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RESEARCH ARTICLE

THE INDICATORS OF SECRETORY AND CELLULAR IMMUNITY OF ORAL FLUID AND PERIODONTAL TISSUE BEFORE AND AFTER COMPLEX TREATMENT IN PATIENTS WITH VIRAL HEPATITIS B

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Abstract

Background: The pathogenetic commonality of many general somatic processes and inflammatory diseases of the oral cavity is due to the development of mechanisms of cellular damage and modification of tissue structures that are common to the whole organism and acquire autoantigenic properties.

The aim of the study was to reveal the immunological changes in the oral cavity with viral hepatitis B and assess the effectiveness of complex treatment.

Material and methods: The study involved 95 patients with HBV with periodontal lesions, as well as 100 patients in the control group non-HBV. The dental status and index assessment of the condition of periodontal tissues were studied in all patients. Oral fluid cytokines IL-2, IL-10, IL-4, ɤ-INF were determined. For morphological studies, tissue samples excised from the gums in the area of direct localization of the pathohistological process were used. Immunohistochemical examination of gingival biopsies was performed using mouse monoclonal antibodies to CD3 to detect T lymphocytes.

Results: An objective examination of the oral cavity of patients with HBV revealed the presence of a generalized inflammatory process in the area of the marginal and alveolar parts of the gums. Pro-inflammatory IL-2 and ɤ-INF in HBV significantly increase: $p<0.001$ and $p<0.0405$, respectively, and anti-inflammatory IL4 sharply decreases compared to the control group by 130 times ($p<0.001$). After complex treatment, pro-inflammatory IL-2 decreased ($p <0.001$), the content of anti-inflammatory IL-4 in OF increased 404 times (<0.002).

Immunohistochemical research of biopsies periodontium tissue taken from patients with HBV us to evaluate the quantitative composition of infiltrate to T-lymphocytes (CD3+).

Conclusion: Thus, the analysis shows that with HBV, gum damage resembles the clinical picture of inflammatory periodontal diseases. Indicators of anti-inflammatory IL4 sharply decrease before complex treatment. A pathomorphological study of periodontal tissues with HBV revealed inflammatory infiltration in all patients. Immunohistochemical study of HBV revealed a positive reaction of lymphocytes for CD3+.

Keywords: *periodontium; cytokines; pathomorphology; immunohistochemistry; HBV*

Introduction

The interest in the study of combined pathology has recently been explained by the accumulation of new facts, the emergence of new information about interorgan, intertissue and intercellular levels of interaction in the system of the whole organism. In this regard, the question of the connection between diseases of internal organs and oral cavity organs is relevant.[1-3]

Among the important problems of modern practical dentistry, the issues of improving the diagnosis, prevention, and treatment of periodontal tissue diseases, despite numerous studies conducted throughout the world, remain relevant and have great social significance.[4-6]

Knowledge of the characteristic pattern of manifestations of certain general somatic diseases in the oral cavity, in particular gastrointestinal and liver diseases, is the most important professional quality of a dentist. Determining the primary etiological factor that caused the development of changes in the oral cavity is one of the key points in the professional activity of a dentist in relation to the diagnosis of the disease. This would greatly help to identify systemic diseases at their earliest stages with a view to subsequently immediately referring the patient to an appropriate specialist.[7]

Periodontal tissues are a complex structural and functional complex and take part in various body functions: chewing, swallowing, speech, breathing. In the structure of the main diseases of organs and tissues of the oral cavity, inflammatory processes in the periodontium occupy one of the leading positions, causing significant functional disorders of the maxillofacial area caused by tooth loss, according to WHO conclusions, 5 times more often than with complicated forms of caries.[8,9]

According to WHO, inflammatory periodontal diseases are one of the most common dental diseases in the world after dental caries. The highest incidence rates occur at ages 15–19 years (55–89%), as well as 35–44 years (65–98%).[10] Periodontal diseases in modern dentistry constitute one of the most important problems due to their wide prevalence, the complex nature of the lesion involving in the pathological process, in addition to the periodontal tissues themselves, other organs and systems, as well as changes in various parts of the homeostasis of the human body, including processes of lipid peroxidation, immune, cytokine systems.[11-13]

The last decades of the 20th century and the beginning of the 21st century were marked by a sharp increase in the number of viral liver diseases, especially parenteral viral hepatitis, characterized by a highly chronic course, the development of many complications and mortality, which determined the medical and social importance of the problem of viral hepatitis.[14, 15]

A significant place in the foreign literature is given to the study of the manifestations of chronic diffuse liver diseases, in particular viral hepatitis B (HBV) in the oral cavity.[16-18] There is sufficient data in the literature indicating the development of numerous disorders in viral hepatitis (VH) not only in the organs of the gastrointestinal tract, liver, immune system, but also in the organs of the maxillofacial region.[19,20]

Recently, there has been a trend towards an increase in the number of patients with chronic liver pathology, which may be due to an increase in the incidence of VH, the use of toxic and medicinal drugs, and poor nutrition.[21]

Studies of the oral cavity in chronic diffuse liver diseases are of great interest to clinicians since

pathological processes developing in the liver, as a rule, lead to organic and functional disorders in the oral mucosa.[22-24,25,26]

In chronic diffuse liver diseases, changes are also observed in the periodontium.[1,27-29]

