DOI: 10.4274/forbes.galenos.2024.59389
Forbes J Med 2024;5(2):116-22

Original Article / Orijinal Araştırma

Investigation of Inflammation and Autophagy due to Biglycan-mediated TLR2/4 Signaling in Oral Lichen Planus Tissues

Oral Liken Planus Dokularında Biglikan Aracılı TLR2/4 Sinyallemesine Bağlı Enflamasyon ve Otofaji Aktivasyonunun Araştırılması

Özlem Ceren GÜNİZİ[1], Hüseyin GÜNİZİ[2], Hamiyet ECİROĞLU[3], Fatma YILDIZ[3]

[1]Alanya Alaaddin Keykubat University Faculty of Medicine, Department of Medical Pathology, Antalya, Türkiye
[2]Antalya City Hospital, Clinic of Otorhinolaryngology, Antalya, Türkiye
[3]University Vocational School of Health Services, Department of Medical Laboratory Techniques, Antalya, Türkiye

Cite as: Günizi ÖC, Günizi H, Eciroğlu H, Yıldız F. Investigation of Inflammation and Autophagy due to Biglycan-mediated TLR2/4 Signaling in Oral Lichen Planus Tissues. Forbes J Med. 2024;5(2):116-22

ABSTRACT

Objective: Oral lichen planus (OLP) is a chronic oral mucosal disease of unknown etiology. Cellular immune response, basement membrane, and extracellular matrix (ECM) molecules are also noteworthy in the pathogenesis. We aimed to investigate the role of Biglycan (BGN) in Toll-like receptors (TLR)2/4-CD14 and TLR4-CD44 mediated signaling mechanisms in the pathogenesis of OLP.

Methods: Twenty-one patients with a previous diagnosis of OLP and 21 patients with normal oral mucosa were included. RNA was isolated from biopsy samples of patients, and the gene expression of BGN, TLR2, TLR4, CD14, and CD44 was analyzed by quantitative real-time polymerase chain reaction and immunohistochemistry.

Results: According to our findings, the fold change rates of BGN, TLR2, TLR4, and CD14 mRNA levels in tissues obtained from patients with OLP were higher compared with the control group. TLR2, TLR4, and CD14 fold change rates were statistically significant ($p<0.05$). CD44 co-receptor mRNA levels were higher in the control group ($p<0.05$). Similar results were obtained by immunohistochemical analysis.

Conclusion: BGN expression has a pro-inflammatory effect in various disease models. The BGN levels in the tissues of patients with OLP were higher than those of the control group, but the difference was not statistically significant. However, TLR2/4-CD14 and CD44 levels were upregulated, as well as CD44 levels were downregulated. We suggest that inflammation signaling is activated and autophagy is inhibited in OLP. BGN-dependent inflammation and autophagy signaling in OLP are evaluated for the first time.

Keywords: Biglycan, oral lichen planus, inflammation, autophagy, TLR

ÖZ

Amaç: Oral liken planus (OLP), etiyolojisi bilinmeyen, kronik enflamatuvar bir oral mukoza hastalığıdır. OLP patogenezinde hücresel immün yanıt, bazal membran ve hücre dışı matriks (ECM) molekülleri de dikkat çekmektedir. Biglikan (BGN), ECM'nin yapısal bir bileşenidir ve aynı zamanda bir sinyal molekülü olarak da görev yapar. Bu çalışmada OLP hastalarının patogenezinde BGN'nin Toll-like reseptörler (TLR)2/4-CD14 ve TLR4-CD44 aracılı sinyalleşme mekanizmaları üzerindeki rolünü, enflamasyon ve otofaji ile ilişkisini araştırmayı amaçladık.

Yöntem: Çalışmamıza daha önceden OLP tanısı almış 21 hasta ve oral mukozası normal olan 21 hasta dahil edildi. Hastaların biyopsi örneklerinden RNA izole edildi ve BGN, TLR2, TLR4, CD14 ve CD44 moleküllerinin gen ekspresyonları kantitatif gerçek zamanlı polimeraz zincir reaksiyonu ve immünohistokimya ile analiz edildi.

Bulgular: Bulgularımıza göre OLP hastalarından elde edilen dokularda BGN, TLR2, TLR4, CD14 mRNA düzeylerinin kat değişim oranları kontrol grubuna göre daha yüksekti. TLR2, TLR4, CD14 kat değişim oranları istatistiksel olarak anlamlıydı ($p<0,05$). CD44 ko-reseptör mRNA düzeyi kontrol grubunda daha yüksekti ($p<0,05$). İmmünohistokimyasal analize göre de benzer sonuçlar elde edildi.

