

Warthin tumor – morphological study of the stromal compartment

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Abstract

Warthin tumor is the second most common benign tumors of the parotid gland, after pleomorphic adenoma. Our study was performed on 21 cases with Warthin tumor diagnosed between 2005–2010, which were analyzed clinically, histologically and immunohistochemically, using anti-CD20 and anti-CD45RO antibodies. The analysis of age distribution within the investigated cases indicated that Warthin tumor incidence is increasing in the seventh decade of life, most patients being male (M/F 5/2). Histopathological, the analysis report of stroma/parenchyma in 14 cases revealed a balanced distribution of the two components, in four cases, the epithelial component was predominant and in three cases, the stromal component was predominant. Immunohistochemical study for the two specific lymphocyte proliferation markers indicated positivity for both epithelial component and stroma. Cell layout of CD45RO and CD20cy at the level of lymphoid stroma had a similar pattern with normal or reactive lymph nodes.

Keywords: Warthin tumor, histopathology, immunohistochemistry.

☞ Introduction

Warthin tumor (cystadenolymphoma) is a benign tumor with nearly exclusive localization in the parotid gland, being the second most common neoplasia, after pleomorphic adenoma.

Tumor incidence varies between 5–11% of all parotid gland salivary malignancies [1]. The most commonly affected area is the caudal region of the gland, only 10% developing at the level of the gland's deep lobe [2]. It can be multi-centrally in 12–20% of cases and for 5–14% of patients they are seen bilaterally [3].

The etiopathogenesis of Warthin tumor is not fully elucidated, but time proved the involvement of certain definite predisposing factors for tumor development. Numerous studies indicate a strong correlation between the incidence of cystadenolymphoma and smoking, the risk of developing such a tumor being eight times higher in smokers than non-smokers [4].

Also, the histogenesis of lymphoid stroma of Warthin tumor remains uncertain [5, 6]. Lymph nodes near the gland may be present within, and on the other hand, salivary gland tissue may be present in the structure of lymph nodes [7, 8]. Therefore, lymphoid stroma often contains many germ centers, which may be the result of an immune response to neoplastic epithelium or may represent residual lymphoid tissue in lymph nodes partially replaced by neoplastic epithelium [9–11].

The aims of this study were to evaluate some clinical and morphological parameters, focusing on the lymphocyte population present in Warthin tumor stroma.

☞ Materials and Methods

The study was performed on 21 cases with Warthin tumor over a period of six years. The studied material was represented by medical records of hospitalized patients diagnosed with Warthin tumor that undergone surgery, during 2005–2011 in Oral and Maxillofacial Surgery Clinic, Emergency County Hospital of Craiova. We were interested in data regarding gender, age and history of patients and lesion topography. After surgery, the pieces were fixed in 10% formalin, processed by usual histological technique and stained with Hematoxylin–Eosin. We followed the quantification between the relationship of epithelial and stromal component.

The selected cases were investigated by immunohistochemical technique, using the visualization system LSAB + System-HRP (code K0690) and as antihuman antibody: CD45RO (mouse monoclonal CD45RO, clone UCHL1, Dako, 1/200) and CD20 (monoclonal mouse CD20cy, clone L26, Dako, 1/1000). The sections were previously processed for antigen retrieval at microwave, in buffer solution Tris-EDTA pH 9, then the endogenous peroxidase was blocked with 3% hydrogen peroxide and the blocking of nonspecific sites was performed with

PBS saline buffer solution that contained 8% BSA (bovine serum albumin). The developing of reactions was performed with DAB (diaminobenzidine). Negative external controls (the primary antibody was omitted) and positive external controls (lymph nodes) were used to validate reactions (data not shown). We followed the quantification of lymphocyte population from the inflammatory infiltration by comparing the number of marked lymphocytes to the total number of lymphoid elements. The evaluation of immunoreactions for the two antibodies was performed by a semiquantitative analysis using a scale with three levels (<25%, 25–75% and >75%) examining microscopically at $\times 40$ at least 10 fields and establishing a mean value. The intensity of immunohistochemical reaction was assessed qualitatively using a scale of three degrees (+, ++ and +++). Statistical analysis was performed using automated software SPSS16, using chi-square test and Pearson index.

