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INVESTIGATION OF WASP GENE EXPRESSION IN PERIODONTITIS

[*1]Kubra SAHIN, [1]Omer ATES

[*1]Department of Medical Biology, Medical Faculty, Tokat Gaziosmanpasa University, Tokat, Türkiye

Research Article

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*Corresponding author: kubra944@hotmail.fr

Abstract

Periodontitis is a chronic inflammatory disease that occurs as a result of an imbalance in microorganisms and the host immune system, causes destruction and bone loss in the periodontium, and also threatens systemic health. Dysregulation of inflammatory and immune pathways causes chronic inflammation, tissue destruction and diseases. For this reason, innate and adaptive immune defects play an important role in immune-mediated inflammatory diseases such as periodontitis. The correct and coordinated movement of immune cells depends on the regulation of the actin cytoskeleton, which perform a role in many processes like migration, cell activation, antigen uptake and recognition. Studies have shown that changes in the expression level of Wiskott-Aldrich syndrome protein (WASP), an important actin cytoskeleton regulator, cause defects in immune and inflammatory response formation. In our study, it was aimed to investigate WASP gene expression in periodontitis and healthy gingival tissue and to reveal its possible relationship with periodontitis. In this direction, 10 volunteers were included in the study and healthy gingival tissue and periodontitis tissue were taken from each patient. Gene expression levels were determined by SYBR Green-based PCR. According to our results, WASP mRNA levels were found to be statistically significantly higher in periodontitis tissues compared to healthy gingival tissue ($p<0.05$). Data suggesting that WASP may be effective in the development of periodontitis have been obtained. However, more research with a large sample size is needed to fully understand the role of WASP in periodontitis

Key Words: Immunity, Inflammation, Periodontitis, WASP

Özet

Periodontitis, mikroorganizmalar ve konak bağışıklık sistemindeki dengesizlik sonucu ortaya çıkan, periodonsiyumda yıkıma ve kemik kaybına neden olan ve aynı zamanda sistemik sağlığı tehdit eden kronik inflamatuar bir hastalıktır. İnflamatuar ve immün yolların düzensizliği kronik inflamasyona, doku yıkımına ve hastalıklara neden olur. Bu nedenle doğal ve adaptif immün defektler, periodontitis gibi immün aracılı inflamatuar hastalıklarda önemli bir rol oynamaktadır. İmmün hücrelerin doğru ve koordineli hareketi, göç, hücre aktivasyonu, antijen alımı ve tanınması gibi birçok süreçte rol oynayan aktin hücre iskeletinin düzenlenmesine bağlıdır. Çalışmalar, önemli bir aktin hücre iskeleti düzenleyicisi olan Wiskott-Aldrich sendromu proteini (WASP) ekspresyon seviyesindeki değişikliklerin, immün ve inflamatuar yanıt oluşumunda kusurlara neden olduğunu göstermiştir. Çalışmamızda periodontitis ve sağlıklı dişeti dokusunda WASP gen ekspresyonunun araştırılması ve periodontitis ile olası ilişkisinin ortaya konması amaçlanmıştır. Bu doğrultuda çalışmaya 10 gönüllü dahil edildi ve her hastadan sağlıklı dişeti ve periodontitis dokusu alındı. Gen ekspresyon seviyeleri SYBR Green temelli PCR ile belirlendi. Sonuçlarımıza göre periodontitis dokularında WASP mRNA düzeyleri sağlıklı dişeti dokusuna göre istatistiksel olarak anlamlı derecede yüksek bulundu ($p<0.05$).

WASP'ın periodontitis gelişiminde etkili olabileceğini düşündüren veriler elde edilmiştir. Bununla birlikte, WASP'ın periodontitisteki rolünü tam olarak anlamak için büyük bir örneklem grubuna sahip daha fazla araştırmaya ihtiyaç vardır.

Anahtar Kelimeler: Bağışıklık, Enflamasyon, Periodontitis, WASP

1. Introduction

Periodontitis is a chronic infectious disease in which the integrity of the periodontium is impaired as a result of local or systemic inflammation (Palm, et al., 2013; Hajishengallis, 2015). The fact that periodontitis is one of the most common diseases and it paves the way for systemic diseases makes periodontitis an important problem (Hajishengallis, 2015; Lee & Lee, 2019). Periodontitis development depends on many environmental and genetic factors (such as microorganisms, age, gender, familial transmission, systemic status, oral care habits, education,

compliance with professional advice and socio-economic status) (Arweiler, et al., 2018; Yılmaz et al., 2019). The disease has a polymicrobial pathogenesis due to the involvement of many different types of bacteria in the inflammation process (Benedetto et al., 2013). Imbalance between host and microorganisms determines the severity of the disease (Cekici et al., 2014). Impaired host immune and inflammatory responses due to the oral microbiota are considered to be the major cause of the initiation, formation and progression of periodontal inflammation and tissue destruction (Bunte & Beikler, 2019). Therefore, how immune and inflammatory responses are regulated has a major role in understanding the pathogenesis of periodontitis (Cekici et al., 2014).

