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Research Article

IMMUNOHISTOCHEMICAL MARKERS IN DIAGNOSTICS OF ORAL PRECANCEROUS DISEASES

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ABSTRACT

Over the past 15 years, the incidence of cancer of the oral cavity, pharynx, and larynx has increased by 15-17%, and almost 90% of patients are working people (30-60 years old). There were 32 patients (15 men and 17 women) aged 35 to 60 years with oral precancerous diseases examined at the department of Therapeutic Dentistry of Tashkent State Dental Institute. An IHC study in patients diagnosed with flat leukoplakia did not reveal neoplastic transformation of epithelial cells; the main changes in the epithelium were characterized by hyperplasia with cell proliferation.

KEYWORDS

Oral precancerous diseases, diagnostics, leukoplakia, immunohistochemistry.

INTRODUCTION

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Nowadays, cancer incidence and the fight against it are important tasks in medicine, and in oral medicine in particular [1,2]. According to the International Cancer Research Foundation (WCRFI) cancer morbidity and mortality is directly related to the level of economic development and, despite the fairly high standard of living in the world, continues to grow constantly [3]. According to WHO (1993), cancer damage to organs and tissues of the mouth is in 4th place, after cancer of the lung, stomach and colorectal area [4]. According to literature sources, about 6,000 patients with oral cancer are diagnosed every year in Russia. About 40% of all head and neck cancer incidence is due to oral cancer. In terms of frequency of occurrence, after laryngeal cancer, oral cancer ranks 2nd. In the structure of oncological morbidity in Russia, oral cancer accounts for 1.5% of all oncological diseases of various organs and systems [3,1]. Over the past 15 years, the incidence of cancer of the oral cavity, pharynx, and larynx has increased by 15-17%, and almost 90% of patients are working people (30-60 years old). Despite the localization of tumors being quite convenient for examination, 60-70% of patients, according to V.A. Lazareva, seek treatment for tumor processes at stages III-IV [5]. Tumors of the maxillofacial region are diverse both in morphological structure and in the variability of clinical manifestations, and therefore early diagnosis and treatment of these diseases are still difficult [6]. Precancerous conditions in most cases precede cancer. The concept of precancer refers to chronic inflammatory processes, benign neoplasms, disturbances in the keratinization process , and atypical keratinization [6]. According to modern literature sources, precancers account for from 15.2 to 84.9% of all diseases of the oral cavity [7]. Precancer does not cause complaints in patients for a long time, and therefore is often not diagnosed in the early stages. Great difficulties arise in differentiating precancer from the onset of malignancy due to the variety of precancerous diseases in clinical course, morphology, and in the early stages of malignancy due to the lack of clear clinical signs [6]. It is well known that there is a high probability of precancerous diseases becoming malignant, so their timely detection and treatment is very important, which increases the chance of preventing the development of cancer and increasing patient survival [4]. Recognizing early forms of cancer prevents the progression of the malignant process, and treating a tumor in the early stages helps reduce mortality, which solves an important medical-biological and social problem in oncology [5]. The standard diagnostic algorithm includes a survey and examination. The most commonly used are visual and visual-instrumental methods, and also use cytological, histological methods, vital staining techniques, stomatoscopy, biomicroscopy. There are also histochemical methods, DNA cytometry , luminescent and radioisotope studies, and electron microscopy. The development of spectroscopy techniques is promising [4]. The histological method is the main

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method of differential diagnosis for a dentist [6]. In this study of the material, two classifications are used: the clinical classification of A.L. Mashkileison (1970) and WHO classification (2005). To assess the degrees of dysplasia, according to the WHO classification, doctors use squamous intraepithelial neoplasia (Squamous Intraepithelial Neoplasia – SIN) from 1 to 3 – depending on the severity of dysplasia [8]. This classification quite clearly describes the stages of malignancy , however, it is not always possible to practically assess the severity of dysplasia, and therefore a variety of additional techniques are used [9]. Currently, immunohistochemical diagnostics make it possible to clearly differentiate various tumors to identify the expression of the proliferation marker Ki-67, the marker of apoptotic activity P53 and cell adhesion proteins. It is this study that helps determine the degree of dysplasia of the epithelium of the oral cavity, since the availability of clinical and histological data does not always allow an accurate assessment of the degree of malignancy. In all phases of the mitotic cycle, except for GO, the Ki-67 protein is observed in the cell - a universal marker of proliferating cells, which has important prognostic significance for varying degrees of dysplasia. For histological examination and immunohistochemistry , sections of the mucous membrane 5 μm thick are mounted on glasses. The Ki-67 proliferation index (Ki67 PI) was determined by the ratio of the number of immunoreactive cell nuclei to the total number of cell nuclei in % [9,10]. In an immunohistochemical study of the mucous membranes in all studied preparations, a pronounced expression of Ki-67 was observed in the nuclei of proliferating cells. In the normal epithelium of the oral mucosa, all immunopositive cells were localized in the basal layer, while in leukoplakia (SIN1, SIN2 and SIN3), an immunohistochemical reaction with antibodies to Ki-67 was detected mainly in the nuclei of cells of the basal and parabasal layers. In the unchanged epithelium of the oral mucosa and in leukoplakia, in the superficial layers the number of these cells was less than 1%. In squamous cell carcinoma, the tissue architecture was completely disrupted and the division of the epithelium into layers was practically absent. Positively stained cells were distributed evenly from the basement membrane to the epithelial surface. To assess proliferative activity in normal epithelium of the oral mucosa, leukoplakia and squamous cell carcinoma, cells positively stained for Ki-67 were counted in all layers of the epithelium (PI 0) and separately. A number of studies have shown the highest proliferation index in squamous cell carcinoma, and also reveal a relationship between an increase in the epithelial proliferation index by Ki-67 and an increase in the degree of epithelial dysplasia. According to Kovyazin V.A. et al . It was found that the proliferative activity of cells in the basal layer of the mucous membrane of the mucous membranes decreases as the degree of neoplasia increases, while in the parabasal layer this indicator increases [9]. In

