An association of the MCP-1 and CCR2 gene polymorphisms with oral lichen planus

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Objective. The purpose of this study was to investigate the frequency of monocyte chemoattractant protein (MCP)-1 CCR2 gene polymorphisms in Turkish patients with oral lichen planus (OLP).

Study Design. A cohort of 50 patients with OLP and 142 control participants without OLP were recruited to investigate the frequency of MCP-1 and CCR2 gene polymorphisms. Chi-square and Fisher's exact tests were used. Student *t* test and analysis of variance were used to compare demographic data between groups.

Results. The MCP AA genotype was less common in the patient group (52%) than in the control group (66.2%; odds ratio [OR] = 0.553; 95% confidence interval [CI], 0.287-1.065; $P = .075$). The MCP G allele was higher in the patient group (48%) than in the control group (33.8%; OR = 1.808; 95% CI, 0.939-3.479; $P = .075$). The frequency of the MCP GG genotype was observed to be higher in the patient group (4%) than in the controls (0.7%; OR: 5.875, 95% CI:0.521-66,24; $p = 0.106$). The CCR2 64I64I genotype was more common in the patient group (6%) than in the control group (2.8%). All results were not statistically significant.

Conclusion. We suggest that the G allele of MCP-1 and 64I64I genotype of CCR2 polymorphisms do not pose an increased risk for Turkish patients with OLP to develop oral squamous cell carcinoma. (Oral Surg Oral Med Oral Pathol Oral Radiol 2021;132:708−714)

Lichen planus (LP) is a chronic inflammatory mucocutaneous disease that has an unknown etiology. It affects skin, nails, and mucosa and may rarely show malignant transformation.[1] It can also affect the scalp, hair follicles, and, less commonly, the eyes, nasal mucosa, and urinary tract.[2] Its oral form, which affects the oral mucosa and progresses with recurrence and remission, is named oral lichen planus (OLP).[3] Unlike the cutaneous lesions of LP, oral lesions are generally chronic and can be an important source of morbidity. OLP affects approximately 0.5% to 2.2% of the population, and the ratio of men to women is 1:2.[4] The most commonly observed type of OLP is the reticular form, which is generally seen bilaterally on the buccal mucosa and tongue. Other types of OLP that are observed less frequently are papular, bullous, erosive, atrophic, and plaque-like forms.[5]

Although the etiopathology of OLP has not been fully elucidated, there are studies showing that the immune response involving both antigen-specific and nonspecific mechanisms plays a role in the pathogenesis of OLP.[6] Supporting these studies, both subepithelial and epithelial lymphocyte infiltration and degeneration of basal keratinocytes were demonstrated histologically in OLP.[7] Indicators of the inflammatory process in OLP such as nuclear factor κ B, tumor necrosis factor α (TNF-α) and various interleukins (ILs) such as IL-lα, IL-6, and IL-8 have been shown to be increased in serum and salivary secretion.[8,9] Important producers of TNF-α are T cells, natural killer (NK) cells, dendritic cells, and macrophages. IL-12, interferon γ (IFN-γ), and TNF-α are significantly elevated over the prototypical Th2 cytokine IL-4.[10] Yet another cytokine identified in OLP is IL-18, which can have similar effects on cytotoxicity and IFN-γ production as IL-12.[11,12]

Chemokines form a family of little cytokines, initially identified by their modulator action on the inflammatory response. Increased attention is being given to these proteins, especially because of their function on endothelial cells.[13] In OLP lesions and in patient peripheral blood, CXCL9, CXCL10, and several general inflammation-associated chemokines have been identified.[14]

One of the most widely studied chemokines is regulated on activation normal T-cell-expressed and -secreted (RANTES), a member of the CC chemokine family, produced by different cell types, including activated T lymphocytes, keratinocytes, and mastocytes.

Statement of Clinical Relevance

There may be a relation between the −2518 A/G monocyte chemoattractant protein-1-V64I CCR2 polymorphisms and oral lichen planus (OLP) pathogenesis. There are some immunotherapy treatments for OLP. Suppression of these gene polymorphisms may help to treat OLP.