The pathogenetic commonality of many general somatic processes and inflammatory diseases of the oral cavity is due to the development of mechanisms of cellular damage and modification of tissue structures that are common to the whole organism and acquire autoantigenic properties. The leading role in the occurrence of these changes is played by failures and dysfunctions of cytokine regulation of immunobiological processes.[30,31] The chronicity of any inflammatory process is based on the relationship of periodontal conditions with pro-inflammatory properties and anti-inflammatory activity.[32,33] The development of inflammatory diseases is determined by the state of cytokine regulation.[34,35] Most of both pro- and anti-inflammatory cytokines (such as IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNFα, ɤ-INF) are present not only in peripheral blood, but also in mixed saliva or oral fluid (OF).[36]

There are a number of works in the literature confirming the idea that the development of chronic periodontitis is accompanied by a violation of local immune mechanisms.[37,38]

In the available literature, insufficient attention is paid to such important problems as the influence of foci of chronic infection in the oral cavity on the general condition of patients with HBV. Despite the progress achieved in the treatment of periodontal tissue diseases in patients with hepatitis B, many issues, including changes in the quality of life in these diseases, remain insufficiently studied.[39-41]

It should be noted that in the available literature no information was found on a comparative single-stage and multilateral study of the periodontal condition, on the study of the cytokine profile of OF, as well as the morphological and immunohistochemical characteristics of periodontal tissues in HBV. There are practically no works devoted to the study of the above indicators before and after complex treatment. In our opinion, this study will be promising for the development of methods for early diagnosis and comprehensive assessment of the dental status of HBV patients with periodontal damage.

The aim of the study

The aim of the study was to reveal the immunological changes in the oral cavity with viral hepatitis B and assess the effectiveness of complex treatment.

Material and Methods

The main base for the study was the «Armenicum» MC, No. 1 dental clinic and the Department of Histology of YSMU after M. Heratsi, immunological laboratory of the Scientific Research Institute of Cardiology named after. L.A. Oganesyan of Yerevan from 2018 to 2022. The study involved 95 patients with HBV with periodontal involvement, as well as 100 control patients without HBV (non-HBV) but with periodontal involvement. The age of the patients ranged from 21 to 64 years: the average age of patients in the main group was 40.17±13.48 (mean ±SD) and 37.99±16.66 (mean ±SD) in the control group.

The dental status of all patients was studied according to pre-developed criteria: the condition of the marginal and alveolar parts of the gums, the dental-periodontal complex. When examining periodontal tissues, attention was paid to the condition of the gingival papillae, the presence of bleeding, swelling, degree of tooth mobility, etc. An index assessment of the condition of periodontal tissues was also carried out.

To assess the condition of periodontal tissues, the periodontal index (PI) according to Russell is used,[42] which makes it possible to take into account the presence of both gingivitis and other symptoms of periodontal pathology: tooth mobility, clinical pocket depth, etc. In the periodontal index, the condition of the gums and alveolar bone is calculated individually for each tooth. The indices of each tooth are summed up, and the result is divided by the number of teeth in the oral cavity. The result shows the patient's periodontal index, which reflects the relative status of periodontal disease in a given oral cavity without regard to the type and causes of the disease.

The periodontal index was calculated using the formula:

$$PI = \frac{\sum}{n}$$

where ∑ - the sum of the highest scores of each

tooth, n - number of teeth examined.

The index value was assessed as follows: 0.1 - 1.5 - initial stage; 1.6 - 4.0 – average; 4.1 - 8.0 – severe stage.

To detect early manifestations of periodontal disease, the sulcus bleeding index (BI) according to Miihlemann and Son is used.[43] The method is sensitive: increased bleeding in clinically healthy periodontal tissue is determined to be 30-40%, which allows the method to be used for early detection of initial inflammatory changes. The method is indicative for both gingivitis and periodontitis.

The bleeding index was calculated using the formula:

$$BI = \frac{\Sigma}{n}$$

where Σ - is the sum of indicators, n - is the number of teeth examined.

BI values were interpreted as follows: 1.0-1.5 - mild bleeding, 1.6-2.5 - moderate bleeding, 2.6 - 3.0 - severe bleeding.

In 18 patients of the main and 30 patients of the non-HBV group, cytokines (IL) OF (IL-2, IL-10, IL-4, ɤ-INF) were determined.

We studied unstimulated mixed saliva (oral fluid), which was collected in the morning on an empty stomach with a sterile syringe into sterile Eppendorf tubes. Samples were frozen and stored at –20ºC. Before testing, the samples were thawed at room temperature and centrifuged at 5000 rpm in the cold. Mucin precipitation was carried out using 6 units of lidase per 1.0 ml of oral fluid (Patent RA No. 3295 A dated May 16, 2019).[44]

The concentrations of cytokines were determined: pro-inflammatory – ɤ - INF (pg/ml, regulator of the cellular immune response); IL-2 (pg/ml, regulator of the inflammatory process) and anti-inflammatory - IL-4 (pg/ml, regulator of the humoral immune response) and IL-10 (pg/ml). We used Vector-Best kits (Novosibirsk, RF), detection was carried out on an enzyme-linked immunosorbent analyzer - photometer Statfax 303 Plus (Florida, USA).

The determination method is based on a three-stage “sandwich” version of an enzyme-linked immunosorbent assay using mono- and polyclonal antibodies to human IL.

In all the groups we examined (20 patients in each group), pathomorphological and immunohistochemical studies were carried out in 30 patients (15 patients in each group).

The material for morphological studies was tissue samples cut from the gums in the area of direct localization of the pathohistological process. To compare the results of pathological studies, gingival tissue was taken as a control from 20 patients with periodontal lesions non-HBV, as noted.

