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BIOLOGICAL MARKERS OF MMP-8 (MATRIX METALLOPROTEINASE 8) AND TIMP-1 (TISSUE INHIBITOR OF METALLOPROTEINASES 1) IN THE ORAL FLUID IN VARIOUS FORMS OF LICHEN PLANUS AND THEIR ROLE IN ASSESSING THE CLINICAL COURSE OF THE DISEASE

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ABSTRACT — Early detection of tumor transformation of lichen planus presents a real challenge, since squamous cell carcinoma can develop both from unchanged epithelium, and against the background of inflammatory or precancerous lesions. Another aspect of untimely diagnosis is the unfavorable long-term prognosis of lichen planus, so after complex treatment, 50% of patients have a relapse: 80% — within 2 years and 20% — within 4 years. The aim of this study was to analyze the levels of MMP-8, TIMP-1 and their ratio of MP-8/TIMP-1 in the oral fluid of patients with varied forms of lichen planus. We examined and treated 24 women (35–70 years old). 14 women with oral lichen planus of the mucosa in a typical form were assigned to Group 1; 10 patients with an exudative-hyperemic form - Group 2. Concentrations of the biological markers MMP-8 and TIMP-1 were determined in the oral fluid before and after treatment. Results of the study showed that to assess the severity of oral lichen planus it is essential to evaluate concentration ratio of biological markers MMP-8/TIMP-1 in mixed saliva. Dynamic changes in MMP-8/TIMP-1 levels should be monitored at least once in a quarter.

KEYWORDS — lichen planus, oral fluid biomarkers, MMP-8, TIMP-1.

INTRODUCTION

Lichen planus is a chronic recurrent inflammatory disease, with a prevalence of 1–2% among the adult population, more common in women [1], has both separate manifestations on the skin, mucous membranes, and combined [2]. The probability of neoplastic transformation of lichen planus is from 0.4 to 6.5%, in this regard, the World Health Organization estimates lichen planus as a precancerous condition [7]. The greatest difficulties are the early detection of tumor transformation of lichen planus, since squamous cell carcinoma can develop both from unchanged epithelium, and against the background of inflammatory or precancerous lesions. Another aspect of untimely diagnosis is the unfavorable long-term prognosis of lichen planus, so after complex treatment, 50% of patients have a relapse: 80% — within 2 years and 20% — within 4 years [9].

Currently, the pathogenetic mechanisms that initiate the development of oral mucosal cancer in patients with lichen planus [2, 4, 17, 19] are not fully investigated. However, it is recognized that endogenous factors can play an important role in the malignancy process. Matrix metalloproteinases (MMP) [18, 8] are considered to be one of these factors.

MMP are a large family of zinc-dependent endopeptidases (about 30) that are capable of destroying all components of the basement membrane and extracellular matrix (ECM). Often there is a cascade activation of several MMP at once, which increases both in inflammatory and malignant diseases [18, 20, 8]. The increased expression of MMP increases the invasive activity of tumor cells, which can penetrate into the surrounding organs and tissues, as well as affect the processes of growth, migration, apoptosis and angiogenesis.

Researchers are particularly interested in the neutrophil collagenase MMP-8, which is involved in reparative processes in ulcerative lesions of the mucous membrane. It is believed that MMP-8 is associated with pathogenetic mechanisms for maintaining long-

term chronic mucosal lesions due to the cleavage of type I collagen, while there is a significant increase in MMP-8 levels [14, 15]. A number of authors believe that the increased collagenolytic activity of MMP-8 is associated with a decrease in the level of the tissue inhibitor of matrix metalloproteinases type 1 (TIMP-1) [9, 15].

TIMP-1 plays an important role in maintaining the integrity of tissues, and has recently become a decisive factor in the assessment of a number of pathological conditions. The versatile effect of TIMP-1 on cellular functions is due to the duality of the structure containing both an MMP inhibitor and a cytokine activator. This feature leads to interactions with numerous cell surface proteins that initiate an exceptionally wide range of effects, which explains the diverse biological consequences of TIMP-1 expression [9, 16].

Almost all components of ECM degrade under the influence of endogenous MMP. The early development of the tumor, as well as distant metastases, may be the result of an imbalance in the MMP/TIMP ratio, which changes the cell structure [5, 8].

