Original Article

Evaluation of Secretory State in Patients with Oral Lichen Planus: A Case-Control Study

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Abstract

Background: Non-secretor individuals lack ABO blood group antigens in their secretions like saliva; these carbohydrate structures play an important role in protection of the oral cavity from exogenous pathogens; therefore these individuals are more susceptible to mucous membrane damages. The aim was to assess the secretory state of patients with oral lichen planus (OLP) in comparison with healthy controls.

Materials and Methods: Fifty patients and 100 age-gender matched control subjects were recruited to the study. Patients were visited in the outpatient clinic of dermatology at Shohada-e-Tajrish Hospital, Shahid Beheshti University of Medical Sciences from 2012 – 2014. Two-milliliter (mL) blood was collected from each subject to detect Lewis phenotypes. According to Lewis phenotype of each subject, secretory state was determined except in subjects with Le (a-b-) phenotype, in whom saliva was collected to determine the secretor status.

Results: Non-secretor status in patients with OLP was more frequent compared with healthy controls (37 out of 50 patients (74%) vs. 24 out of 100 healthy controls (24%), ($P < 0.001$)). There was no association between secretory state, and type of OLP and disease duration ($P > 0.05$).

Conclusion: This study supported the possible role of cell surface histo-blood group antigens in protection of mucosal surface from exogenous pathogens. Therefore, it appears that non-secretor individuals are more prone to oral lichen planus.

Keywords: Cell surface histo-blood group antigens, Lewis phenotypes, non-secretor status, oral lichen planus, secretor status

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Introduction

Lichen planus (LP) is a chronic and an inflammatory disease of the skin and mucosal surfaces. Oral lichen planus (OLP) is a common disease affecting 0.1% to 4% of the population. It is more frequent compared with cutaneous form, and tends to be more persistent and resistant to the treatment. The most commonly affected sites are the buccal mucosa, tongue and gingiva. OLP manifests as white striations, white plaques, erosions, erythema, white papules, or blisters.[1,2]

It is believed that LP results from an abnormal T-cell-mediated immune response in which basal epithelial cells are identified as foreign due to the altered antigenicity of their cell surface or unmasking of an antigen that may be a self-peptide or a heat shock protein. In some individuals, certain factors may trigger an inflammatory response, such as viruses (e.g. Hepatitis C virus), Hepatitis B vaccine, contact allergens (e.g. foods, dental materials or other substances) and medications (e.g. Non- steroidal anti-inflammatory drugs (NSAIDs) or antihypertensive drugs).[3,4]

Carbohydrate structures related to the ABO and Lewis blood-group antigens are the major allogeneic antigens in humans that are distributed in different tissues. Although Lewis antigens are red cell antigens, they are not produced by the erythrocytes. Lewis antigens are components of exocrine epithelial secretions and are absorbed by the erythrocytes. A number of recent studies about the pathogenesis of certain diseases have demonstrated that the patients' secretor status may be a factor influencing the development of several systemic and oral disorders.[5,6] Due to the lack of ABO blood group antigens in their body secretions, the exposure of non-secretor individuals to endogenous and exogenous antigens are more than secretors individuals. Therefore, non-secretor individuals are more prone to autoimmune disorders.[7]

In the present study, we aim to evaluate differences between patients with OLP and the healthy control group in relation to the ABH antigen expression in their saliva. Based on genetic inheritance, Lewis blood group system, ABH (the precursor to the ABO blood group antigens) and secretor status are linked with each other. A secretor is described as a person who secretes blood group antigens into body secretions like the saliva, milk, tear, amniotic fluid, and etc. The Lewis blood group system comprises two most important antigens, Le/a and Le/b, with two categories: Lewis positive (either Le (a+b-), Le (a+b+) or Le (a-b+)) and Lewis negative (Le (a-b-)). The Lewis gene is located on the chromosome 19 closed to secretory gene. Secretory state of individuals can be determined by their Lewis phenotypes (e.g. people with Le (a+b-) phenotype are always non-secretor and those with Le (a-b+) and Le (a+b+) phenotypes are secretors, but secretory state in individuals with Le (a-b-) phenotype should be assessed by presence of blood group antigens in their body fluids like saliva). In this study, we also aim to determine secretor status of subjects by checking Lewis (a) and (b) antigens in their serum and find their Lewis phenotypes. In addition, H antigen of saliva was checked in subjects with Le (a-b-) phenotype.[7]

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Materials and Methods

Fifty patients and 100 age- and gender-matched healthy controls were enrolled in our study. Patients were visited in the outpatient clinic of dermatology at Shohada-e-Tajrish Hospital, Shahid Beheshti University of Medical Sciences from April 2012 till June 2014. Diagnosis of OLP was based on clinical and histopathological features. Exclusion criteria included patients and controls with history of other inflammatory or autoimmune disorders, ischemic heart disease, diabetes mellitus and other oral lesions. Demographic factors of all subjects and the type of OLP were recorded. This study was done in accordance with the declarations of Helsinki, and was approved by the ethical committee of Shahid Beheshti University of Medical Sciences, and all of the subjects gave a written informed consent.