Received/Geliş: 27.03.2024
Accepted/Kabul: 04.06.2024

Corresponding Author/ Sorumlu Yazar:
Hüseyin GÜNİZİ MD,
Antalya City Hospital, Clinic of Otorhinolaryngology, Antalya, Türkiye
Phone: +90 242 513 48 41
drgunizi@gmail.com
ORCID: 0000-0001-8653-0544

Sonuç: Dolaşımdaki çözünür BGN ekspresyonunun çeşitli hastalık modellerinde proenflamatuvar etkiye sahip olduğu bulunmuştur. Bu çalışmada OLP hastalarının dokularındaki BGN düzeyleri kontrol grubuna göre daha yüksekti ancak bu istatistiksel olarak anlamlı değildi. Ancak OLP dokularında TLR2/4-CD14 seviyeleri yukarı doğru düzenlenirken, CD44 seviyeleri de aşağı doğru düzenlenmiştir. OLP'de enflamasyon sinyalinin aktive olduğunu ve otofajinin inhibisyon olduğunu öneriyoruz. OLP'de BGN'ye bağlı enflamasyon ve otofaji sinyali ilk kez değerlendirildi.

Anahtar Kelimeler: Biglikan, oral liken planus, enflamasyon, otofaji, TLR

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory oral mucosal disease of unknown etiology.[1] It is more common in women than men (female/male: 2/1). This disease manifests as white streaks, papules, plaques, erythema, erosions, or blisters that predominantly affect the buccal mucosa, tongue, and gingiva.[2,3] OLP has been defined as a premalignant condition by the World Health Organization, and malignancy has been identified in approximately 10% of cases. Therefore, the elucidation of the pathogenesis of OLP has gained importance.[4,5]

Besides the cellular immune response, the basement membrane, intercellular connections, and extracellular matrix (ECM) molecules draw attention to the pathogenesis of OLP. The ECM is a non-cellular and highly specialized three-dimensional skeletal structure in all tissues and organs. ECM molecular composition and structure can change significantly during normal tissue repair or during the progression of various diseases.[6] Therefore, explaining the relationship between ECM molecules and inflammation in OLP is essential in elucidating the disease's pathogenesis and supporting diagnostic criteria. It was determined that the expression of ECM molecules such as fibronectin, integrin, keratin, collagen, and E-cadherin differs in OLP. Various studies have been conducted on the receptors and signaling pathways of these molecules.[7]

Studies have shown that OLP is a T cell-mediated autoimmune disease and that ECM molecules play important roles in the pathogenesis of the disease.[3,8] In OLP histopathology, oral mucosal basal keratinocyte become sensitized, and the severity of the disease increases with the stimulation of the inflammatory process, which tends to become chronic.[4,9]

Small leucine-rich proteoglycans (SLRP) comprise a large 18-member ECM proteoglycan family.[5] Biglycan (BGN) is a member of the SLRP family of ECM.[10,11] Under normal conditions, BGN is a structural protein. Nevertheless, in situations such as tissue injury and stress, it is cleaved by the proteolytic pathway or released de novo and then acts as a direct signaling molecule in inflammation.[12] It has been reported that BGN stimulates macrophages, some chemokine, and cytokines, especially in inflammation, and causes an increase in inflammation.[6,7,12] In pathogenic inflammation or pathological conditions, BGN binds toll-like receptor (TLR)2 and TLR4 receptors on macrophages and dendritic cells, induces various pro-inflammatory cytokines or chemokine, and causes increased inflammation. Thus, it plays an important role in the endogenous proinflammatory response.[13-15] Recent studies have shown that molecules such as CD14 and CD44 act as co-receptors in BGN-mediated TLR2 and 4 signaling.[12] However, these pathways result in different cellular processes; TLR2/4-CD14 signaling induces inflammation[12] while TLR4-CD44 signaling has been shown to result in autophagy.[10,16,17] CD44 isoform in epithelial cells, active lymphocytes, and tumor cells are associated with inflammation. However, the mechanism by which CD44 expression is signaled remains unclear.[18]

Although BGN has been studied in many inflammatory diseases, only one study has shown its relationship with OLP. This study showed that BGN is overexpressed in OLP and oral squamous cell carcinoma, and it was interpreted that it might increase the release of the immune system and cytokines, thereby causing inflammation.[10] However, there are no studies on the role of BGN in OLP and its effect on inflammation, which is associated with many inflammatory diseases. In this study, we aimed to investigate the role of BGN in TLR2/4-CD14- and TLR4-CD44-mediated signaling mechanisms in patients with OLP and the relationship with inflammation and autophagy.