Tissue pieces were fixed in 10% neutral formalin, dehydrated and embedded in paraffin, according to the standard histological scheme. A series of 5-µm-thick sections were made from the blocks and stained with hematoxylin-eosin and picrofuchsin according to Van Gieson for a general assessment of the condition of the tissues under study. Micro specimens were studied with a Primostar Zeiss trinocular microscope under 100 and 400x (immersion) magnification. Microphotographs were taken using Axio Cam ERc5s (Carl Zeiss - Germany). All signs were studied in accordance with international standards, WHO recommendations and recognized research methods.[45]

The criteria for the main pathomorphological changes in HBV that we have studied are the following: inflammatory infiltration, circulatory disorders, ulceration of the mucous membrane with fibrinous deposits, fibrosis of the mucous membrane, dystrophic changes in the squamous epithelium. Additional criteria included infiltration (lymphoplasmacytic and plasmacytic) and admixture of neutrophils.

All patients with HBV received etiotropic treatment with antiviral drugs.

Immunohistochemical study was performed using reagents produced by Zytomed (Germany) - a manual polymer detection system and a positive control. An immunohistochemical study of gum biopsies was carried out using monoclonal mouse antibodies to CD3 (clone SP7 for determining T-lymphocytes). The listed immunohistochemical marker was selected after control studies as the most informative indicator to assess the functional activity of T-lymphocytes, with high prognostic significance to judge the nature of gum inflammation.

A universal kit containing anti-mouse and anti-rabbit immunoglobulins was used as the second antibodies. Visualization of the color was carried out followed by development of horseradish peroxidase

with diaminobenzidine. To carry out the immunohistochemical reaction, a standard one-step protocol was used with antigen unmasking in citrate buffer (pH 7).

Quantitative assessment of the results of the immunohistochemical reaction was carried out using a computer analysis system for microscopic images. Three microphotographs were taken from each preparation, on which CD3 content was assessed.

In addition to etiotropic treatment, 50 patients of the main group underwent sanitation of the oral cavity and the use of the probiotic "Brefovil" (manufacturer Sacura Italy, S.R.L; active substances: Saccharomyces boulardi 8 mld UFC and zinc 10 mg). The drug was used topically in the form of rinsing and drinking 2 sachets for 10 days.

Statistical analysis

Descriptive analysis (mean ± SD for continuous and frequencies/proportion for categorical variables) were computed for all variables of interest. Differences between two groups were evaluated using “chi-square” or “Fisher’s exact” tests for categorical variables and “Wilcoxon signed rank test” for continuous variables. Spearmen correlation was performed for determination of relationships between continuous variables. P-value was considered significant at <0.05 and <0.001 for highly significant results. Analyses were conducted using Excel 2013 and R software.

Results

The detection rate of periodontal diseases in the HBV patients we examined was 100%. An objective examination of the oral cavity of patients with HBV revealed the presence of a generalized inflammatory process in the area of the marginal and alveolar parts of the gums (Table 1).

Table 1

Clinical examination data of the marginal and alveolar parts of the gums in patients with HBV and non-HBV groups

	Sign	non-HBV n=100		HBV n=95		p-value*
		absolute number	%	absolute number	%	
Hyperemia	Absent	99	99	32	33.7	**<0.001**
	Present	1	1	63	66.3	
Cyanosis	Absent	75	75	65	68.4	>0.308
	Present	25	25	30	31.6	
Edema	Absent	87	87	32	33.7	**<0.001**
	Present	13	13	63	66.3	
Looseness of gingival papillae						
	Absent	99	99	95	100	>1
	Present	1	1	0	0	
Atrophy	Absent	100	100	58	61.1	**<0.001**
	Present	0	0	37	38.9	
Bleeding	Absent	89	89	24	25.3	**<0.001**
	Present	11	11	71	74.7	
Desquamation of the epithelium						
	Absent	100	100	91	95.8	>0.0545
	Present	0	0	4	4.2	

* *p-value test result from the comparison between HBV and non-HBV groups*

A number of pathological changes in the gums were observed. With HBV, hyperemia and edema of the gums were detected in 66.3% (63) of cases, which is statistically significantly higher compared to non-HBV (p<0.001), where this symptom was observed in 1% and 13% of those examined, respectively. Gingival cyanosis was observed in 31.6% (30) of cases (p>0.308). Looseness of gingival papillae was practically not detected either in the non-HBV group (only 1% of those examined) or in the group with HBV (p>1). Gum atrophy was characterized by a decrease in the volume of tissue of the gingival papillae and gingival margin due to the general somatic state of health of the patients. With the above lesions, limited atrophy was observed in the area of one or two teeth, which was visually manifested as a V-shaped defect, roller-like thickened edges of the gums, exposure of the neck and root of the tooth, in particular in the frontal region, with predominant damage to the incisors and canines. In HBV patients, these changes were detected in 38.9% (37) of cases, which is statistically significantly higher compared to non-HBV patients (p<0.001). Bleeding gums were observed in 74.7% (71) of the examined HBV patients, which, when compared with the non-HBV group, was significantly higher by approximately 7 times (p<0.001). Desquamation of the gum epithelium, reminiscent of the clinical picture of desquamative gingivitis, was observed in 4.2% (4) of those examined, which was not statistically different from the non-HBV indicators (p>0.0545), where this symptom was not observed.

According to the results of a comprehensive dental examination, in addition to the main complaints characteristic of periodontitis, abundant dental deposits were observed, both supragingival and subgingival, pathological mobility of teeth of varying degrees, the presence of periodontal pockets (PP) > 3.5 mm, purulent discharge from PP, unpleasant bad breath (Table 2).