In the oral cavity, biomarkers are directly or indirectly released into mixed saliva, so its use as a diagnostic fluid has prognostic value in various diseases [6, 12, 13, 14, 21]. The oral fluid washes the elements of the affected oral mucosa, thereby maintaining the concentration level of the studied proteins.

According to current literature data, the development of lichen planus is due to multifactorial causes, and therefore, there are various treatment protocols and clinical recommendations that lead to temporary improvement and prolongation of the remission period [11]. The polymorphism of the course of lichen planus complicates the determination of the individual prognosis of the course of the disease. The study of biomarkers in the oral fluid allows us to evaluate the effectiveness of therapy and the duration of *light* periods.

The aim of this study was to analyze the levels of MMP-8, TIMP-1 and their ratio of MP-8/TIMP-1 in the oral fluid of patients with various forms of lichen planus.

MATERIALS AND METHODS

In 2019–2020, 24 women (35–70 years old) with a diagnosis of lichen planus of the oral mucosa were examined and treated at the Central State Medical Academy of the Department of Presidential Affairs (Moscow, Russia). Depending on the form of lichen planus, the patients were divided into 2 groups: 1) a typical form (14 patients), 2) an exudative-hyperemic form (10 patients).

The criterion for excluding patients from the study was the presence of pathologies of the oral mucosa: infectious, allergic, benign neoplasms, leukoplakia.

The control group consisted of 19 practically healthy donors aged 45 to 55 years without lesions of the oral mucosa.

The examination was carried out by standard methods: a survey, anamnesis collection, when describing the external status, special attention was paid to the state of the lymph nodes of the regional region. Examination of the oral mucosa included registration of the condition of the mucous membrane of the lips, cheeks, hard and soft palate, gums, teeth, dentition, identification of the source of permanent trauma (dystopian teeth, sharp edges of teeth, fillings, orthopedic structures, the presence of dissimilar metals), determination of the indices: CFR and oral hygiene.

They carried out professional hygiene, eliminated traumatic factors, replaced amalgam fillings and fillings with a broken edge fit, not high-quality orthopedic structures made of dissimilar metals. They gave recommendations on the diet (they excluded hot, spicy, and acidic foods). They were assigned to consult internists to determine the general treatment of lichen planus. Each patient was given an individual therapy plan aimed at the pathogenetic links of the disease and, taking into account the presence of concomitant somatic pathology, the course necessarily included the appointment of antioxidants, vitamin therapy.

Local treatment consisted of anti-inflammatory therapy with solutions of antiseptics based on chlorhexidine 0.05% in the form of oral baths with an exposure of 1 minute. We prescribed applications of oil solutions of vitamin A and E on gauze napkins for 15–20 minutes, which reduce the process of keratinization and affect the proliferation of epithelial cells. An immunocorrective drug was used to activate phagocytosis and produce immunoglobulin A. The course of therapy was 14 days, the drugs were used 3 times a day.

Clinical observations were carried out at the stage of diagnosis, after 14 days of treatment.

At the specified time, unstimulated oral fluid was collected in a graduated tube on an empty stomach in the morning at rest. The mouth was rinsed with water. Oral fluid with a volume of 5 ml was obtained by spitting in the absence of chewing movements, frozen and transported in a refrigerator bag to the laboratory of Clinical Biochemistry of the N.N. Blokhin National Research Center of Oncology of the Ministry of Health of the Russian Federation and stored at-80o before the study for 1–2 months.

The concentration of MMP-8 and TIMP-1 in oral fluid samples was determined using reagent kits for direct enzyme immunoassay "Human MMP-8 Immunoassay "(R&D Systems, USA) and" TIMP-1 ELI-

SA " (Bender Medsystems GMBH, Austria) according to the manufacturer's instructions, as described earlier [13]. The concentration of the studied proteins was expressed in nanograms (ng) per 1 ml of oral fluid.

Statistical processing of the results was carried out using the central characteristic – the median, quartiles were used to estimate the spread of indicators, and nonparametric methods of analysis were used for comparison: the Mann–Whitney test (U test) when comparing independent groups, and the Wilcoxon paired test when evaluating the dynamics of marker levels. The differences were considered statistically significant at $p<0.05$

RESULTS

Patients with a typical form of lichen planus complained of discomfort, roughness of the mucous membrane; with an exudative-hyperemic form-burning when eating irritating food, discoloration of the cheeks, tongue.

