ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Immunohistochemical expression of CK7, CK5/6, CK19, and p63 in Warthin tumor

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Abstract

Our study included a number of 24 cases with Warthin tumor, diagnosed between 2007–2011, which were analyzed in terms of clinical, histopathological and immunohistochemistry point of view, using CK7, CK5/6, CK19, and p63 antibodies. Warthin tumor is most often a tumor with a slow evolution, painless, usually affecting males (M/F 3.2/1) in the seventh decade of life. Histopathologically, it is distinguished the predominance of the typical forms of the tumor, with a balanced ratio epithelium/stroma. The immunostaining for CK7 showed positivity in all the investigated cases both in the columnar luminal cells and basal cells. The immunostaining for CK5/6 was positive in all the investigated cases in bilayer epithelial basal cells, both in the structure of the cysts and the papillae. In the case of the immunostaining for p63 we noticed limited nuclear positivity in the basal cells, while the columnar cells' nucleus were negative. The immunohistochemical study of the bilayer epithelial component of Warthin tumor showed different immunstaining of the two types of epithelia, the oncocytary columnar and the basal on, similar to those found in the salivary gland ducts.

Keywords: Warthin tumor, immunohistochemistry, pathogenesis.

Introduction

Benign tumors of the salivary glands have an important role in OMF pathology with an incidence ranging between 70–80% of all tumors located in the head and neck [1, 2], Warthin tumor representing 11% of salivary tumors benign [3]. It is considered that beside the pleomorphic adenoma this tumor is at least three times more frequently met than the other types of the salivary adenomas [4].

The most accepted hypothesis is that Warthin tumor origin comes from heterotopic salivary duct inclusions in the lymph nodes. This hypothesis is supported by the frequent detection of salivary gland tissue in the lymph nodes peri- and intra-parotidian [5, 6]. Also, the luminal cells on the periphery of the lymphoid stroma in Warthin tumor cases reveals a similar immuno-profile with the striated ducts of normal salivary glands [5].

The study analyzes the distribution of the cyto-keratins and the degree of the epithelial differentiation in Warthin tumor using a panel of specific antibodies.

Materials and Methods

The study included a total of 42 Warthin tumor cases diagnosed in 2007–2011 in the Pathology Laboratory of Emergency County Hospital of Craiova.

The operator pieces were fixed in 10% formalin, processed by the usual histopathological technique and Hematoxylin–Eosin stained.

For the immunohistochemical technique there was used the LSAB+ System–HRP technique (code K0690, Dako), using mouse antihuman monoclonal antibodies (Table 1).

Table 1 – ***Characteristic of used antibodies***

Antibody	Clone	Dilution	Antigen retrieval
CK7	OV-TL 12/30	1:50	Citrate, pH 6
CK5/6	D5/16 B4	1:100	Tris-EDTA, pH 9
CK19	RCK108	1:100	Tris-EDTA, pH 9
P63	4A4	1:50	Citrate, pH 6

The immunoreaction assessment was performed by semiquantitative analysis using a scale with three levels (<25%, 25–75% and >75%), and the reaction intensity was qualitatively assessed by using a scale with three degrees (+, ++ and +++).

To validate the reactions there were used negative external controls. The cases were analyzed clinically, histologically and immunohistochemically.

Results

The cases diagnosed with the Warthin tumor had a

maximum incidence in the seventh life decade (16 cases) including a number of 32 men and 10 women (M/F 3.2/1) the biggest incidence of these tumors being among the smoking persons (36 cases) than in the non smoking. The topographic repartition of the cases with the Warthin tumor has indicated almost exclusively the parotid gland localization, the most affected being the left parotid gland (24 cases).

Histopathologically, the tumor development pattern was a cystic papillary one. We have observed the presence of several cysts with double-layered epithelia formed by oncocytary basaloid or columnar cells, next to a lymphomatosis stroma.

Depending on the rapport between the stromal component and the parenchyma we have histopathologicaly ranged the cases with Warthin tumor in the subtypes: typical Warthin tumors (22 cases) with a balanced rapport between stroma and parenchyma, Warthin tumors with poor stroma (11 cases), Warthin tumors with abundant stroma (six cases) and metaplasic Warthin tumors (three cases) (Table 2).

