ORIGINAL ARTICLE

IMMUNOHISTOCHEMICAL FEATURES OF THE EXPRESSION OF HUMAN PAPILLOMA VIRUS TYPE 16 IN PLEOMORPHIC ADENOMAS OF SALIVARY GLAND

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ABSTRACT

The aim is to reveal the immunohistochemical features of human papilloma virus type 16 expression in various histological variants of pleomorphic adenomas of the salivary gland.
Materials and methods: The material of the study was surgical and biopsy material from 30 patients with pleomorphic adenomas of the salivary glands, among which in 15 cases mesenchymal was detected, in 10 – mixed, in 5 cases – epithelial histological variant, respectively. Immunohistochemical study was performed, using mouse monoclonal antibody to human papilloma virus type 16. Visualization was performed, using an EnVision™ FLEX detection system. Histological sections of grade III cervical intraepithelial neoplasia (CIN III) were used as a positive control; for a negative control, the procedure was performed without primary antibodies. The immunohistochemical reaction was assessed by a semi-quantitative method by counting the percentage of positively stained cells in the field of view of a microscope × 400. Microspecimens were studied, photoarchived on an Olympus BX-41 microscope.
Results: Expression of human papilloma virus type 16 of varying severity was determined in 26 cases of pleomorphic adenomas of the salivary glands, which was 86.7%. The epithelial component of the pleomorphic adenoma of the salivary gland was characterized by a more pronounced expression of the monoclonal antibody to human papilloma virus type 16 compared to the mesenchymal component of the tumor. The severity of the immunohistochemical reaction with a monoclonal antibody to human papilloma virus type 16 depended on the histological variant of the pleomorphic adenoma of the salivary gland. Epithelial, mixed and mesenchymal variants of pleomorphic adenoma of the salivary gland were characterized, respectively, by the most pronounced, pronounced and moderately pronounced expression of a monoclonal antibody to human papilloma virus type 16.
Conclusions: A comprehensive immunohistochemical study with a monoclonal antibody to human papilloma virus type 16 revealed the presence of a causal relationship between the infection of a patient with human papilloma virus type 16 and development of pleomorphic adenoma of the salivary gland in him.

KEY WORDS: human papilloma virus type 16, immunohistochemistry, pleomorphic adenoma of the salivary gland

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INTRODUCTION

Salivary gland tumors are rare forms of head and neck tumors and benign cases constitute the greatest frequency since only 20 % are malignant. An overall European standardized rate of 4.2-4.9 per 100,000 person-years was reported with a female preponderance (1:1.43) and with an annual 1 % rise in female incidence [1].

Pleomorphic adenoma is the most common salivary gland neoplasm worldwide, accounting for 70-80 % of abnormal growths. It mainly occurs in the superficial lobe of the parotid gland but can also affect the submandibular and minor salivary glands [2].

Etiological factors, causing pleomorphic adenomas, should be well known to minimize their incidence [3]. The importance of human papilloma viruses in the development of pleomorphic adenomas of the salivary glands is a controversial issue. Some scientists note in their studies possible effect of human papilloma virus in pleomorphic adenoma development. On the other hand, some studies do not imply human papilloma virus as a causative agent of salivary gland pleomorphic adenoma [1].

Human papilloma viruses belong to the Papillomaviridae family. Papillomaviruses are one of the most heterogeneous groups of viruses that infect humans and animals. To date, more than 250 papilloma virus types have been identified, and each of these genotypes are associated with infection at particular anatomical sites [4]. Human papilloma viruses, due to differences in DNA sequence, are divided into alpha-, beta-, gamma-, mu- and nu-groups. Human papilloma viruses, taking into account the risk of developing malignant tumors, are classified into viruses of high carcinogenic risk (16, 18, 31, 33-35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70 types) and low carcinogenic risk (6, 11, 42, 43, 44 types) [5].

Oncogenic potential of papilloma virus type 16 is mainly due to the E6 and E7 oncoproteins, as they are key regulators of the cell cycle [6].

Oncoprotein E6 binds protein p53, leading to degradation of the latter by ubiquitin-dependent proteolysis, which results in a violation of the mechanisms controlling proliferation, apoptosis and DNA repair. Interaction of E6 protein with telomerase also enhances the proliferative activity of cells. The E7 oncoprotein action mechanism is associated with functional inactivation of the tumor suppressor pRb, resulting in the release of the transcription factor E2F, which activates genes whose protein products stimulate the cell entry into the S-phase of the cell cycle [7].

THE AIM

The aim is to reveal the immunohistochemical features of human papilloma virus type 16 expression in various histological variants of pleomorphic adenomas of the salivary gland.

