interacting protein (WIP), which protects WASP and N-WASP from protease degradation, following a basic region and a GTPase-binding domain (GBD; or CDC42/Rac-interactive binding (CRIB) domain). On the other hand, the amino-terminal domain of the WAVE sub-family of proteins is characterized by having a WAVE homology/SCAR homology domain (WHD/SHD) followed by a sequence of basic units; however, they lack the GBD/CRIB domain, hence they don’t have surface for direct linking with Rho GTPases (Mughees et al., 2021; Kurisu & Takenawa, 2009). The WHD domain of WAVEs does not bind to WIP but mediates direct binding to Abl-interacting protein 1 (Abi1), which is involved in Ras to Rac signaling (Fernando et al., 2009). Inside the cells, WAVE proteins form a heteropentameric complex with HSPC300/Brick1, Sra1/CYFIP1, Abi1/2/3, and Nap1,

called the WAVE regulatory complex (WRC) (see Figure 2.11) (Kurisu & Takenawa, 2009).

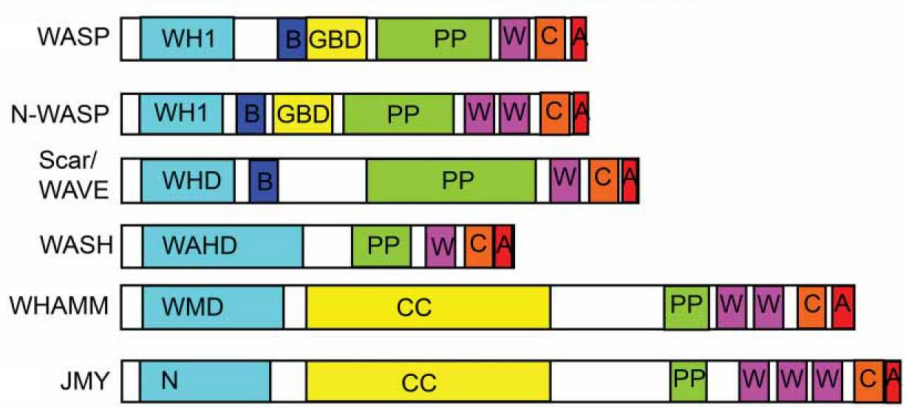

Figure 2.11. Schematic diagram showing domains of WASP family proteins (Tyler et al., 2016).

2.11.4. Activation of the WASP-WAVE Family

The WASP sub-family proteins are autoinhibited in the absence of extracellular signals. When inactive, these proteins exist in a closed conformation state where GBD at the amino-terminal binds to the VCA motif at the C-terminal, preventing the activation of the ARP 2/3 complex (Fernando et al., 2009). Activated upstream Cdc42 or Rac1 is shown to bind with the CRIB motif of WASP sub-family proteins and stop their autoinhibition by releasing the GBD from the VCA; therefore, opening the closed conformation and allowing the activation of the ARP 2/3 complex (Spiering & Hodgson, 2011).

Meanwhile, WAVE proteins exist as a heteropentameric complex (WRC). The activation mechanism of WAVE proteins has been controversial since their discovery. Initially, the WAVE sub-family of proteins were thought to be not autoinhibited and are inherently active (Fernando et al., 2009). Meanwhile, in recent years, it has been explained that, similar to WASP proteins, WAVE proteins are inactive in nature and exist in a closed conformational state via the masking of the VCA region (Frugtniet et al., 2015). WAVEs do not form direct links with Rho GTPases as they lack the GBD/CRIB

domain of WASP proteins. However, they still act as a downstream effector of Rac (Fernando et al., 2009). In order for Rac1 to have an effect on WAVE downstream effectors, Rac1 has to bind to the adapter molecule IRSp53/ Sra component, and this complex will then bind to WAVE proteins, inducing conformational change and exposing the VCA region for ARP 2/3 activation. Additionally, Abi1 binding seems to be necessary for WAVE activity (see Figure 2.12) (Spiering & Hodgson, 2011; Mughees et al., 2021).