The data obtained from our study will make important contributions to the literature regarding whether BGN is associated with inflammation in OLP and evaluate the signaling mechanisms associated with BGN as a biomarker.

METHODS

Patient Selection and Criteria

Ethical approval was obtained from the Clinical Research Ethics Committee of the Alanya Alaaddin Keykubat University (ALKU) Faculty of Medicine (date: 19.11.2020, decision no: 25/10). This study applied the principles of the Declaration of Helsinki and local laws. Written informed consent forms were obtained from the participants included in the study.

Our study included 21 patients previously diagnosed with OLP at the ALKU Faculty of Medicine Ear Nose and Throat Polyclinic and oral mucosal tissue samples of 21 patients whose biopsies were previously taken with the preliminary diagnosis of OLP and reported as normal tissue. Patients who were pregnant, <18 years of age, or diagnosed with any

malignancy were excluded from the study. The expression levels of BGN, TLR2, TLR4, CD14, and CD44 genes were evaluated by quantitative real-time polymerase chain reaction (RT-qPCR) and immunohistochemistry analyses of paraffin-embedded tissues from patients and volunteers.

Immunohistochemical Analyzes

Sections of 5 µm thickness were taken from each paraffin block of the oral mucosa in all groups using a microtome. After being flattened in a water bath at the appropriate temperature, the sections were transferred onto superfrosted slides. The following primary antibodies were used: anti-BGN (Cat#FNab00895, Fine Test, China) (1:50 dilution), anti-TLR2 (Cat#BT-AP09061, BT Lab, China) (1:100 dilution), anti-TLR4 (Cat#BT-AP09062, BT Lab, China) (1:100 dilution), anti-CD14 (Cat#FNab01426, Fine Test, China) (1:50 dilution), and anti-CD44 (Cat#BT-AP01504, BT Lab, China) (1:50 dilution). Samples were classified as stained with strong density, medium density (similar to normal laryngeal squamous epithelium), and weak density. All immunohistochemical sections were scored according to staining intensity as follows: (-): No Staining, (+): Poor Staining, (++): Medium staining, (+++): Strong staining.

Determination of Gene Expression Levels

Total RNA Isolation from Paraffin-embedded Tissue

To study mRNA expression profiles in formalin-fixed and paraffin-embedded (FFPE) tissues, 8 sections of 10 µm thickness were taken for each patient. According to the manufacturer's instructions, total RNA was extracted from the prepared samples using the PureLink™ FFPE Total RNA Isolation Kit (Cat#K156002, Invitrogen, USA). After measuring the purity and concentrations (ng/µL) of the isolated RNAs with an ELISA Plate Reader (Synergy H1, BioTek, USA) (260/280=1.8-2.1), total RNA samples were stored at -80 °C until use.

cDNA Synthesis

Using reverse transcriptase PCR (RT-PCR), cDNA synthesis was performed from mRNAs. For cDNA synthesis, an ABT cDNA synthesis kit (Cat#C03-01-20, ABT, Türkiye) was used, and cDNA synthesis was performed in a total volume of 20 µL according to the manufacturer's instructions. The obtained cDNAs were stored at -20 °C for real-time PCR reaction.

Gene Expression Measurement by Quantitative Real-time PCR

The gene expression levels of BGN, TLR2, TLR4, CD14, and CD44 genes, which are believed to play a role in the pathogenesis of OLP, were determined using the relative gene expression RT-qPCR method. Beta-actin (β-actin) was used as the housekeeping gene. RT-qPCR reactions were performed using a QRT-PCR master mix kit (Cat#Q03-02-01, ABT. 2X qPCR SYBR Green Master Mix, Türkiye) and a LightCycler 96 system (Roche Diagnostics, Basel, Switzerland). The relative expression values were calculated using the cycle threshold (Ct) method according to the $2^{-\Delta\Delta Ct}$ formula and the fold change in the mRNA expression of the target genes was determined.[19]

Statistical Analysis

Statistical Package for the Social Sciences, a Windows version 17.0 computer program, was used for statistical analysis. The immunohistochemical analysis was performed using Fisher's exact test. In addition, differences between the paired groups were compared with the independent sample t-test or Mann-Whitney U test for parametric or nonparametric samples. Chi-square analysis and frequency analysis were used for categorical variables. The significance level was set as $p<0.05$ in all statistical comparisons.