Results

Study results revealed that Warthin tumor occur at patients aged between IV–VIII decades of life, the maximum of incidence being in the seventh decade of life (nine cases). Gender distribution of Warthin tumor predominantly showed that in most cases the patients were male (15 cases). History of living and working conditions could establish a correlation with risk factors, namely higher incidence of these tumors at smoker to the nonsmokers. Thus of 21 people with Warthin tumor 18 were smokers, 13 of which were male (Table 1). We observed that the topography of neoplasia was mostly located in the parotid glands and in one case the tumor was bilateral.

Table 1 – Clinical and pathological profile of the studied group

Sex	Age [years]	Smoke	Tumor/Stroma ratio
Males (15)	<50 (2)	Smokers (18)	1 (14)
Females (6)	50–60 (6)	Non-smokers (3)	>1 (4)
	60–70 (9)		<1 (3)
	>70 (4)		

*(No. of cases).

Histopathological analysis of studied cases showed proliferation of oncocytary epithelium with bistratified, basaloid or columnar pattern, which made cystic structures. Oncocytary columnar cells formed a continuous layer, which limited the cysts of lumen, unlike basaloid cells, which were present among light cells or formed a discontinuous layer under the luminal layer. The stroma component determined a thin capsule in the peripheric area that separated them completely from the rest of the gland. The stroma had a lymphoid character made up of small lymphocytes, plasma cells, histiocytes and rarely mast cells. In three cases was observed the necrosis and fibro-hyaline areas, papillary cystic proliferation, being separated in places by a series of bands of hialined collagen fibers.

The analysis of quantitative report between stroma and parenchyma revealed in 14 cases (66.7%) a balanced

distribution of the two components, in four cases (19%) the predominance of epithelial component and in three cases (14.3%) the predominance of stromal component (Table 1, Figure 1, a–c).

The lymphocytic stroma component varied. In cases of epithelial component predominance, the infiltrate of lymphocytes was reduced to small lymphocytic aggregates arranged in the papillae axis or the walls of cystic formations and in case of equal report between stroma and parenchyma and especially the ones with stroma predominance there were numerous lymphoid follicles with presence of reactive germ centers.

The study of immunoreactions for those two specific markers of lymphocyte type proliferation (CD20cy and CD45RO) revealed membrane positivity for all analyzed cases (Table 2).

Table 2 – Immunostain evaluation for CD20cy and CD45RO

Immunostain evaluation	CD20cy	CD45RO
Semi-quantitative (qualitative)	<25%	5/21 (+++)
	25–75%	12/21 (++/+++)
	>75%	4/21 (+)
		4/21 (+++)
		12/21 (++/+++)
		5/21 (+)

Comparative analysis of immunostain reactions for CD20cy and CD45RO revealed a predominance of B-lymphocytes specifically marked with CD20cy in five cases (23.8%) respectively T-lymphocytes marked with CD45RO in four cases (19%). In both cases, the stain presented a high intensity (+++).

In most analyzed cases the immunoreactions for the two sets of lymphocytes had mean positive values of stromal elements between 25–75% and the reaction was moderate to intense (++/+++). This aspect was identified in 12 cases, which accounted for 57.2% of studied lesions. In these cases, regarding the average percentage of positivity, the immunostain with CD20cy was higher than CD45RO.

Reactions with extreme values of the immunoreactions (<25% or >75%) belonged to lesions with well-represented epithelial component or with balanced parenchyma/stroma tumor ratio. Intermediate values of immunoreactions (25–75%) belonged to predominantly stromal tumors and lesions with balanced parenchyma/stroma tumor ratio.

