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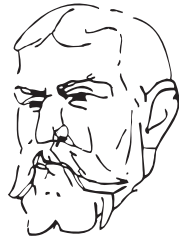
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ORIGINAL ARTICLE

INDICATORS OF DENTAL HEALTH AND LOCAL IMMUNITY IN YOUNG ADULTS WHO HAVE SUFFERED FROM CORONAVIRUS INFECTION

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ABSTRACT

The aim: To determine the dental status and state of local immunity in young adults who have suffered from the coronavirus disease.

Materials and methods: The main group consisted of 30 people aged 20–22 years, who suffered from the coronavirus infection Covid19 6.1±1.2 months ago. The comparison group included 20 people who did not have a coronavirus infection. The control group consisted of 35 people, randomized by age and sex, who did not have signs of caries and periodontal tissue disease and did not have coronavirus disease. All patients were examined for dental status and local immunity.

Results: The analysis of indicators of dental status revealed the possibility of the existence of a relationship between the signs of acute SARS-Cov2 viral infection and the development of caries and periodontal tissue diseases. Significant changes in the local immunity of the oral cavity were found in the examined patients, which had a pathogenetic influence on the development and progression of caries and periodontal tissue diseases: a significant increase in the level of Ig G, as well as a probable decrease in the concentration of SIg A relative to the comparison group, a probably higher normative value of pathogenic small- and medium-molecular CICs with a significant decrease in the level of physiological large-sized CICs relative to the comparison group, a decrease in the content of anti-inflammatory IL-4, as well as increased concentration of pro-inflammatory cytokines.

Conclusions: Young adults who have suffered a coronavirus infection during the last 6 months have significantly higher caries prevalence, bleeding index, PMA index and hygiene index, halitosis, which indicates deeper tissue damage and tooth pathology with the formation of dentition defects than in the comparison group. Indicators of local immunity of the oral fluid have a deep and specific character.

KEY WORDS: caries, coronavirus disease, young adults, local immunity, cytokines, secretory immunoglobulin A, circulating immune complexes

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INTRODUCTION

Since December 2019, the novel coronavirus disease 2019 (COVID-19) has spread rapidly around the world, causing various complications that can be fatal [1-3]. The impact of the transmitted coronavirus disease on various organ systems is of considerable interest today [4].

The pathogenesis of COVID-19 involves selective virulence and pathogenicity factors that are specific to β -CoV viruses in general or unique to the SARS-CoV-2 virus that caused the COVID-19 pandemic. One of its pathogenic strategies is the ability of the virus to provoke aggression of the immune system against its own tissues in the form of autoimmune and auto-inflammatory processes, which is considered a unique feature of the survival of β -CoV in the body of an infected person [5].

In general, diseases of the oral cavity are considered as signs of SARS-CoV-2 infection: [6]. Preliminary observations indicate the expression of ACE-2 in the epithelial

cells of the oral cavity and raised the question of its ability to contribute to the fact that the periodontal pocket serves as a reservoir for severe infectious diseases, in particular, SARS-CoV-2 [7]. There are several potential sources of viral infection in these pockets, such as direct exposure of the gingival epithelial tissue to the oral environment or virus movement in the blood or via infected immune cells in the periodontium [8]. Natto prove the hypothesis that samples obtained from periodontal pockets or carious lesions will show positivity for SARS-CoV-2 and this may serve as an alternative means of detecting the virus, comparable to other methods such as nasopharyngeal swab, oropharyngeal swab and saliva examination [9].

SARS-CoV-2 infection can cause changes in the organs and tissues of the oral cavity, which is associated with a significant representation of angiotensin-converting enzyme type 2 (ACE2) receptors in the epithelial cells of the oral mucosa, gums and fibroblasts of the periodontal ligament [10, 11]. Thus, the oral mucosa can

be a gateway for the virus and function as a reservoir for SARS-CoV-2. The role of mucous membranes in the implementation of the immune function explained by the fact that a large part of antigens enters the body, overcoming this physiological barrier [12].

S-IgA of saliva in combination with specific epitopes of cariogenic bacteria causes a site-specific immune response. [13-16]. Conditions associated with hypofunction of the salivary glands, impaired oral clearance, low pH of saliva, and changes in the composition of saliva often lead to a disturbance in the function and composition of the oral microbiota, which causes dysbacteriosis and the associated risk of oral diseases [16-18]. The levels of s-IgA in the saliva of caries patients fluctuate [19, 20]. Therefore, the heterogeneity of s-IgA levels in saliva observed due to regional differences, differences in age, types of teeth, and methods of detecting s-IgA in saliva [21].