RESULTS

The study included 42 individuals; there were 15 female and 6 male patients in the patient group and 11 female and 10 male patients in the control group. The lowest age was 19 years, and the oldest was 68 years. Mean age were 44.5 (±11.08) and 49.2 (±13.51) in the patient and control groups. To gain an understanding of the relationship between inflammation and BGN in OLP, we evaluated BGN, TLR2, TLR4, CD14, and CD44 gene expression levels in paraffin-embedded tissues using immunohistochemistry and RT-qPCR in all study groups.

Quantitative Real-time PCR Findings

The mRNA expression levels of BGN, CD14, CD44, TLR2, and TLR4 proteins in the tissues of patients with OLP and the control group were compared using RT-qPCR. The relative expression levels of these genes are presented in Figure 1. In the results obtained, there was no statistically significant difference between the OLP patients and the control group in terms of BGN mRNA expression level (1.14 fold) (p=0.438) (Figure 1A). The relative expression levels of CD14, TLR2, and TLR4 mRNA in the OLP samples were significantly elevated to 1.95 (p=0.001), 2.34 (p=0.04), and 1.96 (p=0.005) folds, respectively, compared with the control group (Figure 1B, 1D, 1E). In contrast, CD44 gene expression levels were significantly decreased (3.7 fold) in OLP patients compared with controls (p=0.001) (Figure 1C).

Immunohistochemical Findings

In histological examinations, intense subepithelial inflammatory cell infiltration, epithelial atrophy, and basement membrane degeneration were observed in OLP

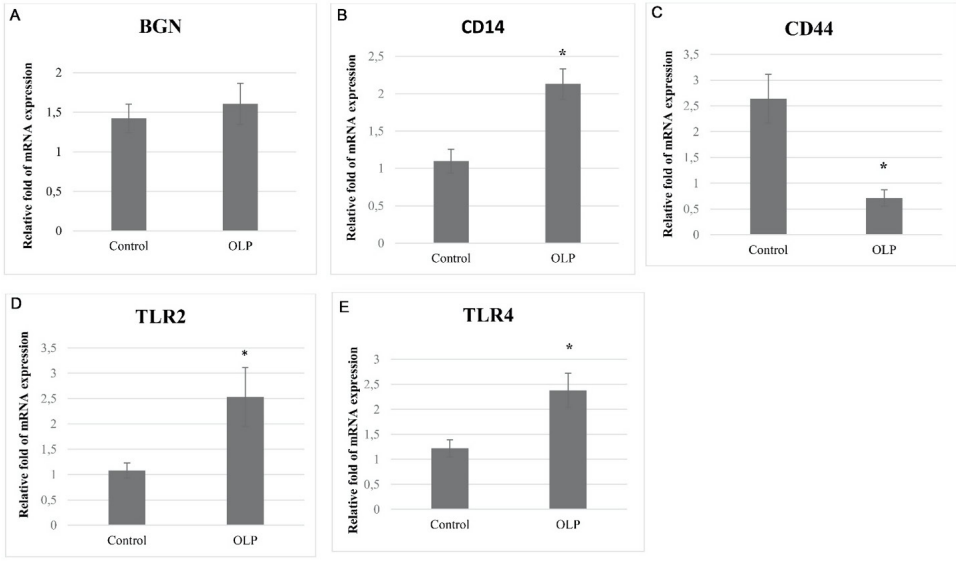

Figure 1. Relative fold to mRNA expression levels of BGN, CD14, CD44, TLR2, and TLR4 in tissues of OLP patients and the control (mean value±standard error). Significance level compared to control **p<0.05 vs. significant differences in CD14, CD44, TLR2, and TLR4 expression between OLP and control were found

OLP: Oral lichen planus, BGN: Biglycan

tissues. BGN, TLR2, TLR4, CD14, and CD44 findings obtained from the OLP and control groups by immunostaining are shown in Figures 2 and 3. Accordingly, no significant immunohistochemical staining was obtained for BGN in both groups (-) (Figure 2A, Figure 3A). CD14 (Figure 2B, Figure 3B), TLR2 (Figure 2D, Figure 3D), and TLR4 (Figure 2E, Figure 3E) stained weakly (+) in normal tissues and moderately (++) in OLP tissues. CD44 stained strongly (+++) in normal tissues and weakly (+) in OLP tissues (Figure 2C, Figure 3C).