Immunity is a complex process based on the concerted and correct action of all immune cells. Innate immunity, which is effective in the initial stages of inflammation, creates the first immune response against pathogens, tissue damage, and injury. Adaptive immunity activated by innate immunity is necessary for memory formation and long-term protection. Both forms of immunity act together and in coordination to protect and maintain tissue homeostasis. Irregularities in their interactions cause chronic inflammation and diseases such as periodontitis (Bouma et al., 2009; Bunte, & Beikler, 2019). Immune system cells need actin cytoskeleton rearrangements for an accurate and effective immune response (Vicente-Manzanares & Sanchez-Madrid, 2004). Actin cytoskeleton rearrangements play a role in many immune processes such as migration of neutrophils and macrophages to the inflammatory region (Cekici et al., 2014), uptake of antigens by dendritic cells, neutrophils and macrophages (Banchereau & Steinman, 1998; Swanson, 2008), phagocytosis of antigens in the inflammatory region (Swanson, 2008).

Wiskott-Aldrich syndrome protein (WASP) is the principal actin-cytoskeletal reorganizer. Expressed mostly on hematopoietic cells, WASP is involved in signal transduction from cell surface receptors to the actin skeleton and is required for many hematopoietic and immune cell functions, including normal cell movement, inflammatory cell migration, immune synapse formation, and phagocytosis. (Thrasher & Burns, 2010; Yapıcı & Kılıc, 2008). WASP expression errors; It causes many defects including impaired T cell proliferation, impaired macrophage phagocytosis, impaired movement and activation of immune system cells, platelet abnormalities (Bouma et al., 2007; Yapıcı & Kılıc, 2008). These defective functions lead to a complex cellular and humoral immunodeficiency, resulting in susceptibility to serious and life-threatening infections (Ochs & Thrasher, 2006). Loss of WASP protein activity causes Wiskott-Aldrich syndrome, an X-linked disease that results in complex immunodeficiency, increased autoimmunity, eczema, recurrent infections, and microthrombocytopenia (Bouma et al., 2007; Thrasher & Burns, 2010).

Studies have shown that in WASP deficiency, neutrophils, which form the first line of defense with pathogens, are defective, cause disruption of normal T-cell function and B-cell tolerance, macrophages cannot form podosomes, and cell migration in dendritic cells is defective (Oliver et al., 2006; Zhang et al., 2006; Tsuboi, 2007; Westerberg et al., 2012). Taken all together, most immune cell line migrations are thought to be imperfect as a result of a lack of WASP expression and presumably due to errors in the efficiency of immune cells to reach sites of infection and transport antigens to the secondary lymphoid tissue that drains them (Bouma et al., 2009).

In this study, we hypothesized that WASP-mediated actin cytoskeleton rearrangements involved in inflammatory cell migration may have a central role in the development of the inflammatory disease periodontitis. For this purpose, we investigated WASP gene expression levels in periodontitis tissues.

2. Materials and Methods

2.1. Specimens from patients

Periodontitis and healthy gingival tissue samples were taken from 10 people who were diagnosed with periodontitis by applying to the Department of Oral, Dental and Maxillofacial Surgery of Tokat Gaziosmanpasa University. 6 women and 4 men were included in the study. The mean age of the volunteers included in the study was 56.8±7.34. The study was accepted by the Tokat Gaziosmanpaşa University Clinical Research Ethics Committee (project number: 21-KAEK-008). Written informed consent form was obtained from all participants for the study. The study was conducted in accordance with the Declaration of Helsinki. Demographic data of the individuals included in the study are given in Table 1. The financing source of the project is Gaziosmanpasa University Scientific Research Projects (Project No: 2021/56).