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Its biological effect, when RANTES is linked to different receptors, such as CCR-1, CCR-3, CCR-4, CCR-5, CCR-9, and CCR-10, found on the cell surface.[15]

Monocyte chemoattractant protein (MCP)-1 is one of the important chemokines involved in the migration and infiltration of monocytes, NK cells, and memory T lymphocytes.[15] The MCP-1 receptors are most closely related to the receptor for the chemokines macrophage inflammatory protein 1α and RANTES. The chemokine family has been divided into 2 subfamilies based on arrangement of the first 2 cysteines. CXC family members are potent neutrophil agonists, and CC members are potent monocyte agonists.[16,17]

CCR2 is the receptor for MCP-1 and is widely observed in defense cells such as monocytes/macrophages, NK cells, basophils, dendritic cells, T lymphocytes, and B lymphocytes.[18] CCR2 belongs to the family of class A G protein−coupled receptors, and there are 2 splice variants, CCR2A and CCR2B. Although CCR2A is the main isoform expressed by mononuclear cells and vascular smooth muscle cells,[19] monocytes and activated NK cells predominantly express CCR2B.[20] The MCP-1/CCR2 axis has been shown to play a role in a number of disorders, such as rheumatoid arthritis, psoriasis, atopic dermatitis, atherosclerosis, multiple sclerosis, and insulin-resistant diabetes.[20] In a recent study, expression of MCP-1 and CCR-2 in OLP was demonstrated.[21] Because of the probable importance of chemokines in a variety of inflammatory and disease processes, attention has recently focused on the leukocyte receptors that mediate chemokine responses.[16] Therefore, in this study, we aimed to investigate whether −2518 A/G MCP-1 and V64I CCR2 single-nucleotide polymorphisms have a role in OLP pathogenesis.

MATERIALS AND METHODS

The institutional human study review committees of Istanbul University approved this prospective study (2010/221-29). This study was conducted in accordance with the tenets of the Declaration of Helsinki, and written informed consent was obtained from all patients. A total of 50 patients with OLP who were treated in our clinic and diagnosed as OLP according to biopsy and 142 control participants without OLP were included in the study. Biopsy specimens were taken from the patients and a diagnosis of OLP was made. Blood was taken from patients who were diagnosed later. The criteria determined by van der Meij and van der Waal[22] were used for the clinical diagnosis of OLP.

These criteria include the presence of (1) bilateral and more or less symmetric lesions; (2) a lacelike network of slightly raised gray-white lines (reticular pattern); and (3) erosive, atrophic, bullous, and plaque-type lesions, which are accepted only as a subtype in the presence of reticular lesions elsewhere in the oral mucosa. In addition, microscopic diagnosis was made by performing a biopsy.

Those who met any of the following criteria were excluded from the study: patients with diagnosed systemic lupus erythematosus or graft vs host disease, patients who were pregnant, patients who clinically mimicked OLP but were considered to have an oral lichenoid lesions reaction when other parameters are considered, patients with drug reactions that clinically mimic OLP, patients using local or systemic corticosteroids, and those who did not want to participate in the study.

DNA isolation from peripheral blood

Ten milliliters of peripheral blood sample were drawn into a sterile Ethylenediaminetetraacetic acid (EDTA) tube and transferred to a centrifuge tube. An erythrocyte lysis solution was added to the samples at a ratio of 1:3 (30 mL) and mixed. Samples were kept at +4°C for 15 minutes. The samples were then centrifuged at 1500 rpm for 10 minutes and supernatant discarded. Fifteen to 20 mL of erythrocyte lysis solution was added to the pellets, and the samples were kept at +4°C for 15 minutes. They were then centrifuged again at 1500 rpm for 10 minutes and the supernatant was discarded. Then 500 μL 10% SDS 50 μL proteinase K (20 mg/mL) and 9.4 mL leukocyte lysis solution were added to the suspended pellet and incubated overnight in a 56°C water bath. After incubation, 0.37 mL of 9.5 M ammonium acetate solution was added per milliliter of sample, and the samples were mixed slowly, followed by centrifugation for 25 minutes at 3000 rpm to precipitate the proteins. After centrifugation, the supernatant portion was transferred to a clean centrifuge tube and 99% absolute alcohol was added in a ratio of 1:2 to precipitate the DNA. The condensed DNA was expected to come to the surface of the alcohol, and the DNA was taken with a sterile micropipette tip. DNA was washed in 70% alcohol and dissolved in Tris-EDTA solution.