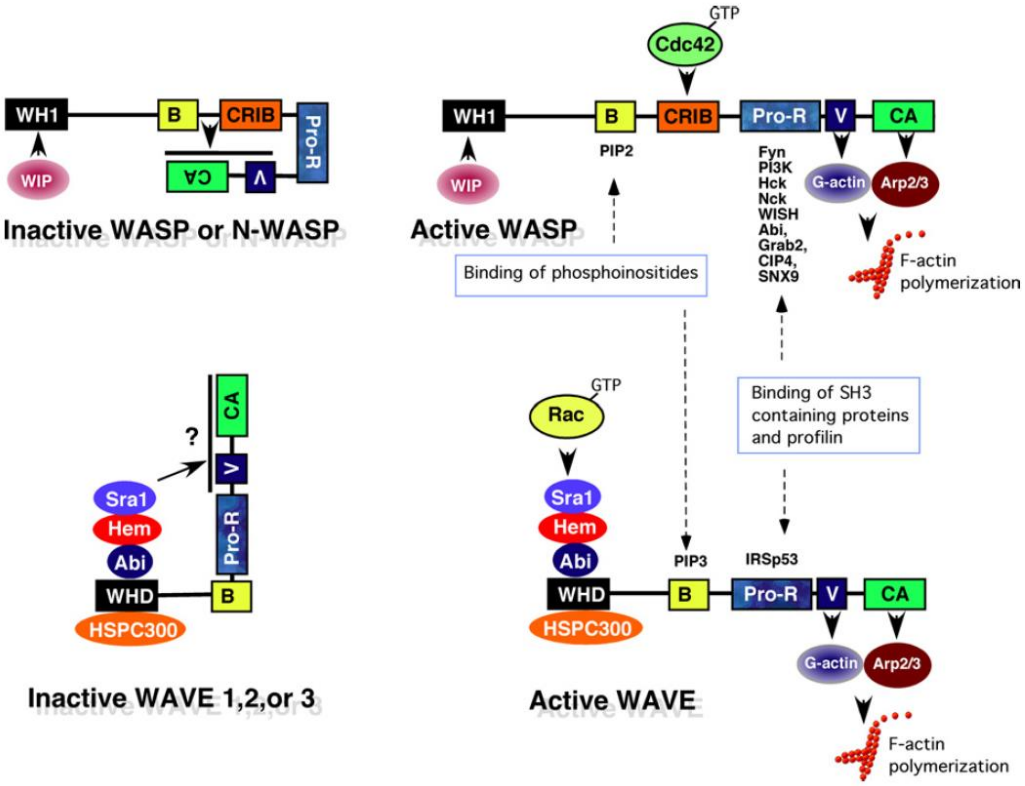

Figure 2.12. Activation of WASP and WAVE complexes (Park et al., 2010).

2.11.5. Activation of ARP 2/3 Complex By WASP-WAVE Family of Proteins

In the active state of WASP/WAVE family of proteins members, the carboxyl-terminal of these proteins makes changes in the actin cell skeleton by starting the growth of new actin filaments through the ARP 2/3 complex via causing conformational change and bringing actin ARP 2 and 3 subunits (forming ARP 2/3 complex) into close proximities, binding and recruiting 2 G-actin monomers, and then moving them to the side of already existing actin filaments (the V domains acts as G-actin binding site and

the CA acts as the ARP 2/3 binding site), which will act as a seed in promoting F-actin branching in a 70-degree angle manner which is vital in the initiation of membrane protrusions including filopodia and lamellipodia (Caron, 2002; Weeks et al., 2016; Luan et al., 2018).

2.11.6. WASP-WAVE Roles in Diseases

According to many studies, impairment or deficiency of the WASP-WAVE family of proteins is involved in the progression of many conditions or the dysregulation of many cellular processes. A study by Lee et al. showed that WASP deficiency can lead to the dysregulation of autophagy and inflammatory function (Lee et al., 2017). In another study by Nguyen et al., it was revealed that WASP deficiency leads to colitis and mucosal immune dysregulation in mice (Nguyen et al., 2012). In 2019, Wang et al. showed that knock down of N-WASP will upregulate the expression of inflammatory cytokines in gingival fibroblasts of humans, therefore they suggested that N-WASP may have a role in periodontitis pathogenesis (Wang et al., 2019). Regarding WAVE protein deficiency, Jiang and colleagues showed that abnormal expression of WAVE3 is linked to abnormality in wound healing (Jiang et al., 2010).

2.11.7. The Role of WASP-WAVE Family Proteins in Cancer Progression

The actin cytoskeletal structures, such as lamellipodia and filopodia, have a crucial role in cellular migration as they are associated with the extracellular matrix (ECM). Several studies have indicated the involvement of WASP-WAVE family proteins in several human cancers, such as prostate, breast, cervical, ovarian, brain, lung cancers, and others (Mughees et al., 2021). They have been found to act both as tumor suppressors as well as tumor enhancers based on the cancer type and pathological stage (Kurisu & Takenawa, 2010). A study by Menotti et al. showed that WASP acts as a tumor suppressor in T cell lymphoma (Menotti et al., 2019). Kurisu et al. revealed an increased expression of WAVE1 and 2 proteins in mouse melanoma cells (Kurisu et al., 2005). Overexpression of WAVE2 proteins was also observed in breast cancer and hepatocellular carcinoma (Fernando et al., 2007; Fernando et al., 2009). In addition, overexpression of the WAVE3 protein has also been reported in grade III breast tumors (Fernando et al., 2009).

2.12. WAVE1 protein

One of the important proteins of the WAVE family is WAVE1, which forms one of the subunits of WRC and is encoded by the WASP Family Member 1 (WASF1) gene that is located at chromosomal region 6q21. The WASF1 gene is 80,186 bps in length and translates into a 559-amino acid protein (Figure 2.13) (Srivastava et al., 2021). It was first discovered in 1996, and in 1998 it was first found to be an actin cytoskeleton regulator through interactions with ARP2/3 downstream of Ras-related C3 botulinum toxin substrate (Rac) (Frugtniet et al., 2015).

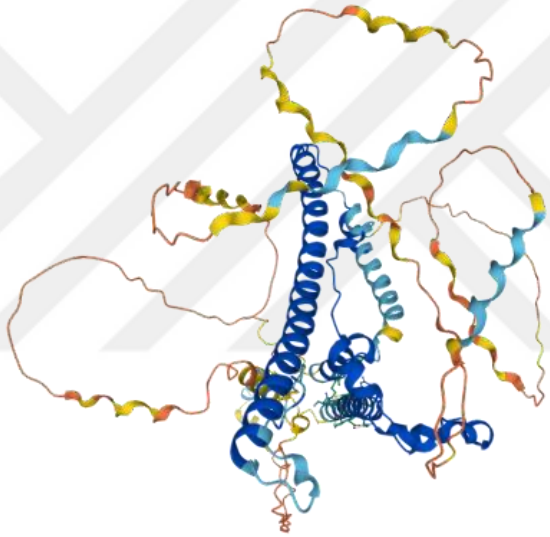

Figure 2.13. Predicted WAVE1 3D structure based on AlphaFold.