Table 2

Clinical examination data of the dental-periodontal complex in patients with HBV and non-HBV groups

	Sign	non-HBV n=100		HBV n=95		p-value*
		absolute number	%	absolute number	%	
Subgingival dental plaque	Absent	61	61	28	29.5	**<0.001**
	Present	39	39	67	70.5	
Tooth mobility degree I		29	29	22	23.2	>1
Tooth mobility degree II		7	7	49	51.6	**<0.001**
Tooth mobility degree III		5	5	3	3.2	**>1**
No tooth mobility		59	59	21	22	**<0.001**
Periodontal pockets >3.5 mm	Absent	61	61	12	12.6	**<0.001**
	Present	39	39	83	87.4	
Purulent discharge from pathological pockets	Absent	91	91	55	57.9	**<0.001**
	Present	9	9	40	42.1	
Having bad breath	Absent	26	26	27	28.4	**>0.704**
	Present	74	74	68	71.6	

* *p-value test result from the comparison between HBV and non-HBV groups*

Subgingival dental deposits, detected on both the lingual and vestibular surfaces of the central incisors and canines of the lower jaw and the buccal surface of the upper premolars and molars, were detected in 70.5% (67) of cases, while non-HBV were detected in 39% (39) patients, which statistically has a significant difference (p<0.001). Soft plaque was observed in 100% of cases. The presence of PP > 3.5 mm occurred in 87.4% (83) of patients, purulent discharge from PP was observed in 42.1% (40) of patients. Both

indicators had a high degree of reliability (p<0.001). Complaints about the presence of bad breath were made by 71.6% (68) of those examined, which practically coincided with the presence of this symptom in the control group - 74% (74) (p>0.704).

When analyzing data regarding pathological tooth mobility of degrees I and III in the group of patients with HBV and the non-HBV group, no statistically significant difference was revealed (p>1). II degree of pathological mobility in HBV patients was detected in 51.6% (49) of those examined, which was statistically significantly higher compared to the non-HBV group (p<0.001), where this sign was detected in 7% (7) of patients.

To assess the degree of periodontal damage in patients with hepatitis B, the PI indices according to Russel and SBI according to Mühlemann and Son were determined as the most objective criteria for judging the state of inflammatory changes in the periodontium (Table 3).

Table 3
Index assessment of the condition of periodontal tissues in patients with HBV and non-HBV groups (mean ± SD)

Index/points	non-HBV	HBV	p-value*
PI, point	0,95±0,48*	4,08±0,41*	**<0.001**
SBI, point	1,68±1,66*	2,82±0,21*	**<0.001**

** p<0,001; ** p<0,0104*

As can be seen from the table, in patients with HBV, the periodontal indices were statistically significantly different from the periodontal indices in non-HBV patients. The PI index averaged 4.08±0.41 points, which is 4.3 times higher than the PI index in patients in the control group (Figure 1), and the SBI index averaged 2.82±0.21 points, which is also 1.68 times higher than the value of the SBI index in patients of the non-HBV group. The difference in data is statistically significant with a high degree of confidence (p<0.001) (Figure 2).