DNA purity determination

DNA samples were diluted 1:3 with Tris-EDTA solution. The absorbance of DNA at 260 nm and the absorbance of RNA and protein at 280 nm were measured by resetting the spectrophotometer with Tris-EDTA solution. To evaluate DNA purity, the OD 260/OD 280 ratio was calculated, and DNA with a ratio of 1.7 to 1.8 was considered pure.

Identification of MCP-1 polymorphism by polymerase chain reaction

In genomic DNA samples, alleles of the MCP-1 A −2518 locus were amplified by polymerase chain reaction (PCR). A total of 25 μL of the PCR mixture was prepared for the amplification of each DNA sample. This

PCR mixture comprised 100 to 200 ng DNA, 0.5 mL 0.2 mM dNTP, 1.5 mM $MgCl_2$, and 1.0 U Taq DNA polymerase from each primer. Primers for MCP-1 −2518 and CCR-2 V64I were designed from previous work.[23] Primers for amplification and quantification of the MCP-1 −2518 were as follows: forward, (5′)-TCT CTC ACG CCA GCA CTG ACC-(3′) and reverse, (5′)-GAG TGT TCA CAT AGG CTT CTG-(3′); and the primers for CCR-2 V64I were forward, (5′)-CAT TGC AAT CCC AAA GAC CCA CTC-(3′) and reverse, (5′)-TTG GTT TTG TGG GCA ACA TGA TGG-(3′). Amplification reactions were carried out in a thermal cycler as follows: the reaction was conducted in a MJ Research Techne (Bio-Rad) for 35 cycles. Conditions of the reaction were as follows: denaturation at 95°C for 3 minutes, annealing of primers at 55°C for 35 seconds, and elongation at 72°C for 10 seconds. All samples were tested in duplicate. PCR products were cut with PvuII restriction endonuclease and subjected to 2% agarose gel electrophoresis. DNA fragments were imaged under ultraviolet light and genotyped after staining with ethidium bromide. The A allele gives 234 bp and the G allele gives 159 bp and 75 bp. Those in the AA genotype have a band at 234 bp, those in the GG genotype at 159 bp and 75 bp, and those in the AG genotype at 234 bp, 159 bp, and 75 bp.

Detection of CCR2 gene polymorphism by PCR

In the genomic DNA samples of individuals, alleles of the CCR2 locus were amplified by PCR. A total of 25 μL of PCR mixture was prepared for the amplification of each DNA sample. The PCR mixture was prepared as 100 to 200 ng DNA, 0.5 μL of each primer, 0.1 mM dNTP, 1.5 mM $MgCl_2$, and 1.0 U Taq DNA polymerase. Amplification reactions were carried out in thermal cycler as follows: the reaction was conducted in a MJ Research Techne (Bio-Rad) for 33 cycles. Conditions of the reaction were as follows: denaturation at 94°C for 5 minutes, annealing of primers at 56°C for 30 seconds, and elongation at 72°C for 30 seconds. All samples were tested in duplicate. The fragment of CCR2 locus amplified by PCR was cut with BsaBI restriction endonuclease and then subjected to 3% agarose gel electrophoresis (Figure 1). After DNA fragments were stained with ethidium bromide, they were visualized under ultraviolet light and genotyped. The CCR2 wt allele gives 173 bp and the 64I allele 149 bp and 24 bp bands. Those with the CCR2 wt/wt genotype have a band at 173 bp, those in the 64I/64I genotype have bands at 173 bp and 149 bp, and those in the wt/64I genotype have bands at 173 bp, 149 bp, and 24bp.

Statistical analysis

Statistical analysis was performed using SPSS 11.0. Statistical significance was taken as $P < .05$. Chi-square and Fisher's exact tests were used to evaluate the differences between the frequency of genotypes and alleles. Student *t* test and analysis of variance were used to compare demographic data between groups. Allele frequencies were determined according to the gene counting method.