WAVE1 is primarily expressed in neuronal cells, however, it is also expressed in low levels in various cell types in the body (Tang et al., 2020). Despite its role in nervous system development, it also functions as a very crucial molecule for regulating tumor growth, invasion and metastasis (Zhang et al., 2016). WAVE1 acts as a suppressor of elongation and not an enhancer of actin filaments (Mughees et al., 2021). It is reported that the knockout of WAVE1 results in severe neurological defects (Moulding et al., 2013). Another study reported that mutations in WAVE1 results in a rare form of intellectual disability (Ito et al., 2018). Soderling et al. revealed that WAVE1 deficiency

leads to behavioral deficits in mice (Soderling et al., 2003). WAVE1 plays a crucial role in the central nervous system (CNS) as it mediates dendritic spine morphogenesis, growth cone dynamics, synaptic plasticity, neurite outgrowth, and oligodendrocyte myelination (Ceglia et al., 2010). Kim et al. reported that oligodendrocytes that were isolated from mice that lacked WAVE1 had less processes in comparison to normal oligodendrocytes (Kim et al., 2008).

WAVE1 is a critical pro-autophagic protein that is able to enhance the cell survival of leukemia cells and function as a negative regulator of apoptosis (Zhang et al., 2016). It has been revealed that WAVE1 is expressed in the bone marrow–derived macrophages; however, its function there is yet to be known (Dinh et al., 2008). It is reported that WAVE1 mediates phagocytosis inhibition caused by oxidized phospholipids, and it has a role as a vital modulator of the innate immune response to severe infections caused by bacterial pathogens (Matt et al., 2013). Dinh et al. also reported in their study that WAVE1 plays an important role in macrophage biology (Dinh et al., 2008).

It has been found that WAVE1 plays a key part in the metastasis and migration of multiple types of cancers, such as prostate and breast cancers. Knocking down WAVE1 expression resulted in reduced invasiveness of prostate cancer. Meanwhile, increased expression led to a higher level of invasiveness and growth (Lane et al., 2014). Similar results were observed in diseased patients with breast cancer as they were associated with an increased level of WAVE1 (Fernando et al., 2007).

A study showed that WAVE1 deficiency decreases dorsal ruffle formation (which are involved in extracellular matrix degradation) and MMP secretion (Fernando et al., 2009). WAVE1 and WAVE2 have been reported to have distinct and overlapping roles in the control of actin assembly at the leading edge. The role of WAVE1 protein in the regulation of actin polymerization by activating ARP 2/3 complex seems to be important in the formation of actin-based membrane protrusions that are observed in cell migration. However, their respective roles there are still not completely understood. Hence, WAVE1's activity in cell migration has received attention in recent years (Tang et al., 2020).

3. MATERIALS AND METHODS

3.1. Study setting and sampling

Eighteen periodontitis patients (10 males and 8 females) were included in the current study in order analyze the mRNA expression of the WAVE1 gene in periodontitis tissues. Those periodontitis cases were included who applied for tooth extraction due to advanced dental support tissue loss at the outpatient clinic of Tokat Gaziosmanpasa University, Faculty of Dentistry, Department of Oral, Dental and Jaw Surgery. The research was conducted in the Medical Biology and Genetics laboratories.

For each of the patients, a gum tissue sample with 2 mm periodontitis (Study group, n = 18) was obtained during surgery from areas that require soft tissue correction. The same amount of tissue was obtained from healthy tissues (Control group, n = 18) of the same patient from the lateral area during flap operation to close the soft tissue defect formed in the tooth extraction area.

Oral and written consent was acquired from all the recruited participants of the study before tissue collection, and they were informed about the study being conducted using their tissue samples. The necessary ethical committee approval for the current study was obtained from Tokat Gaziosmanpasa University, Faculty of Medicine, Ethics Committee for Clinical Research with the decision number of 21.KAEK-008. The project was funded by the Scientific Research Center of Tokat Gaziosmanpaşa University with the project number of 2022/03.

3.2. Storing tissues

After the collection of 19 periodontitis and 19 control tissue samples, some of the periodontitis tissues were removed and sent for histopathological examination. The remaining tissues were quickly cut into smaller desired pieces, separated in different tubes, and quickly frozen with liquid nitrogen stored at -80 degrees Celsius (°C) to be later used in RNA isolation, complementary DNA (cDNA) synthesis, and gene expression analysis of the WAVE1 gene.

3.3. Devices used in the study

Refrigerator (Vestel, Turkey)

Shaker (Ika, Germany)

-20 ° C freezer (Arcelik, 2052DY, Turkey)

-80 ° C Deep Freezer (Nuaire, NU-9483E, USA)

Distilled Water Device (Elga-option Q7, UK)

Oven (Memmert, Germany)

Power Supply (Biorad, 1645070, USA)

Precision Balance (Kern ABT, WB0750631, Germany)

Homogenizer (Heildolph Silent Crusher, D91126, Germany)

Magnetic Stirrer (Velp Scientifica, F20520162, Italy)

Microwave (Arcelik, MD554, Turkey)

Automatic pipettes (Transferpette, Germany)

pH Meter (Hanna Instruments, HI2020W, USA)

Plate (Applied Biosystems, 4346907, England)

Plate adhesive (Applied Biosystems, 201405092, UK)

Qubit 2.0 Fluorometer (Invitrogen by life technologies, Australia)

Real-time polymerase chain reaction (PCR). (Applied Biosystems, UK)

