ORIGINAL ARTICLE

MORPHOLOGICAL FEATURES OF BIOPSY SAMPLES OF THE ORAL MUCOSA IN PATIENTS WITH VIRAL HEPATITIS B AND C

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Abstract

Background: Currently, diseases of the oral mucosa and periodontal disease are an important problem in dentistry. 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.

Purpose: The aim of the study was to reveal the morphological features of biopsy samples of the oral mucosa in patients with HBV and HCV.

Materials and methods: The study involved 95 patients with HBV, 96 patients with HCV with lesions of the oral cavity and periodontium, as well as 100 patients in the control group without HBV and without HCV, but with lesions of the oral cavity and periodontium. The age of the patients ranged from 21-64 years. In all the groups we examined, 20 morphological studies were carried out.

Results: To study the dental status, patients' complaints and data from a clinical examination of the oral cavity were taken into account, which included: external examination of the lips and corners of the mouth, assessment of the condition of various parts of the oral mucosa. To confirm the results of clinical data, we conducted a morphological study of a biopsy sample of the oral mucosa. Biopsies were taken from the affected areas of the oral mucosa.

Conclusion: During a pathomorphological study of OM in viral hepatitis B and C, inflammatory infiltration, circulatory disorders and dystrophic changes in the squamous epithelium were detected in all patients, fibrosis - in all patients with HCV. Lymphoplasmacytic infiltration was detected in almost all patients with HBV and HCV.

Keywords: oral mucosa; periodontium; morphology; HBV; HCV.
Introduction

Currently, diseases of the oral mucosa (OM) and periodontal disease are an important problem in dentistry. This is due to the wide prevalence of lesions of the oral cavity and periodontium, the variety of nosological forms and their connection with systemic pathologies, difficulties in diagnosis and treatment, the success of which depends on the correctness of the diagnosis.1-3

According to WHO, inflammatory periodontal diseases are one of the most common dental diseases in the world after dental caries.4,5

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 human body, including processes of lipid peroxidation, immune, cytokine systems.6-8

Many authors point to the high frequency and varied clinical picture of oral lesions in diseases of the gastrointestinal tract.9,11 Due to diseases of the digestive organs, the functional activity of the salivary glands, the composition and properties of saliva change, which leads to a violation of the dynamic balance in the oral cavity. An increase in the content of mucin, C-reactive protein, and a decrease in the activity of lysozyme in the oral fluid have been established, which indicates a violation of the nonspecific resistance of the immune system in diseases of the digestive organs.12

Diseases of the oral mucosa (OMD) against the background of concomitant general somatic diseases represent one of the most difficult problems in dentistry due to difficulties in diagnosis and treatment.13 The development of lesions of the oral mucosa aggravates the course of the underlying disease and determines the characteristics of therapeutic measures.14,15 Correct and timely assessment of the condition of the oral cavity and the doctor’s choice of modern means of rational treatment are pressing issues in dentistry.2

From the last decades of the twentieth century to the present time, there has been an increase in the incidence of viral hepatitis.16,17 The global coverage of territories and the high epidemic potential of this group of diseases retain their social and economic significance. The WHO report “Global hepatitis report” (2017) noted that about 325 million people in the world suffer from viral liver diseases, and mortality from them, unlike HIV infection, tuberculosis and malaria, continues to rise.18 According to WHO data, about a third of the world's population may have contact with the hepatitis B virus (HBV) during their lifetime; 257 million people are chronically infected with the hepatitis B virus, 71 million with the hepatitis C virus (HCV).21,22

Studies of the oral cavity in chronic diffuse liver diseases are of great interest to clinicians since pathological processes developing in the liver,23-25 as a rule, lead to organic and functional disorders in the oral mucosa.26, 27

Literature data indicate the possibility of extrahepatic replication of hepatitis B and C viruses in cells of the skin and mucous membranes, vascular intima, tissues of the bone marrow, blood, lymph nodes and spleen. Particular attention is drawn to the evidence of active replication of the virus in circulating macrophages - monocytes. The possibility of extrahepatic replication of hepatitis B and C viruses does not exclude the possibility of damage to the skin and mucous membranes, including the oral mucosa and periodontal tissue, as evidenced by the detection of hepatitis C virus RNA in the minor salivary glands and saliva itself.30

Timely and effective diagnosis of various diseases of the oral mucosa still remains an important problem in dentistry, since they are characterized by a wide variety of both morphological structure and clinical manifestations.1,31

It should be noted that in the available literature no information was found on a comparative one-time study of the state of the oral organs in HBV and HCV. In our opinion, this study will be promising for the development of methods for early diagnosis and comprehensive assessment of the dental status in patients with HBV and HCV with lesions of the oral cavity.

