

Micronuclei Comparison in Lichen Planus and Oral Lichenoid Responses

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ABSTRACT

BACKGROUND AND OBJECTIVE: Lichen planus is a mucocutaneous chronic inflammatory disease with unknown etiology. Malignant potential of oral lichen planus (OLP) and lichenoid reactions (OLR) are controversial. Since micronucleus frequency of cells is representative of risk of malignancy, the aim of present study was to evaluate micronucleus frequency in these lesions.

METHODS: In this cross-sectional study, study group consisted of 20 OLP and 20 OLR which clinically and histopathologically had been confirmed and 20 healthy individuals without oral lesions and systemic disease who presented in oral medicine department of Babol dental college. After receiving written consent, smears were prepared from lesion site at buccal mucosa by cytobrush and were stained at laboratory using Papanicolaou stain. In each slide 500 cells were assessed under light microscope at 400X magnification; mean number of micronucleated cells and mean total numbers of micronuclei were calculated.

FINDINGS: Mean number of micronucleated cells in OLP, OLR and normal mucosa were 5.20 ± 3.73 , 5.65 ± 3.66 and 0.95 ± 1.19 and number of micronuclei were 6.75 ± 4.94 , 8 ± 4.66 and 1.30 ± 1.72 respectively. Mean number of micronucleated cells and number of micronuclei were significantly greater in OLP and OLR than normal mucosa ($p < 0.001$) but there were no significant differences between OLP and OLR ($p = 0.67$ and $p = 0.36$ respectively). There were no significant differences in mean number of micronucleated cells between reticular and erosive subtypes of OLP and also OLR ($p = 0.96$). There were also no significant differences in mean number of micronuclei between these subtypes ($p = 0.96$ and $p = 0.93$ respectively).

CONCLUSION: The results of this study indicated that significant increase in micronucleus frequency of OLP and OLR are probably indicative of higher risk of malignancy in these lesions.

KEY WORDS: Lichen planus, Lichenoid Eruptions, Micronuclei.

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Introduction

Lichen planus is a muco-cutaneous chronic inflammatory with unknown etiology (1). Oral and dermal mucosa may have clinical and microscopic changes similar to those seen in lichen planus, which are called lichenoid reactions and are caused by topical or systemic etiologic agents (2, 3). The potential for malignant oral lichen planus and oral lichenoid reactions are controversial (8-4). Micronuclei are round to oval-shaped bodies whose examination in tufted cells is a reliable tool for studying the risk of malignancy (12-9). In studies of microscopic frequency of micronuclei in buccal mucosa cells or peripheral blood lymphocytes in patients with oral lichen planus, there was a significant increase in micronuclei compared to the control group (10,13-15) and there was no significant difference in the frequency of Micronuclei between two sub-types of erosive and reticular Lichen planus (14, 10).

There is only one study in this regard in patients with oral lichenoid responses in which micronuclei frequency in oral mucosal epithelial cells of patients with contact lens and lichen planus lesions is significantly higher than that of the control group but the difference was no meaningful between two groups of oral lichen planus and contact lichenoid reactions (3), while there was no comparison between the type of lichenoid reactions. In previous studies, generally, a variable (micronuclei frequency) was used to evaluate DNA damage and perform comparisons, but in the present study, two variables (micronuclei frequency and frequency of cells with micronuclei) have been used. In addition, our emphasis in this study is on the comparison between oral lichen planus and oral lichenoid responses and the comparison between subtypes, especially in oral lichenoid responses, which are discussed only in one study, while no comparison was made in this study. Furthermore, in the mentioned study there is no comparison of subtypes of oral lichenoid responses, and from the wide range of oral lichenoid responses, only contact lichenoid reactions have been reported, while our study covers a wider range of these reactions and our study results are more general. Therefore, the aim of this study was to evaluate

and compare the frequency of micronuclei in patients with oral lichen planus and oral lichenoid responses in order to evaluate the extent of DNA damage and the risk of malignancy in these lesions and subtypes.