Mostly immunomarked B-lymphocytes revealed diffuse distribution at the level of lymphoid stromal infiltrate. In cases of stromal predominance and presence of lymphoid follicles, they occupied mainly cortical area. Also, in most cases B-lymphocyte elements came into contact with suprajacent epithelium, which sometimes crossed it, further found inside the cysts in the intraluminal secretion (Figure 1, d–f).

The immunomarked T-lymphocytes also showed diffuse distribution at stromal level, this being less evident compared to B-cell elements. In cases characterized by the presence of lymphoid follicles, the T-marked lymphocytes mainly occupied paracortical area. The distribution of T-lymphocytes from the suprajacent epithelium was one of indifference, although in some cases, particularly the ones with underrepresented stroma the adjacent epithelium immunostained areas were identified (Figure 1, g–i).

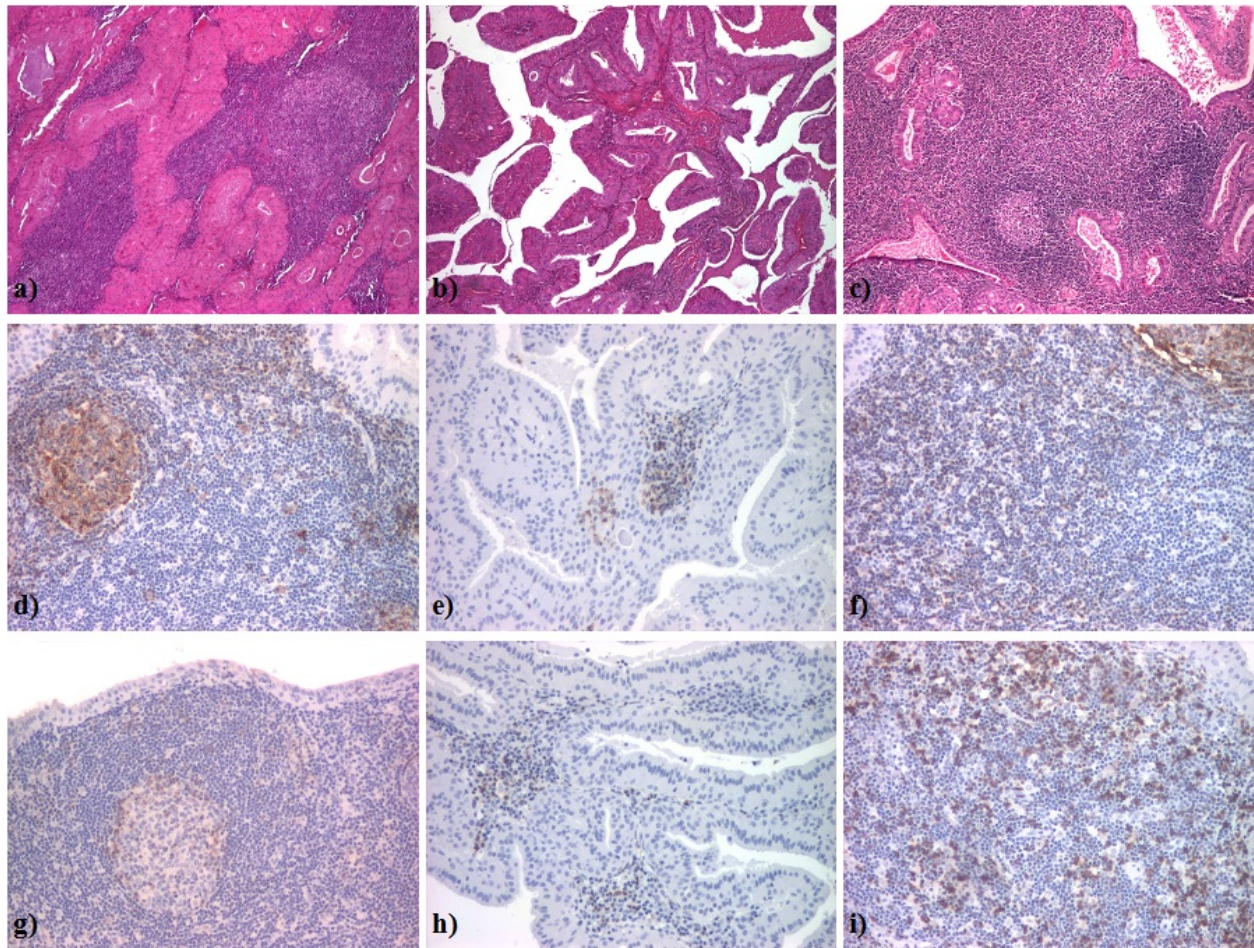


Figure 1 – Warthin tumor, $\times 100$: (a) Balanced distribution of epithelial and stroma component (HE stain); (b) Epithelial component predominance (HE stain); (c) Stromal component predominance (HE stain); (d) Cortical distribution of B-lymphocytes (CD20cy stain); (e) Intraepithelial B-lymphocytes (CD20cy stain); (f) Diffuse distribution of B-lymphocytes (CD20cy stain); (g) Paracortical distribution of T-lymphocytes (CD45RO stain); (h) T-lymphocytes adjacent to the epithelium (CD45RO stain); (i) Diffuse distribution of T-lymphocytes (CD45RO stain).

This study did not find any correlation between immunohistochemical stain made with CD45RO or CD20cy and parenchyma/stroma tumor report ($p > 0.05$, chi-square). There was a negative linear distribution of B- and T-lymphocytes following the quantification of markings on serial sections, Pearson index is -0.99 (Figure 2).

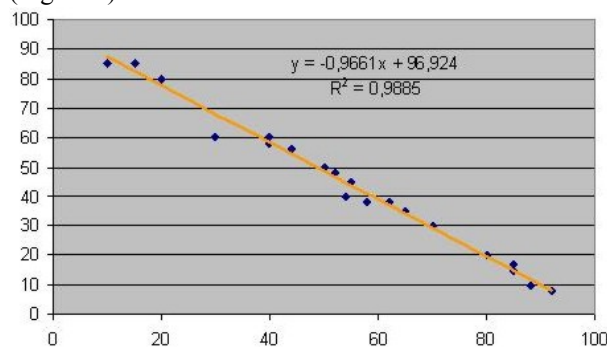


Figure 2 – Negative linear distribution of B- and T-lymphocytes.