The aim of the study was to reveal the morphological features of biopsy samples of the oral mucosa in patients with HBV and HCV.

Materials and Methods

The main base for the study was the Violeta MC,
No. 1 dental clinic and the Department of Histology of Yerevan State Medical University after M. Heratsi (YSMU) from 2019 to 2021. The study involved 95 patients with HBV, 96 patients with HCV with lesions of the oral cavity and periodontium, as well as 100 patients in the control group without HBV and without HCV, but with lesions of the oral cavity and periodontium. The age of the patients ranged from 21-64 years. The final diagnosis of HBV was established based on the detection of hepatitis B virus surface antigen (HbsAg) in blood serum by enzyme-linked immunosorbent assay (ELISA) and hepatitis B virus DNA by polymerase chain reaction (PCR). The final diagnosis of HCV was established based on the detection of hepatitis C virus RNA in the blood using PCR. All patients' dental status was studied according to pre-developed criteria, which included an external examination of the lips and corners of the mouth, assessment of the condition of various parts of the oral cavity, as well as the tongue. In all the groups we examined, 20 morphological studies were carried out. To compare the results of morphological studies, tissues of the oral cavity from 20 non-HBV and non-HCV patients were taken as a control. For the histological study of the oral cavity, the cheek area was selected as an example of tissue covered with stratified squamous epithelium and having a pronounced connective tissue subepithelial layer.

Tissue pieces were fixed in 10% neutral formalin, dehydrated and embedded in paraffin, according to the standard histological scheme. A series of sections 5 μm thick were made from the blocks and stained with hematoxylin-eosin and picrofuchs in according to Van Gieson for a general assessment of the condition of the tissues under study. It is known that the diagnosis and prognosis of the outcome of diseases is primarily based on the study of hematoxylin-eosin sections. Microspecimens were studied with a Primostar Zeiss trinocular microscope at 200, 400 and 1000x 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.

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. Spearman 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.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Yerevan State Medical University (Approval No. 2 dated 24 October 2019).

Results

To study the dental status, patients' complaints and data from a clinical examination of the oral cavity were taken into account, which included: external examination of the lips and corners of the mouth, assessment of the condition of various parts of the oral mucosa. Patients complained of discomfort in the oral cavity, dry mouth, tightness of the lips, soreness in the corners of the mouth, burning and tingling sensations in the tongue, changes in taste, coated tongue, and roughness of the oral cavity. Clinical examination data are presented in Tables 1 and 2.

**Table 1.**

*Clinical examination data on the oral mucosa in patients with HBV and non-HBV groups*

<table>
<thead>
<tr>
<th>Sign</th>
<th>non-HBV n=100</th>
<th>HBV n=95</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>absolute number</td>
<td>%</td>
<td>absolute number</td>
</tr>
<tr>
<td>Erosion on the lips</td>
<td>99</td>
<td>99</td>
<td>92</td>
</tr>
<tr>
<td>Present</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Cracks in the corners of the mouth


<table>
<thead>
<tr>
<th>Sign</th>
<th>non-HCV n=100</th>
<th>HCV n=96</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>absolute number</td>
<td>%</td>
<td>absolute number</td>
</tr>
<tr>
<td>Erosion on the lips</td>
<td>Absent 99</td>
<td>99</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Present 1</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Cracks in the corners of the mouth</td>
<td>Absent 99</td>
<td>99</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Present 1</td>
<td>1</td>
<td>41</td>
</tr>
<tr>
<td>Disorders in the mucous membrane relief</td>
<td>Absent 97</td>
<td>97</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Present 3</td>
<td>3</td>
<td>86</td>
</tr>
<tr>
<td>Hemorrhages on the buccal mucosa and the hard palate</td>
<td>Absent 100</td>
<td>100</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Present 0</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>Telangiectasia on the buccal mucosa</td>
<td>Absent 100</td>
<td>100</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Present 0</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
<td>Coated tongue</td>
<td>Absent 100</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Present 0</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>Foci of epithelial desquamation on the surface of the tongue</td>
<td>Absent 100</td>
<td>100</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Present 0</td>
<td>0</td>
<td>60</td>
</tr>
</tbody>
</table>

