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RISK FACTORS FOR MALIGNANCY OF PAPILOMAS IN THE OROPHARYNGEAL MUCOUS MEMBRANE ASSOCIATED WITH HUMAN PAPILOMAVIRUS (HPV)

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INTRODUCTION

More than 150 HPV subtypes have been identified in individuals with persistent HPV infection [1]. Oral precancerous lesions (OPL) and changes in the mucous membrane remain unresolved due to the etiopathogenetic complex of HPV infection depending on the virus strains and the background of patients' immunity [3, 7]. In particular, human papillomavirus lesions are known as possible risk factors or cofactors for malignant neoplasms; however, most of the worldwide research is devoted to the diagnosis of HPV strains, their prevalence and possible oncopathogenesis [9]. Anyway, the role of local immune homeostasis (LIH) of the oral mucosa (OPM) in HPV infection remains unresolved as well as the degree of pathological changes depending on LIH of the OPM and predicting the recurrence of HPV infection. Human papillomavirus (HPV) has a significant tropism for epithelial and mucosal tissues and can be found in several anatomical regions of the oral cavity [5, 8]. An increase in the number of somatic and endocrine pathologies, an unfavorable environmental situation and pandemics that reduce the immune protection of the barrier structures of the integumentary tissues in the human body lead to an increase of pathogenicity of HPV infection in mucous membranes, particularly, the oropharynx [6]. Located in anatomically significant places, papilloma can lead

ABSTRACT — Analysis of the HPV positive papilloma structures at different levels of pathological process development was carried out. Classical staining of preparations with hematoxylin and eosin was used for immunohistochemical determination of Ki67-positive cells and phenotyping of CD positive cells. We found that the process of papilloma formation begins with a local increase in the proliferative activity of keratinocytes which contributes to the formation of a local epithelial convex above the surface of the oropharyngeal mucosa (OPM) in the form of papilloma.

The connective tissue of the OPM adjacent to the epithelium grows at the second stage. At the same time here is a decrease in immunocytes/macrophages number of the oral epithelium not only in the growth zone of the papilloma but in the adjacent neoplasm tissue.

The third stage is characterized by the destruction of the basal membrane of the OPM. Apoptotic cells in the cambial layer and forming leukocytes infiltrate the OPM lamina propria. Virus-infected keratinocytes are phagocytized by macrophages or exfoliated from the surface of the epithelial layer. The emerging in the middle layers defect of tissue of the epithelial layer and the absence of Langerhans cells indicate a relationship between the migration of antigen-presenting cells expressing CD68 with impaired differentiation and specialization of keratinocytes.

The conclusion is based on the analogy of "leukemic failure" in leukemia and on the absence of differentiating epithelial cells between the cambium and the specialized surface layer in the long-existing papilloma. The disappearance from the epithelium of CD68-positive cells specific to the epithelial layer is a prognostic sign of malignization in the mucous membrane of the oropharynx.

KEYWORDS — HPV; HPV-associated oral epithelial dysplasia; oral mucosa, CD cells, malignant neoplasms, papilloma, risk factors for malignant neoplasms, oropharyngeal mucosa.

to substantial dysfunctions and therefore its removal requires not only conservative treatment but also surgical removal [2]. Despite numerous studies, the reasons for the spontaneous regression of neoplasms on the mucous membrane of the oropharynx related to HPV infection are not properly investigated well so far and the mechanisms of their spread and progression to malignancy indicate the necessity for research in this direction [4].

Aim of the research:

to establish morphological criteria estimating the risk of malignancy for papillomas located at the mucous membrane of the oropharynx. In this regard, the following objectives were achieved:

1. To establish most character and diagnostically significant phenotypes in the composition of cell ensembles of papilloma structures.
2. To determine the morphological prognostic criteria indicating the possibility of spontaneous recovery or the transformation of papilloma into a chronic form during the HPV infection.
3. To establish the stages of papilloma formation.