The immunohistochemical study observed the expression CK7, CK5/6, CK19, and p63 at the level of the epithelial, luminal one and/or basal component of the Warthin tumor.

We have observed the positivity of the antibody used in all the investigated cases (Table 3).

Table 2 – ***Clinico-pathological parameters of the patients included in the study***

Clinico-pathological characteristics		No. of cases	%
Age [years]	30–50	10	23.81
	50–70	28	66.67
	>70	4	9.52
Gender	Female	10	23.81
	Male	32	76.19
Smoke	Smoker	36	85.71
	Non-smoker	6	14.29
Localization	Parotid gland	41	97.62
	Other localization	1	2.38
Histopathology	Stroma-rich	6	14.28
	Stroma-poor	11	26.19
	Typical	22	52.38

Table 3 – ***Immunostain evaluation for CK7, CK5/6, CK19 and p63***

Immunostain		CK7			CK5/6			CK19			P63		
		+	++	+++	+	++	+++	+	++	+++	+	++	+++
<25%	Luminal cells	-	-	-	-	-	-	-	-	-	-	-	-
	Basal cells	36	-	-	-	-	-	26	3	-	-	-	-
25–75%	Luminal cells	-	5	4	-	-	-	-	3	4	-	-	-
	Basal cells	6	-	-	-	2	9	11	12	-	-	12	11
>75%	Luminal cells	-	12	21	-	-	-	-	13	22	-	-	-
	Basal cells	-	-	-	-	4	27	-	-	-	-	12	7

The cytokeratins became positive at the membranary/cytoplasmatic level and p63 at the nuclear level.

The analysis of the immunostain at CK7 for the luminal columnar cells has indicated positivity in a percentage of more than 75% in 33 cases (78.6%), and for the rest of nine cases (21.4%) the luminal cells were positive in a percentage between 25 and 75%, while the immunostain of the basal cells has indicated in the majority of the cases positivity in under 25% of the cells (36 cases, which is 85.7%).

When speaking about the assessment of the immunostain intensity of the columnar cells we have noticed the presence of an intense immunostaining for most of the cases (21 cases).

For the basal cells, the immunostaining was in all the cases with reduced intensity. We may say that on the whole the stain for CK7 was more intense and better expressed at the level of the luminal epithelial component in comparison with the basal one (Figure 1, a and b).

The immunostain for CK5/6 has indicated that in most of the cases (31 cases, which is 69.9%) the basal cells were positive in a high percentage, over 75% of the cells, most of them showing raised intensity staining (+++) in 27 cases. Only in 11 cases (26.1%), the immunostain was present in 25–75% of the cells, having also risen intensity staining. The marking CK5/6 was absent at the level of the luminal cells (Figure 1, c and d).

The immunostain evaluation of the columnar cells at CK19 showed the fact that the great majority of the cases (35 cases – 83.3%) were positive in over 75% of the cells, with big and moderate intensity. In a reduced number of cases (seven cases, which is 16.6%), the positivity was present between 25–75% of the cells (Figure 2, a and b).

About the immunostain of basal cells, we observed that in the most of the cases (29 cases, namely 64%) there were positive in fewer than 25% of the cells. Also, we observed that the immunostain intensity at CK19 of this cell type was weak in most of the cases.

The immunostain for p63 showed positivity in 12 cases in a percentage between 25–75% and in other 12 cases over 75% of the bases cells.

About the immunostain intensity of the basal cells, we observed the presence of a moderate reaction (++) in 11 cases (26.2%) and in other nine cases (21.8%), the immunostain was highly positive (+++) (Figure 2, c and d).