In the oral cavity with a typical course of the disease, grayish papules were recorded on the unchanged oral mucosa (Fig. 1).

The exudative-hyperemic form of lichen planus was characterized by the presence of single or multiple elements of the lesion, connecting in various patterns in the form of a grid, a ring, leaves against the background of a hyperemic mucosa (Fig. 2).

After the treatment, there was a regression of the lesion elements in the typical form of lichen planus, an unexpressed lichenoid reaction was noted (Fig. 3).

In group 2, the papules were pronounced, but they were located on the pale pink mucous membrane of the oral cavity (Fig. 4).

A comparative analysis of the medians of the concentration of TIMP-1 in the oral fluid of patients with typical and exudative-hyperemic forms of lichen planus before treatment and in the control did not reveal statistically significant differences (p=0.49). The median concentrations of TIMP-1 were almost the same, amounting to 619 and 625 ng/ml, respectively. The median TIMP-1 in the group of patients with the typical form of PL was 616; 612-637 ng / ml (Table. 1; Fig. 5).

There were statistically significant differences in the concentration of MMP-8 in the oral fluid of the general group of patients with lichen planus and in the control group (p=0.0006), the medians were 311 and 210 ng / ml, respectively (Table. 2; Fig. 6). The frequency of detection of MMP-8 levels above the upper limit of control (335 ng/ml) in the general group of patients with lichen planus was 43% (p=0.014 according to the exact Fisher criterion), that is, almost half of the examined patients with lichen planus observed an excess of MMP-8 concentrations in the oral fluid relative to healthy donors.

At the same time, the median MMP-8 in the oral fluid of patients with typical lichen planus was the lowest 303; 285–556 ng/ml, while in exudative-hyperemic patients it corresponded to 370; 310–429 ng/ml.

The excess of MMP-8 concentrations relative to the control group was recorded, which was lower in group 1 (37.5%), and in group 2 was 50%.

Against the background of treatment, the values of the MMP-8 index decreased by 2 times during the repeated study.

At the same time, the median concentration of MMP-8 in the oral fluid of patients with lichen planus in the typical form was more than 2 times higher than in the exudative-hyperemic form of lichen planus (medians 311 and 117 ng/ml, respectively), but this difference did not reach the level of statistical significance.

Statistically significant differences were also found when comparing the ratio of MMP-8 and TIMP-1 levels in the oral fluid of patients with lichen planus and in the control (p=0.006). It should be noted that in 43% of patients with this pathology of the oral mucosa, the ratio of MMP-8/TIMP-1 was exceeded compared to the maximum value of this ratio in the control (p=0.0016). At the same time, the median ratio of MP-8/TIMP-1 in the group with a typical form of PL reached 0.55 and was significantly lower than in the group with exudative-hyperemic — 1.06 (Table 3; Pic. 7).

When repeated studies were performed in patients with lichen planus, the concentration of MMP-8 decreased by 1.3–6.2 (median 3.3; p=0.028), and the concentration of TIMP-1 — by 1.1–3.1 (median 1.57; p=0.028). There was a statistically significant decrease in the ratio of MP-8/TIMP-1 (Fig. 7; p=0.043), in three patients this indicator increased.

DISCUSSION

The study demonstrated an increase in the concentration of MMP-8 in the oral fluid of patients with typical and exudative-hyperemic forms of lichen planus at the stage of diagnosis of the disease compared to healthy donors, and the increase in this marker was most pronounced in patients with exudative-hyperemic forms of lichen planus.

The levels of TIMP-1 in patients with the studied forms of lichen planus did not differ from those in the control group.

At the same time, the ratio of MMP-8 and TIMP-1 concentrations was significantly higher than in the control group. It can be assumed that the low level of TIMP-1 in patients with lichen planus does not suppress the collagenolytic activity of MMP-8,

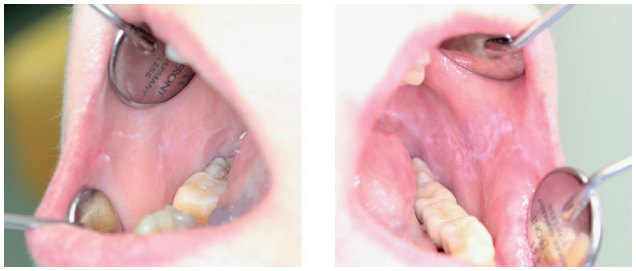

Fig. 1. Patient P., 53 years old. The clinical picture of the typical form of lichen planus before treatment. Papules are located on the mucous membrane of the cheeks on the right and left along the line of closing of the teeth.

