DYNAMICS OF UREASE AND LYSOZYME ACTIVITY IN THE ORAL FLUID AND THE DEGREE OF ORAL DYSBIOSIS IN PERSONS WITH PERIODONTAL TISSUE DISEASES AGAINST THE BACKGROUND OF E-CIGARETTE SMOKING

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Abstract

Introduction. A new challenge for humanity and medicine, in particular, is the sharp increase in the use of modern systems for heating tobacco among young people. Electronic cigarettes are often marketed as a safe alternative to regular ones. However, as far as oral health is concerned, modern studies state that smoking e-cigarettes is just as harmful as smoking. Smoking is a well-known risk factor for the inflammatory diseases of the periodontal tissues. It has been proven that the probability of developing periodontal disease in a smoker is 2.5-6 times higher than in non-smokers. However, the role played by smoking in the etiology of periodontal diseases is still not fully understood. The nature and mechanism of action of harmful tobacco smoke and the changes that occur in smokers' periodontal tissues, small salivary glands and oral fluid are of both theoretical and practical interest.The aim of the present study was to investigate the dynamics of inflammation markers in oral fluid and to determine the degree of oral dysbiosis in patients with periodontal tissue diseases, and also in patients which smoke e-cigarettes. **Materials and methods**. Study of inflammatory markers (urease and lysozyme activity) in oral fluid and determination of the degree of oral dysbiosis were performed for 90 patients, divided into three groups. The activity of urease was determined by the reaction with urea, with the formation of ammonia. The activity of lysozyme was determined by the bacteriolytic method. Conclusions about the degree of insemination by the microflora of the oral cavity were based on the degree of dysbiosis of the oral cavity, determined by the enzymatic method, according to Levytskyi. **Results and discussion**. In patients with periodontal tissue diseases who smoke e-cigarettes, a significant imbalance of the inflammatory markers in the oral fluid was noted, characterized by: a 4.5-fold increase in urease activity, a 8.3 times increase in microbial insemination, against the background of a 44.6% decrease in lysozyme activity, compared to the data in the control group, $p<0.01$. At the same time, in people who did not have this bad habit, the values of the above parameters, although they differed from the data in the control group, were lower – as to urease activity and the degree of oral dysbiosis, and higher, respectively – as to lysozyme activity, than in the main group, $p1 <0.01$, $p1<0.05$. **Conclusions**. An increase in the activity of urease and a decrease in the activity of lysozyme in the oral fluid of people with periodontal tissue diseases who smoked e-cigarettes led to an increase in the degree of oral dysbiosis. This caused the growth of the inflammatory process in the periodontal tissues and contributed to the increase in tissue permeability, which in turn produced disturbances in the periodontal components, particularly in its microcirculatory channel.

Keywords: *chronic catarrhal gingivitis, generalized periodontitis, oral fluid, urease, lysozyme.*

1. INTRODUCTION

A healthy lifestyle of a person is assured by his/ her activity, aimed at preserving health and at fighting against bad habits. One of such harmful habits is smoking. All forms of tobacco use, including smoking cigarettes, cigars, pipes, chewing tobacco, or snuff, as well as exposure to its combustion products on nonsmokers (passive smoke), significantly increase morbidity and premature mortality, caused by more than 20 different diseases [1,12]. Most studies have shown that the risk of developing diseases depends on both number of cigarettes smoked and duration of smoking [9].

A person who smokes one pack of cigarettes a day makes more than 70,000 puffs a year, while the components of tobacco smoke can affect the human body directly through the mucous membranes of the oral cavity, respiratory departments of the lungs, and also indirectly - through absorbtion in blood [16]. At the same time, the organs and tissues of the oral cavity are the place of primary contact of smoker's body with the components of tobacco smoke. The resulting changes occurring in periodontal tissues, oral mucosa and small salivary glands

can be the first early symptoms for diagnosing diseases caused by smoking [14].