DISCUSSION

OLP is an inflammatory disease, and it is thought that the basement membrane and ECM molecules also play a role in its pathogenesis in addition to the cellular immune response. Many endogenous and exogenous inflammatory processes are known to be involved in the pathogenesis of OLP, but the mechanisms involved in these processes need to be elucidated.[2,3,9] It has been determined that the expression of ECM molecules such as fibronectin, integrin, keratin, collagen, and E-cadherin differ in OLP.[6,7,12] In this study, we investigated the effects of BGN on TLR2/4-CD14- and TLR4-CD44-mediated signaling mechanisms in patients with OLP as a relationship with inflammation and autophagy. To understand the relationship between these signaling mechanisms and the pathogenesis of the disease, we examined the gene expression of these molecules at the mRNA level by RT-qPCR and protein level by IHC staining.

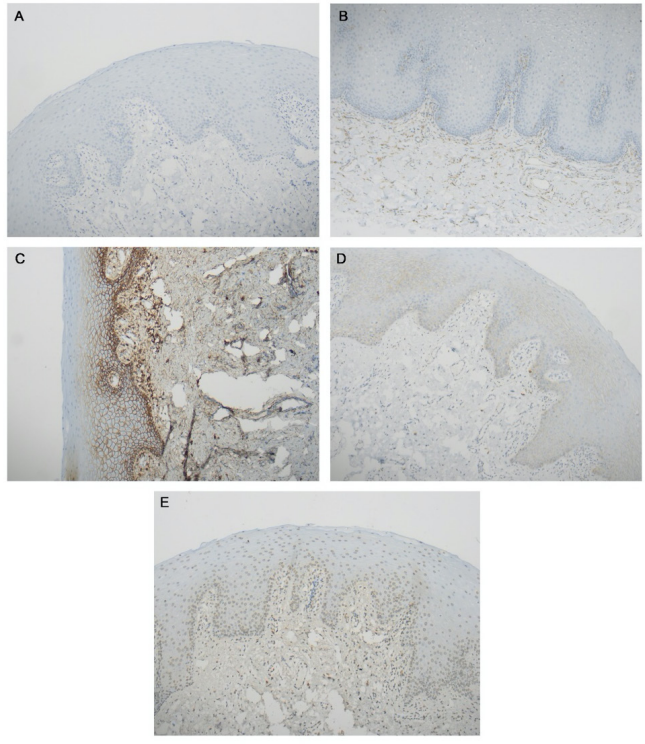

Figure 2. Representative images of the IHC staining of BGN, CD14, CD44, TLR2, and TLR4 from the control group. IHC staining of NOM tissue sections showed BGN (-) (A), CD14 (+) (B), CD44 (+++) (C), TLR2 (+) (D), and TLR4 (+) (E)

Original magnification ×10, IHC: Immunohistochemistry, NOM: Normal oral mucosa

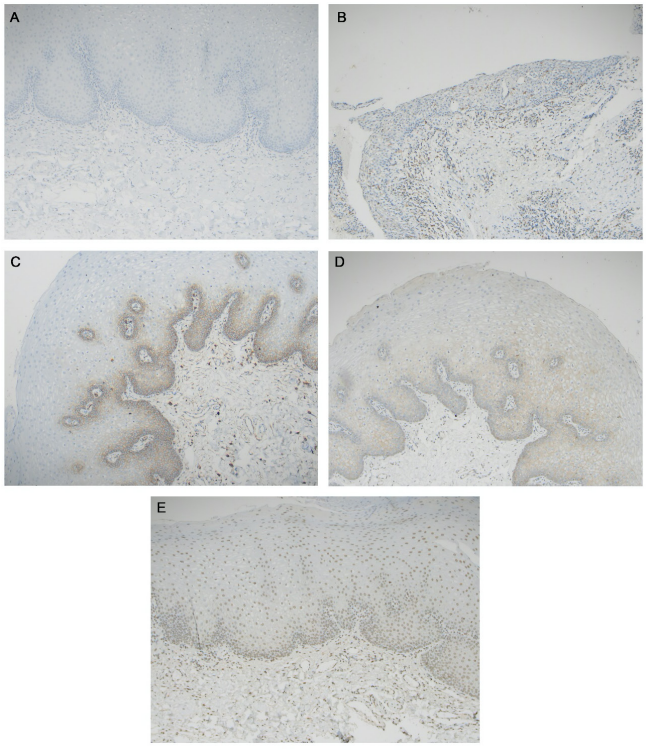

Figure 3. Representative images of the IHC staining of BGN, CD14, CD44, TLR2, and TLR4 from the OLP group. IHC staining of OLP tissue sections showed BGN (-) (A), CD14 (++) (B), CD44 (+) (C), TLR2 (++) (D), and TLR4 (++) (E)