MATERIAL AND METHODS

Biopsy material of papillomas located on the mucous membrane of the oropharynx was examined in 58 patients. The distribution of material by the topography of papillomas, age groups and duration of clinical manifestations of human papillomavirus infection is shown in Table 1.

Table 1. Distribution of clinical material of papilloma depending on age and localization

Age groups (years old)	Localization for extraction of the biopsy specimens from the mucous membrane at:				
	para-nasal sinuses	mouth	nasal cavity	pharynx	vocal cords
20–39	4	6	5	7	3
40–59	3	3	1	4	5
60 and more	2	4	4	3	4
Total	9	13	10	14	12
Total examined	58				

125 biopsies of the oropharyngeal mucosa from 58 patients were studied, obtained in accordance with fundamental ethical principles Declaration of Helsinki, GCP Rules (Good Clinical Practice) and approved by the ethical commission of Far Eastern Federal University, (Vladivostok, Russia). Classical staining of preparations with hematoxylin and eosin was used for immunohistochemical determination of Ki67-positive cells and phenotyping of CD effector cells according to DAKO protocols, PCR diagnostics to identify HPV etiology of papillomas. The analysis of the preparation and the production of illustrations were carried out using an Olympus Bx52 microscope and Olympus DP25 digital camera with original software.

RESULTS

All cases of HPV-positive dysplasia had a clinically specific visualized macro picture and histological appearance. Macroscopically, it has the shape of hemispheres of different sizes, sometimes reaching 6 mm in height. The edges of papillomas could not be clearly defined. Epithelial eminences could pass into tissues with signs of infiltration along the edges, but more often, had clear boundaries (Fig. 1).

We noted that structural features of papillomas correlate with the duration of the disease recorded in the patient's history. With a short period of the disease and doctor visits at earlier stage uneven thickenings prevail due to the keratinized layer of epithelial cells in the structure of papilloma. The basal membrane is well identified, the cambial and spinous layers are located parallel to the surface of the oropharynx mucous membrane. However, keratinocytes of the granular layer can protrude into the surface of the mucous membrane of the oropharynx (Fig. 2a).

In this case, the proper plate of the OPM does not protrude into the epithelial layers, the stroma is well expressed, but together with the epithelium it does not grow above the surface (Fig. 2a, b). The forming local outgrowths of the epithelium are formed due to an increase in the regenerative potential of the cells of the spinous layer and invagination of the granular layer.

With a chronic process and the absence of treatment, the growth of the connective tissue plate along with the epithelium is observed (Fig. 2c), with the formation of sometimes significant elevations above the surface of the OPM (Fig. 2d).

The basal membrane becomes folded, poorly identified, but the cell polarity remains. Crest-like growths of the epithelium are revealed, signs of apoptosis appear in the basal and spinous layers. Not only the epithelial surface of the mucous membrane acquired tuberosity and folding, but also the relief of the basement membrane with the adjacent connective tissue changes. There is infiltration into the spinous layer of large granulocytes with eosinophilic granules in the cytoplasm. We noted that despite the proliferation of the epithelium and then the connective tissue with the formation of a papilloma rising above the surface, the proliferative activity of the epithelium decreases, and the number of cells with signs of apoptosis increases. CD-positive cells disappear from the epithelial layer and are identified in the connective tissue of the lamina propria (Fig. 3).

This indicates a decrease of the local immunity in the mucous membrane and its barrier properties and the chronicity of HPV infection.