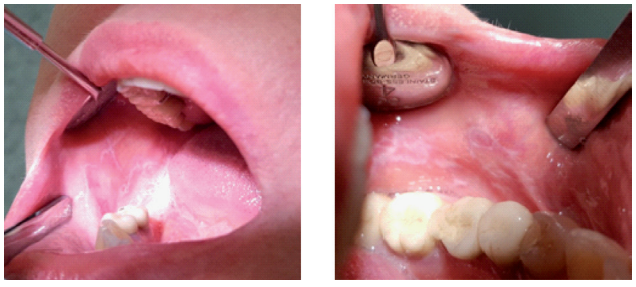

Fig. 2. Patient H., 47 years old. Clinical picture of exudative-hyperemic form of lichen planus before treatment. Elements of the lesion on the hyperemic mucous membrane of the cheeks on the left and right sides.

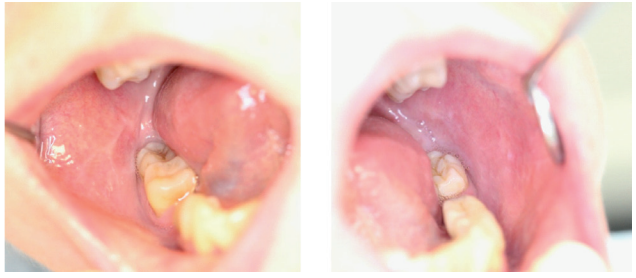

Fig. 3. Typical form of lichen planus after treatment.

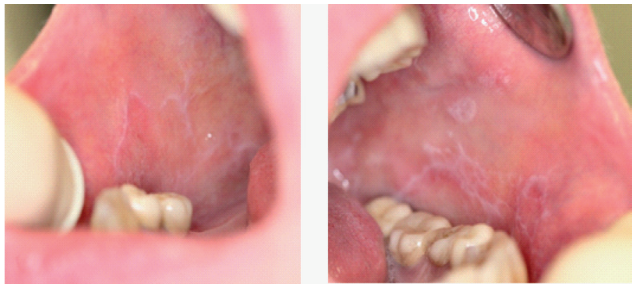

Fig. 4. Exudative-hyperemic form of lichen planus after the treatment.

***Table 1.** The content of TIMP-1 in the oral fluid of patients with lichen planus and in the control before treatment*

Group	N	TIMP-1, ng/ml			P
		Limits	Median	Quartiles	
Control	19	91,1-1825	625	610-1128	>0,05
Lichen planus (general group)	24	141-1659	619	610-776	

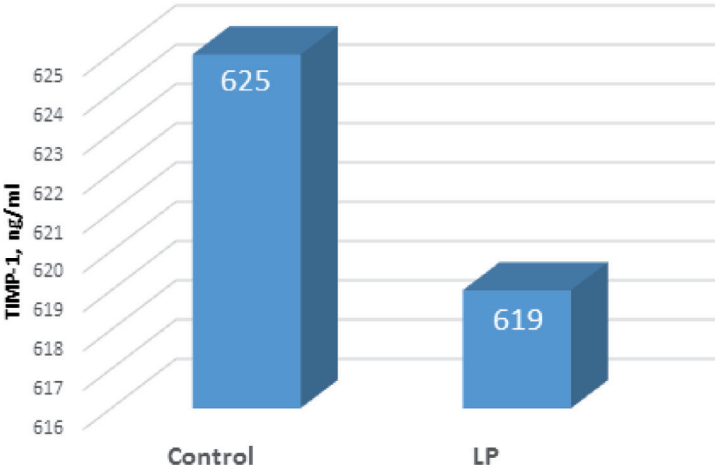

Fig. 5. Concentrations of TIMP-1 in the oral fluid of patients with lichen planus and in the control group

***Table 2.** The content of MMP-8 in the saliva of patients with lichen planus and in the control*

Group	N	MMP-8, ng / ml			P
		Limits	Median	Quartiles	
Control	19	28,7-335	210	115-262	0,0006
Lichen planus (general group)	24	144-3294	311	284-742	

which causes the severity of clinical manifestations, especially in group 2.