A new challenge for humanity and medicine, in particular, has become the sharp increase in the use of modern systems for heating tobacco among young people. Electronic cigarettes are often marketed as a safe alternative to regular cigarettes [11]. However, as far as oral health is concerned, modern studies state that smoking e-cigarettes is just as harmful as classic smoking [17].

Smoking is a well-known risk factor for inflammatory diseases of the periodontal tissues [3]. It has been proven that the probability of developing periodontal diseases in a smoker is 2.5-6 times higher than in non-smokers. However, the role played by smoking in the etiology of periodontal diseases is still not fully understood [8].

The nature and mechanism of action of harmful tobacco smoke and the changes occurring in the periodontal tissues, small salivary glands and oral fluid of smokers are of theoretical and practical interest [19].

The **aim** of the study was to investigate the dynamics of inflammation markers in the oral fluid and to determine the degree of oral dysbiosis in patients with periodontal tissue diseases, and also in those who smoked e-cigarettes.

2. MATERIALS AND METHODS

The investigation was conducted at the Bukovinian State Medical University, Chernivtsi, Ukraine. The study of inflammatory markers (urease and lysozyme activity) in oral fluid and determination of the degree of oral dysbiosis were performed in 25 persons without somatic and dental diseases and with no bad habit of smoking regular and e-cigarettes – the control group; 30 people with chronic catarrhal gingivitis (CCG) and generalized periodontitis (GP) of the initial Ist degree, who did not smoke - the comparative group; 35 subjects with chronic catarrhal gingivitis and generalized periodontitis of the initial Ist degree, who smoked e-cigarettes - the main group.

The activity of urease was determined by the reaction with urea, with the formation of ammonia, while the activity of lysozyme was determined by the bacteriolytic method [4]. Conclusions on the degree of insemination by the microflora of the oral cavity were based on the degree of dysbiosis of the oral cavity, as determined by the enzymatic method, according to Levytskyi [5]. The method consists in determining the ratio of the specific weight of urease activity to the specific weight of lysozyme activity in the oral fluid. The specific activity was taken as the ratio of enzyme activity in pathology to the activity of the same enzyme in healthy individuals. Statistical processing of the obtained results was performed on a personal computer using the licensed programs "Microsoft Excel 2021" and "Statistica" [6].

3. RESULTS

As a result of the conducted studies, it was established (Table 1) that the activity of urease in the oral fluid of the control group was 0.11±0.01 µcat/l. In patients with chronic catarrhal gingivitis and generalized periodontitis of the initial Ist degree, an increase in the activity of urease in the oral fluid was observed, compared to the data from the control group, $p<0.01$. At the same time, the maximum values of the studied parameter were determined in persons with periodontal tissue diseases who smoke e-cigarettes (the main group): 4.5 times higher than in the control, $p<0.01$, and 1.4 times exceeding the similar values in the subjects of the comparative group, $p<0.01$.

Table 1. Indicators of the activity of urease, lysozyme and degree of oral dysbiosis of the oral cavity in the patient groups under study

Indicators	Control group, n=25	Comparative group, n=30	Main group, n=35
Urease, µcat/l	0.11±0.01	0.33±0.02•	0.49±0.04•*
Lysozyme, unit/l	96.0±3.20	62.30±2.77•	54.68±2.30•**
Degree of dysbiosis, unit.	1.0±0.02	5.14±0.03•	8.84±0.04•*

•$p<0.01$ – a significant difference in values compared to the data of the control group;
*$p_1<0.01$; **$p_1<0.05$– a significant difference in values relative to the data of the comparative group

At the same time, in patients with periodontal tissue diseases from the comparative and main groups, a decrease in the activity of lysozyme in the oral fluid was determined, compared to the data in the control group - by 36.18% and 44.56%, respectively, $p<0.01$. Quite noteworthy, in the patients of the main group who had a bad habit (smoking e-cigarettes), the activity of lysozyme in the oral fluid was 13.12% lower than in the examined subjects of the comparison group who did not smoke e-cigarettes, $p<0.05$.