At the same time, there are significant gaps in our knowledge regarding the interaction between patient immunity, the development and progression of major dental diseases, and SARS-CoV-2, although Wyllie et al. (2020) demonstrated that saliva analysis is an accurate screening tool for SARS-CoV-2 infection, equivalent to nasopharyngeal swab analysis [22]. Nowadays, saliva considered as unique sample for evaluating the secretory immunity of the oral cavity [23]. There is evidence that age affects immune functioning associated with periodontitis and (less studied) dental caries, however, the mechanistic changes in the functioning of the immune system are complex and not fully explored [24].

THE AIM

To determine the dental status and the state of local immunity in young adults who suffered a coronavirus disease.

MATERIALS AND METHODS

The main group consisted of 30 people aged 20-22 who had Covid19 coronavirus infection, mostly mild to moderate severity. The comparison group included 20 people who did not have a coronavirus infection. The control group consisted of 35 people, randomized by age and sex, who did not have signs of caries and periodontal tissue disease and did not have coronavirus disease. In the main group of patients, the duration from the moment when there was a disease with coronavirus disease to the moment of inclusion in the study was 6.1 ± 1.2 months.

The performed dental examination for these patients included: determination of the prevalence and inten-

sity of dental caries, Average of Decayed, Missing and Filled Teeth index (DMFT); Standard debit of seized, shooting, and imprinted text index (SD of DMFT); quality of restorations according to a modified version of the clinical and radiographic criteria of Hickel et al. (2010); halitosis was assessed using the Winkler WTCI (Winkler Tongue Coating Index) plaque index; the intensity of the inflammatory process in periodontal tissues was assessed by the papillary-marginal-alveolar index (PMA) (G. Parma, 1960; Masler, 1967); oral hygiene status Average of Oral Hygiene Index Score (OHIS) (OHI-S); degree of bleeding sulcus bleeding index (SBI). A specially developed questionnaire was made specifically to included 40 questions on oral care, living conditions, and diet.

To assess the state of local immunity of the oral cavity, the following were evaluated in the oral fluid: the phagocytic activity of neutrophils by the degree of absorption of latex particles with the calculation of the Hamburg phagocytic index and Wright's phagocytic number; the functional state of B-lymphocytes by examining the level of the main classes of immunoglobulins Ig G, Ig A, Ig M by the method of simple radial immunodiffusion in a gel according to G. Mancini et al., 1965; the concentration of circulating immune complexes (CIC) - by the method of precipitation in a solution of polyethylene glycol (PEG-6000) on a microspectrophotometer «Specol-21» (Germany) at a wavelength of 450 nm [24]; the level of cytokines was studied by the immunoenzymatic method according to the methods of the developing companies with kits certified in Ukraine.

The results of the descriptive analysis presented in the form of percentage distribution, mean value and standard deviation (SD). Chi-square and Fisher's exact test were used to determine any differences in the distribution of categorical variables. Because continuous variables did not follow a normal distribution, Kruskal-Wallis analysis of variance and the Mann-Whitney U test used to detect differences in means between two or three groups. The level of significance was set at $p < 0.05$. Pearson's correlation coefficient used for non-normally distributed data or Spearman's for non-normally distributed data to assess the correlation between measures. For interpretation, the Chaddock scale used: the strength of the relationship was determined by the value of the correlation coefficient r (0.00-0.29 - "very weak", 0.30-0.49 - "weak", 0.50-0.69 - "medium", 0.70-0.89 - "strong", 0.90-1.0 - "very strong"). Statistical data processing was also determined by the method of variational statistics using the Microsoft XP "Excel" application program package and the specialized "STATGRAPHICS Plus version 2.1" program.

Table I. Mean number of decayed surfaces (DS), decayed teeth (DT) and decayed filled surfaces (DFS) in persons who suffered from coronavirus disease ($p < 0.05$)

Indicators	Main (Mean (SD))	Control (Mean (SD))
DS	2,1 (3,0)	1.4 (2.6)
DT	1.2 (2.1)	0.8 (1.4)
DFS	25.9 (16.6)	11.6 (12.1)

Table II. Distribution of subjects according to the quality of the poorest dental restoration

Indicators	Main	Control
Individuals with restorations	24 (80%)	8 (40%)
Quality of poorest filling		
Good	1 (4,2%)	5 (65%)
Acceptable	14 (58,3%)	3 (37,5%)
Poor	7 (29,2%)	0
Unacceptable	2 (8,4%)	0

RESULTS

DENTAL STATUS OF THE EXAMINED PATIENTS

Tables I and II show some indicators of the dental status of the examined young adults who suffered from the coronavirus disease.