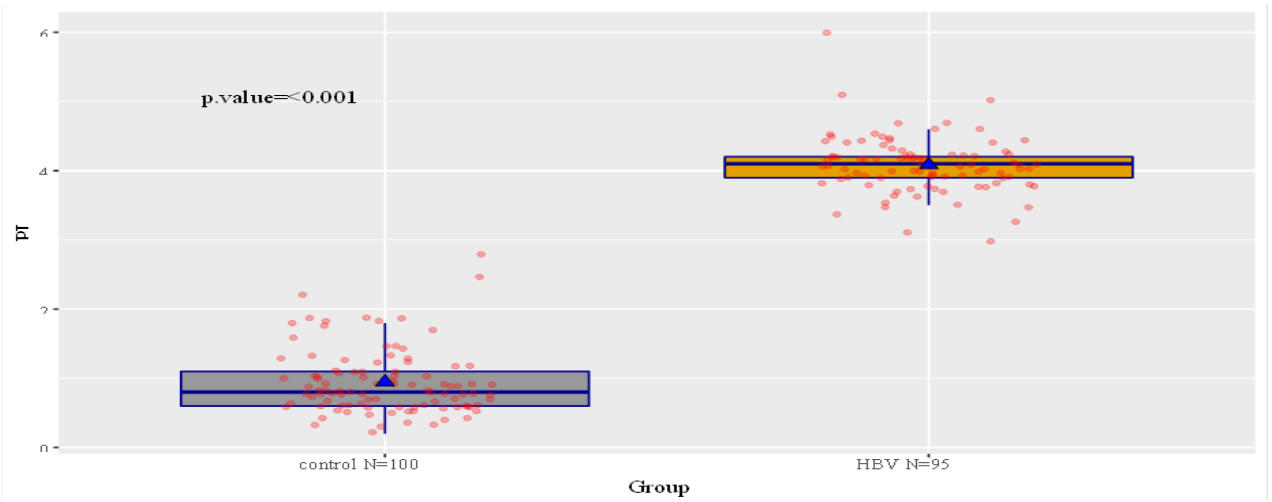


Figure 1. *PI index in patients with HBV and non-HBV group*

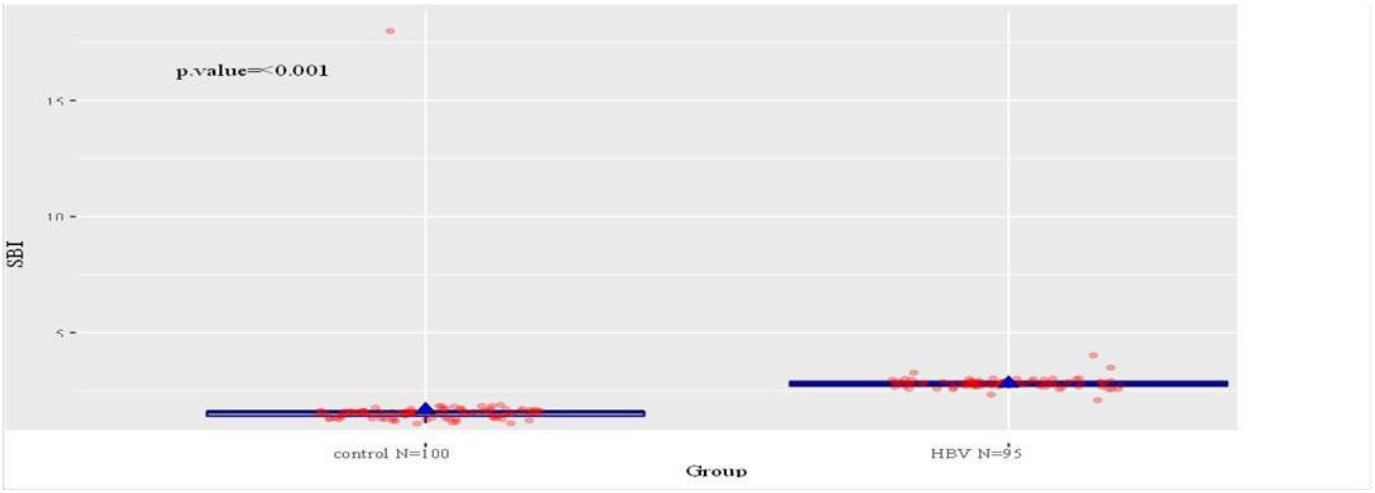


Figure 2. *SBI index in patients with HBV and non-HBV group*

At the second stage of the study, we studied the content of pro-inflammatory cytokines - IL-2, ɤ-INF and anti-inflammatory cytokines - IL-4 and IL-10 (Table 4).

Table 4

Oral fluid cytokine levels in the non-HBV group and in patients with HBV before complex treatment (mean ± SD)

Cytokines	Non-HBV (n=30)	HBV (n=18)	Odds Ratio	95% CI	p value*
IL2	2.83±5.67	31.1 ± 23.59	-28.28	[-40.15; -16.41]	**<0.001**
IL10	0.94 ±1.33	8.38 ±15.51	-7.44	[-15.16; 0.29]	**<0.001**
IL4	14.29 ±26.11	0.11±0.3	14.18	[4.43; 23.93]	**<0.001**
ɤ-INF	0.72±3.04	2.49±4.24	-1.77	[-4.11; 0.57]	**<0.0405**

∗ *p-value test result from the comparison between non-HBV and HBV groups*

A comparative analysis of the indicators of OF cytokines in HBV and in the non-HBV group revealed that the number of pro-inflammatory cytokines IL-2 and ɤ-INF in HBV significantly increases: IL-2 - 11 times (p <0.001), ɤ-INF - 3 times .5 times (p<0.0405). The amount of IL-10 also increases significantly by 8.9 times (p <0.001). The levels of anti-inflammatory IL4 sharply decrease compared to non-HBV - by 130 times (p <0.001).

After complex treatment (as indicated in Materials and Methods paragraph) for HBV, the content of the proinflammatory cytokine IL-2 in OF decreased almost 36 times (p<0.001). The level of ɤ-INF also decreased, however, the difference between the data before and after treatment was statistically insignificant (p>0.071). The content of anti-inflammatory IL-10 in OF decreased by 8.1 times (p<0.006), and anti-inflammatory IL-4, on the contrary, increased by 404 times with a high degree of confidence (p<0.002) (Table 5 and Figure 3).

Table 5

Oral fluid cytokine levels in the patients with HBV before and after complex treatment (mean ± SD)

Cytokines	HBV (n=18) before	HBV(n=18) after	Odds Ratio	95% CI	p value*
IL2	31.1 ± 23.59	0.87±1.24	30.23	[18.49; 41.97]	**<0.001**
IL10	8.38 ±15.51	1.03±1.79	7.35	[-0.4; 15.09]	**<0.006**
IL4	0.11±0.3	44.41±41.9	-44.3	[-65.14; -23.47]	**<0.002**
ɤ-INF	2.49±4.24	0.27±0.78	2.22	[0.09; 4.36]	0.071