Methods

This cross-sectional study was approved by the Ethics Committee of Babol University of Medical Sciences with the code MUBABOL.REC.1396.155. According to similar studies (15-13, 10, 3), this study was performed on 20 patients with oral lichen planus (including 10 cases of reticular type and 10 cases of erosive type), 20 patients with oral lichenoid responses (including 13 reticular and 7 erosive types) and 20 healthy subjects from Babol dental department and specialized clinics and private clinics in Babol. As much as possible, groups were matched based on the age and gender. Diagnosis of selected patients was confirmed by a dermatologist during a clinical examination. After diagnosis and biopsy, their diagnosis should be confirmed by the pathologist. Clinical and histopathological diagnosis of lichen planus and lichenoid responses are based on the criteria of Neville et al. (1). Patients who did not receive drugs for oral lichen planus or oral lichenoid responses, were included in the study (10). Healthy people were also people without oral lesions and certain systemic diseases and volunteered to participate in the study. Five general exit criteria were used to exclude conditions that affect oral mucosa: 1. Systemic diseases such as leukemia, lymphoma, rheumatoid diseases, diabetes mellitus and megaloblastic anemia. 2. Treatment history, such as irradiation to the head and neck., Immunosuppressive drugs or cytotoxic drugs. 3. Local pathologic changes such as macroscopic abnormalities, gum and periodontal disease. 4. Patients with harmful habits, such as smoking and alcohol consumption (at any rate). Also, people with infectious and inflammatory diseases (such as inflammatory bowel disease) and pregnant women and those with moving prosthesis were excluded from the study (16). In each of the study groups, oral cytological smears were extracted from the oral mucosa by exfoliative method. Before smear

preparation, the purpose of the study was explained to the participants and the written consent was obtained. Samples were prepared from buccal mucosa lesions for oral lichen planus and oral lichenoid responses (in healthy subjects, the smear of buccal mucous membrane was prepared); hand pressure during the preparation of the smear was such that only epithelial surface cells be isolated and bleeding does not occur (16). Initially, each person was asked to rinse the oral cavity with water. A piece of gauze was then slowly drawn onto the target area (buccal mucosa), and then smear was prepared by cytobrush (cytobrush, padtan teb, Iran) (16). The cytobrush was rotated 10-15 times at constant and medium pressure, then dried on a glass slide. Immediately, the cells present in the smear were stained with 95% ethanol spray (pathofix, padtan, Iran) from 25 cm distance with a maximum of 2 bar spray pressure. On each slide, a code for each patient was written and sent to the pathology lab for staining. The fixed smears were stained for a maximum of up to three days according to the standard staining method of papanicolaou (16). In each slide, 500 cells were examined by a pathologist with 400x magnification of the OlympusCX21 optical microscope (Olympuscorporation, Tokyo, Japan), and the micronuclei frequency was evaluated in these cells. Only cells with known cell specifics were selected. In cases of observation of cell collapse and membrane insufficiency, those cells were not included in the study. For counting 500 cells, the counting was started from one side of smear and counting were carried out, and so the smear was swept up and down, then left or right, until finally 500 cells in the examined fields were detected and examined. (10). The following criteria were used to identify the micronuclei (Mn): 1. Their color intensity should be similar to the core. 2. It must be completely recognizable and separate from the core and there should be no overlapping or communication with the core. 3. Its size should be less than or equal to 1.3 (one third) of the core. 4. It has a circular or elliptical shape. 5- texture is similar to the original core (10). Finally, two parameters were measured, which included the mean frequency of total number of Mn per 500 cells and the mean frequency of micronucleated cells in 500 cells

of the mean frequency of micro nucleated cells per 500cells (10). Data were analyzed using SPSS 20 software, Kruskal-Wallis and Mann-Whitney tests and $p < 0.05$ was considered significant.