According to a modified version of the clinical and radiographic criteria described by Hickel et al. (2010), the quality of the restorations in the examined patients was recorded clinically and, if possible, radiographically.

During the clinical examination of patients of the main group, 16 (53.33%) patients were diagnosed with bleeding gums, and 12 (40.0%) patients were diagnosed with halitosis and bleeding gums.

Patients with bleeding gums had dental deposits, which were found in 11 (68.75%) of the examined. In the second place, it should be noted the presence of carious cavities and their irrational filling, which were found in 3 (18.75%) patients. Orthodontic pathology was detected in 2 (12.50%) persons.

The indicators of the condition of the gums in the patients were assessed as mild and moderate severity of gingivitis, the indicators of the PMA index were around $39.41 \pm 1.3\%$. The state of individual oral hygiene was mostly assessed as unsatisfactory: (OHI-S index was on average 1.8 ± 0.12 points). SBI index was on average 1.48 ± 0.1 points.

In 8 (66.6%) examinees with the clinical symptom of halitosis and bleeding gums, dental deposits are the main etiological factor affecting the periodontal tissues. Among local traumatic factors it should be noted the presence of carious cavities and their irrational filling, which occurred in 2 (16.66%) cases. Crowding of teeth was found in 1 (8.34%) examinee.

Indicators of the PMA index around $42.2 \pm 1.9\%$. The state of individual oral hygiene was mostly assessed as unsatisfactory: (OHI-S index around 2.10 ± 0.25 points). SBI index around 1.68 ± 0.33 points.

It has been found a direct correlation between the state of oral hygiene, the presence of caries on the contact and cervical surfaces of the teeth, and the prevalence of periodontal diseases (gingivitis) ($r=0.75$).

The report results of the main group of examinees intended to show the level of oral care, some aspects of eating behavior, some conditions and lifestyle that can affect oral care, the nature of changes in oral care after the coronavirus infection. From the survey data, 20% were senior students, 53.3% were junior students, and 26.6% were studying at a medical college. 70% worked as dental assistants. 43.3% of healthy eating behavior was noted by those individuals who had previously observed a nutrition schedule. From the questionnaire data, a direct correlation was found between the indicators of the change in the nature of nutrition after the transferred coronavirus infection with the increased KPV index ($r=0.63$; $p<0.001$). It was also found a statistically significant direct correlation between the number of meals per day and the intensity of dental caries ($r=0.91$; $p<0.001$). The respondents noted that they began to consume sweets more often after suffering a coronavirus infection, which is confirmed by a direct statistically significant correlation ($r=0.66$; $p<0.001$). Young adults living in a dormitory used sweets more often ($r=0.74$; $p<0.001$). Only 6.6% of respondents added vitamins and trace elements to their daily diet, and 83.3% started doing so only after contracting the coronavirus infection. Regarding the frequency of visits to the dentist: 53.3% visited annually, 46.6% - once or twice in the previous 5 years, the rest - as needed. 43.3% claim that they did not receive oral hygiene instructions from their dentist.

Table III. Indicators of local immunity in the oral fluid of examined patients (M±m)

Immunological indicators	Main group (n=30)	Comparison group (n=20)	Control group (n=35)
Ig G, g/L	2,75 ± 0,22*	1,96±0,23* x	1,03±0,04
Slg A, g/L	0,67 ± 0,01*	1,05 ± 0,02* x	1,29±0,03
CIC large (>19S), cu.	29,43±1,18*	36,08 ± 1,15*x	43,5±3,13
CIC middle (11-19S), cu.	51,46±1,97*	42,89± 1,16 x	36,32±2,18
CIC small (<11 S), cu.	31,42±1,06*	23,11 ± 1,14*x	15,83±1,44
TNF-α, pg/mL	88,6 ± 3,7*	63,9±3,1*x	42,3±4,9
IL-1β, pg/mL	87,6±2,6*	62,1±2,4 *x	39,42±4,5
IL-4, pg/mL	17,2±1,6 *	18,5±0,9 *x	25,42±3,3
IL-6, pg/mL	11,9±0,6	13,2±0,5	12,8±1,3
Phagocytic number	5,08±0,22 *	6,97±0,17 *x	9,53 ± 0,56
Phagocytic index, %	38,71±2,36 *	46,21±1,43 *x	52,82 ± 2,21

Notes: * – the difference between the indicator and the control group is significant ($p < 0.05$);

x - the difference in the indicator between the groups is significant ($p < 0.05$);

n - the number of patients.