Table 1. Demographic data of patients

	Periodontitis n=10
Age	56.8 ± 7.34
Gender	6 F/4 M

Abbreviations: M, male; F, female

2.2. Detection of gene expression

Total RNA isolation from tissue was performed by Thermo Scientific GeneJET RNA Purification Kit (Thermo; ABD) in accordance with the manufacturer's procedure. Isolated total RNAs were translated into cDNA using the Applied Biosystems High Capacity cDNA Reverse Transcription Kit (Thermo; USA). cDNA concentrations were measured with high precision using the Invitrogen Qubit™ 1X dsDNA HS Assay Kit (Invitrogen, USA) according to the manufacturer's protocol. SYBR Green-based quantitative Real Time PCR (Applied Biosystem StepOnePlus) was used to determine the WASP gene expression level. Beta Actin (ACTB) was used as the reference gene in the study. WASP gene data were normalized with the ACTB gene. The PCR reaction was prepared by adding 2x Master Mix Green High ROX™, WASP and ACTB Primers, cDNA sample, dH2O in a total volume of 25 µl. PCR program consisted of 95 °C for 15 min, 40 cycles of 95 °C for 20s and 60 °C for 1 min. 2-ΔΔCt method were used to calculate the mRNA levels of the WASP gene (Livak & Schmittgen, 2001).

3. Statistical analysis

Values were shown as Mean ± SDM. Statistical analyses were implemented with SPSS software version 22.0 (SPSS, Inc., Chicago, IL). Paired sample test was used in the analysis of WASP gene expression. p values less than 0.05 were considered significant.

4. Results and Discussion

4.1. Gene expression results

RT-PCR results have shown that, WASP gene expression decreased in 20% (2/10) and increased in 80% (8/10) of periodontal tissues. These data show a 2.02-fold change in WASP gene expression compared to healthy gingival tissue samples (Figure 1). A range of 0.9-1.1 was used in the analysis of the data. Analysis results showed that the increase in WASP gene expression level was statistically significant in periodontitis tissue and healthy gingival tissues (p=0.032) (Table 2).

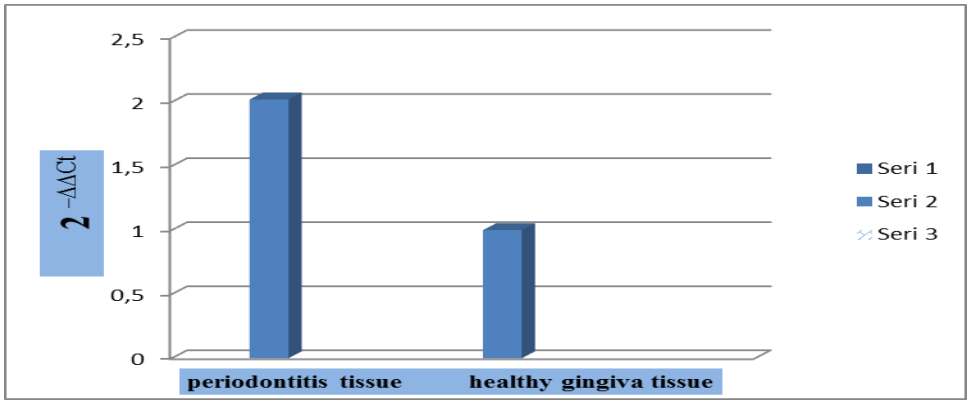

Figure 1. Graphic showing fold change of WASP gene in periodontitis tissues (n= 10) and relative to the normal gingiva tissues.

Relatife quantification values of WASP genes were measured using RT-PCR. Quantitative expression data of WASP genes were normalized with ACTB gene (fold change of periodontitis tissues=2.02, fold change of healthy tissues=1)

Table 2. Expression level of WASP

	WASP (mean ± SD)	*p* volue
Periodontitis tissue (n= 10)	1.02±1.27	
		* *p*=0.032
Healthy Gingiva tissue (n=10)	1	

Abbreviations: SD, standard deviation

* $p < 0.05$.

4.2. Discussion

Periodontitis is a chronic inflammatory disease of multifactorial etiology, which is one of the important causes of tooth loss in adults, showing immune dysregulation (abnormal immune function) at its base, characterized by deepening of periodontal pockets, loss of alveolar bone loss and connective tissue attachment (Chrysanthakopoulos, 2011; Grönkjaer et al., 2018; Loos & Van Dyke, 2020). Periodontitis causes gingival infection, destruction of the periodontium that supports the tooth, and often gingival recession (Grönkjaer et al., 2018). The disease occurs as a result of the interaction of many genetic and environmental factors like microbial biofilms and host response. Due to the combination of many causative factors, individual differences are very important in the course of the disease (Loos & Van Dyke, 2020). In addition to the availability of

periodontopathogens like *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythia*, genetic and environmental factors appear to enhancement sensitivity in developing the disease. Previous studies have shown that immune system imbalance caused by pathogens is a major factor in the onset and development in periodontitis. In addition, an increasing number of studies have demonstrated the importance of immune imbalance and systemic inflammation resulting, including the abnormality of cytokines in the host immune response (He at al., 2021).Chronic inflammation, cause changes in tissue homeostasis, including the uncontrolled activation of immune and non-immune cells, leading to disease complications such as abnormal tissue repair. Current anti-inflammatory treatments are often insufficient to prevent or reverse these complications. Actin cytoskeleton remodeling is critical for cell activation in inflamed tissues; however, the cytoskeleton has not been adequately studied as a therapeutic target in inflammation (Ivanov et al., 2021).