PCR Thermal Cycler (Analytik Jena‘s Biometra, Germany)

Centrifuge (Hettich, D78532, Germany)

Refrigerated centrifuge (Hettich, Germany)

Vortex (Velp Scientifica, F20220176, Italy)

3.4. Chemical materials, kits and kit contents

Isolation of RNA kit (ThermoFisher Scientific GeneJet RNA Purification kit, USA, LOT: 01125929)

- Washing Buffer 1
- Washing Buffer 2
- Lysis Buffer
- RNase-free Water
- Proteinase K
- RNA Purification Column Collection Tube

cDNA synthesis kit (ThermoFisher Scientific High-Capacity cDNA Reverse Transcription Kit, USA, LOT: 01152470)

- Reverse Transcriptase Enzyme
- 10X RTase Reaction Solution
- Mix 10 mM dNTP
- RNase Inhibitor
- Oligo (dT) 20
- Random hexamer (primer)
- Nuclease-free water

Qubit ™ 1X dsDNA HS Assay Kit (Invitrogen, USA, LOT: 2346191)

Other materials:

- TE Buffer
- 2-Mercaptoethanol
- Ethanol (Sigma-Aldrich Catalog No: E7023, USA)
- Test tube
- Distilled water
- Pasteur pipette
- Eppendorf tube
- Lancet

3.5. RNA isolation from the tissue samples

The stored tissue pieces were unfrozen to be used for RNA isolation. From the sample tissues, ThermoFisher Scientific GeneJet RNA Purification kit was used according to the manufacturers protocol:

1- Using a homogenizer, periodontitis specimens (30 mg) were disintegrated.

2- Using a Pipette the disintegrated tissues were transferred into a 1.5 ml eppendorf tube.

3- The specimens were added 300 µl lysis buffer without waiting and were mixed with a vortex for 10 seconds (lysis buffer; 2-MERCAPTOETHANOL).

4- 600 µl of diluted Proteinase K was added to the tubes and vortexed.

5- The resulting mixtures incubated for 10 minutes at 25 °C.

6- The mixture was centrifuged for 5 minutes a 12,000 rpm.

7- Supernatants were separated put into new RNAase-free microcentrifuge tubes.

8- 450 µl of ethanol was added to each of the samples and was mixed by pipetting.

9- Samples were transferred into filtered tubes and up to 700 µl of lysate added to them.

10- The mixture was centrifuged for 1 minute at 12,000 rpm.

11- The tubes were discarded and filters were taken into new 2 µl tubes.

12- 700 µl of Wash Buffer 1 added to the tubes and centrifuged for 1 min at 12000 rpm.

13- Filters were retrieved and placed back into the new 2 µl tubes.

14- 600 µl of Wash Buffer 2 added to the tubes and centrifuged for 1 min at 12000 rpm.

15- The filters were retrieved, transferred to the new tubes.

16- Then the filtered container and column were centrifuged at max speed for 1 minute.

17- The filters were transferred to 1.5 µl tubes.

18- Added 100 µl RNAase Free Water and centrifuged for 1 min at 12000 rpm, and then the RNA was ready.

3.6. cDNA synthesis

For the synthesis of cDNA from isolated RNA, ThermoFisher Scientific High-Capacity cDNA Reverse Transcription Kit was used in accordance to the manufacturers protocol. In order to obtain the best performance from the cDNA synthesis kit, we made sure that the RNA samples do not contain PCR inhibitor, reverse transcription inhibitor, RNase activity, and genomic DNA. The kit's components were mixed in a microtube and was then added to each isolated RNA samples in recommended amounts (Table 3.1). The mixture was centrifuged immediately to remove any air bubbles and spin down the contents. Finally, the microtube were put into a thermal cycler in order for 33 cycles of reverse transcriptase PCR (RT-PCR) to take place (Table 3.2).

Table 3.1. Reverse transcriptase reaction components per sample

Components (20 µl)	**Volume (µl)**
10X Reaction Buffer	2 µl
dNTP mixture	1 µl
Randomized hexamer	2 µl
Reverse Transcriptase	1 µl
RNase Inhibitors	0.5 µl
RNase free Water	3.5 µl
RNA template	10 µl
Total volume	20 µl

Table 3.2. PCR program applied for cDNA synthesis (33 cycle)

RT Steps	**Temperature (°C)**	**Time**
Step 1	25 °C	10 minutes
Step 2	37 °C	120 minutes
Step 3	85 °C	5 minutes
Step 4	4 °C	∞

3.7. Real-time or quantitative RT-PCR (qRT-PCR)

The kit used for real-time RT-PCR was RealQ Pluss2x Master Mix Green High Rox. The expression analysis of the WAVE1 gene was performed using the SYBR Green-based qRT-PCR method. Real-time PCR (or qPCR) allows the simultaneous observation of cDNA amplification. This technique is based on the measurement of a fluorescent signal that increases in proportion to the amount of DNA. Thus, the amount of amplification is determined by the binding of fluorescent dyes (such as SYTO9, SYBR Green) or probe sequences, that generate signals due to the degradation, to the DNA helix. In the real-time PCR analysis, beta-actin (ACTB) was used as a control.