Regarding daily teeth cleaning, the interviewees used the following set of tools: electric toothbrush, dental floss, brushes, mono-bundle brush, irrigator - 13.3%; manual toothbrush, dental floss, monobundle brush - 16.6%, manual toothbrush, dental floss - 36.6%, others - only manual toothbrush. 73.3% of the respondents noted that they did not take care of oral hygiene as usual during the illness due to coronavirus infection and during the first month after. 59% of them cited improper physical condition as the reason, 27.2% cited a temporary change in taste sensations.

From the data of the questionnaire survey, it can be concluded that the peculiarities of the course of the coronavirus infection can have a negative impact on individual oral hygiene care in the direction of its temporary deterioration.

INDICATORS OF LOCAL IMMUNITY IN THE EXAMINED PERSONS

The analysis of literary sources showed the leading role of local immunity of the oral cavity in the pathogenesis of dental diseases. Local immunity of the oral cavity is represented by the phagocytic activity of macrophages, specific bactericidal or bacteriostatic and virus-neutralizing activity of immunocompetent substances of the oral cavity, primarily cytokines present in the oral fluid. In addition, the T- and B immune systems play a significant role in the formation of the immune response.

Scientific works shows that phagocytosis is an important mechanism of resistance of the mucous membrane to various bacterial infections.

In the available literature, there are data on changes in indicators of local immunity in dental patients, especially in patients with generalized periodontitis. As it is known from the literature [2, 15, 25], TNF-α is considered

as the main mediator that initiates and prolongs the development of

Table III shows the results of examination of indicators of local immunity in examined patients.

The analysis of the data presented in Table III showed that in the main group of patients, found significant increase in the level of Ig G in the oral fluid, as well as a probable decrease in the concentration of Slg A relative to the comparison group. A violation of the ratio of the concentration of CICs with a probably higher normative value of pathogenic small- and medium-molecular CICs with a significant decrease in the level of physiological large-sized CICs compared to the comparison group was established. In both groups of patients, was detected a low level of IL-4 in the oral fluid. A decrease in the content of anti-inflammatory IL-4 is a compensatory immunological reaction to the development of dental caries. Established that the patients of the main group also had a probable increase in the concentration of pro-inflammatory cytokines TNF-α, IL-1β in the oral fluid, and an increase in the content of IL-6.

In patients from the comparison group with caries and periodontal tissue diseases, who did not suffer from coronavirus infection, immunological indicators, had the same direction changes, but their degree of expression was probably less.

The study of the phagocytic activity of phagocytes in the oral fluid established a significant inhibition of this function in all groups of patients.

DISCUSSION

Therefore, we conducted an examination of the condition and data of local immunity in young adults who suffered a mild and moderate coronavirus infection.

The analysis of indicators of dental status revealed that within 6 months after experiencing the coronavirus disease in the examined patients, its indicators significantly worsened: the prevalence of caries was significantly higher, the quality of restorations was unsatisfactory than in the comparison group, were detected halitosis and bleeding gums (chronic catarrhal gingivitis).

This indicates the existence of a relationship between the signs of acute SARS-CoV-2 viral infection and the development of caries and periodontal tissue diseases. In the studies of Matsuyama Y. et al. shows similar results: "A degree-cohort interaction criterion for caries using a difference-in-differences analysis showed that dental caries among the cohort exposed to COVID-19 showed a significant increase in caries" [26].

Data from Sari A. et al. (2021) report: "In general, diseases of the oral cavity are considered signs of SARS-CoV-2 infection: among 5342 respondents from 43 countries of the world (8.1% reported infection with COVID-19), 42.7% had oral manifestations" [6]. Natto et al. (2022) used real-time polymerase chain reaction (RT-PCR) to assess SARS-CoV-2 in periodontal carious pockets. From the scientists' data: "A total of 180 samples from 72 patients were examined by RT-PCR for amplification of the E and S genes of SARS-CoV-2. SARS-CoV-2 was present in 41.7% of patients with COVID-19 and periodontal pockets and in 16.7% with carious lesions".