*p-value test result from the comparison between HCV and non-HCV groups*
HBV was 52.6%, which is significantly and significantly higher than that in the non-HBV group (p <0.001). Disorders in the mucous membrane relief of the oral cavity in the HBV group was observed in 93.7%, which with a significant degree of reliability was many times higher than the frequency of occurrence of this indicator in non-HBV patients (p <0.001). Hemorrhages on the buccal mucosa and hard palate were detected in 91.6% of the examined patients with HBV, and telangiectasia on the buccal mucosa in the HBV group was detected in 26.3% of cases. In both cases, they were statistically significantly different from the non-HBV group (p<0.001). The examination of the tongue also revealed symptoms that were absent in the non-HBV group. The presence of coated on the surface of the tongue was found in 84.2% of examined HBV, and foci of epithelial desquamation of the surface on the tongue - in 18.9% (p<0.001).

A similar picture was observed in patients with HCV. And so, erosions on the lips were detected in 13.5% of cases, the presence of cracks in the corners of the mouth was observed in 42.7%. The incidence of these two indicators in the non-HCV group was 1%.

Disturbances in the relief of the oral cavity in the examined population with HCV were detected in 89.6%, in the non-HCV group - in 3%. When analyzing the above-described indicators, the difference in data was statistically significant compared with the non-HCV group (p <0.001). Hemorrhages on the buccal mucosa and hard palate were observed in 78.1% of those examined and in 67.7% of cases, telangiectasia on the buccal mucosa. These two signs were absent in the non-HCV group and were statistically significant (p<0.001). Obviously, these signs are pathognomonic for HCV.

The examination of the tongue also revealed symptoms that were absent in the non-HCV group. The presence of coated on the surface of the tongue was found in 93.8% of examined HCV, and foci of epithelial desquamation of the surface on the tongue - in 62.5% (p<0.001). Compared to the non-HCV group, the difference in data in both cases was statistically significant (p<0.001).

To confirm the results of clinical data, we conducted a morphological study of a biopsy sample of the oral mucosa. Biopsies were taken from the affected areas of the oral mucosa. All signs of damage to the oral cavity were presented in Table 3.

### Table 3.
The main morphological changes in the oral mucosa with HBV and HCV

<table>
<thead>
<tr>
<th>Sign</th>
<th>HBV n=20</th>
<th>HCV n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>absolute number</td>
<td>%</td>
</tr>
<tr>
<td>Inflammatory infiltration</td>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>20</td>
</tr>
<tr>
<td>Circulatory disorders</td>
<td>Absent</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>19</td>
</tr>
<tr>
<td>Ulcerations of the mucous membrane with fibrinous deposits</td>
<td>Absent</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>1</td>
</tr>
<tr>
<td>Fibrosis of the mucous membrane</td>
<td>Absent</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>3</td>
</tr>
<tr>
<td>Dystrophic changes in squamous epithelium</td>
<td>Absent</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>18</td>
</tr>
<tr>
<td>Lymphoplasmacytic infiltration</td>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>20</td>
</tr>
<tr>
<td>Neutrophil admixture</td>
<td>Absent</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>8</td>
</tr>
</tbody>
</table>
In addition to the main pathomorphological changes, we identified such criteria as lymphoplasmacytic infiltration and an admixture of neutrophils. In the oral cavity, the main localization of the pathological process was the mucous membrane of the cheek - in 55%, gums - in 45% of patients. Inflammatory infiltration was determined in the form of lymphoplasmacytic in 90% (18) of patients with HCV and with HBV, this sign was observed in all patients (Fig. 1 a, b). An admixture of neutrophils was observed in 40% (8) of patients with HBV and 20% (4) of patients with HCV. Ulcerations of the mucous membrane were also noted. 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. Dystrophic changes in the squamous epithelium were in the form of acanthosis, parakeratosis and thickening.