** p-value test result from comparing HBV patients before and after treatment*

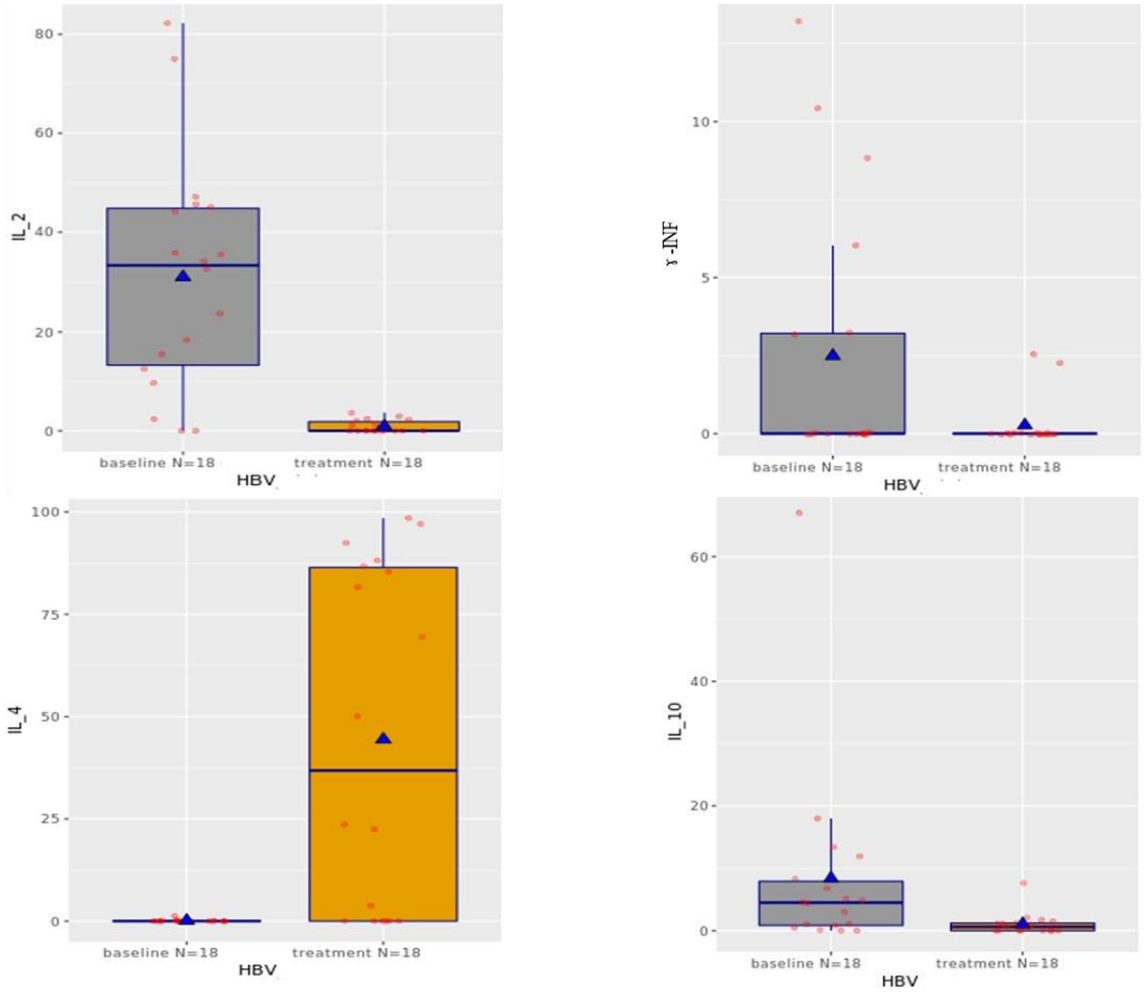


Figure 3. *Oral fluid cytokine levels in the patients with HBV after complex*

To confirm the results of the clinical data, at the third stage of the study, we conducted a morphological study of the gum biopsy. Biopsies were taken from the affected areas of the alveolar and marginal gingiva. All signs of damage were presented in Table 6.

Table 6
The main morphological changes in the oral mucosa with HBV

Sign		HBV n=20	
		absolute number	%
Inflammatory infiltration	Absent	0	0
	Present	20	100
Circulatory disorders	Absent	1	5
	Present	19	95
Ulcerations of the mucous membrane with fibrinous deposits	Absent	19	95
	Present	1	5
Fibrosis of the mucous membrane	Absent	17	85
	Present	3	15
Dystrophic changes in squamous epithelium	Absent	2	10
	Present	18	90
Lymphoplasmacytic infiltration	Absent	0	0
	Present	20	100
Neutrophil admixture	Absent	12	60
	Present	8	40

In addition to the main pathomorphological changes, we identified such criteria as lymphoplasmacytic infiltration and an admixture of neutrophils. Inflammatory infiltration was determined in the form of lymphoplasmacytic in all HBV patients in 100% (20) of cases, and an admixture of neutrophils was noted in 40% (8) of patients. Circulatory disorders were manifested by edema, hemorrhage, stasis in the capillaries, plethora, and angiomatosis. Obliteration of the lumen of blood vessels, fibrinoid necrosis and fibrinoid swelling of the vessel walls were observed. Changes in the squamous epithelium included acanthosis, parakeratosis, and thickening (Figures 4 and 5). The results of the histological study show that practically in the group of patients with HBV, combinations of the pathohistological signs described above were found.

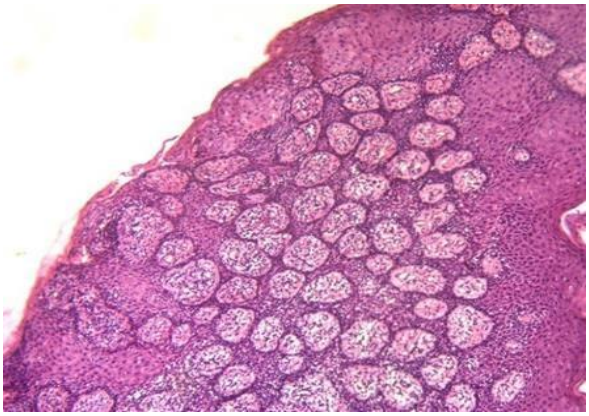


Figure 4. *Signs of focal keratinization in the thickness of the epithelial layer. (Hematoxylin and eosin staining x400)*