Activation of WASP, a cytoskeletal rearrangement protein, has been implicated in many biological processes that require upregulation of the actin cytoskeleton, such as vesicle trafficking, microbial infection, and cell substrate adhesion (Bompart & Caron, 2004). WASP activity is regulated by its structural conformation and exists as an inactive auto-inhibited conformation at rest. WASP activity is regulated by the combined effect of phosphorylation and post-translational modification, apart from the structural conformation. Phosphorylation of WASP appears to increase its activity during many cell processes, including chemotaxis, proliferation, and formation of adhesion structures, phagocytosis, and immunological synapse. In human WASP, Tyrosine 291 has been identified as an important regulator of WASP activity. This phosphorylation is thought to alter the stability of the auto-inhibited form of WASP. In addition, mice expressing a non-phosphoryltable Tyr293Phe WASP mutant were shown to have immune defects as in completely WASP-deficient mice, resulting in a WASP deficiency-like phenotype. Therefore, Tyr293 phosphorylation is thought to be important for normal WASP activity and function. These results shown that phosphorylation may provide a mechanism for controlling WASP activity (Thrasher & Burns, 2010).

Multiple functional domains of WASP have been determined to be important for cell/cell interaction, actin polymerization, chemotaxis, cell signaling, NK cell activity, synapse formation, and Treg function. Therefore, the clinical phenotype of individuals with modifications in the WASP gene has been reported to highly depending on the type and location of the mutation (Ochs, 2009). Gain-of-function mutations in the WASP GTPase-binding domain lead to X-linked

neutropenia (XLN), characterized by congenital neutropenia and recurrent major bacterial infections, and induce constitutively active WASP by blocking folding into the automatically inhibited conformation (Keszei et al., 2018). Overactive mutations of WASP in XLN human and animal models result in excessive actin dynamics and incremented migration of neutrophils and B cells to chemokines. Macrophages expressing overactive WASP have incremented levels of abnormal podosome aggregation and F-actin (Westerberg et al., 2010).

It was thought that WASP deficiency causes host cell death associated with increased inflammation, impaired autophagic p62/LC3 and bacterial clearance, defective canonical autophagosome formation, and therefore may form the basis for new treatment strategies (Lee et al., 2017). Zhang et al., showed that WASp deficiency in both human and murine neutrophils leads to problems in clustering of β2 integrins and imperfect adhesion and transendothelial migration. In addition, it was thought that impaired β2 integrin function due to WASP deficiency may contribute to immunodeficiencies (Zhang et al., 2006). Studies have shown that WASP downregulation inhibits podosome formation and impairs motility of dendritic cells. A decrease in the number of podosomes causes abnormal cell motility (Oliver et al., 2006).

In related to our study; it was shown that the loss of N-WASP in psoriasis, which has the same pathogenesis as periodontitis, causes an increase in inflammation by increasing interleukin (IL-23, IL-17) (Li et al., 2018; Wang et al., 2020). As far as the literature was scanned, there is only one study associated with the cytoskeletal rearrangements and periodontitis, and it has been reported that N-WASP inadequacy caused periodontal tissue inflammation in mice (Wang et al., 2020). In our study, the expression WASP in periodontitis tissue were found to be significantly higher than normal gingiva ($p = 0.032$). Contrary to our results, Wang et al. showed that decreased expression levels of another cytoskeletal protein, N-WASP, increased periodontal inflammation (Wang et al., 2020). We think that the current contradiction may be due to the fact that WASP activation is regulated at translational rather than transcriptional levels, phosphorylation mechanisms play a role in WASP activation, or because of working with a small sample group. At the same time, we believe that non-coding RNAs that play a role in the regulation of gene expression at epigenetic, transcriptional and translational levels may cause differences in WASP expression levels.

In this context, we think that our results should be supported by further studies such as mutation analysis, analysis of mass spectrophotometer, and Western blot analysis with a larger sample group.

5. Conclusion

In this study, a significant relationship was found between periodontitis and WASP, and data suggesting that it may be effective in the development of periodontitis were obtained. Our results may provide a molecular basis for a better understanding of the pathogenesis of periodontitis and may be useful in developing targeted treatment strategies.

Conflicts of interest

The authors declare that there are no potential conflicts of interest relevant to this article.

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