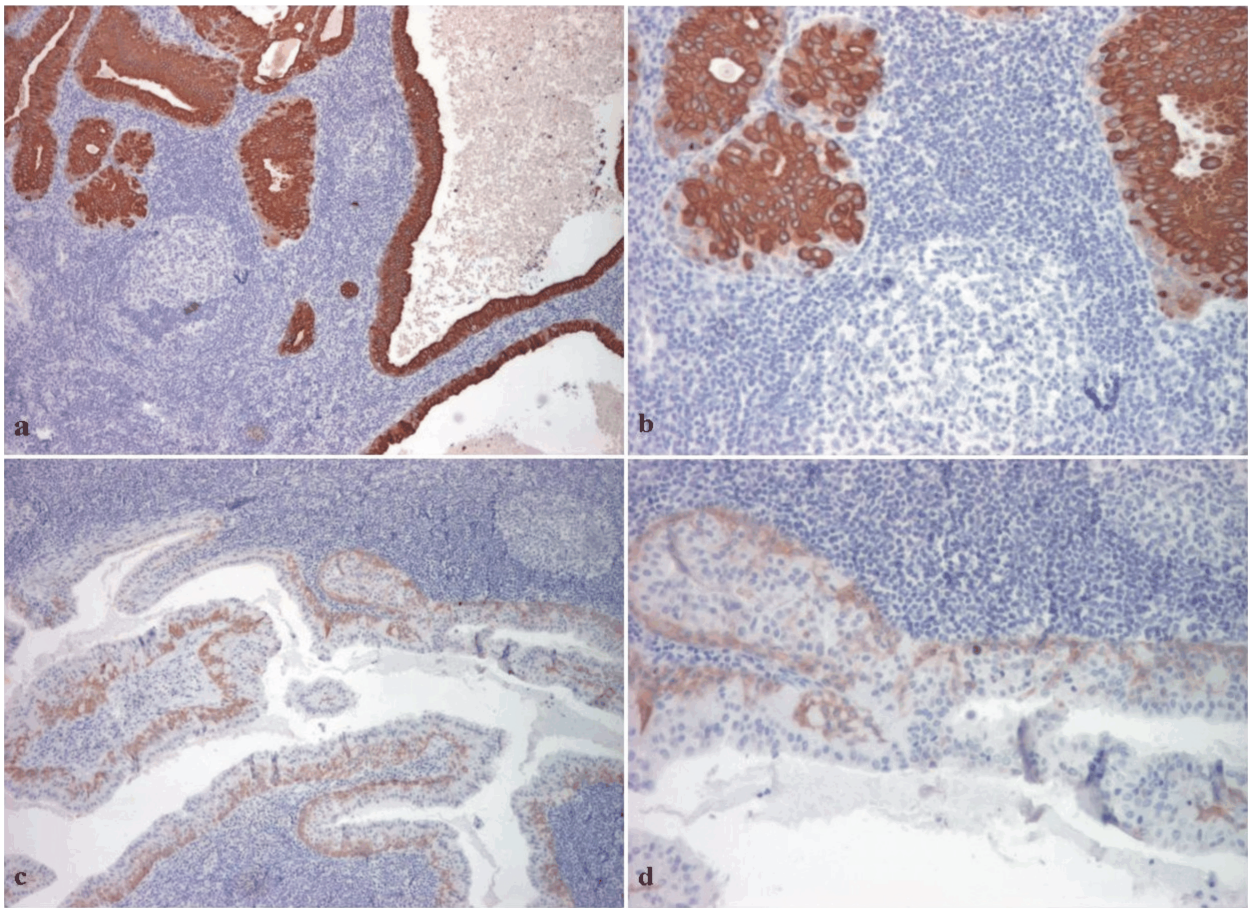

Figure 1 – ***Warthin tumor. CK7 immunostain: ×40 (a), ×100 (b); CK5/6 immunostain, ×40 (a), ×100 (b).***