The presented observations support the hypothesis that samples obtained from periodontal pockets or carious lesions will show positivity for SARS-CoV-2, and this may serve as an alternative means of detecting the virus, comparable to other methods such as nasopharyngeal swab, oropharyngeal swab and saliva examination. [9]. Due to the tropism of the virus to mucous membranes, Paradowska-Stolarz attempted to describe oral manifestations of SARS-CoV-2 infection. The author emphasizes that there are many oral symptoms in COVID-19, but the coexistence with the underlying disease is not fully established.

It is still not clear whether the oral symptoms are manifestations of the disease or its arise from a loss of immune response. Of the identified symptoms in the oral cavity, the most common are: dysgeusia (disturbance of taste), toothache, exacerbation of autoimmune diseases, herpes simplex, chicken pox viruses, ulcers and aphthous stomatitis [11].

From the answers that we have from questionnaire of the main group of examinees about the level of their oral care, some aspects of eating behavior, some conditions and lifestyle that can affect the care of the oral cavity after suffering a coronavirus infection, it follows that such changes are observed in the direction of

deterioration, which is confirmed by direct statistically significant correlations.

The use of the above-mentioned data can help the dentist in forming a further set of individual preventive measures in the development of dental caries and periodontal tissue diseases in young people who have suffered a coronavirus infection.

Significant changes in local immunity were found in the examined patients, which contributed to the pathogenetic role in the development and progression of the carious process and periodontal tissue diseases.

They were manifested by changes of different degrees of severity: in the main group of patients was found a significant increase in the level of Ig G in the oral fluid, as well as a probable decrease in the concentration of SIg A relative to the comparison group, a probably higher normative value of pathogenic small- and medium-molecular CICs with a significant decrease in the level of physiological large CICs size relative to the comparison group, a decrease in the content of anti-inflammatory IL-4, as well as an increased concentration of pro-inflammatory cytokines.

Zinchuk O.M. et al. showed that the vast majority of the examined (86.1%) had a high level of anti-coronavirus antibodies, which exceeded the limits of quantitative indication. At the same time, there was no statistically significant difference in the level of IgG to SARS-CoV-2 among patients and persons with an asymptomatic course of the infectious process [3].

In particular, research in recent years has shown that secretory immunoglobulin A (s-IgA) of saliva plays an important role in local immunity, preventing microbial adhesion to the surface of hard tooth tissues, neutralizing certain enzymes and bacterial toxins of cariogenic bacteria, and also synergizes with other saliva proteins. such as lactoferrin or lysozyme, preventing caries [13-15, 27, 28]. Salivary s-IgA levels in the group of children with caries were significantly lower than in healthy control children, and adults did not show significant differences [20].

According to the review of scientific literature Preshaw P.M. et al. notes about age-related changes in immune function in the context of periodontitis, which should not be considered a decrease in immune functioning: "A systematic literature search revealed aspects of innate immunity (antimicrobial peptides, neutrophils and NEO, dendritic cells, natural killer cells, Toll-like receptors) and adaptive immune response (B and T cells, secretory IgA and IgG), which may play a role in susceptibility to caries with age" [23].

It can be assumed that a history of coronavirus disease can be attributed both to local factors of the occurrence of caries and periodontal tissue diseases, as local immu-

nological factors in saliva change, and to general causes, as there are disorders in various somatic organs. In the available literature, there is little data about the global pandemic on the course of dental diseases, especially in young adults. Therefore, there is a need for a more detailed study of the impact of SARS-CoV-2. on the prevalence and parameters of dental diseases among young adults infected with COVID-19.

CONCLUSIONS

1. In young adults who have suffered a coronavirus infection during the last 6 months, the prevalence of caries and its intensity are significantly higher than in the comparison group, unsatisfactory quality of res-

torations, significantly higher values of the bleeding index, PMA index and hygiene index, halitosis, which indicates a significant deterioration of dental health in these individuals and the further development of a strategy for the prevention of hard dental tissues and periodontal disease for this category of patients.

2. A study of local immunity has shown that 6 months after suffering from the COVID-19 disease, pronounced changes persist in young adults. It consist in an imbalance of circulating immune complexes with a predominance of the content of small-sized pathogens, as well as an increased concentration of pro-inflammatory cytokines against the background of a decrease in the phagocytic activity of immunocompetent cells of the oral fluid.

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