Results

Of the total samples of oral lichen planus, 12 samples (60%) belonged to female and 8 samples (40%) were male. Of the total samples of oral lichenoid responses, 11 samples (55%) belonged to female subjects and 9 samples (45%) were related to male subjects. The mean age of patients with lichen planus and oral lichenoid responses was 47.2 ± 10.74 years and 43.7 ± 8.7 years, respectively. In the healthy group, 10 samples (50%) belonged to female subjects and 10 samples (50%) were related to male subjects. The mean age of healthy subjects was 40.34 ± 5.67 years. The mean number of cells with micronuclei in the normal group, oral lichen planus, and oral lichenoid responses was significantly different ($p < 0.05$). Table 1 and Figure 1 shows the mean number of micronuclei in the oral lichen planus group ($p < 0.001$) and the oral lichenoid reaction ($p < 0.001$) was significantly higher than normal mucosa. However, the difference between the mean number of micronuclei in the lichen planus group and the lichenoid reactions was not significant. The mean number of micronuclei was significantly different in normal mucosa, oral lichen planus and oral lichenoid responses ($p < 0.05$). The mean number of micronuclei in the lichen planus group was significantly higher than the healthy group ($p < 0.001$) and in the oral lichenoid reaction group compared with the healthy group ($p < 0.001$). However, the difference in mean micronuclei between oral lichen planus and oral lichenoid responses was not significant (Table 1). The average number of cells with micronuclei in reticular and erosive oral lichen planus were 5.10 ± 3.78 and 5.30 ± 3.88 , respectively. There was no significant difference between the number of micronuclei cells in reticular lichen planus and erosive. The mean number of micronuclei in reticular and erosive lichen planus were 6.50 ± 5.10 and 7 ± 5.03 , respectively. There was no significant difference between the average number of

microorganisms in lichen planus, reticular and erosive. The mean micronuclei number in reticular and erosive oral lichenoid responses was 5.69 ± 3.75 and 5.57 ± 3.78 , respectively. The average number of cells with micronuclei was not significantly different in reticular and erosive oral lichenoid responses. The mean number of micronuclei in reticular and erosive oral lichenoid responses was 8.08 ± 4.76 and 7.86 ± 4.84 , respectively. There was no significant difference between the average number of micronuclei in reticular and erosive oral lichenoid responses. There was no significant difference

between the number of cells with micronuclei in the reticular oral lichen planus and the reticular lichenoid reaction. There was no significant difference between the mean number of micronuclei in oral lichen planus and reticular oral lichenoid responses. There was no significant difference between the mean number of micronuclei in oral lichen planus and erosive oral lichenoid responses. There was no significant difference between the average number of micronuclei in oral lichen planus and erosive oral lichenoid responses.

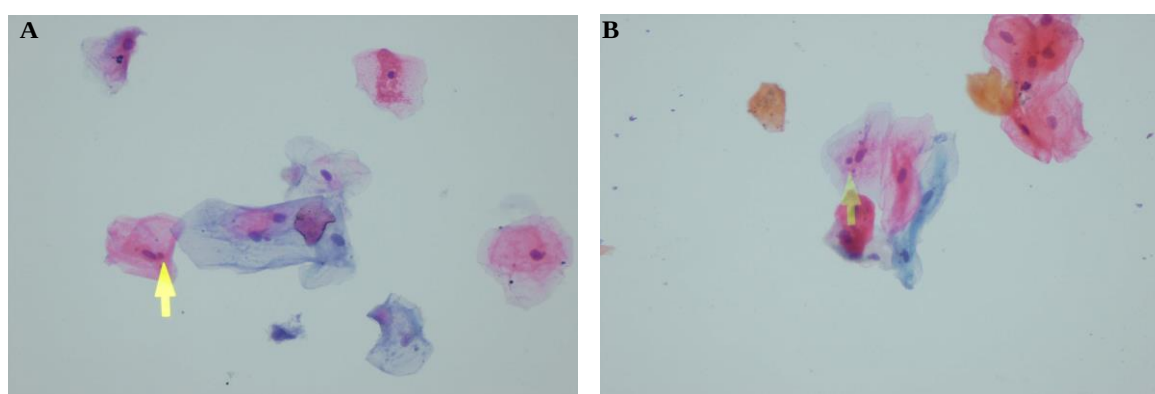


Figure 1. micronuclei in smear from a person with a) oral lichen planus, b) oral lichenoid reaction (400x magnification)

Table 1. Comparison of the average number of micronuclei and cells with micronuclei in the studied groups

