ARTICLE

DEVELOPMENT OF PERSONALIZED DIAGNOSTIC IN LEUKOPLAKIA (CLINICAL AND PREVENTIVE ASPECTS)

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ABSTRACT

Leukoplakia is considered one of the most common varieties of keratosis, characterized by chronic course and affecting the oral mucosa and the vermilion border. Early methods of diagnosis of this disease is the key to a successful prognosis. In order to improve the quality of diagnostic features of the oral mucosa, immunohistochemical study of biopsy material in patients with different forms of leukoplakia was carried out. As a result, the results of the immunohistochemical method of diagnosis, allowing to timely determine the transformation of the cells of the oral mucosa with the identification of tissue antigens using a set of monoclonal mouse antibodies.

KEY WORDS
oral mucosa, public health, leukoplakia, immunohistochemical study, precancerosis, immunohistochemical markers.

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INTRODUCTION

Leukoplakia is considered one of the most common varieties of keratosis, characterized by chronic course and affecting the oral mucosa and the vermilion border [1-3]. Leukoplakia is characterized by the presence of foci of hyperkeratosis with the phenomena of chronic inflammation in areas of the oral mucosa, which normally are not keratinized [1, 4, 5]. In the general structure of medical care for patients in dental medical organizations, leukoplakia affects age groups from 21 to 34 years old, from 35 to 44 years old, and over 55 years old, mainly men (4.3% compared with 1.9% of women) [6].

Mucosal leukoplakia of the oral mucosa is an optional precancer, the degree of probability and frequency of malignancy are not clearly defined and, according to various domestic and foreign scientists, vary from 15 to 70%. 100% of cases of oral leukoplakia treat 5.6% of precancerous conditions and 4.87% of cases of early cancer. These are patients with a verrucous and erosive-ulcerative form of leukoplakia, whose precancer condition can transform into invasive squamous cell cancer [1]. Therefore, any case of leukoplakia that cannot be clearly defined and is not clearly benign requires research to diagnose a precancerous condition or cancer on time [1, 7, 8] It is extremely important to make medical workers aware on time of the emergence of new medical technologies that improve the quality of medical services provided [9]. One of such diagnostic methods is immune histochemical. Immunohistochemistry is a highly accurate modern diagnostic method that allows you to identify and identify antigens in the clinical material that are inherent in pathological conditions [2, 3, 7].

The relevance of the topic of this work is due to the high prevalence of leukoplakia of the oral mucosa and the complexity of diagnosing its various types and the beginning of the malignancy process based on only clinical data. Therefore, any case of leukoplakia that cannot be clearly defined and is not clearly benign requires research to diagnose a precancerous condition or cancer on time [1, 4, 5]. It seems promising to use modern diagnostic technologies to accurately assess the clinical picture of the oral mucosa, to differentiate the type of its lesion, to establish the correct diagnosis [2, 5].

The objective of the study was to determine the most important immune histochemical signs of inflammation of the oral mucosa, as well as the state of the microvascular bed in patients with leukoplakia.

Tasks during the study are to compare the immune histochemical data of the features of the mucous membrane of the cheeks of practically healthy individuals with the data in patients with leukoplakia.

MATERIALS AND METHODS

To achieve this goal, research biopsy material was studied, obtained by a selective method from archival material from 52 patients with various forms of leukoplakia of the oral mucosa, living in Kazan and being treated at the Republican Clinical Oncologic Dispensary of the Ministry of Health of Russia for the period of 2017-2019. The control objects of the study were fragments of the mucous membrane of the cheeks of 52 healthy individuals who died from accidental causes (forensic autopsy). Sectional material was sampled at the pathology department of the RKOD of the Ministry of Health of Russia. Written consent from the subjects was taken and the study was approved by the Kazan University ethical committee.

The criteria for the selection of sectional material were: age from 20 to 55 years, a section of the entire thickness of the mucous membrane of the vestibule of the mouth in the area of premolars with sizes within 3

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cm. Histological material was collected in the accessible region of transition of the attached mucous membrane of the alveolar process of the upper and / or lower jaw into the mucosa the shell of the cheeks or lips in the area. The material was fixed in 10% neutral formalin according to Lilly's method or in Bowen liquid. According to the generally accepted technique, after appropriate posting with increasing concentration of alcohols, processing in xylene followed by embedding into paraffin [4].

4-5 μm thick sections were obtained on a LeicaSM 2000R sled microtome. The obtained specimens were stained with hematoxylin, eosin, according to van Gieson, and also according to picro-Mallory, which allows identifying "young", "mature", and "old" fibrin [5].

For immune histochemical studies, paraffin sections were straightened in a HistobatLEICAHI 1210 water bath and placed on slides treated with poly-L-lysine and dried at 35 ° C for one hour [2]. After dewaxing, washing, and dewatering, antigens were unmasked in citrate buffer (DAKO: Target Retrieval Solution, pH 6.0, code S 169984-2). Suppression of endogenous peroxidase was carried out using a 3% hydrogen peroxide solution. For the production of immune histochemical reactions with the identification of tissue antigens, a set of monoclonal mouse antibodies was used [Table 1].