RESULTS

The demographic characteristics of the patients are given in Table I. Thirty-six of the patients were female and 14 were male, and the mean age was 52.17 ± 11.96 years. The lesions were the erosive form in 16 patients (32%), reticular form in 13 patients (26%), reticular and erosive forms in 10 patients (20%), and reticular and plaque forms together in 11 patients (22%).

There was no significant difference between the patient and control groups in terms of MCP-1 A −2518G genotype and allele distributions ($P = .083$, $P = .057$; Table II). There was no significant difference in CCR2 V64I genotype and allele distributions between patients with OLP and control participants without OLP ($P = .580$; $P = .379$; Table III).

There were no significant changes in the frequency of MCP G, MCP GG, and MCP AA genotypes between the patient and control groups. There was no significant difference between patients with OLP and those in the control group in MCP-1 A −2518G and CCR2 V64I polymorphism (Table IV).

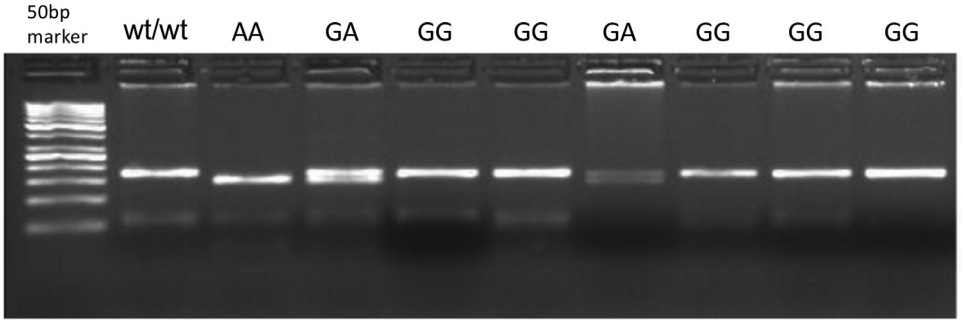

Fig. 1. View of CCR2 genotypes on 3% agarose gel.

Table I. Demographic characteristics of the patient and control groups.

Variables	*Patient group (n = 50)*	*Control group (n = 142)*	*P*
Sex, n (%)			
Female	36 (72)	93 (65.49)	.399
Male	14 (28)	49 (34.51)	
Age (years)	52.17 ± 11.96	50.16 ± 12.46	.314
Smoker, n (%)			
No	39 (78)	80 (56.34)	.008
Yes	11 (22)	62 (43.66)	
Alcohol use, n (%)			
No	46 (92)	130 (91.54)	.921
Yes	4 (8)	12 (8.46)	
Systemic disease, n (%)	19 (38)	0	—
No	31 (62)		
Yes			
Hypertension, n (%)	38 (76)	0	—
No	12 (24)		
Yes			
Diabetes, n (%)		0	—
No	39 (78)		
Yes	11 (22)		
Clinical type of lesions	13	—	—
Reticular form	16		
Erosive form	11		
Reticular and plaque	10		
Reticular and erosive			
Location of the lesions			
Buccal mucosa	28	—	—
Tongue	1		
Lips	1		
Buccal mucosa and tongue	8		
Buccal mucosa and gingiva	6		
Gingiva	2		
Buccal mucosa, tongue, and gingiva	4		
Any lesions on other parts of body, n	—	—	
(%)	43 (86)		
No	7 (14)		
Yes			

Table II. MCP-1 A −2518G genotype and allele distributions in patient and control groups.

MCP-1 A −2518 Ggenotype and allele	*Patient group (n = 50), n (%)*	*Control group (n = 142), n (%)*	*P*
AA	26 (52.0)	94 (66.22)	
AG	22 (44.0)	47 (33.1)	
GG	2 (4.0)	1 (0.7)	.083
A	74 (74%)	235 (82.74)	
G	26 (26)	49 (17.25)	.057

MCP, monocyte chemoattractant protein.

Table III. CCR2 V64I genotype and allele distributions in patient and control groups.