Two mL of venous blood sample were obtained from each subject in a tube with EDTA as an anticoagulant. For Lewis blood group antigen typing, red blood cells washed 3 times with 5% saline suspension. Lewis blood grouping were done by a tube test using a standard commercial antiserum. For each specimen, two tubes containing one drop of anti Le/a and anti Le/b, as well as two drops of 5% RBC suspension were used. After 10-minute incubation at room temperature, the tubes were centrifuged at 3000 rpm for 30 seconds and observed for agglutination. With regard to the reaction pattern of anti-Le/a and anti-Le/b with red cell suspension, four phenotypes were detected: Le (a-b-), Le (a-b+), Le (a+b-) and Le (a+b+). The saliva was checked for the presence of H antigen for determination of secretor status in Le (a-b-) subjects. For this aim, 3 mL of saliva was collected in a glass tube and boiled for 5 min to destroy salivary enzymes. Then, for detection of secretory state, the haemagglutination inhibition method was used. Two drops of the filtrated saliva and one drop of anti-H (extracted from *ulex europaeus* lectin) were mixed and incubated for 20 min at room temperature. In the second step, group O cell (an indicator cell) was added, then the tubes were centrifuged at 3000 rpm for 30 sec. Anti-H agglutinates O cells, but if saliva contains H substance, it is neutralized and cannot agglutinate O cells. Therefore, agglutinated and non-agglutinated tubes were categorized as non-secretor and secretor, respectively.[8]

Statistical analysis

All statistical analysis were performed using the statistical software SPSS 16.0.0. (SPSS Inc. Chicago, IL, U.S.A.). Two-sided *P*-values less than 0.05 were considered statistically significant. Continuous variables are expressed as mean SD or as median with minimal to maximal range (min-max). Categorical data are presented as number (percentage).

Results

Fifty patients with OLP and 100 age- and sex-matched healthy controls were included in this study. Baseline demographics and clinical features of two groups are presented in Table 1.

Patients and controls had significant differences in distribution of secretor status. Non-secretory state in patients with OLP was more frequent compared with healthy individuals; 37 of 50 patients (74%) *vs.* 24 of 100 healthy controls (24%), ($P < 0.001$, Table 2) (Chi-square test). All five healthy subjects with Le (a-b-) phenotype were non-secretors based on the haemagglutination inhibition test in their saliva. In patient group, this phenotype was

Table 1. Baseline demographics and clinical characteristics of patients with oral lichen planus and healthy controls

Characteristic	Patients with lichen planus (n = 50)	Healthy controls (n = 100)	*P*-value	Test
Gender			0.34	Chi-square
Female	32 (64%)	56 (56%)	- - -	- - -
Male	18 (36%)	44 (44%)	- - -	- - -
Age, years			0.94	*t*-test
Mean SD	43.12 ± 10.89	43.25 ± 11.26	- - -	- - -
Median (range)	42.5 (23–63)	43 (23–68)	- - -	- - -
Age at onset of disease, year			- - -	- - -
Mean SD	37.93 ± 12.00	- - -	- - -	- - -
Duration of disease , year				
Median (range)	3 (0.5–20)	- - -	- - -	- - -
Values are no. (%) unless otherwise noted.				