Original magnification ×10, IHC: Immunohistochemistry, OLP: Oral lichen planus

BGN is known to play a role in maintaining the integrity of the ECM under normal physiological conditions. In addition, proteolytic cleavage from the ECM stimulates macrophages, some chemokine, and cytokines, especially in tissue damage and inflammation. Increases inflammation by sending a signal via TLR4.[7,10,12] According to our studies, only one study investigated the relationship between BGN and OLP. This study showed that the BGN level was increased in tissues adjacent to OLP tissues but histologically remained unchanged, whereas it was lower in OLP tissues. Based on these findings, it has been suggested that high BGN expression in adjacent tissues of the OLP contributes to the enhanced infiltration of immune cells in the OLP region.[10] In our study, BGN was less stained in OLP tissue immunohistochemically than in control tissue, but the difference was not statistically significant. In addition, the mRNA expression level of BGN was decreased in OLP tissues compared with the control group, but the results were not statistically significant. Our results show that BGN is less in OLP patients, which supports the current study, but it would be useful to examine samples from adjacent tissues for mRNA expression. This study contributes to the evaluation of the reduction of BGN, which supports the mucosal structure in terms of its effect on the etiology of OLP, independent of signaling molecules.

Previous studies have shown that BGN plays a role in initiating and maintaining the inflammatory response. This molecule has been implicated in proinflammatory signaling in various diseases, causing an increase in cytokines such as tumor necrosis factor-α, IL-6 and IL-1β.[20] It is involved in the pathogenesis of rheumatoid arthritis, lupus nephritis, ischemic acute kidney injury,[14] osteoarthritis,[21] and insulin-dependent diabetes mellitus type 1.[12,20] By stimulating various signaling mechanisms and increasing proinflammatory activity. Based on these findings, we examined the mRNA levels and immunoreactivity of BGN in OLP, an inflammatory disease. The BGN levels in the tissues of patients with OLP were higher than those of the control group, but the difference was not statistically significant. According to the study by Lonar-Brzak et al.[10], BGN was increased to a greater extent in healthy adjacent tissues than in inflammation-positive tissues in patients with OLP. This was interpreted as supporting the progression of the disease and increasing the risk of malignancy. Increases in BGN levels in adjacent tissues may be intended to modulate inflammation. We directly studied the lesioned tissues, so a comprehensive examination of the adjacent tissues is recommended.

Various studies have reported that TLR2 and TLR4 are important in regulating the inflammatory response in the epithelial tissues of patients with OLP. However, different studies have reported that TLR2 or TLR4 are downregulated or upregulated, and no definite conclusion has been reached on this issue.[22,23] In the present study, TLR2 and TLR4 transcripts and protein levels were significantly increased in OLP samples with inflammation. This supports the idea that inflammatory mediators play an active role in inflammation.

In some disease models, BGN has been reported to act as a ligand of TLR2 and TLR4 in inflammation, whereas CD14 and CD44 molecules act as co-receptors and stimulate different signaling mechanisms.[12,24] In recent studies, binding of BGN with a TLR2/4-CD14 co-receptor with high affinity was reported to induce immune cell infiltration and increase inflammation in inflamed tissues.[15,17] Conversely, BGN overexpression in macrophages has been shown to activate autophagy through TLR4-CD44 signaling which is very interesting.[17] Accordingly, the selection of BGN to TLR2/4 receptors and co-receptors may be decisive in directing tissue toward chronic inflammation or regeneration.[15] According to our research, the effects of TLR2/4 receptors and CD14-CD44 co-receptors on OLP in the BGN-induced inflammation pathway have not been

studied. Our results showed that TLR2/4 and CD14 levels were increased at the mRNA and immunohistochemical levels in OLP tissues. In support of the data from various diseases previously analyzed, we found that the TLR2/4-CD14 signaling pathway increased inflammation in patients with OLP. Although the course of BGN was insignificant, our results suggest that it is increased in OLP tissues and may be related to the TLTR2/4-CD14 inflammation signaling pathway.