CCR2 V64I genotype and allele	*Patient group (n =50), n (%)*	*Control group (n =142), n (%)*	*P*
wt/wt	38 (76.0)	113 (79.6)	
wt/64I	9 (18.0)	25 (17.6)	
64I/64I	3 (6.0)	4 (2.8)	.580
wt	85 (85)	251 (88.38)	
64I	15 (15)	33 (11.61)	.379

DISCUSSION

In this study, we investigated the frequency of MCP1, CCR2 gene polymorphisms in the pathogenesis of OLP as an immune-mediated disease associated with T-lymphocyte immunologic dysfunction.

OLP is an autoimmune and chronic inflammatory disease that affects the oral mucosa. The exact etiology of OLP is still unknown, but it is believed to be associated with a cell-mediated immune disorder in which genetic and environmental factors play a role.[6] The prevalence of OLP is around 2.0% in the general population, and rates are significantly higher in women than in men.[24] OLP is most commonly observed in the buccal mucosa in the oral cavity and less often in the lingual, labial, and gingival mucosa. Typical feature of lesions in OLP is bilateral symmetric distribution, especially in the posterior regions of the oral cavity. According to the Andreasen classification system, 6 clinical OLP forms—papular, reticular, atrophic (erythematous), plaque-like, bullous erosive, and erosive ulcers—have been described.[7] Papular, reticular, and plaque-like forms are clinically present as white keratotic lesions and are generally painless. In erosive, bullous, and atrophic forms, symptoms can be very painful in advanced cases.[25]

Chemokines are essential in cell recruitment and trafficking during inflammation.[26] They play a role in the selective recruitment and chemotaxis of neutrophils, monocytes, and lymphocytes. It has been reported that MCP-1 chemokines and CCR2 receptors are increased in inflammatory diseases.[5,27-29] MCP-1 is one of the most important chemokines involved in the extravasation of monocytes and macrophages.[30] M1 macrophages can aid OLP progression by 3 main mechanisms: initiation of inflammation, activation and priming of T cells, and direct destruction of the basal membrane. They can exacerbate inflammation through the production of pro-inflammatory cytokines (TNF-α and IL-1β), which can upregulate cell adhesion

Table IV. Odds ratios and 95% confidence intervals for the −2518 A/G MCP-1 and V64I CCR2 genotypes investigated in patients with oral lichen planus and control participants.

MCP-1 genotype/allele	*Patient group (n = 50)*	*Control group (n = 142)*	*Odds ratio*	*95% Confidence interval*	*p*
AA	26	94	0.553	0.287-1.065	.075
AG	22	47	ref		
GG	2	1	5.875	0.521-66.24	.106
A	74	235	ref		
G	26	49	1.808	0.939-3.479	.075
CCR2 V64I genotype/allele					
64I/64I	3	4	2.202	0.475-10.201	.302

MCP, monocyte chemoattractant protein.

molecules on endothelial and keratinocyte surfaces and induce chemokine expression (RANTES; MCP-1 for monocytes) by oral keratinocytes, which results in increased recruitment of inflammatory cells into the lesion.[31,32] CR2 is involved in both anti-inflammatory (T-cell-mediated) and pro-inflammatory (antigen-presenting cell- and T-cell-mediated) processes.[20] OLP is also a T-cell−mediated autoimmune disease in which the auto-cytotoxic CD8+ T cells trigger apoptosis of the basal cells of the oral epithelium.[33]

It is assumed that MCP-1 plays an important role in the pathogenesis of various diseases characterized by mononuclear cell infiltration, such as atherosclerosis, rheumatoid arthritis, diabetes mellitus, and OLP.

Both MCP-1 and its receptor CCR2 have been found to play an important role in the development of atherosclerosis.[34] In addition, MCP-1 levels are significantly increased in individuals with rheumatoid arthritis.[35] In an experimental study on animals, MCP-1 was reported to cause insulin resistance in diabetes mellitus.[36]

Several studies[15,16,20,21,23,26,31,37,38] have reported MCP-1, CCR2 chemokine expressions on a cellular basis. Our study is the first study on polymorphisms to examine the frequency of risky alleles and genotypes on patients with OLP and controls.