Table 2. Frequency of Lewis phenotypes and secretor status in patients and healthy controls

Lewis phenotypes / Secretor status	Patients, N (%)	Controls, N (%)	*P*-value
Le (a+b-)	28 (56%)	19 (19%)	($P < 0.001$)
Le (a-b+)	6 (12%)	68 (68%)	($P < 0.001$)
Le (a-b-)	12 (24%)	5 (5%)	($P < 0.001$)
Le (a+b+)	4 (8%)	8 (8%)	1
Non-secretor	37 (74%)	24 (24%)	($P < 0.001$)
Secretor	13 (26%)	76 (76%)	

Table 3. Distribution of oral lichen planus and frequency of secretor and non-secretor status in patients

Type of mucosal lichen plan	Number (%)	Non secretor (total 37)	Secretor (total 13)	*P*-value
Erosive	10 (20%)	7	3	0.794
Atrophic	3 (6%)	3	0	0.309
Plaque type	1 (2%)	1	0	0.554
Reticular	35 (70%)	25	10	0.792
Bullous	1 (2%)	1	0	0.554

found in 12 of 50 subjects (24%), haemagglutination inhibition test of saliva were negative in 3 out of 12 patients with Le (a-b-) phenotype. Therefore, in this patient group, 9 of 50 subjects (18%) were non-secretors (Table 2).

Distribution of the types of OLP is presented in Table 3. There was no significant association between the type of lesions and secretor status. Also, there was no significant association between non-secretory state and disease duration in patients.

Discussion

ABO blood group antigens are fucosylated carbohydrates present on human erythrocytes and body fluids. Their presence in body secretions depends on the expression of a dominant allele of secretor gene (Fucosyltransferase2 (FUT2)). These cell-surface fucosylated oligosaccharides are associated with a number of biological processes, such as bacterial adhesion, inflammation and tissue differentiation. These functions may be due to body secretions of ABH secretors which compose more diverse carbohydrates than those of non-secretors.[7] Diversity of carbohydrates in body secretions is mainly influenced by the ABH secretor status and this can have an important effect on bacterial adhesion and persistence.[7]

Complement activation is effective in microbial clearance. Gunput, et al.[9] in their study concluded that complement activation in secretors was significantly higher compared with non-secretors because fucose-rich oligosaccharide side chains, such as Lewis b antigens, in the context of mucosal inflammation induced activation of complement via lectin pathway.

The prevalence of chronic periodontitis and gingivitis in non-secretors were higher compared with secretors in the study conducted by Tabasum, et al.[10]

Several studies reported that the prevalence of Candida sp. carriers and persistent Candida infection in ABH non-secretors is higher compared with secretors.[11] Also, females with recurrent idiopathic vulvovaginal candidiasis are much more likely to be non-secretors.[12] Nurjadi, et al. demonstrated that group O/ non-secretors were at increased risk of carrying S. aureus in their throat. They also proposed that histo-blood group antigen type carbohydrates appear to act as ligands for S. aureus and may play a role in modulating S.aureus colonization. Oligosaccharide epitopes in secretor's saliva have an important role in recognition of some microorganisms and clearance of oral mucosa by entrapping them.[13]

Above-mentioned studies demonstrated that the innate immune system function is different between secretors and non-secretors. Secretory state has a protective role for oral mucosa by accelerated clearance of microorganisms. According to the study conducted by Vidas, et al. there is a high incidence of oral disease and epithelial dysplasia in non-secretors.[14] Also, Campi, et al. concluded that non-secretor status may be an independent risk factor for development of oral cancer.[15]

Creuzot-Garcher, et al. evaluated the association of Lewis blood group expression with ocular cicatricial pemphigoid (OCP). They showed that anti-Le/a and anti-Le/b immunoreactivities of the goblet and/or epithelial cells were markedly decreased, and 41% of the patients had non-secretor phenotype which was significantly more than the same phenotype in the normal French population (20%).[16]

These studies show that non-secretors are at risk of mucous membrane disorders. The saliva in secretors contains different kinds of oligosaccharides with different terminal carbohydrates. Oligosaccharide epitopes have an important role in clearance of oral mucosa; also they have a significant role in prevention of mucosal damage by bacterial enzymes. Although the exact mechanism of association between the presence of histo-blood group antigens and oral lichen planus is not known, but it appears that non-secretors are more susceptible to mucosal barrier breakage compared with secretor individuals; therefore it leads to better accessibility of the immune system for initiation of an immunologic reaction with keratinocyte surface antigens.[7,13]

The results of our study are consistent with those of previous studies, which show a high prevalence of non-secretor status in mucous membrane disorders. We found that frequency of Lewis phenotype with non-secretory state was higher in patients with oral lichen planus than in healthy controls. Our findings supported that the secretory state may have a protective effect on mucous membrane, due to the key role of cell surface histo-blood group antigens.

Limitations

In our study, the number of cases with severe forms of OLP (erosive and bullous) was low; therefore we could not evaluate the possible association between disease severity and secretory state. Further studies with larger sample sizes are needed to clarify this association.

Conflict of interest

There are no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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