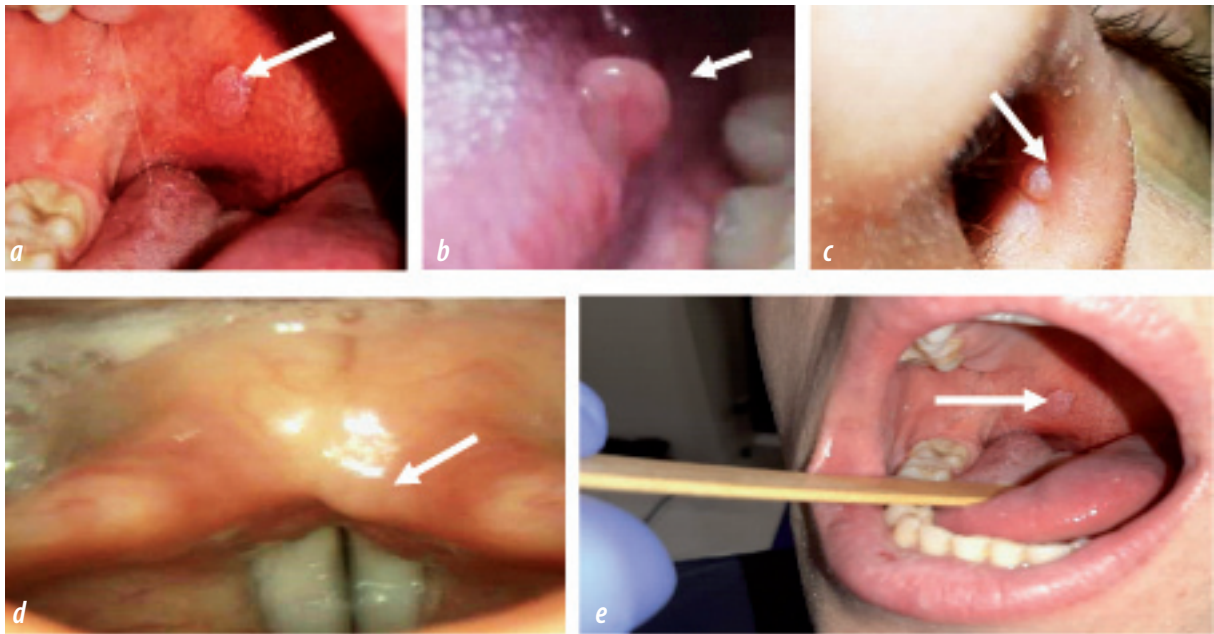


Fig 1. Papillomas of the mucous membrane: a — woman, 62 years old, palate; b — man, 45 year old, tongue c — man, 22 year old, the nasal cavity d — man 65 year old, oropharynx e — man 34 year old, palate

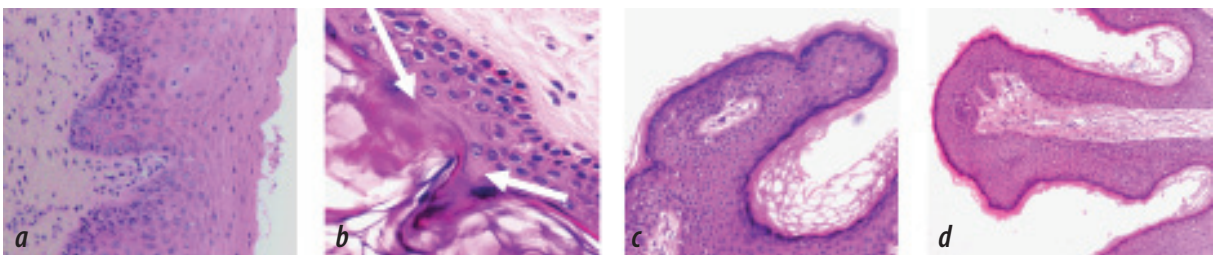


Fig. 2. a — mucous membrane in normal condition; b — 34-year-old patient with papilloma on the mucous membrane of the soft palate, which appeared 2 months later; c — 5 months later; d — 1.5 years later. Histological section of papilloma. Staining with hematoxylin and eosin. Zoom: a — ×200, b — ×400, c — ×200, d — ×200