Tao et al.[39] showed that the expression of MCP-1 genes was increased in patients with OLP compared with those without OLP. Later, Yin et al.[21] determined that MCP-1 mRNA and CCR2 mRNA levels in OLP lesions were higher than in the control group, according to reverse transcription PCR results. These findings were consistent with the immunohistochemical findings, which showed a marked increase in the OLP lesions in MCP-1+ and CCR2+ cells compared with the control group.

In our study we found a positive association between the MCP GG genotype polymorphism of the MCP-1 gene and the presence of OLP in patients. The risk of OLP increases 1.8 times in the presence of the MCP-1 G allele and 5.8 times in the presence of the GG genotype. The CCR2 64I64I genotype was observed to increase in the patient group (6%) compared with the control group (2.8%), and this increased the risk of disease by 2.20 times. None of these findings were statistically significant. Moreover, Yin et al.[21] observed that there were no significant differences in the expression of MCP-1 and CCR2 between erythematous, erosive, and reticular OLP lesions. In our study, comparable to these findings, no statistically significant difference was observed between the clinical appearances of the lesions according to MCP1 CCR2 gene polymorphism.

OLP has been defined by the World Health Organization as a potential precancerous condition, representing a generalized state associated with a significantly increased risk of oral cancer. It is hypothesized that inflammatory mediators such as cytokines and chemokines released from infiltrating T cells induce fundamental changes in proteins in oral epithelial cells, leading to the progression of OLP to oral squamous cell carcinoma (OSCC). The proteomic alteration in epithelial cells may in turn cause the release of cytokines/chemokines, attracting more T cells into the epithelium.[40]

Many studies have investigated the genetic effect of MCP-1 and its receptor, CCR2, polymorphisms on the susceptibility and clinic pathologic status of oral cancer but not in OLP.[7,37,38,41]

Based on OLP's premalignant status and chronic inflammatory character, we expected to find connections between OLP prevalence and active MCP-1 CCR2 gene polymorphisms in patients with OLP compared with those in the control group.

A study[23] revealed that the G allele and GG genotype of MCP-1 and 64I allele of CCR2 pose an increased risk for OSCC. In our study on Turkish patients with OLP 35.2%, the MCP G allele was higher in the patient group (48%) than in the control group and the frequency of the MCP GG genotype was observed to be higher in the patient group (4%) than in the control group (0.7%). These results were not statistically significant. Our study may be the first to examine the frequency of MCP GG and MCP G+ genotype polymorphisms in patients with OLP.

The −2518 A/G MCP-1 has a higher expression of MCP-1 as a result of single nucleotide polymorphism, which has been shown to affect carcinogenesis.[37] Walczak et al.[38] reported a relationship between colorectal cancer and the GG genotype of −2518 A/G MCP-1 single nucleotide polymorphism. Chen et al.[41] determined that there was a correlation between V64I CCR2 gene polymorphism and oral cancer development in a Taiwanese population. Similar to these findings, in our study CCR2 64I64I genotype frequency was 2.2 times higher in the patient group than in the control group.

The results showed that there was no statistical difference between patients with OLP and control participants without OLP in the frequency of higher-risk genotypes. These results would suggest that there is no increased risk for Turkish patients with OLP to develop OSCC.

It is important to follow up OLP cases to determine the effects of these genotypes and alleles on the malignant transformation potential of OLP. This could be regarded as a limitation of this study because there were no cases that underwent malignant transformation during the study period and the relatively low number of patients in the study group did not allow investigation of malignant transformation potential, which accounts for 1% to 2% of total cases per year worldwide.[42]

CONCLUSIONS

We suggest that the G allele of MCP-1 and 64I allele of CCR2 do not pose an increased risk for Turkish patients with OLP to develop OSCC.