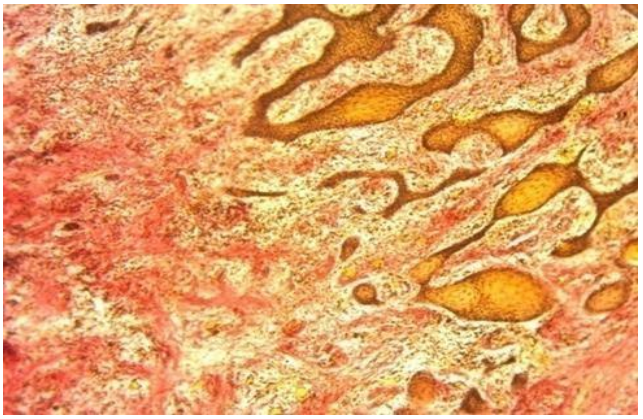
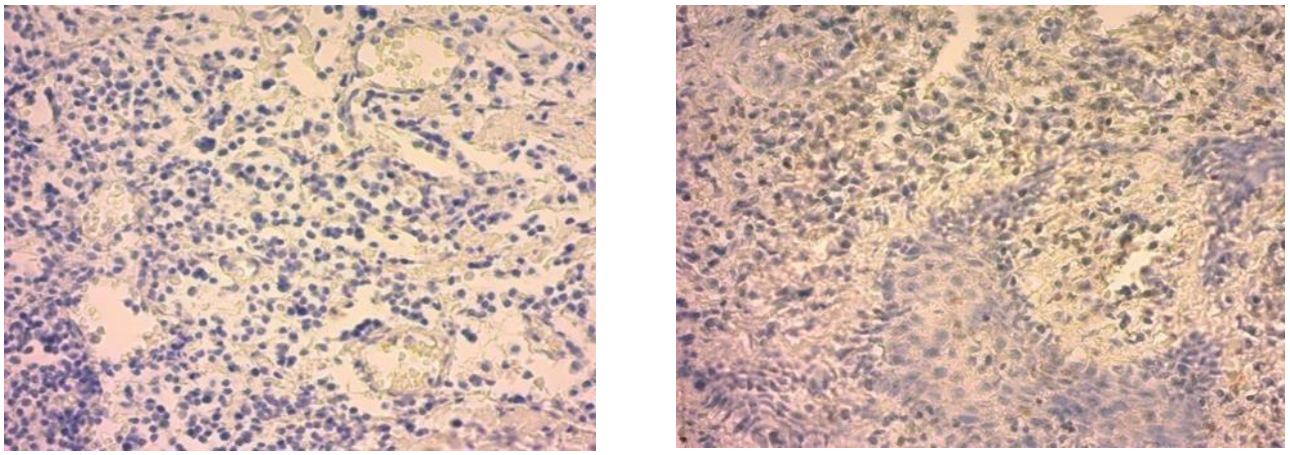


Figure 5. *Acanthosis of the squamous epithelium of the transitional gum. (Staining with picrofuchsin according to Van Gieson x100)*

The results of immunohistochemical studies show that periodontal damage due to HBV occurs with the participation of cellular and humoral immunity and is accompanied by certain immune disorders. This indicates not only a decrease in immune anti-infective reactions, but also their disunity.

Immunohistochemical research of biopsies periodontium tissue taken off from patients with HBV us to evaluate the quantitative composition of infiltrate to T-lymphocytes (CD3+).

Diffuse lymphocytes in its plate of periodontal tissue are represented mainly with T-cells, though T-lymphocytes were singly localized in the thick epithelial stratum (Figure 6).



a **b**

Figure 6. *Focal positive reaction to CD 3+ in single cells of the inflammatory infiltrate in HBV (a) x400. Diffuse pronounced positive reaction to CD 3+ in the cells of the inflammatory infiltrate of HBV in remission (b) x 400*

Keratoconjunctivitis was observed in 67 patients with HBV, who were sent for consultation to an ophthalmologist.

Thus, numerous morphological signs can be divided into those that are indicators of the severity and activity of inflammation, and those that are associated with a long-term chronic course of inflammation in the oral c11avity.

Discussion

There is practically no pathology that does not affect the condition of the oral mucosa and periodontal disease. At the same time, the similarity of clinical manifestations in the oral cavity of diseases that differ in etiology and pathogenesis contributes to difficulties in making a final diagnosis.[46,47].

Dental care for patients, even with an established diagnosis of viral hepatitis, is provided mainly on the basis of referral due to acute pain. There are very few developments on dental tactics for managing patients with hepatitis. In countries with a high level of dental service, experience on this problem has also not been accumulated.[26,48]

The data regarding the condition of the marginal and alveolar parts of the gums in patients with HBV are interesting. According to Fedeli U. et al. (2017), who studied the characteristics of periodontal damage in patients with chronic liver diseases of viral etiology, with chronic hepatitis and liver cirrhosis caused by the hepatitis B virus, more severe degenerative and inflammatory changes are observed in periodontal tissues compared to those caused by the hepatitis C virus.[49] Our data indicate that it is probably difficult to make an unambiguous conclusion regarding the comparison of the severity of periodontal damage with HBV, because only some symptoms are significantly more often detected with HBV.

In the available literature there are few works where the symptoms of periodontal damage in HBV, especially early manifestations of the disease, have been studied and systematized. The reliability of the frequency of occurrence of one or another symptom of the lesion has also not been studied. Some authors even point out the contradictory data indicating a connection between periodontal lesions and viral hepatitis. In our opinion, these conclusions are related

to the incorrectness of the research. Thus, Nagao Y. et al. (2014) studied the nature of periodontal lesions in patients suffering from chronic hepatitis (HBV - 20) and liver cirrhosis caused by HBV infection (15 patients). Presenting data from a few cases of periodontal disease using descriptive analysis, the authors concluded that there was no association between chronic HBV and disease stage with the frequency and nature of periodontal disease.[50] Given the small number of patients and the lack of proper statistical analysis, one has to doubt the reliability of the authors' conclusions. According to Bagewadi S.B. et al. (2015) describe 3 clinical cases with a review of the literature, where, together with periodontal damage in the form of bleeding, swelling and friability of the gingival papillae, lichen planus was diagnosed in 2 cases.[51]