MATERIALS AND METHODS

The material of the study was surgical and biopsy material from 30 patients with pleomorphic adenomas of the salivary glands, among which in 15 cases mesenchymal was detected, in 10 – mixed, in 5 cases – epithelial histological variant, respectively. The cut pieces were placed in cassettes. With the help of a cassette holder they were placed in a container for 24-36 hours for fixation in a buffered 10% formalin solution with a pH of 7.4. Conduction and filling the tissue with paraffin was performed using a histoprocessor «HISTOS-5» («Milestone», Italy) on the program for the operating material. After completion of the paraffin impregnation program, the cassettes were removed from the histoprocessor paraffin unit, and at the «HESTION TEC-2800 Embedding Center», the tissue pieces were filled with molten paraffin into molds, followed by solidification on the «HESTION TEC-2800 Cryo Console» refrigeration module. From the obtained paraffin blocks histological sections 3-4 μm thick were made, using a microtome «MICROM HM 325» («Thermo Fisher Scientific», Germany). Immunohistochemical study was performed, using mouse monoclonal antibody (MCA) to human papilloma virus type 16 (clone CAMVIR-1, «Diagnostic BioSystems», USA). Brown staining of cell nuclei characterized positive expression of the marker. Visualization was performed, using an EnVision™ FLEX detection system (Dako, Denmark). Antigen was unmasked in citrate buffer pH 6.0 at 95 °C. Primary antibodies have been incubated at room temperature for 30 minutes, secondary – for 20 minutes. Sections were counterstained with Gill hematoxylin. Histological sections of grade III cervical intraepithelial neoplasia (CIN III) were used as a positive control; for a negative control, the procedure was performed without primary antibodies. The immunohistochemical reaction was assessed by a semi-quantitative method by counting the percentage of positively stained cells in the field of view of a microscope × 400. Microspecimens were studied, photoarchived on an Olympus BX-41 microscope (Japan).

The obtained digital data were statistically processed using the Statistica 10.0 program. The average values were compared, using the nonparametric Mann-Whitney U test. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Among all cases of pleomorphic adenomas studied by us, the immunohistochemical reaction with MCA to human papilloma virus type 16 was positive in 26 cases, i.e. 86.7 %. Analyzing the severity of immunohistochemical expression among 26 cases, it was noted that in 7 – this reaction was mild, in 4 – moderately pronounced, in 15 cases – pronounced. Our data indicate that human papilloma virus type 16 can act as one of the exogenous trigger factors involved in the development of pleomorphic adenoma of the salivary glands.

In our earlier immunohistochemical study with polyclonal rabbit antibody to p16, in 23 cases out of 30 (76.7 %) this marker was found to be expressed in pleomorphic adenomas [8], which is known to be an indirect sign of the human papilloma virus integration of high oncogenic risk into the genome and transformation of epithelial cells into tumor cells [9].

In 26 cases of pleomorphic adenomas, nuclear expression of MCA to human papilloma virus type 16 was determined both in the epithelial (parenchymal) and mesenchymal (stromal) components of the tumor (figure 1). In the tumor parenchyma, a positive immunohistochemical reaction was detected in the nests and cords that formed epithelial cells, as well as in solid, trabecular, cystic, glandular, ductal and tubular structures. A few myoepithelial cells also expressed the above MCA. In the tumor stroma fibroblastic cells, immune cells, vascular endotheliocytes, as well as cellular elements of myxoid and mucoid zones were expressed MCA to human papilloma virus 16.

The positive immunohistochemical reaction found by us in vascular endothelial cells may indicate tropism of human papilloma virus type 16 to the vascular endothelium. Domestic scientists have also revealed a positive reaction in the vessels of the fallopian tubes, which, according to their data, indicates hematogenous spread of the virus in the human body [7].

In the course of a morphometric study, when calculating the percentage of positively stained cells in various pleomorphic adenomas, we found that the maximum, moderate and minimum values, respectively, were in epithelial, mixed and mesenchymal variants of the tumor (table 1). It was also revealed that in epithelial, mixed and mesenchymal variants of pleomorphic adenoma, the epithelial component of the tumor compared with the mesenchymal one was characterized by a large ($p<0.05$) number of positively stained cells.

An equally important issue is the study of human papilloma viruses' effect on the clinical course of pleomorphic adenoma of the salivary glands [9]. In our research, we did not study regularities of the clinical course of salivary glands pleomorphic adenomas, depending on the presence or absence of human papilloma virus type 16.