The results of the histological study show that almost all groups of patients showed combinations of the pathohistological signs described above. Signs of inflammation of the oral cavity mucous were observed in biopsy specimens of all examined patients in the form of pronounced inflammatory cell infiltration mainly in the upper layers of the mucous membrane (inflammation was predominantly productive in nature). The inflammatory infiltrate was represented mainly by lymphocytes, plasma cells and segmented leukocytes. This is a manifestation of both severe chronic inflammation and the participation of immune mechanisms in the development of the pathological process.

Figure 1. Angiomatosis and lymphoplasmacytic infiltration in the lamina propria of the mucous membrane in patients with HBV (a) and HCV (b). Lymphoplasmacytic infiltrate is located predominantly under the squamous epithelium (hematoxylin and eosin staining x 100)

The severity of cellular reactions is an indicator of the activity of the immune system in relation to cellular surveillance. Circulatory disorders occurred with almost the same frequency as inflammatory infiltration. Lymphoid infiltration was also detected around unevenly full-blooded microvessels, at the border of the epithelium with the underlying tissue, in some cases with migration of the infiltrate into the thickness of the epithelial layer (Figure 2a, b).

Figure 2. Migration of the inflammatory infiltrate into the thickness of the epithelial layer during exacerbation of stomatitis (staining with hematoxylin and eosin x 400)
In the squamous epithelial layer, signs of thickening, focal keratinization, acanthosis (Figure 3. a, b) and dystrophic changes (hydropic and vacuolar degeneration) in the cells were found - in 90% (18) of cases with HBV and HCV (Figure 4. a, b).

A morphological examination of the oral mucosa revealed swelling and hyperemia with multiple erosions of irregular shape. Segmented leukocytes predominated in the infiltrate: with HBV in 40% (8) and with HCV in 20% (4) of cases. In this case, damage to epithelial cells and the appearance of vacuoles in their cytoplasm were detected, i.e. vacuolar, up to balloon dystrophy, death and desquamation of the epithelium with the formation of microerosions, which were often detected only by microscopic examination. Erosions or microulcers were covered with fibrinous deposits in 5% (1) of cases with HBV (Figures 5 a, b).

**Figure 3.** Leukoplakia of the oral mucosa (a). Signs of focal keratinization in the thickness of the epithelial layer (b). (Staining with hematoxylin and eosin x400)

**Figure 4.** Hydropic (a) and vacuolar (b) degeneration in the cells of the epithelial layer (staining with hematoxylin and eosin x 400)

**Figure 5.** Aphthous stomatitis (a). Microerosions of the mucous membrane are covered with fibrin deposits (b). Superficial ulcers of the oral mucosa are covered with fibrinous deposits (hematoxylin and eosin staining x 100)
As the inflammatory process in the oral cavity weakened, bleeding decreased and erosions became epithelialized. Changes caused by the development of sclerosis (proliferation of connective tissue) of the mucous membrane were determined in 15% (3) of cases with HBV and in 95% (19) with HCV. Healing of the oral mucosa occurred against the background of a higher density of newly formed vessels and the appearance of collagen fibers (Figures 6 a, b).

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

Discussion

There is practically no pathology that does not affect the condition of the oral mucosa (OM). 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. Lesions of the oral mucosa aggravate the course of diseases and serve as an important addition to the characteristics of the general clinical picture of viral hepatitis. Viral liver damage plays an important role in assessing the dental health of patients. At the same time, dentists and doctors of other specialties do not pay due attention to the condition of the oral cavity in liver diseases. The experience of dentists in infectious diseases departments has shown that the effectiveness of diagnosis and treatment of lesions of the oral cavity depends on the earliest possible examination of the admitted patient.

In the available literature, there are few works that would study and systematize the symptoms of damage to the oral cavity in HBV and HCV, especially early manifestations of the disease. 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 OM 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 OM lesions in patients suffering from chronic hepatitis (HBV - 20, HCV - 23 patients) and liver cirrhosis caused by HBV (15 patients) or HCV infection (16 patients). Presenting data on a few cases of OM lesions using descriptive analysis, the authors came to the conclusion that there is no connection between chronic HBV and HCV, as well as the stage of the disease with the frequency and nature of OM lesions. Given the small number of patients and the lack of proper statistical analysis, one has to doubt the reliability of the authors’ conclusions.