Autophagy promotes cell survival against various stressors, negatively regulates inflammation, and prevents excessive tissue damage.[25] In addition, the inhibition of autophagy leads to increased inflammation and cellular damage.[26] Zhang et al.[25] showed that autophagy is activated via the Akt/mTOR pathway in OLP tissues. In contrast, Tan et al.[26] reported that autophagy-related gene expression was downregulated in OLP. Recent studies suggest that TLR4/CD44 signaling activates autophagy.[17] Ghazi et al.[27] showed increased CD44 immunoexpression in the epithelium of dysplastic OLP and interpreted that it may contribute to inflammation. However, some CD44 isoform were reported to be decreased in OLP tissue compared with controls or did not differ between these two groups in different studies.[16,18,28] In our study, although TLR4 expression was increased, the level of CD44 co-receptor, which regulates the autophagy pathway, was significantly decreased in OLP tissues. This result shows that TLR/4 CD44 signaling is downregulated in OLP, suggesting the inhibition of autophagy.

Study Limitations

There are two main limitations to our study. First, paraffin-embedded tissues from 21 patients previously diagnosed with OLP were studied. Increasing the number of patients will increase the study reliability. Additionally, studies examining signaling pathways at the molecular level are needed to better understand how BGN, TLR2, TLR4, CD14, and CD44 affect the molecular mechanisms underlying OLP.

CONCLUSION

According to the results of the present study, BGN-mediated TLR2/4 and CD14 signaling may promote inflammation in OLP. In addition, we suggest that inflammation signaling is upregulated by increased TLR2/4-CD14 levels in OLP tissues, and autophagy inhibition may occur by downregulating TLR2/4-CD44 signaling. We recommend that inflammation and autophagy in the OLP should be investigated in more comprehensive studies.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Clinical Research Ethics Committee of the Alanya Alaaddin Keykubat University (ALKU) Faculty of Medicine (date: 19.11.2020, decision no: 25/10).

Informed Consent: Written informed consent forms were obtained from the participants included in the study.

Authorship Contributions

Surgical and Medical Practices: H.G., Concept: Ö.C.G., H.E., F.Y., Design: Ö.C.G., H.E., F.Y., Data Collection or Processing: H.G., Analysis or Interpretation: Ö.C.G., H.G., H.E., F.Y., Literature Search: Ö.C.G., H.G., H.E., F.Y., Writing: Ö.C.G., H.G., H.E., F.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was financially supported by the Alanya Alaaddin Keykubat University Scientific Investigations Unit (project code: 2021-04-03-MAP02).

REFERENCES

1. Louisy A, Humbert E, Samimi M. Oral lichen planus: an update on diagnosis and management. Am J Clin Dermatol. 2024;25:35-53.
2. Casparis S, Borm JM, Tektas S, et al. Oral lichen planus (OLP), oral lichenoid lesions (OLL), oral dysplasia, and oral cancer: retrospective analysis of clinicopathological data from 2002-2011. Oral Maxillofac Surg. 2015;19:149-56.
3. El-Howati A, Thornhill MH, Colley HE, Murdoch C. Immune mechanisms in oral lichen planus. Oral Dis. 2023;29:1400-15.
4. González-Moles MÁ, Keim-Del Pino C, Ramos-García P. Hallmarks of Cancer Expression in Oral Lichen Planus: A Scoping Review of Systematic Reviews and Meta-Analyses. Int J Mol Sci. 2022;23:13099.
5. Shearston K, Fateh B, Tai S, Hove D, Farah CS. Oral lichenoid dysplasia and not oral lichen planus undergoes malignant transformation at high rates. J Oral Pathol Med. 2019;48:538-45.
6. Theocharis AD, Manou D, Karamanos NK. The extracellular matrix as a multitasking player in disease. FEBS J. 2019;286:2830-69.
7. Nastase MV, Zeng-Brouwers J, Beckmann J, et al. Biglycan, a novel trigger of Th1 and Th17 cell recruitment into the kidney. Matrix Biol. 2018;68:293-317.
8. Wang H, Deng Y, Peng S, et al. RNA-Seq based transcriptome analysis in oral lichen planus. Hereditas. 2021;158:39.
9. Payeras MR, Cherubini K, Figueiredo MA, Salum FG. Oral lichen planus: focus on etiopathogenesis. Arch Oral Biol. 2013;58:1057-69.
10. Lonar-Brzak B, Klobuar M, Veliki-Dali, et al. Expression of small leucine-rich extracellular matrix proteoglycans biglycan and lumican reveals oral lichen planus malignant potential. Clin Oral Investig. 2018;22:1071-82.