Table 3.3. qRT-PCR reaction components per sample

Components (20 µl)	**Volume (µl)**
A.B.T.™ 2X qPCR SYBR Green Master Mix (with ROX)	12.5 µl
Forward Primer	0.5 µl
Reverse Primer	0.5 µl
Template cDNA	3 µl
RNase-free Water	8.5 µl
Total volume	25 µl

Table 3.4. Implemented qRT-PCR program (40 cycles)

PCR Steps	**Temperature (°C)**	**Time**	**Cycle**
Step 1	50 °C	20 seconds	1
Step 2	95 °C	10 minutes	1
Step 3	95 °C	15 seconds	40
Step 4	55 °C	1 minute	40

Table 3.5. Primers used in the study

Primers	Sequence
Beta-Actin	5'-GCATGGGTCAGAGAGATTCC-3' 5'-CACGCAGCTCATTGTAGAAGG-3'
WAVE1 Forward primer	5'-TCTCTTGCACTTGCGGATGA-3'
WAVE1 Reverse primer	5'-TTTGTGCCAGTTCACCTAGTCT-3'

3.8. RNA quantification

For measuring the concentrations of RNA samples, Qubit 2.0 device and RNA HS-Assay kit (Q32852) was used.

3.9. Calculation of cDNA concentration

The amount of cDNA was measured using the Qubit 2.0 fluorometer device and the ssDNA Assay Kit from Qubit (Invitrogen, Q10212). The amount of cDNA to be added to each assay was calculated to be 60 ng, and also the amount of cDNA to be added to each sample was calculated independently.

3.10. Calculating the expression of the WAVE1 gene

Calculating amplification efficiency (E) is an important stage to make sure that the primers and components perform well and provide accurate results to make a safe comparison between the samples. The formula for this calculation is $E = 10^{(-1/slope)}$. The percentage of qPCR efficiency is calculated by E% = (E-1)*100. Typically, desired amplification efficiencies range from 90% to 110%. The expression level of the WAVE1 gene was calculated using the $2^{-\Delta\Delta CT}$ method. Data from periodontitis tissue and normal gum tissue of the same patient were compared to each other, and the amount of WAVE1 gene expression in these tissues were analyzed by using StepOne v2.3 program.

3.11. Statistical analysis

The data of the WAVE1 gene were normalized with the Beta ACTIN gene and statistical analysis was performed with the data from the $2^{-\Delta\Delta CT}$ formula to calculate the fold change. Statistical analysis was performed using SPSS 22.0 software. Student t-test and Mann-Whitney U test were used to analyze the level of significance of the data. Statistical significance level was accepted as 0.05, hence, a *p-value* of ≤ 0.05 was considered significant and a *p-value* of > 0.05 was considered non-significant.

4. RESULTS

In this study 18 periodontitis patients were recruited, of whom 8 (44.4%) were female and 10 (55.6%) were male. They were associated with a mean age of 53.1 years, ranging from 34 to 65 years old (Figure 4.1).

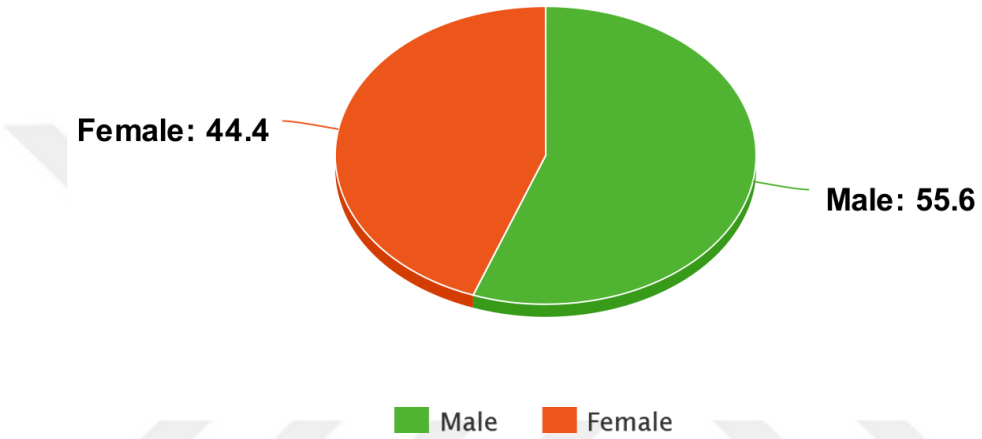

Figure 4.1. Gender distribution of the studied patients

4.1. qRT-PCR image and evaluation of WAVE1 gene

SYBR Green based quantitative Real-Time PCR technique was used to determine the level of WAVE1 mRNA expression. For WAVE1 and the reference gene (Actin: ACTB), serial dilution was performed and samples of cDNA were prepared in different concentrations (1/10:1/100:1/1000 and 1/10000) so as to optimize qPCR efficiency and to also determine the efficiency of the used primers. The qPCR efficiency was found to be about 91.5%. Figures 4.2-4.5 show the amplification plot and melt curve of WAVE1 and ACTB genes, which were acquired via qRT-PCR.