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REFERENCES

1. Parashar P. Oral lichen planus. *Otolaryngol Clin North Am*. 2011;44:89-107. vi.
2. Farhi D, Dupin N. Pathophysiology, etiologic factors, and clinical management of oral lichen planus, part I: facts and controversies. *Clin Dermatol*. 2010;28:100-108.
3. Lodi G, Scully C, Carrozzo M, Griffiths M, Sugerman PB, Thongprasom K. Current controversies in oral lichen planus: report of an international consensus meeting. Part 1. Viral infections and etiopathogenesis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2005;100:40-51.
4. Aghahosseini F, Arbabi-Kalati F, Fashtami LA, Fateh M, Djavid GE. Treatment of oral lichen planus with photodynamic therapy mediated methylene blue: a case report. *Med Oral Patol Oral Cir Bucal*. 2006;11:E126-E129.
5. Sugerman PB, Savage NW, Walsh LJ, et al. The pathogenesis of oral lichen planus. *Crit Rev Oral Biol Med*. 2002;13:350-365.
6. Roopashree MR, Gondhalekar RV, Shashikanth MC, George J, Thippeswamy SH, Shukla A. Pathogenesis of oral lichen planus—a review. *J Oral Pathol Med*. 2010;39:729-734.
7. Andreasen JO. Oral lichen planus. 1. A clinical evaluation of 115 cases. *Oral Surg Oral Med Oral Pathol*. 1968;25:31-42.
8. Gu GM, Martin MD, Darveau RP, Truelove E, Epstein J. Oral and serum IL-6 levels in oral lichen planus patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2004;98:673-678.
9. Rhodus NL, Cheng B, Bowles W, Myers S, Miller L, Ondrey F. Proinflammatory cytokine levels in saliva before and after treatment of (erosive) oral lichen planus with dexamethasone. *Oral Dis*. 2006;12:112-116.
10. Kurago ZB. Etiology and pathogenesis of oral lichen planus: an overview. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2016;122:72-80.
11. Zhang Y, Liu W, Zhang S, et al. Salivary and serum interleukin-18 in patients with oral lichen planus: a study in an ethnic Chinese population. *Inflammation*. 2012;35:399-404.
12. Bai J, Zhang Y, Lin M, et al. Interleukin-18 gene polymorphisms and haplotypes in patients with oral lichen planus: a study in an ethnic Chinese cohort. *Tissue Antigens*. 2007;70:390-397.
13. Kiefer F, Siekmann AF. The role of chemokines and their receptors in angiogenesis. *Cell Mol Life Sci*. 2011;68:2811-2830.
14. Xavier GM, de Sa AR, Guimaraes AL, da Silva TA, Gomez RS. Investigation of functional gene polymorphisms interleukin-1beta, interleukin-6, interleukin-10 and tumor necrosis factor in individuals with oral lichen planus. *J Oral Pathol Med*. 2007;36:476-481.
15. Bose S, Cho J. Role of chemokine CCL2 and its receptor CCR2 in neurodegenerative diseases. *Arch Pharm Res*. 2013;36:1039-1050.
16. Charo IF, Myers SJ, Herman A, Franci C, Connolly AJ, Coughlin SR. Molecular cloning and functional expression of two monocyte chemoattractant protein 1 receptors reveals alternative splicing of the carboxyl-terminal tails. *Proc Natl Acad Sci U S A*. 1994;91:2752-2756.
17. Oppenheim JJ, Zachariae CO, Mukaida N, Matsushima K. Properties of the novel proinflammatory supergene "intercrine" cytokine family. *Annu Rev Immunol*. 1991;9:617-648.
18. Murphy PM, Baggiolini M, Charo IF, et al. International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev*. 2000;52:145-176.
19. Bartoli C, Civatte M, Pellissier JF, Figarella-Branger D. CCR2A and CCR2B, the two isoforms of the monocyte chemoattractant protein-1 receptor are up-regulated and expressed by different cell subsets in idiopathic inflammatory myopathies. *Acta Neuropathol*. 2001;102:385-392.
20. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res*. 2009;29:313-326.
21. Yin J, Yang X, Zeng Q, Yang L, Cheng B, Tao X. The positive correlation of the CCL2-CCR2 axis with the disease activity may indicate the fundamental role in the pathogenesis of oral lichen planus. *J Oral Pathol Med*. 2016;45:41-47.
22. van der Meij EH, van der Waal I. Lack of clinicopathologic correlation in the diagnosis of oral lichen planus based on the presently available diagnostic criteria and suggestions for modifications. *J Oral Pathol Med*. 2003;32:507-512.
23. Bektas-Kayhan K, Unur M, Boy-Metin Z, Cakmakoglu B. MCP-1 and CCR2 gene variants in oral squamous cell carcinoma. *Oral Dis*. 2012;18:55-59.