Accordingly, in patients with periodontal tissue diseases, an increase in the degree of oral dysbiosis was determined: up to 5.14±0.03 units – in the comparative and up to 8.84±0.04 units – in the main groups, against 1.0±0.02 units - in persons of the control group, $p<0.01$. At the same time, the degree of oral dysbiosis in patients of the main group was 1.6 times higher than in the comparative group, $p<0.01$ (Fig. 1).

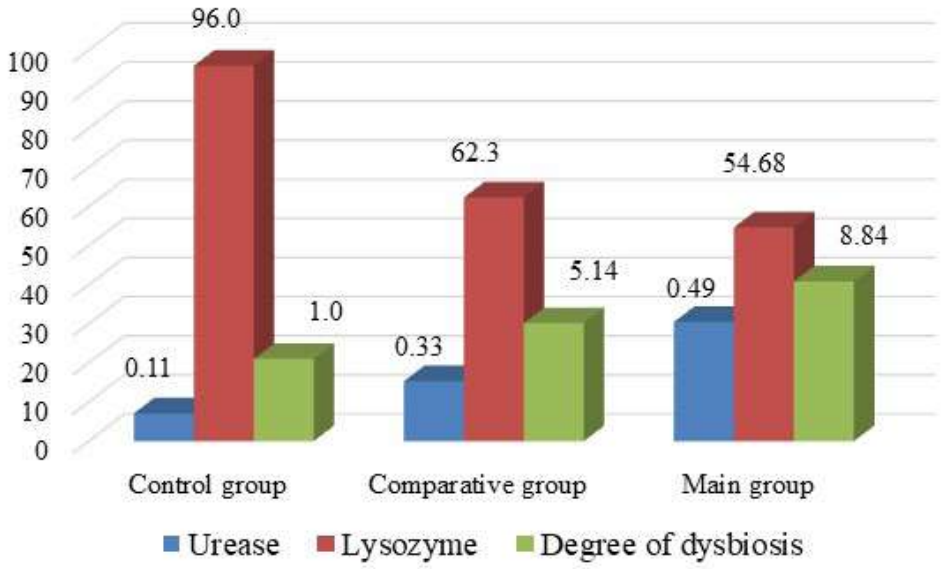

Fig. 1. Average values of urease activity, lysozyme and indicators of oral cavity dysbiosis in the patients of the study groups

In order to detail the obtained data, we tracked the dynamics of markers of inflammation of the oral fluid and the degree of oral dysbiosis in patients of the main and comparative groups with chronic catarrhal gingivitis and generalized periodontitis of the initial Ist degree (Table 2).

As a result of the research, it was established that, in the subjects smoking e-cigarettes (the main group) with chronic catarrhal gingivitis and generalized periodontitis of the initial Ist degree, the urease activity in the oral fluid was probably higher compared to similar values in patients who did not have this bad habit, namely: 1.4 times with CCG and 1.5 times with GP of the initial Ist degree, $p<0.01$.

Table 2. Dynamics of urease activity, lysozyme and indicators of the degree of dysbiosis of the oral cavity in patients of the study groups with chronic catarrhal gingivitis and initial forms of generalized periodontitis

Indicators Urease, µcat/l	Comparative group, n=30		Main group, n=35	
	Chronic catarrhal gingivitis	Generalized periodontitis of the initial - I degree	Chronic catarrhal gingivitis	Generalized periodontitis of the initial – I degree
Lysozyme, unit/l	0.22±0.01	0.44±0.03	0.30±0.02*	0.68±0.05*
Degree of dysbiosis, unit.	74.0±2.95	50.60±2.65	66.20±2.35**	43.15±2.25**
Indicators	2.59±0.02	7.69±0.04	3.94±0.03*	13.73±0.05
*p<0.01; **p<0.05– a significant difference in values relative to the data in the comparative group				

At the same time, it was observed that the activity of lysozyme in the oral fluid of patients of the main and comparative groups decreased, this tendency being most significant in patients with periodontal tissue diseases who smoked e-cigarettes. Also, the activity of lysozyme in the oral fluid was lower in the subjects of the main group: in chronic catarrhal gingivitis - by 10.54% and, in the case of generalized periodontitis of the initial Ist degree - by 14.73%, compared to the corresponding data in patients of the comparative group, p< 0.05.