The basis of the inflammatory process of any etiology is the launch of the cytokine cascade, which includes, on the one hand, pro-inflammatory cytokines, and on the other, anti-inflammatory mediators. The balance between the two opposite groups largely determines the nature of the course and outcome of the disease.[52] The main problem is the lack of available laboratory diagnostic methods that would clearly reflect a shift in the cytokine balance towards inflammatory or anti-inflammatory / immunosuppressive reactions. Considering the multiplicity, as well as the synergism and pleiotropy of the cytokines involved in these reactions, it is clear that determining the concentration in the blood of any one of them will not adequately reflect the state of the entire cytokine balance. Perhaps, only a one-time assessment of the level of several mediators (at least 2-3 of the opposing subgroups) may be more correct. In connection with the above, we consider it appropriate to discuss some methodological aspects of our work in the study of cytokines. The latter can come from the blood serum as a result of their transudation, but the content of cytokines in saliva does not correlate with their level in the blood, which indirectly indicates the predominance of their local synthesis.[53] Taking into account the recommendations of the literature, we studied the content of both pro-inflammatory cytokines - IL-2, ɤ-INF, and anti-inflammatory cytokines - IL-4 and IL-10 in OF, which is more accessible and non-invasive. There is a sufficient number of works in the literature devoted to the study of the content and ratio of pro- and anti-inflammatory cytokines in OF in various pathologies.[33,54,55] However, it should be noted that the available data are very contradictory. This also applies to data from control groups, which creates certain difficulties regarding a clear understanding of normal cytokine levels in OF. There are isolated studies in the literature devoted to the study of this issue in HBV, but there are no studies on the simultaneous study of pro-inflammatory (IL-2, ɤ-INF) and anti-inflammatory cytokines (IL-10, IL-4) in the same group of patients. Considering the high variability of normal levels of cytokines in GC, we found it interesting to analyze the data from the available literature regarding those cytokines that were identified in our work. The content of IL-4 in OF in practically healthy people, according to various authors, ranges from 2.3 (1; 8.5) to 15.2±1.5 pg/ml,[56,57] IL -10 – from 4.83 ± 0.40 to 22.59 pg/ml (11.04-43.74) [56], IL-2 – from 0.1 ± 0.02 pg/ml to 10.0 (8 .5; 28.5),[58] ɤ-INF from 18.35 ± 0.47 to 23.8 ± 1.5 pg/ml.[57] Our data regarding IL-4 and IL-2 indices in the control group coincide with the literature data, however, there are discrepancies regarding IL-10 and ɤ-INF indices, which once again proves the high variability of normal cytokine levels in OF. Thus, in chronic catarrhal gingivitis and chronic generalized periodontitis of mild and moderate severity, an increased content of pro-inflammatory cytokines IL-1β, IL-6, IL-8, IL-17 IL-18, ɤ-INF, TNF-α, receptor antagonist IL-1 (RAIL) against the background of a decrease in the level of anti-inflammatory cytokines IL-4 and IL-10. In chronic generalized periodontitis, the immune response is activated, mediated by 2nd order T-helpers with activation of B-lymphocytes and plasma cells; in the gingival fluid, an increase in the level of all pro-inflammatory and anti-inflammatory cytokines (IL-4, IL-10), immunoregulatory IL-2 is determined, IFα, RAIL.[33,59] There are a number of works in the literature devoted to the development of periodontitis with HBV.[10,50] However, there is practically no work on the study of cytokines in OF with HBV. Therefore, the results of our studies partly coincide, and in some cases diverge from the literature data.

In our work, we carried out a comparative analysis of all the indicators we studied before and after complex treatment. It should be noted that we have not found similar works in the available literature. There are works devoted to the study of the dynamics of

single indicators after antiviral therapy. In our work, we studied the results of complex treatment.

Our study has some strengths and limitations. One of the strengths of this research is that to the best of our knowledge there has not been carried out similar research in Armenia so far.

Moreover, the study used a comprehensive approach looking at the lesions of periodontium from clinical, biochemical, pathohistological, as well as from the immunohistochemical points of view. Thus, the paper contains more detailed results which are few or lacking in the similar publications available, i.e. our research is the first to do a immunohistochemical study of biopsies of the oral mucosa in patients with HBV to assess the qualitative composition of the infiltrate to T (CD3+) lymphocytes.

One of the limitations of the study is that despite the fact that the HBV group had 95 participants and the non-HBV group – 100, only 18 of them from the HBV group and 30 patients from the non-HBV group agreed to pass the test of cytokines of the oral fluid (OF).

Another limitation was that the initial raw data from which the Excel database was created was a paper-based registry; and as the data were not double entered in the process of the data transmission errors could have happened.

The third limitation of our study was the fact that after consultation with an ophthalmologist, we do not present the data in this article. We will address this issue in our subsequent studies.

Conclusion

Thus, the analysis shows that with HBV, gum damage resembles the clinical picture of inflammatory periodontal diseases, in particular catarrhal and hypertrophic gingivitis, as well as chronic generalized periodontitis. Indicators of anti-inflammatory IL4 sharply decrease before complex treatment. In a pathomorphological study of periodontal tissues with HBV, inflammatory infiltration, circulatory disorders and dystrophic changes in the squamous epithelium were detected in all patients, and lymphoplasmacytic infiltration was identified in almost all patients with HBV. An immunohistochemical study of HBV revealed a positive reaction in lymphocytes for CD3+.

Declarations

Conflicts of interest and financial disclosures

The author declares that he has no conflict percent and there was no external source of funding for the research in question.

Ethical approval

The study was approved by the University ethics committee and was conducted in accordance with the Declaration of the World Medical Association.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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