Discussion

Warthin tumor was originally described by Albrecht and Arzt (1910) by using a descriptive term, namely as a papillary cystadenoma lymphomatosum. Subsequently,

(1929) it was recognized as a distinct tumor entity [1].

Analysis of distribution in accordance to age factor for various cases showed that Warthin tumor develops between decades IV–VIII, with maximum of incidence in the seventh decade of life and most patients affected by it are males (M/F 5/2). These data are similar to those obtained by other studies that have reported the development of neoplasia at a rate of 71% in men in the seventh decade of life [12, 13]. A recent study which included a total of 45 cases of Warthin tumor reported that this tumor is present in patients aged 35 to 83 years, the average age being 60.5 years and male/female ratio being 43/2 [2].

For all 21 cases, the location of tumors was in the parotid glands with similar aspects to other studies, which show their development almost exclusively in the parotid gland [13].

Numerous studies regarding contributory factors to Warthin tumor were considered to be smoking, seen as an important factor in the etiology of this tumor [5, 10, 12, 20]. It was considered that patients with Warthin tumor are more inveterate smokers than patients with other salivary neoplasias [14]. Specific literature indicated a higher incidence of Warthin tumor: 78–98% among smokers [14–16]. Knoke JD *et al.* [17], in a study with results later confirmed by other studies [18],

concluded that the duration of smoking for a patient is more important to take into account in terms of risk for developing Warthin tumor than the number of smoked cigarettes. Meza R *et al.* [19] assess in their study the likelihood for appearance of Warthin tumor both for smokers and for people who have stopped smoking. They concluded that after the cessation of smoking Warthin tumor risk decreases over a period of 15–25 years.