Group	Micronuclei Mean $\pm$ SD	Average rate	cells with micronuclei Mean $\pm$ SD	Average rate
Normal mucosa	0.95 $\pm$ 1.19	14.18	1.3 $\pm$ 1.72	13.98
Lichen planus	5.2 $\pm$ 3.73	37.95	6.75 $\pm$ 4.94	36.95
Lichenoid reactions	5.65 $\pm$ 3.66	39.38	8 $\pm$ 4.66	40.58

P<0.001

Discussion

In our study, the mean number of cells with micronuclei and the mean number of micronuclei in oral lichen planus group and oral lichenoid reaction group was significantly higher than normal oral mucosal group. Given the fact that micronuclei are a reliable tool for studying the risk of malignancy, this finding is likely to be due to a significant increase in the risk of malignancy in lichen planus lesions and oral lichenoid responses than the normal mucosa, and indicating Lichen planus lesions and oral lichenoid responses could be classifying in malignant lesions group. Based

on the results and increased micronuclei of oral lichen planus and oral lichenoid responses, a microscopic frequency evaluation in these patients and a prediction of the risk of malignancy and appropriate treatment are suggested. Also, since patients with oral lichen planus may be at increased risk or potential for oral malignancy, microscopic assessment and evaluation can be used to control and monitor the beneficial effects of long-term interventions and prognosis. In our study, the difference between the oral lichen planus and oral lichenoid responses was not significant in terms of the mean number of cells with micronuclei. This finding

suggests that the risk of malignancy in oral lichen planus lesions and oral lichenoid responses is not significantly different and is somewhat similar. In our study, there was no significant difference between the mean number of cells with micronuclei and the average number of micronuclei in reticular and erosive lichen planus.

This finding suggests that the risk of malignancy in the reticular and erosive forms of oral lichen planus is not significantly different. In some sources (1) it has been argued that If there is a risk of malignancy for lichen planus, its erosion type is more likely to have malignant potential which is in contrast with the findings of our study. Perhaps the reason for this contradiction is related to the relative small sample size, and considering the larger sample size and further studies, the difference between the different types of lichen planus is significant.

It can also be argued that, given that the micronuclei shows cytogenetic damage in the early stages, and considering that the samples used in our study are selected from the lesions that are at the beginning of the diagnosis and have not been treated so far, there is no significant difference between the average number of micronuclei and the risk of malignancy in different types of reticular and erosive lichen planus, and if this investigation is carried out in subsequent years, results of this study may be different from the results of this study. In our study, there was no significant difference in the mean number of cells with micronuclei in the reticular and erosive lichenoid responses. This finding suggests that the risk of malignancy in reticular and erosive types of oral lichenoid responses is not significant. In the study of Buajeeb et al., a significant increase in micronuclei frequency was observed in cells from buccal mucus lesions of people with atrophic-erosive lichen planus compared to normal mucus in healthy subjects (13).

These findings are in line with our results. In the study of Ergun et al., the frequency of micronuclei in peripheral blood lymphocytes in lichen planus patients

was significantly higher than that of the control group, and there was no significant difference in the frequency of micronuclei between reticular and erosive lichen planus (14). Their findings are consistent with our findings, with the difference that their study was conducted on peripheral blood lymphocytes and in our study, epithelial cells were used. In the study of Saruhanoğlu et al., the frequency of micronuclei in buccal epithelial cells of people with oral lichen planus and contact lichenoid reaction was significantly higher than control group, but there was no significant difference in the mean micronuclei in epithelial cells of buccal mucosa and peripheral blood lymphocytes. between the two groups of oral lichen planus and contact lichenoid reactions (3).

Their findings are consistent with our results, with the difference that in our study, the comparison of the mean micronuclei between the lichen planus subtypes as well as the subtypes of the lichenoid reactions was made. In the study of Vidyalakshmi et al., the mean number of micronuclei cells in the lichen planus group was significantly higher than healthy subjects (10). There was no significant difference between the different clinical types of oral lichen planus. Our findings are in line with these findings. Significant increase in the micronuclei in oral lichen planus and oral lichenoid responses to normal mucus in healthy subjects may indicate a higher risk of malignancy in this type of lesions, and it can be concluded micronuclei could be an appropriate tool to predict the risk of malignancy and long-term prognosis of lichen planus and lichenoid reaction lesions and as well as the monitoring of the effects of therapeutic interventions.

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