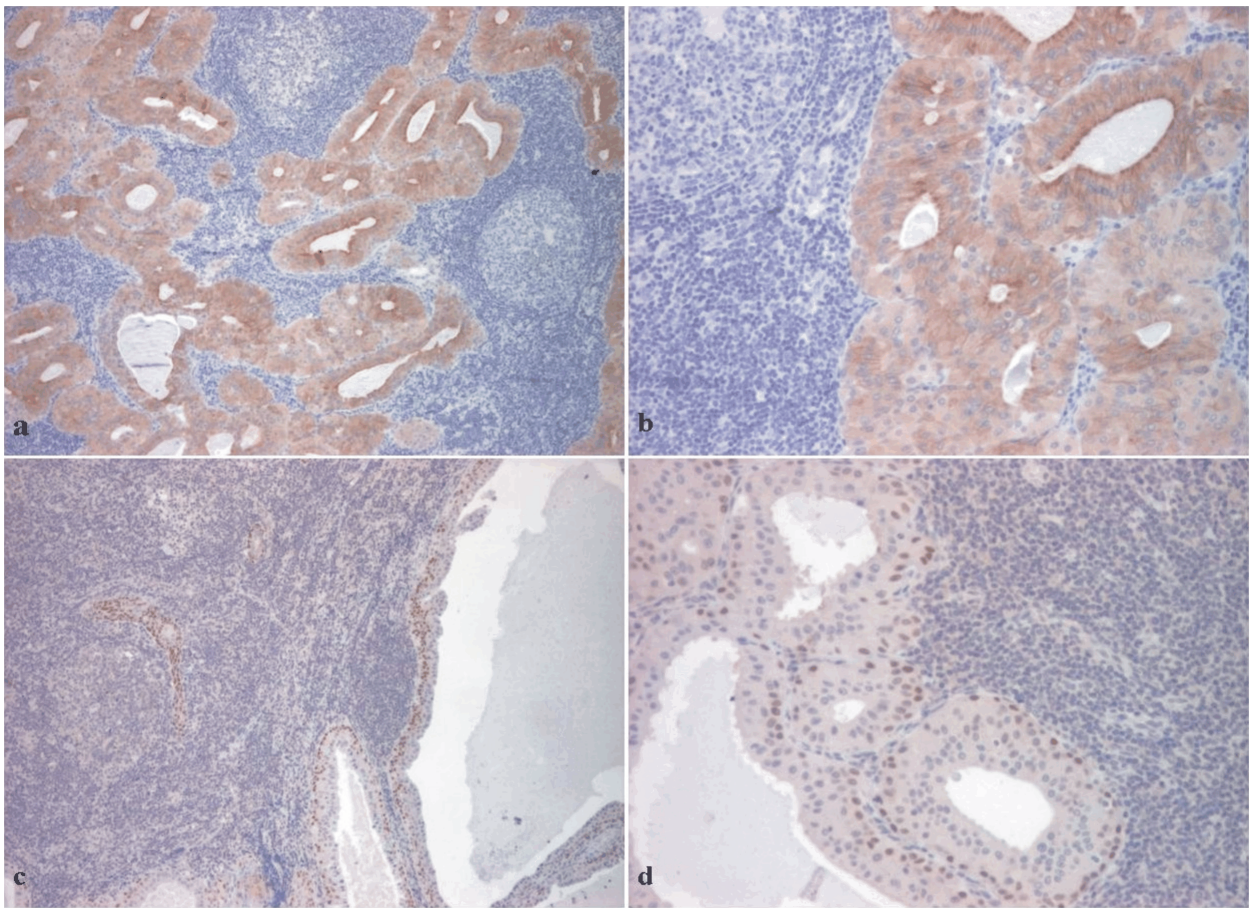

Figure 2 – ***Warthin tumor. CK19 immunostain: ×40 (a), ×100 (b); p63 immunostain: ×40 (a), ×100 (b).***

Discussion

The Warthin tumor represents 15% of all the epithelial tumors of the parotid gland [7], which is the second tumor after the pleomorphic adenoma [8].

The data obtained through the statistic analysis, realized according to the age categories, which showed that the Warthin tumor has the maximum incidence situated in the seventh life decade, overlap those from the medical literature, several authors considering that the Warthin tumor develops in most of the cases at patients between 60 and 70-year-old [9–11].