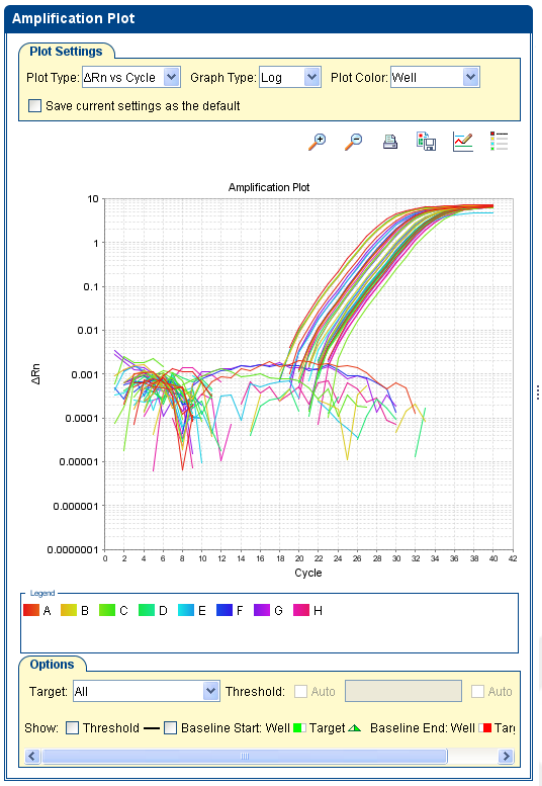

Figure 4.2. WAVE1 amplification plot

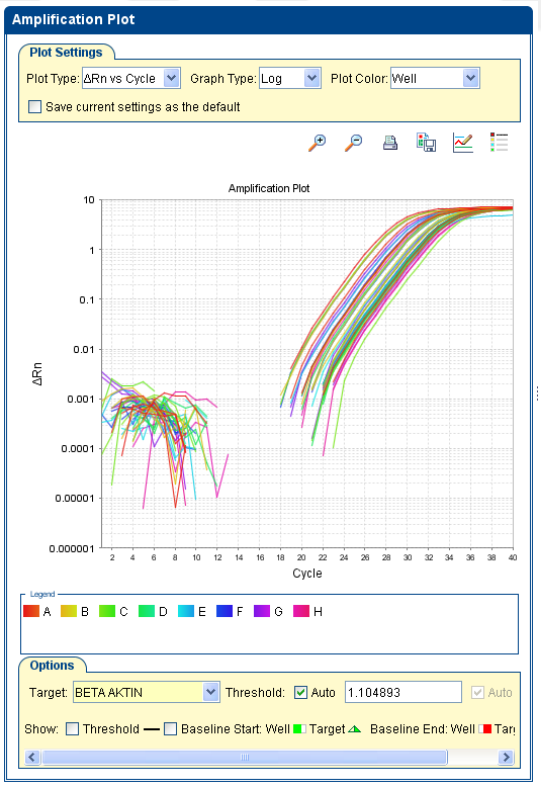

Figure 4.3. ACTB amplification plot

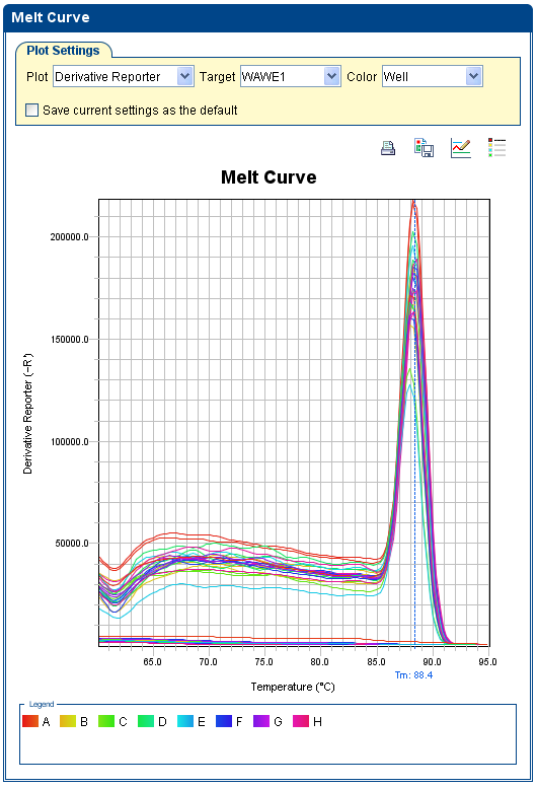

Figure 4.4. WAVE1 melt curve

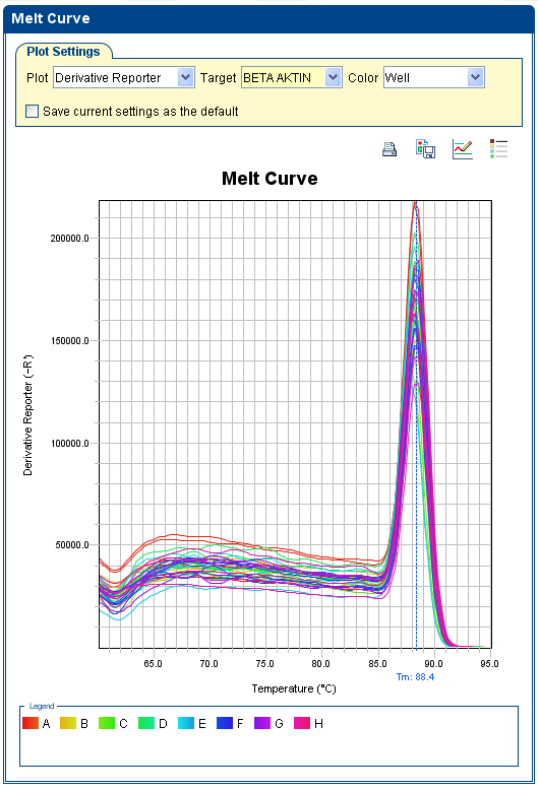

Figure 4.5. ACTB melt curve

4.2. Statistical analysis of qRT-PCR findings of WAVE1 mRNA

According to the qRT-PCR analysis results, WAVE1 was overexpressed in periodontitis tissues when compared to control tissues and the difference was statistically significant (*p-value* = 0.0412). Hence, the findings of this research suggest that WAVE1 may have a role in the development and progression of periodontitis (Table 4.1) (Figure 4.6).