DISCUSSION

Thus, we have established the stages of the papilloma development. The process of papilloma formation begins with a local increase in the proliferative activity of keratinocytes, which contributes to the formation of a local epithelial prominence above the surface of the mucous membrane of the oropharynx. At the second stage, the growth of the adjacent connective tissue of the OPM into the epithelial cap of the papilloma joins the process. At the third stage of the pathological process, the number of immunocytes/macrophages in the epithelial layer of OPM decreases not only in the papilloma growth zone, but also in the surrounding tissue. The fourth stage is characterized by the destruction of the basement membrane of

the OPM, apoptosis of the cells of the cambial layers, and the formation of a leukocyte infiltrate. Keratinocytes with contaminated viruses are phagocytized by macrophages or desquamated onto the surface of the epithelial layer. The emerging tissue defect in the differentiating layers of the epithelium and the absence of Langerhans cells indicate the relationship between the impaired differentiation and specialization of keratinocyte differentiation cells and the migration of effector cells expressing CD68 into the connective tissue of the OPM lamina propria.

CONCLUSION

A decrease of proliferative activity, an increase in the process of apoptosis and impaired differentia-

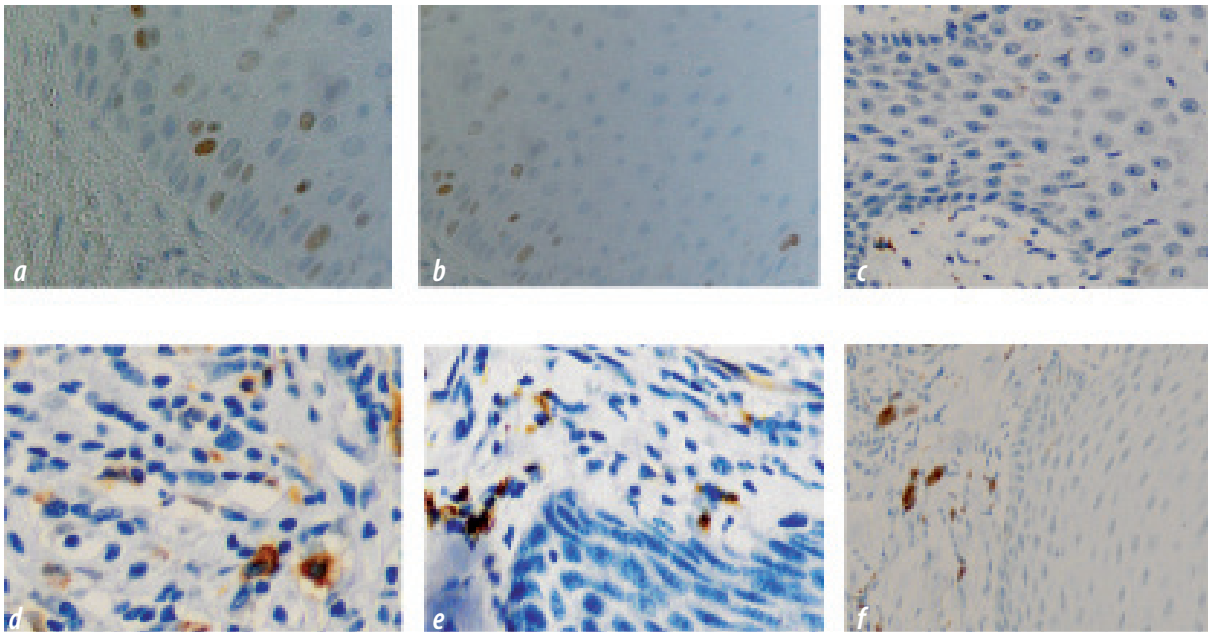


Fig. 3. 34 year old patient with papilloma on a mucous membrane of the palate. The duration of disease from the beginning of clinical manifestations: a — normal mucous membrane; b — 2 months; c — 5 months; d — 1.5 year; e, f — 2 years. Histological section of papilloma. Immune staining: a, b to identify Ki67; c, d, e, f — to identify CD68 and CD163 Immune staining. Zoom — $\times 400$

tion and specialization of epithelial cells resulting in a decrease of the barrier properties of the integumentary epithelium are a risk factor for carcinogenesis in the mucous membrane of the human oropharynx. The absence of differentiating layers of epithelial cells is similar to leukemic failure in leukemia.

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