11. Berdiaki A, Giatagana EM, Tzanakakis G, Nikitovic D. The landscape of small leucine-rich proteoglycan impact on cancer pathogenesis with a focus on biglycan and lumican. Cancers (Basel). 2023;15:3549.
12. Roedig H, Nastase MV, Wygrecka M, Schaefer L. Breaking down chronic inflammatory diseases: the role of biglycan in promoting a switch between inflammation and autophagy. FEBS J. 2019;286:2965-79.
13. Ilie M, Khambata-Ford S, Copie-Bergman C, et al. Use of the 22C3 anti–PD-L1 antibody to determine PD-L1 expression in multiple automated immunohistochemistry platforms. PLoS One. 2017;12:e0183023.
14. Moreth K, Frey H, Hubo M, et al. Biglycan-triggered TLR-2- and TLR-4-signaling exacerbates the pathophysiology of ischemic acute kidney injury. Matrix Biol. 2014;35:143-51.
15. Roedig H, Damiescu R, Zeng-Brouwers J, et al. Danger matrix molecules orchestrate CD14/CD44 signaling in cancer development. Semin Cancer Biol. 2020;62:31-47.
16. Siponen M, Kullaa A, Nieminen P, Salo T, Pasonen-Seppänen S. Altered expression of hyaluronan, HAS1-2, and HYAL1-2 in oral lichen planus. J Oral Pathol Med. 2015;44:401-9.
17. Poluzzi C, Nastase MV, Zeng Brouwers J, et al. Biglycan evokes autophagy in macrophages via a novel CD44/Toll?like receptor 4 signaling axis in ischemia/reperfusion injury. Kidney Int. 2019;95:540-62.
18. Chaiyarit P, Thongprasom K, Satayut S, et al. Alteration of the expression of CD44 isoforms in oral epithelia and saliva from patients with oral lichen planus. J Clin Immunol. 2008;28:26-34.
19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods. 2001;25:402-8.
20. Nastase MV, Young MF, Schaefer L. Biglycan: a multivalent proteoglycan providing structure and signals. J Histochem Cytochem. 2012;60:963-75.
21. Barreto G, Soininen A, Ylinen P, et al. Soluble biglycan: a potential mediator of cartilage degradation in osteoarthritis. Arthritis Res Ther. 2015;17:379.
22. Amin NR, Yussif N, Ahmed E. The effect of smoking on clinical presentation and expression of TLR-2 and CD34 in Oral lichen Planus patients: Clinical and immunohistochemical study. BMC Oral Health. 2020;20:129.
23. Ohno S, Tateishi Y, Tatemoto Y, Morishita K, Sasabe E, Yamamoto T. Enhanced expression of Toll-like receptor 2 in lesional tissues and peripheral blood monocytes of patients with oral lichen planus. J Dermatol. 2011;38:335-44.
24. Rider D, Furusho H, Xu S, et al. Elevated CD14 (Cluster of Differentiation 14) and Toll-Like Receptor (TLR) 4 Signaling Deteriorate Periapical Inflammation in TLR2 Deficient Mice. Anat Rec (Hoboken). 2016;299:1281-92.
25. Zhang N, Zhang J, Tan YQ, Du GF, Lu R, Zhou G. Activated Akt/mTOR-autophagy in local T cells of oral lichen planus. Int Immunopharmacol. 2017;48:84-90.
26. Tan YQ, Zhang J, Du GF, Lu R, Chen GY, Zhou G. Altered autophagy-associated genes expression in T cells of oral lichen planus correlated with clinical features. Mediators Inflamm. 2016;2016:4867368.
27. Ghazi N, Saghravanian N, Ghazi A, Shakeri MT, Khajehbahrami H. CD44 expression in dysplastic and non-dysplastic oral lichen planus. Int J Cancer Manag. 2020;13:e98061.
28. Neppelberg E, Loro LL, Øijordsbakken G, Johannessen AC. Altered CD40 and E-cadherin expression–putative role in oral lichen planus. J Oral Pathol Med. 2007;36:153-60.