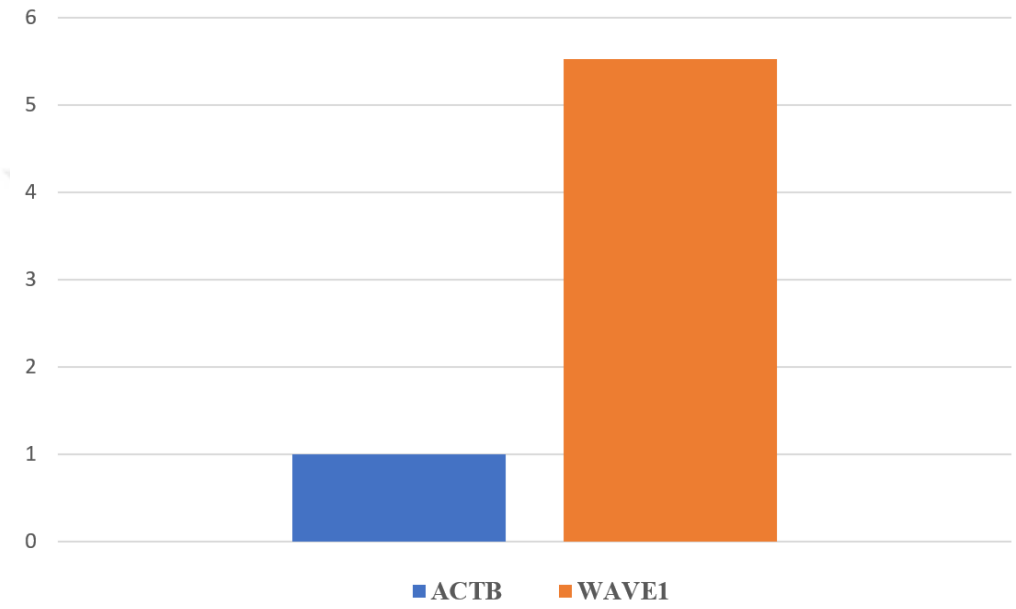

Figure 4.6. Average $2^{-\Delta\Delta Ct}$ values of WAVE1 and ACTB genes

Table 4.1. Expression of WAVE1 gene in periodontitis compared to normal tissue

Gene	Sample	
	Periodontitis tissue (n=18)	Normal tissue (n=18)
WAVE1 gene [AVG (SD)]	5.54 (6.263)	
P-value	0.0442	

5. DISCUSSION AND CONCLUSION

Periodontitis is a common chronic multifactorial inflammatory disorder characterized by periodontium destruction (Hajishengallis, 2015). The condition is a result of the interaction between host inflammatory response and supragingival and subgingival biofilms produced by oral bacterial pathogens (Oppermann et al, 2012). It is one of the most frequent conditions of the oral cavity and has a global prevalence of 11%, by affecting more than 743 million people, making it the 6th most prevalent human disease on the planet (Eke et al., 2018; Tonetti et al., 2017). The condition frequently leads to gum infection and withdrawal, destruction of the periodontium that supports the teeth, and even tooth loss, which significantly affects the quality of life in these patients (Grønkjær et al., 2018; Borges et al., 2013).

The severity and progression of the condition depend on multiple interacting risk factors, including: genetic, microbiological, environmental, and immunological factors, as well as race, gender, and age. Some people are more genetically at risk of developing periodontitis (Guzeldemir-Akcakanat et al., 2016). Today, the molecular or cellular mechanisms responsible for periodontitis pathogenesis are yet to be established, and there are currently no therapeutic targets or pre-diagnostic markers for the condition (Lundmark et al., 2018).

Even though periodontitis has been extensively researched, to date, specific genes directly involved in the condition have not been described (Davanian et al., 2012). Gene expression profiling is an effective method for generating complete genome-level data on pathologic conditions such as rheumatoid disorders, periodontitis, asthma, and cancer, and gives valuable insight regarding these disorders. Genetic analysis could provide evidence for the involvement of genes in periodontitis pathogenesis (Guzeldemir-Akcakanat et al., 2016). Some studies have sought to evaluate gene expression profiles in healthy periodontal tissues and compare them to diseased tissues. This has aided in the identification of genes involved in the homeostasis of these tissues, as well as the discovery of new biological markers that may aid in determining why some people are predisposed to periodontitis (Sánchez-Muñoz et al., 2018). The majority of differentially expressed genes in periodontitis in comparison to normal gingival tissues were found to

be elevated in periodontitis tissues. Moreover, gene ontology enrichment analysis showed that the majority of these differentially expressed genes were associated with inflammatory and immunological processes (Davanian et al., 2012). Investigations are still being conducted to analyze the expression of candidate genes for periodontitis in order to assess their impact and further investigate the potential therapeutic targeting of these genes.

Few studies have used microarrays or RNA sequencing to examine gene expression in gingival tissue from individuals with periodontitis. These investigations have demonstrated changes in gene expression between periodontitis and healthy tissues, with apoptosis, inflammation, and cell death being substantially represented in periodontitis tissues (Lundmark et al., 2018).