A comparative study of damage to the oral mucosa clearly shows that symptoms such as cracks in the corners of the mouth, disturbances in the relief of the oral cavity, plaque on the surface of the tongue, which often occur with viral hepatitis B and C, after complex treatment undergo almost the same reverse dynamics...
with a high degree of significance (p<0.001). Similarly, indicators for the presence of hemorrhages on the buccal mucosa and hard palate improve in viral hepatitis B and C. Desquamation of the epithelium on the surface of the tongue, on the contrary, is detected more often in HCV with significant dynamics towards reverse development after complex treatment. With HBV, the number of patients with desquamation was small and, accordingly, the difference in data before and after complex treatment was unreliable. As for telangiectasia, the latter is a pathognomonic symptom of HCV, undergoing significant reverse dynamics after treatment.

The problem of liver diseases of viral etiology is still extremely relevant due to their widespread distribution. The global coverage of territories and the high epidemic potential of this group of diseases retain their social and economic significance. Pathologies caused by HBV and HCV most often occur in young people of working age, leading to disability and a fairly high mortality rate. Thus, the study of the state of OM in viral hepatitis B and C, the characteristics of pathomorphological changes is very important, and therefore our work was carried out.

The paper contains more detailed results which are few or lacking in the similar publications available.

The weakness of the study was the limited number of biopsies, which was associated with the high cost of the study and the refusal of patients to undergo a biopsy.

Conclusion

In viral hepatitis B and C, the leading positions are occupied by two leading pathognomonic symptoms - Disorders in the mucous membrane relief OM and the presence of plaque on the surface of the tongue. Hemorrhages on the buccal mucosa and hard palate are characteristic of viral hepatitis B and C, telangiectasia and foci of desquamation of the epithelium on the surface of the tongue are characteristic of HCV. During a pathomorphological study of OM in viral hepatitis B and C, inflammatory infiltration, circulatory disorders and dystrophic changes in the squamous epithelium were detected in all patients, fibrosis - in all patients with HCV. Lymphoplasmacytic infiltration was detected in almost all patients with HBV and HCV.

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.

Source of funding

The work was not funded.

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Patients with Viral Hepatitis B and C.


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МОРФОЛОГИЧЕСКИЕ ОСОБЕННОСТИ БИОПТАТОВ СЛИЗИСТОЙ ОБОЛОЧКИ ПОЛОСТИ РТА У ПАЦИЕНТОВ С ВИРУСНЫМ ГЕПАТИТОМ В И С

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Резюме

Введение: В настоящее время заболевания слизистой оболочки рта и пародонта являются важной проблемой стоматологии. Исследования полости рта при хронических диффузных заболеваниях печени представляют большой интерес для клиницистов так как патологические процессы, развивающиеся в печени, как правило, приводят к органическим и функциональным нарушениям в слизистой оболочке полости рта.

Цель исследования явило изучение морфологических особенностей биоптатов слизистой оболочки полости рта у пациентов с ВГВ и ВГС.

Материал и методы: В исследование участвовали 95 пациентов с ВГВ, 96 пациентов с ВГС с поражением СОР и пародонта, а также 100 пациентов контрольной группы без ВГВ и без ВГС, но имеющие поражения СОР и пародонта. Возраст пациентов колебался 21-64 лет. Во всех обследуемых нами группах проведены по 20 морфологических исследований.

Результаты: Для изучения стоматологического статуса учитывались жалобы пациентов и данные клинического осмотра полости рта, который включал: внешний осмотр губ и углов рта, оценку состояния различных отделов слизистой оболочки рта. Для подтверждения результатов клинических данных нами было проведено морфологическое исследование биоптата слизистой оболочки полости рта. Биоптаты брались с пораженных участков слизистой полости рта.

Выводы: При патоморфологическом исследовании СОР при вирусных гепатитах В и С воспалительная инфильтрация, расстройства кровообращения и дистрофические изменения плоского эпителия выявлены у всех пациентов, фиброз – у всех пациентов с ВГС. Лимфоплазмоцитарная инфильтрация определена почти у всех пациентов с ВГВ и ВГС.