Table 1. Results of the immunohistochemical reaction in different types of pleomorphic adenoma of the salivary gland

Histological variant of the tumor	Results of the immunohistochemical reaction		
	% positively stained cells	% positively stained cells in epithelial tumor component	% positively stained cells in mesenchymal component of the tumor
Mesenchymal	27.2±3.22 *, ***	45.6±4.02	19.6±2.08 ****
Mixed	56.9±2.87 **, ***	50.5±3.95	21.5±3.19 ****
Epithelial	75.8±4.12 *, **	49.6±2.19	20.9±3.14 ****

* – differences are significant compared to the mixed variant of pleomorphic adenoma,
** – differences are significant compared to the mesenchymal variant of pleomorphic adenoma,
*** – differences are significant compared to the epithelial variant of pleomorphic adenoma,
**** – differences are significant compared to the epithelial component of the tumor.

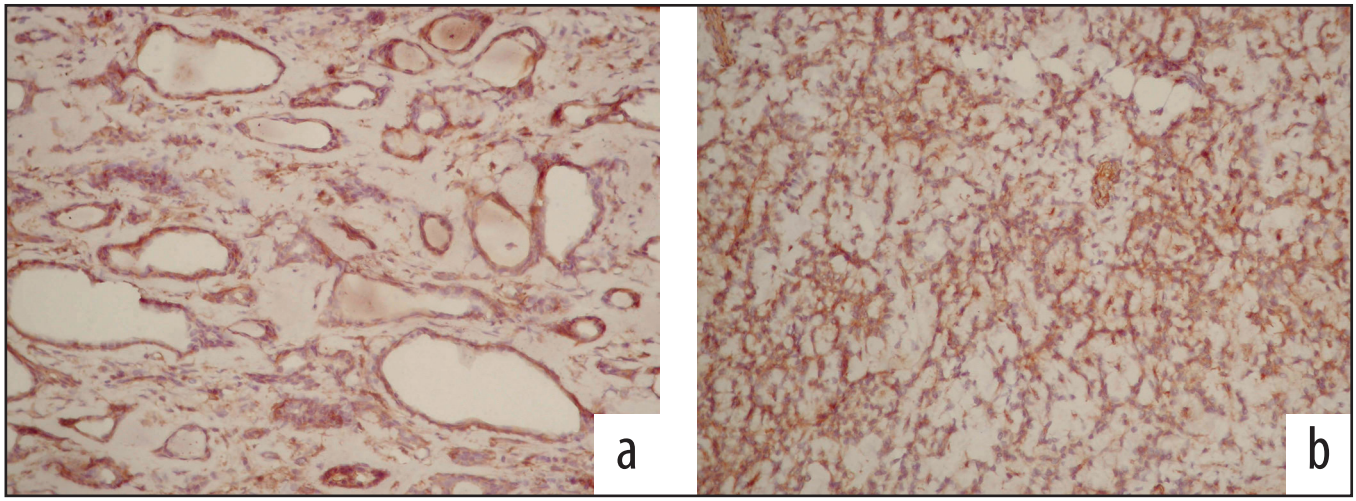

Fig. 1. Expression of MCA to human papilloma virus type 16 in the epithelial and mesenchymal components of the pleomorphic adenoma. Immunohistochemical reaction with MCA to human papilloma virus type 16, a) × 200, b) × 200.

Studies by foreign scientists show that presence or absence of human papilloma viruses in a patient is an independent predictor of survival in malignant tumors of the oropharynx and larynx [5]. It has been noted that human papilloma virus-associated oropharyngeal cancer has a significantly better prognosis compared to other cancers caused by alcohol and tobacco [10].

CONCLUSIONS

Based on our comprehensive immunohistochemical study, the following conclusions can be drawn:

1. Expression of human papilloma virus type 16 of varying severity is determined in 26 cases of pleomorphic adenomas of the salivary glands, which is 86.7%. The obtained data indicate the presence of a causal relationship between the infection of a patient with human papilloma virus type 16 and development of pleomorphic adenoma of the salivary gland in him.
2. The epithelial component of the pleomorphic adenoma of the salivary gland is characterized by a more pronounced expression of the monoclonal antibody to human papilloma virus type 16 compared to the mesenchymal component of the tumor.
3. The severity of the immunohistochemical reaction with a monoclonal antibody to human papilloma virus type 16 depends on the histological variant of the pleomorphic adenoma of the salivary gland. Epithelial, mixed and mesenchymal variants of pleomorphic adenoma of the salivary gland are characterized, respectively, by the most pronounced, pronounced and moderately pronounced expression of a monoclonal antibody to human papilloma virus type 16.

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Conflict of interest:
The Authors declare no conflict of interest.

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