The analysis of the data concerning the distribution of the studied cases by gender, showed the prevalence of these lesions to the male persons (76.19% namely M/F 3.2/1). The data from the medical literature show for the Warthin tumor a rapport 3.3/1 men/women [12, 13] and recent studies indicate even a biggest discrepancy when it comes to the distribution by gender, so the rapport men/women is 9/1 for this tumor [2].

About the topographic distribution of the studied Warthin tumors, it can be observed an almost exclusive development at the level of the parotid gland, the left parotid gland being affected in a higher percentage than the right parotid gland [10, 14].

In our study, we could establish an etiopathogenetic correlation namely a higher incidence of these tumors in the smoking persons (36 patients) than in the not smoking persons. The medical studies consider that between smoking and the Warthin tumor etiology there is a high correlation being speculated that the cigarettes action like a local irritating agent producing metaplasia to the epithelial conducts of the parotid glands [2, 15].

The analysis of the CK7 immunoexpression has indicated positivity in all the investigated cases both in the columnar luminal cells and in the basal cell. Overall, the marking was more intense and better expressed at the level of the luminal epithelial component. The investigation of the expression CK5/6 has also indicated positivity in all the studied cases but this was limited to the basaloid cell level, with raised intensity. It is to be remarked that the immunoexpression for the investigated cytokeratins was similar to that in the normal salivary glands but with marked heterogeneity when it comes to the proportion and the intensity of the immunostaining for the two epithelia types.

More studies dealed with the assessment of the different cytokeratins expression at the level of the epithelial component of the Warthin tumor. Among those, thinking of their expression heterogeneity, CK7 and CK20 have an important role. The data in the medical literature concerning the immunoexpression of the tandem CK7 and CK20 in the Warthin tumor indicate positivity for CK7 and negativity for CK20 similarly to other tumors derived from the salivary glands [16–18].

One of the studies says that the immunostaining distribution in the tumor cell compartments is important since the cell subtypes that form the tumor population have a heterogeneous character [18]. The authors find the positive CK7 diffused in the epithelial structures of the normal salivary glands, more intensely spread in the ductal luminal cells and less intense in the basal ductal cells, the myoepithelial and acinary ones, while for CK20 all the epithelial cell elements of the normal salivary glands are negative for CK20.

Some studies constantly communicate the expression CK7, CK8, CK18 and CK19 in the tumor epithelial cell, having an immunoprofile which has the tendency of being similar to that in the normal ductal epithelia. Meanwhile, the authors communicate a divers and heterogeneous distribution in the basal and luminal cells of the neoplasia, which are different even in the different zones of the same tumor [19].

CK5/6 is positive in the basal epithelial layers of the cysts and in the papillary projection in the Warthin tumor [20]. There are other studies concerning the reactivity of the tumors for the CK6, which tell that the basal cells were strongly positive for CK6 but the reactivity of the columnar cells was apparently reduced [21].

The analysis of the immunostain for the p63 has indicated nuclear positivity in all the investigated cases, with variable intensity but limits at the level of the basal cell level. P63 is essential for the epithelial cells surviving and can function as an oncogene. P63 in comparison with p53 and p73 plays an essential part in the identification of the stem cells, the cell development and differentiating process [22]. Medical studies that have observed the p63 immunoexpression in the Warthin tumors communicate similar aspects to those obtained by this study, namely they communicate for all the investigated cases strong and constant p63 reactivity in the basal cells [23, 24], in comparison with the palisadic nuclei of the columnar cells that were negative [23]. In the same time, it is mentioned nuclear positivity also for the rare stromal lymphoid cells [23]. The fact that p63 is expressed only in the basal cells of the benign tumors of the salivary glands suggests the role of the p63 in the oncogenesis of these tumors [23].

Weber A *et al.* (2002) considers that even if the salivary glands tumors present a large spectrum of histopathological forms, the origin cells could be unique, meaning from the basal epithelial cells that generate the salivary ducts [22].

Conclusions

The differences that appear in the immunoprofiles of the luminal and basal cells of the Warthin tumor can be ranged with those of the salivary glands ducts system and indicate the fact that the tumor could develop from any level of those.

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Received: March 25th, 2012

Accepted: July 20th, 2012