Table 1: Characterization of primary antibodies

Antibody	Clone	Specificity	Working dilution	Manufacturer
CD 45	MS355-R	all leukocytes	1:200	Thermo
myeloperoxidase	polyclonal, RB-373-A code	neutrophilic leukocytes	1:800	Dako
CD 3	SP7	T-lymphocytes	1:150	Lab Vision
CD 20	L26	B-lymphocytes	1:250	Lab Vision
CD 38	AT13/5	plasma cells	1:100	Diagnostic Biosystems
CD 68	PGM1	macrophages	1:200	BioGenex
CD 31	9611	vascular endothelium	1:20	BioGenex
collagen IV	PHM-12 + CIV22	basal membranes	1:150	Lab Vision
pancytokeratins (CKR)	AE1/AE3	epithelium	1:300	Lab Vision
vimentin	V9	fibroblasts, connective tissue	1:300	Lab Vision
Ki 67	B56	proliferative cells	1:50	Pharmingen

The primary antibodies were diluted with a special buffer with a component that prevents the non-specific binding of antibodies (DAKO: Antibody Diluent with Background Reducing Component, code S3002). The exposure of the first MCAD was 1 hour at a constant (30°C) temperature, supported by a heating plate (LEICAHI 1220 histological plate). Then, the glasses with slices were washed for 10 minutes in Tris buffer. The binding of the primary antibodies to cellular and structural elements was determined using the standard biotin-streptavidin-peroxidase method (DAKO: LSAB® + System-HRP, K0690 code) with diaminobenzidine as a chromogen. After washing in distilled water, the specimens were additionally stained with Mayer gamatoxylin for 1-2 minutes. Then followed by repeated washing in water (15 min.), dehydration in 96% alcohol (10 min.) and clarification in carbol-xylene (5 min.). Slices were enclosed in Canadian balsam or in special media from DAKO (Ultramount, Far amount, code S302580-2).

To assess the detected changes, recommendations were used on the pathomor phological diagnosis of pathological manifestations in accordance with modern requirements of evidence-based medicine [10].

RESULTS

Given the generally accepted classification by A.L. Mashkilleison (1984), we identified 2 research groups: 1 - control (intact mucous membrane of the cheek); 2 - forms of leukoplakia [1].

Examination of the control material of the first study group revealed the intact mucous membrane of the cheek in the form of a multilayer squamous non-keratinizing epithelium and its own plate, which boundlessly flows into the submucosal base. Immunohistochemically, the entire epithelial layer, consisting of the basal, intermediate, and surface layers, is evenly MCAD-stained against pan-cytokeratins. The basement membrane is contoured as a continuous thin line of MCAD against type IV collagen. The nuclei of mitotically active cells of the basal layer express MCAD against Ki 67.

The connective tissue of the own plate of the mucous membrane contains fibroblasts, lymphocytes, macrophages, individual plasmocytes, white blood cells, and mast cells that do not penetrate the epithelial layer. Most of the cellular elements that infiltrate the connective tissue base express CD 45 antigen. An immune histochemical analysis of their own platelet of the mucous membrane and submucosa shows high expression of MCAD against vimentin - V9. Neutrophilic leukocytes are found as MCAD-detected single cells against myeloperoxidase.

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DISCUSSION

The conducted immune histochemical analysis with monoclonal mouse antibodies in squamous leukoplakia testified to the expression of MCAD against CD 45 (total leukocyte antigen, clone MS355-R), CD 3 (T-lymphocytes, clone SP7), CD 20 (B lymphocytes, clone - L26), CD 68 (macrophages, clone - PGM1), CD 38 (plasma cells, clone - AT13/5) and myeloperoxidase (neutrophilic leukocytes, code - RB-373-A). In addition, compared with the control, the connective tissue has the increased expression of MCAD against vimentin (fibroblasts, connective tissue, clone - V9). Changes in the blood vessels of the mucous membrane and submucosa were determined, which confirms the reaction with MCAD against CD 31 (vascular endothelium, clone - 9611), showing swelling of the endothelium, and with MCAD against type IV collagen (basement membranes, clone - PHM-12 + CIV22) - uneven coloring and thickening of the vascular basement membranes.

In the case of verrucous leukoplakia, immune histochemical analysis clearly demonstrated chronic diffuse inflammatory cell infiltration of the lamina propria and submucosa with a predominance of CD3 (T-lymphocytes, clone SP7), CD 20 (B-lymphocytes, clone L26), CD38 (plasma cells, clone - AT13/5) and CD 68 (macrophages, clone - PGM1) and the formation of "pearls" as a result of cell polymorphism. In some areas, pronounced sclerosis of the plate of the mucous membrane and submucosa is observed with the increased expression of MCAD against vimentin (fibroblasts, connective tissue, clone - V9). The reaction with MCAD against CD 3 (T-lymphocytes, clone - SP7) confirms T-lymphocyte infiltration. own plate of the mucous membrane with penetration of cells into the epithelial layer. Microcirculatory disorders progress, accompanied by the deposition of masses of “mature” and “old” intra- and extravascular fibrin, which is confirmed by the reaction with MCAD against CD 31 (vascular endothelium, clone - 9611).

CONCLUSION

Distinctive features in erosive-ulcerative leukoplakia is the formation of erosion with a violation of the epithelial lining or deeper ulcerative defects reaching the muscular layer of the cheek. In cell infiltrate, along with lymphocytes, plasma cells and macrophages, a large number of neutrophilic leukocytes appears, sometimes with the formation of leukocyte-necrotic masses. An immune histochemical study in this case shows a significant increase in the expression of MCAD against myeloperoxidase, especially on the surface of a mucosal defect. Summarizing the analysis of studies, we can conclude that immune histochemical markers can become determinative in the prognostic aspect for the early diagnosis and determination of tactics of therapeutic measures for leukoplakia of the oral mucosa. Orientation in the stages of precancerous changes is important for the oncological alertness of a practitioner.

CONFLICT OF INTEREST

There is no conflict of interest.

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