In the study by Davanian et al., microarray analysis was conducted for 50 genes, of which IRF4 and CCL18 were detected to be most significantly overexpressed in periodontitis affected-tissues (Davanian et al., 2012). Another study by Duarte et al. discovered that IL-17, RAGE, RANKL, TLR-2, and TLR-4 were all upregulated in gingival tissue samples from periodontitis patients when compared to normal tissues (Duarte et al., 2012). Zhan and colleagues sought to identify candidate genes related to periodontitis among 21 genes and reported that chemokine [CXC motif] ligand 1 IL-18, CD44, MMP3, IL-6 signal transducer, MMP7, MMP13, TLR9, and chemokine [C-C motif] receptor 1 genes were associated with periodontitis (Zhan et al., 2014). In a study by Lundmark and associates, 92 genes were shown to be substantially overexpressed in inflamed gingival connective tissue in comparison to non-inflamed tissues. Four of the most strongly up-regulated genes were X-box binding protein 1, signal sequence receptor subunit 4, immunoglobulin lambda-like polypeptide 5, and marginal zone B and B1 cell specific protein. These genes were likewise shown to be considerably more expressed in periodontitis tissues compared to healthy tissues (Lundmark et al., 2018).

The actin cytoskeleton is fundamental in many aspects of cell biology. Regulation of the actin cell skeleton is vital for intracellular activities, particularly for inflammatory cell migration, and the formation of cytotoxic T cell apparatus and immune synapses. Inadequate or abnormal expression of proteins involved in regulating the actin cell skeleton increases immunodeficiency and/or autoimmune/inflammatory diseases, such as

WASPs and WASP WAVEs (Lafouresse et al., 2013). In the current study, we attempted to analyze the role of WAVE1 gene in the development of periodontitis.

WAVE1 is most abundant in mouse brain tissue and leukemia cells, and it is expressed at very low levels in other tissues such as the peripheral blood, liver, heart, kidney, lung, and pancreas (Zhang et al., 2016). Few studies have investigated its function in other tissues and systems. WAVE1 appears to regulate actin polymerization in response to T cell activation, especially in the formation of the immunologic synapse. However, the underlying processes remain unknown (Dinh et al., 2008). WAVE1 is a very poorly studied protein. To date, no other study has been conducted regarding the role of WAVE1 or any other members of WASP family of proteins in periodontitis or in any other inflammatory diseases.

WAVE1 is essential for CNS development and function. In neurons, WAVE1 is localized to dendrites and dendritic spines, as well as to axonal growth cones and mitochondria. Therefore, WAVE1 is important for synaptic plasticity, neurite outgrowth, growth cone dynamics, and dendritic spine morphogenesis (Ceglia et al., 2010). Additionally, WAVE1 is localized to oligodendrocytes and has a function in CNS myelination (Kim et al., 2006). It has been reported that WAVE1-null mice have CNS abnormalities such as sensorimotor retardation, neuroanatomical deformities, and behavioral abnormalities such as decreased anxiety, limb weakness, and deficiencies in hippocampal-dependent learning and memory. WAVE1 homozygote knockout mice have a smaller body size and a lower viability (Ceglia et al., 2010). These electrophysiological and behavioral deficiencies have been linked to changes in dendritic spine morphology and dynamic actin polymerization (Matt et al., 2013). In a study of WAVE1 knock-out mice, it was found that during development WAVE1 absence results in postnatal lethality (Dahl et al., 2003). Ito et al. reported that WASF1 de novo truncating mutations induce intellectual impairment as well as seizures (Ito et al., 2018). Srivastava also added further evidence that WASF1 de novo mutations produce an autosomal dominant neurodevelopmental disease (Srivastava et al., 2021).

The majority of WAVE1 activity has so far been investigated in the brain. It has been reported that WAVE1 expression is controlled during primary macrophage development and by adhesion. Because several macrophage activities, such as

phagocytosis, movement, and secretion, need cytoskeletal modification (Dinh et al., 2008). Matt et al. described the role of WAVE1 in macrophages and its function in innate immunity by mediating suppression of phagocytosis. It is crucial to note that WAVE1 only altered phagocytosis in the presence of oxidized phospholipids, indicating a necessity for oxidative stress as found in severe inflammatory illnesses or infections (Matt et al., 2013). An investigation that looked at the functions of WAVE1-deficient cells discovered that dorsal ruffles are involved in ECM breakdown (Fernando et al., 2009).

WAVE1 acts as a suppressor of elongation and not an enhancer of actin filaments, which was also observed in our study (Mughees et al., 2021). Altered expression of WAVE1 has been noted in multiple types of cancer. WAVE1 is also a key molecule in the regulation of tumor growth, invasion, and metastasis. A study by Zhang et al. showed that WAVE1 knockdown promotes leukemia cell apoptosis through negative regulation of autophagy. Furthermore, suppressing WAVE1 expression improved leukemia cells' susceptibility to chemotherapy and apoptosis (Zhang et al., 2016). It has been noted that patients who died of breast cancer due to metastasis had a high level of WAVE1 (Fernando et al., 2007). In a study regarding WAVE activity in invasion and metastasis, WAVE1 and WAVE2 protein levels, as well as Rac activity, were shown to be elevated in highly invasive mouse melanoma cells (Kurisu et al., 2005). The loss of endogenous WAVE1 led to a considerable decrease in prostate cancer's invasive capacity (Frugtniet et al., 2015). It has been shown that WAVE1 is upregulated in primary BMMCs and human hematological cancer cell lines from pediatric leukemia patients (Zhang et al., 2016).

Despite the current study having its own strengths, it also has it is own limitations, with having a small sample size being the major one.

This is the first study investigating the relationship between WAVE1 and periodontitis. The findings of the current study implied WAVE1 was overexpressed in periodontitis tissues when compared to healthy control tissues, and this difference was found to be statistically significant ($p = 0.0442$), suggesting that WAVE1 may have a significant role in the development and progression of periodontitis. Further studies with larger sample sizes are recommended to confirm our findings.

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