24. Chrcanovic BR, Cruz AF, Trindade R, Gomez RS. Dental implants in patients with oral lichen planus: a systematic review. *Medicina (Kaunas)*. 2020;56:53.
25. Payeras MR, Cherubini K, Figueiredo MA, Salum FG. Oral lichen planus: focus on etiopathogenesis. *Arch Oral Biol*. 2013;58:1057-1069.
26. O'Connor T, Borsig L, Heikenwalder M. CCL2-CCR2 signaling in disease pathogenesis. *Endocr Metab Immune Disord Drug Targets*. 2015;15:105-118.
27. Gerber JS, Mosser DM. Reversing lipopolysaccharide toxicity by ligating the macrophage Fc gamma receptors. *J Immunol*. 2001;166:6861-6868.
28. Stein M, Keshav S, Harris N, Gordon S. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med*. 1992;176:287-292.
29. Vazquez-Torres A, Jones-Carson J, Mastroeni P, Ischiropoulos H, Fang FC. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. I. Effects on microbial killing by activated peritoneal macrophages in vitro. *J Exp Med*. 2000;192:227-236.
30. Takada Y, Hisamatsu T, Kamada N, et al. Monocyte chemoattractant protein-1 contributes to gut homeostasis and intestinal inflammation by composition of IL-10-producing regulatory macrophage subset. *J Immunol*. 2010;184:2671-2676.
31. Li J, Farthing PM, Thornhill MH. Oral and skin keratinocytes are stimulated to secrete monocyte chemoattractant protein-1 by tumour necrosis factor-alpha and interferon-gamma. *J Oral Pathol Med*. 2000;29:438-444.
32. Yamamoto T, Osaki T, Yoneda K, Ueta E. Cytokine production by keratinocytes and mononuclear infiltrates in oral lichen planus. *J Oral Pathol Med*. 1994;23:309-315.
33. Eversole LR. Immunopathogenesis of oral lichen planus and recurrent aphthous stomatitis. *Semin Cutan Med Surg*. 1997;16:284-294.
34. Namiki M, Kawashima S, Yamashita T, et al. Local overexpression of monocyte chemoattractant protein-1 at vessel wall induces infiltration of macrophages and formation of atherosclerotic lesion: synergism with hypercholesterolemia. *Arterioscler Thromb Vasc Biol*. 2002;22:115-120.
35. Rantapaa-Dahlqvist S, Boman K, Tarkowski A, Hallmans G. Up regulation of monocyte chemoattractant protein-1 expression in anti-citrulline antibody and immunoglobulin M rheumatoid factor positive subjects precedes onset of inflammatory response and development of overt rheumatoid arthritis. *Ann Rheum Dis*. 2007;66:121-123.
36. Kamei N, Tobe K, Suzuki R, et al. Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. *J Biol Chem*. 2006;281:26602-26614.
37. Rovin BH, Lu L, Saxena R. A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. *Biochem Biophys Res Commun*. 1999;259:344-348.
38. Walczak A, Przybylowska-Sygut K, Sygut A, et al. An association of the MCP-1 and CCR2 single nucleotide polymorphisms with colorectal cancer prevalence. *Pol Przegl Chir*. 2017;89:1-5.
39. Tao XA, Li CY, Xia J, et al. Differential gene expression profiles of whole lesions from patients with oral lichen planus. *J Oral Pathol Med*. 2009;38:427-433.
40. Liu Y, Messadi DV, Wu H, Hu S. Oral lichen planus is a unique disease model for studying chronic inflammation and oral cancer. *Med Hypotheses*. 2010;75:492-494.
41. Chen MK, Yeh KT, Chiou HL, Lin CW, Chung TT, Yang SF. CCR2-64I gene polymorphism increase susceptibility to oral cancer. *Oral Oncol*. 2011;47:577-582.
42. Giuliani M, Troiano G, Cordaro M, et al. Rate of malignant transformation of oral lichen planus: a systematic review. *Oral Dis*. 2019;25:693-709.

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