After the treatment, 75% of patients showed a statistically significant decrease in the studied parameters (MMP-8, TIMP-1, MMP-8/TIMP-1), which correlates with the elimination of the inflammatory reaction of the oral mucosa.

A decrease in the levels of MMP-8, TIMP-1 and their ratio after the therapy indicates a favorable course of the disease due to the inhibition of the functional activity of neutrophil collagenase

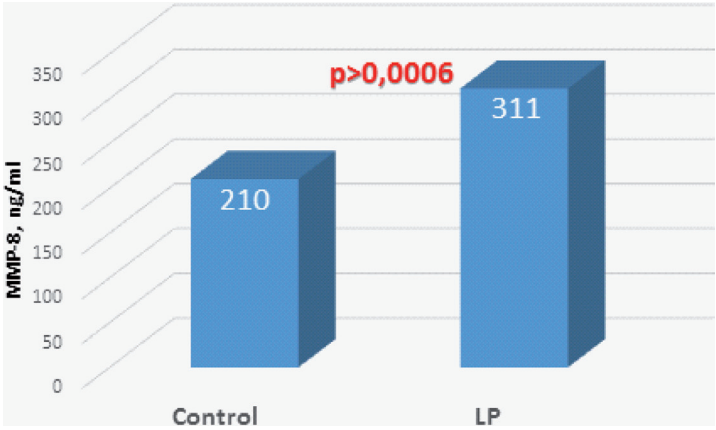

Fig. 6. Concentrations of MMP-8 in the oral fluid of patients with lichen planus and in the control.

Table 3. The ratio of MMP-8/TIMP-1 in the oral fluid of patients with lichen planus and in the control

Group	N	MMP-8/ TIMP-1 ratio			P
		Limits	Median	Quartiles	
Control	19	0,03-0,85	0,25	0,17-0,42	0,006
Lichen planus (general group)	24	0,15-5,21	0,55	0,34-1,78	

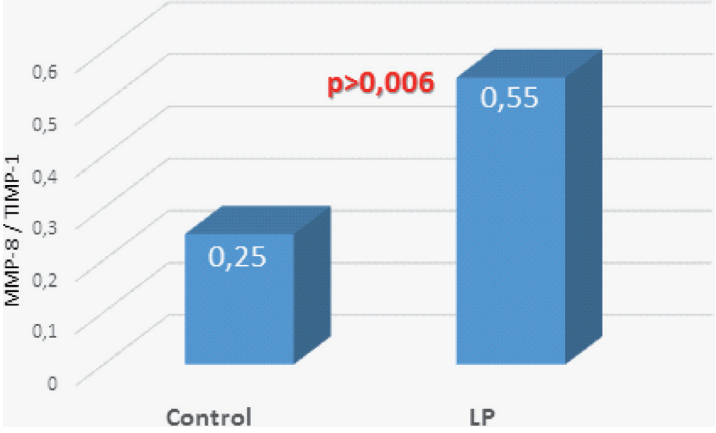

Fig. 7. The ratio of MMP-8/TIMP-1 in the oral fluid of patients with lichen planus and in the control.

MMP-8, which contributes to the preservation of the integrity of the mucosal tissues and prevents the development of the tumor process.

By the ratio of MMP-8/TIMP-1, we can judge the prognosis of the disease: the duration of remission and possible relapses. In the group with exudative-hyperemic form, the MMP-8/TIMP-1 imbalance was 2 times higher than in the typical course, this fact explains the appearance of a relapse of the disease after 3 months in the form of the addition of a pain factor and hyperemia of the oral mucosa along the periphery of the lesion elements.

CONCLUSION

To determine the prognosis of the course of the disease in patients with various forms of lichen planus of the oral mucosa, it is necessary to evaluate the ratio of the concentration of biological markers MMP-8/TIMP-1 in mixed saliva.

Dynamic monitoring of changes in the oral fluid level of MMP-8/TIMP-1 should be carried out at least once a quarter to assess the possible risks of relapse.

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