Histopathological, Warthin tumors are considered neoplasias with monotonous aspect, composed by associating the proliferation in various proportions of bilayered oncocytary epithelium with lymphoid-type stroma. However, Seifert G *et al.* (1980) describes the variability of the stroma/parenchyma ratio, depending on which he appreciated the existence of four subtypes: subtype 1 (classic Warthin tumor) with epithelial component representing 50% of tumor (77% of cases), subtype 2 (stroma poor) in which the epithelial component represents 70–80% of tumors (14% of cases), subtype 3 (stroma rich) with epithelial component representing only 20–30% of the tumor (2% of cases) and subtype 4 characterized by extensive squamous metaplasia [20]. In the study, the number of 21 tumors falls in the following categories: 14 cases for subtype 1, four cases for subtype 2 and three cases for subtype 3. The incidence of different tumor subtypes for 21 analyzed cases is similar to that reported in other studies [5, 6, 20].

Immunohistochemical study for the two specific markers of lymphocyte proliferation (CD20cy and CD45RO) indicated positivity in all cases, with the predominance of B-lymphocytes immunostained specifically with CD20cy at stromal level and in relation to the suprajacent epithelium. The predominance of B-type CD20 positive at stromal level was observed in other studies as well [13, 17, 20, 21].

CD20cy positive cells were present both in the diffuse lymphoid infiltration and lymphoid follicles predominantly in the cortical area. Bruce H reached to the conclusion that substantial composition of lymphoid stroma for Warthin tumors of B-type cells are arranged in a pattern similar to that of reactive lymph nodes [22]. Simpson RH and Eveson JW showed the predominance of B-lymphocytes in tumor stroma, suggesting that the composition of tumor stroma is made of normal lymphoid structures and reactive lymph-ganglionic area [2]. Song K *et al.* describes the predominance of B-lymphocyte cells in the lymphoid stroma, which are often concentrated at the level of germ centers at the expense of T-lymphocyte cells [1].

Immunoreaction for CD45RO at the level of stromal component was evident in all investigated cases, positive T-lymphocytes are present both in the diffusely lymphoid infiltrate and in the lymphoid follicles, predominant in the paracortical area; the study also communicates the presence predominant of T-cells between germ centers [1].

It was conceptualized the fact that Warthin tumor is a neoplasm that develops from heterotopic salivary ducts of the lymphoid tissue within or periparotidian. A more likely explanation is that the tumor is a metaplasial process with secondary lymphoid reaction,

aspect supported by the fact that several other benign and malign tumors of salivary gland appear to stimulate an associated lymphoid proliferation [12, 13]. The number and topography of B- and T-cells are comparable to those of lymph nodes, observations that are combined with morphological data to support the concept that the lymphoid component of Warthin tumor is derived from a pre-existing lymph node [5.11]. Recent studies have reported the presence of B-cells (CD20), NK (CD56) and T (CD3), including helper subtypes (CD4) and suppressor (CD8) in the tumor's stroma, something similar to that of normal or reactive lymph nodes [23]. Also, it was found that CD20-positive B-lymphocytes were located in the germ centers and peripheral B-area while CD3-positive T-lymphocytes are located inter-follicularly [6]. The authors suggest that intraparotidian lymph nodes may undergo reactive hyperplastic modifications and Warthin tumor may begin in these structures.

☐ Conclusions

The analysis of age distribution in the investigated cases indicated that Warthin tumor occur between decades IV–VIII, the maximum of incidence being present in the seventh decade of life and the most patients affected are males (M/F 5/2). Of 21 people with Warthin tumor 18 were smokers and 13 are male.

Histopathological analysis of the stroma/parenchyma report revealed a balanced distribution of the two components in 14 cases (66.7%), a predominantly epithelial component in four cases (19%) and a preponderance of stromal component in three cases (14.3%).

The immunostain of stromal component was intense at specific type markers for lymphocytic proliferation and lymphomatous-type stroma with predominantly type B-lymphocytes (specific CD20cy immunostained) constitute a non-reactive proliferation in response to epithelial proliferation that modulates it.

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