According to the obtained results, it was established that the microbial insemination of the oral cavity increased in the patient groups of the study, as confirmed by the indicators of the degree of oral dysbiosis, the value of which increased from 2.59±0.02 units, with chronic catarrhal gingivitis up to 7.69±0.04 with generalized periodontitis of the initial Ist degree in persons of the comparative group, and from 3.94±0.03 with CCG to 13.73±0.05 units with GP of the initial Ist degree in patients of the main group, p<0.01.

4. DISCUSSION

Several authors outlined the relationship between smoking and the development of gingivitis and periodontitis, showing that tobacco smokers have more severe lesions of the mucous membrane of the oral cavity and periodontal tissues [2,21]. According to other researchers, in tobacco smokers there is a certain relationship between the frequency of gingivitis and periodontitis and the amount of tobacco consumption, as well as a more severe and widespread damage of the periodontal tissues, more tartar and plaque [7].

Despite some studies denying the influence of smoking on the composition of gingival microflora, latest data allows us to conclude that the number of bacterial complexes of "smokers" increases in smokers. For example, an increase in the number of *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia* and *Porphyromonas gingivalis* was evidenced in smokers [10]. Some scientists noted that *Treponema denticola* is more often cultured from periodontal pockets in smokers. According to [13], periodontal pathogens including *P. gingivalis* are increased in smoking patients, and [18] showed higher numbers of *T. forsythia*, *Campylobacter rectus*, *P. gingivalis* and *Peptostreptococcus* micros in the dental plaque of smoking patients.

The researchers note that nicotine is a known factor in gum disease, while e-cigarette flavorings exacerbate cell damage caused by their vapors, the menthol-containing vapor contained in e-cigarettes causing the highest damage [15]. Although further research is needed to examine the long-term effects of e-cigarette use, [22] believe that such devices may have negative effects on oral health. In general, their data indicate a pathogenetic role of (e-cigarette) vapor for the

cells and tissues of the oral cavity, which leads to the deterioration of periodontal health [20].

Therefore, our research established that, in patients with periodontal tissue diseases who smoke e-cigarettes, a significant imbalance of the inflammatory markers in the oral fluid was noted, characterized by: a 4.5-fold increase in urease activity, a 8.3 times increase in microbial insemination, against the background of a decrease in lysozyme activity by 44.6%, compared to the data in the control group, $p<0.01$. At the same time, in people who did not have this bad habit, the values of the above parameters, although they differed from the data in the control, were lower – as to urease activity and degree of oral dysbiosis, and higher - as to lysozyme activity than in the subjects of the main group, $p1 <0.01$, $p1<0.05$.

Consequently, with the increase in the intensity of the damage to the tooth-holding complex, in the subjects of the main and comparison groups, an increase in the activity of urease and degree of oral dysbiosis was noted, alongwith a decrease in the activity of lysozyme in the oral fluid, even if, in patients who smoked e-cigarettes, the imbalance of inflammatory markers was more pronounced.

5. CONCLUSIONS

An increase in the activity of urease and a decrease in the activity of lysozyme in the oral fluid of persons with periodontal tissue diseases who smoked e-cigarettes led to an increase in the degree of oral dysbiosis. This caused the growth of the inflammatory process in the periodontal tissues and contributed to the increase in tissue permeability, which in turn caused disturbances in the periodontal components, particularly in the microcirculatory channel.

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