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immunohistochemistry of intact stratified squamous epithelium, pronounced expression of claudin-1 is noted in the cells of the basal, parabasal and spinous layers. Thus, in hyperplasia, a high degree of cell proliferation is manifested by a decrease in the level of claudin-1 expression, and severe neoplasia is manifested by the complete absence of this protein on the cell surface [10].

According to modern studies, the presence of the P53 protein is noted in the nuclei of cells of all layers of the epithelium of the oral cavity, in normal epithelium and in all types of leukoplakia. With the increase of dysplasia in all layers of the epithelium, the number of cells with this protein increases, their maximum number in squamous cell carcinoma [6]. Immunohistochemistry is also used to identify HPV16 antigens and proteins associated with HPV-P16INK4a in epithelial cells in various types of leukoplakia and cancer. Thus, according to a number of authors, increased expression of P16INK4a is an indirect indicator of HPV and thereby reflects a violation of the mechanisms responsible for cell proliferation. This indicator also confirms the presence of an infection with a high risk of developing neoplasia [11]. Optical coherence tomography (OCT) is a diagnostic method based on imaging the microstructure of tissues using near-infrared light [11,12]. According to modern literary sources, this research method is based on the difference in the optical properties of tissues depending on their structure. With OCT, it becomes possible to obtain images of subsurface structures at a depth of up to 2 mm. This method is used in clinical practice for the differential diagnosis of clinically similar diseases, precancers and cancer, fixing the boundaries of a malignant neoplasm, determining the optimal location for a biopsy, and also dynamic monitoring of the state of the oral cavity during treatment [12]. According to Rabinovich O.F. et al ., to describe OCT images of the SOP, concepts such as layering, structure, boundary characteristics, surface character, optical inhomogeneity, image depth, brightness, contrast are used. The main sign of malignancy is loss of structure, which is confirmed by a homogeneous homogeneous image with a shallow signal depth or its absence [11]. Currently, cancer screening methods are becoming increasingly popular . According to modern literature sources, screening is a system of primary selection of individuals with a latent disease through simple, safe and inexpensive methods for the purpose of further in-depth examination [6,13].

MATERIAL AND METHODS OF RESEARCH

There were 32 patients (15 men and 17 women) aged 35 to 60 years with oral precancerous diseases examined at the department of Therapeutic Dentistry of Tashkent State Dental Institute. The diagnosis of leukoplakia was made based on clinical examination, optical coherence tomography, histological and IHC studies. Histological examination is considered the “gold standard” for diagnosing diseases of the oral

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mucosa, providing objective information about structural changes in the tissue. The advantage of a biopsy is the ability to study the pathological process at the cellular level, but the main disadvantage is its invasiveness . The success of histological examination and the objectivity of the diagnosis largely depend on the correct location for taking the biopsy [1, 9]. According to the WHO classification (2005), leukoplakia without atypia , leukoplakia SIN1, SIN2 and SIN3 (Squamous Intraepithelial Neoplasia). IHC research makes it possible to characterize the pathological process in various layers of the oral mucosa epithelium at the molecular level [2, 3]. The collection of biopsy material for research was carried out with the written consent of the patients. The IHC study was carried out in accordance with the standard protocol. Tissues were fixed in 10% neutral formaldehyde (pH 7.4) and, after soaking in alcohol, embedded in paraffin with a melting point of 54 °C. For histological and IHC studies, serial sections 5 μm thick were mounted on poly- L-lysine- coated glass . Using mouse monoclonal antibodies, tissue antigens to Ki-67 were detected (clone - MM 1, Diagnostic Biosystems - 1:200), to keratin-8 (clone - TS1, Thermo scientific - 1:100) and using purified rabbit antiserum antibodies to claudin-1 (Thermo scientific - 1:200). Immune complexes were determined using a biotin-free detection system based on horseradish peroxidase (BioGenex , USA); sections were counterstained with Mayer's hematoxylin [3].

Results and discussion Of the 32 patients we examined, 20 were diagnosed with flat leukoplakia and 12 with verrucous form. Histological mucosal lesions in the clinical diagnosis of leukoplakia can range from hyperplasia to invasive cancer. The superficial keratin layer can be located above benign, mature stratified epithelium of the squamous or pseudoepitheliomatous type or on mucosa with mild, moderate or severe dysplasia (SIN 1, SIN 2 or SIN 3). With hyperplasia, thickening of the epithelium is noted due to an increase in one of its components - basal, spinous (acanthosis) or superficial (hyper- , parakeratosis) cell layers, without cellular atypia . Slight increase in cell density and cellular atypia possible due to inflammation. Histological examination of foci of verrucous leukoplakia against the background of thickening of the surface keratin layer reveals an expansion of the layer of spinous cells with dyskeratic changes. In the subepithelial connective tissue base, individual lymphomacrophage infiltrates are found. An IHC study in patients diagnosed with flat leukoplakia did not reveal neoplastic transformation of epithelial cells; the main changes in the epithelium were characterized by hyperplasia with cell proliferation (nuclear localization of the Ki-67 protein) in the basal and parabasal cell layers, the absence of ectopic expression of keratin-8 in epithelial cells and well-developed intercellular contacts (claudin-1). In patients with verrucous form of leukoplakia, according to IHC diagnostics, the following results were obtained: in 9 - SIN 1, in 1 - SIN 2

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and in 2 - SIN 3. In SIN 1, expression of the Ki-67 protein was noted in the nuclei of epithelial cells of the lower third of the mucous membrane , in the same zone, ectopic expression of keratin-8 and the absence of membrane staining of cells for claudin-1 were detected. In patients with SIN 2, nuclear expression of Ki-67 protein, keratin-8 and decreased expression of claudin-1 in the lower 2/3 of the mucosa were determined. In 2 patients with SIN 3, cell proliferative activity of the Ki-67 protein and ectopic expression of keratin-8 were observed in all layers of the mucosal epithelium in the absence of expression of the intercellular contact protein claudin-1. Thus, clinical examination and IHC examination biopsy material for proteins Ki-67, keratin-8 and claudin-1 are the most informative methods for diagnosing various forms of leukoplakia, in which malignancy is possible.

REFERENCES

1. A retrospective 20-year analysis of proliferative verrucous leukoplakia and its progression to malignancy and association with high-risk human papillomavirus /J. D. Upadhyaya, S. G. Fitzpatrick, M. N. Islam [et al.] // Head Neck Pathol. – 2018. –Dec., Vol. 12(4). – P. 500–510.
2. A study on the intrapapillary capillary loop detected by narrow band imaging system in early oral squamous cell carcinoma / R. Sekine, T. Yakushiji, Y. Tanaka [et al.] //J. Oral Maxillofac. Surg. Med. Pathol. – 2015. – Vol. 27. – P. 624–630.
3. Accuracy of autofluorescence in diagnosing oral squamous cell carcinoma and oral potentially malignant disorders: A comparative study with aero-digestive lesions / X. Luo, H. Xu, M. He [et al.]. – Text: electronic // Scientific Reports. – 2016. –Vol. 6. – 29943. – URL: https://www.researchgate.net/publication/305369840 (date of access: 09.10.2021).
4. Advances of salivary proteomics in oral squamous cell carcinoma (OSCC) detection: an update / R. Sannam Khan, Z. Khurshid, S. Akhbar [et al.]. – Text: electronic // Proteomes. – 2016. – Dec. 15, Vol. 4(4). – 41. – URL: https://pubmed.ncbi.nlm.nih.gov/28248250/ (date of access: 09.10.2021).
5. Alsarraf, A. Liquid-based oral brush cytology in the diagnosis of oral leukoplakia using a modified Bethesda Cytology system / A. Alsarraf, O. Kujan C. S. Farah // Journal of Oral Pathology & Medicine. – 2018. – Oct., Vol. 47(9). –P. 887–894.
6. Alsarraf, A. H. The utility of oral brush cytology in the early detection of oral cancer and oral potentially malignant disorders: A systematic review /A H. Alsarraf, O. Kujan, C. S. Farah // Journal of Oral Pathology & Medicine. – 2017. – Vol. 47(2). – P. 104–116.

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7. Berman, J. Candida Albicans: A molecular revolution built on lessons from budding yeast / J. Berman, P. Sudbery // Nature Reviews Genetics. – 2002. – Vol. 3(12). – P. 918–930.

8. Bombeccari, G. P. Oral Candida colonization and oral lichen planus /G. P. Bombeccari, A. B. Giannì, F. Spadari // Oral Disease. – 2017. – Vol. 23(7). –P. 1009–1010.