CLINICAL, HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF WARTHIN TUMOR

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INTRODUCTION

This study has as goal to go further in the knowledge of Warthin’s tumor from the point of view of the clinical, histopathological and immunohistochemical character, aiming to establish the most significant correlations between the clinical, morphological and immunohistochemical studied parameters and the their prognostication role. The investigation of the immunomarkers and the setting of possible correlations between the studied parameters may influence the early detection and treatment, having a direct impact on the prognostication.

CHAPTER IV. MATERIAL AND METHOD

STUDIED MATERIAL

The performed study is analytical, retrospective and prospective, and within the study I have compared the clinical, morphological and biomolecular traits of cystadenolymphoma of salivary glands, diagnosed within the Pathological Anatomy Laboratory of the County Clinical Emergency Hospital in Craiova during 2007-2011. The tested group was formed of 42 patients admitted in the Clinic of Oral Maxillary Facial Surgery of the Clinical Emergency Hospital no. 1 Craiova, having as diagnosis Warthin’s tumor.

METHODS USED IN RESEARCH

For the clinical and statistical study, I have investigated the retrospective and present observation sheets of the patients diagnosed with Warthin’s tumor.

For the retrospective histopathological study, I have used paraffin blocks from the histo library of the Pathological Anatomy Laboratory of the County Clinical Emergency Hospital in Craiova and we have performed series sections, hematoxylin-eosin stain and PAS, Giemsa, Gomory and Masson trichrome (Bio-Optica kit). For the retrospective study, out of the biological material obtained after the surgery we chose 3 area of interest from each tumor, including the safety margins of the lesion.

The immunohistochemical analysis was performed in all the cases that were investigated histopathologically. The method is based on the use of a secondary unmarked antibody, this one having specificity against the unmarked primary antibody, attached to the antigen tissue and that becomes at its turn an antigen. The panel of used antibodies are: CK7(OV-TL12/30), CK19(LE-
CD/9), CK5/6(D5/16B4), CEA(II-7), p63(4A4), CD117(polyclonal), AAM(MUC213-UC9, CD20cy(Clone L26), CD45R0(UCHL1), S100(polyclonal), D2-40(D2-40).

CHAPTER V. STUDY OF CLINICAL AND PARACLINICAL PARAMETERS IN WARTHIN’S TUMOR

V.A. RESULTS

The clinical study was performed on a number of 42 cases diagnosed with Warthin’s tumor between 2007-2011. For this period of time, the distribution was random, the maximum of incidence being registered in 2010.

By studying the data written on the observation sheets, I could do a statistical analysis that revealed that the cased with Warthin’s tumor diagnosis had a maximum of incidence during 61-70 years of age in male patients (M/F=3,2/1), the majority of them being smokers (36 patients), the tumors were almost exclusively at the level of the parotid gland (41 cases).

V.B. DISCUSSIONS

Data from the specialized literature show that the Warthin’s tumor represent 15% of all the epithelial tumors of the parotid gland and 11% of the total benign salivary tumors. Many authors consider that Warthin’s tumor develops in the great majority of cases in patients with the age between 60 and 70, their data overlapping the data in our study.

Regarding the distribution of the clinical cases on gender and age groups, we notice that the age segment with the greatest incidence of this medical condition (61-70 years old), the greatest percentage is represented by males (30,96 %). Data from specialized literature confirm the great incidence of Warthin’s tumor in males in the VI-VII decades.

As for the topographical distribution of the studied Warthin’s tumor, we notice the almost exclusive appearance at the level of the parotid gland. Data from specialized literature show that tumors develop more frequent at the level of major salivary glands (77-91%), the thyroid glands being the most affected (70-80%).

We have established an etiopathogenic correlation – the greatest incidence of this tumor in smokers when compared to non-smokers. Thus, out of 42 persons presenting Warthin’s tumor 36 patients were smokers and 28 were males. Many studies show a strong connection between
the incidence of the cystadenolymphoma and smoking, the risk of developing such a tumor being 8 times greater in smokers than in non-smokers.

CHAPTER VI. STUDY OF THE HISTOPATHOLOGIC PARAMETERS IN WARTHIN’S TUMOR
VI.A. RESULTS

When they were analyzed on the microscope, the studied Warthin’s tumors showed a specific structure, being formed by a dual layered epithelial component with oncocytary and basaloid cells and by a lymphoid stroma component.

The development pattern of the epithelial component was: mainly cystic in 8 cases, mainly papillary in 5 cases or cystic and papillary in 29 cases.

The dual layered epithelia showed metaplasia modifications in 16 cases of the squamous, mucous or oncocyotoma. The metaplasia modifications were noticed in 16 cases, that is 38% of all the analyzed tumors.

The epithelial and the lymphoid stroma component were present in various percentages. Thus, according to the ratio between the two components, the 42 analyzed cases of Warthin’s tumor were divided in: 28 cases of typical form, 14 cases of epithelial prevalence and 3 cases of stroma prevalence.

VI.B. DISCUSSIONS

The epithelial component was represented in all the cases by dual layered epithelia of the columnar oncocyotary and basaloid type. The epithelial component form a double cellular layer, presenting a typical aspect, formed of smooth granular columnar cells, oncocyotary and basaloid type that cover the papillary projections in macro and micro cysts.

A particular aspect noticed in the studied tumors was represented by the presence of metaplasia modifications observed in 16 cases. Data from specialized literature report the presence of metaplasia modifications on the level of epithelial component of the tumor, of metaplasia type presenting squamous cells, mucous or even ciliated cells, especially as a response to the inflammatory infiltrate or to the existence of some strokes.
The analysis of the 42 cases diagnosed with Warthin’s tumor revealed the fact that the epithelial and the lymphoma components of the tumor were present in various ratios, obtaining three different architectural patterns. Data from specialized literature in this field report quite similar results, most of the cases having a balanced stroma/parenchyma ratio and the fewest cases having an abundant stroma.

CHAPTER VII. STUDY OF IMMUNOHISTOCHEMICAL MARKERS INVOLVED IN THE PATHOGENESIS OF WARTHIN’S TUMOR

VII.A. RESULTS

For the immunohistochemical study of the 42 cases diagnosed with Warthin’s tumor I have used markers that addressed the epithelial and stromal component in order to understand at least partially the pathogenesis of the tumors and to identify the markers involved in the development of these tumors.

For the epithelial component of the tumors I have observed the evaluation of the quantitative and qualitative immune expression for CK7, CK19, CK5/6, CD117, CEA, p63, and AAM, and the markers addressed to the stromal component were represented by CD20cy and CD45RO specific to the proliferations of the lymphocyte type, and also S100 and D2-40.

VII.B. DISCUSSIONS

Several studies paid attention to the evaluation of different expressions of the cytokeratins at the level of the epithelial component of Warthin’s tumor. Data from specialized literature regarding the immune expression of the tandem CK7 and CK20 in Warthin’s tumor show positivity for CK7 and negativity for CK20, similar to other tumors derived from the salivary glands. CK5/6 is positive in basal epithelial layers on the cysts level and on the papillary projections in Warthin’s tumor.

The study of the immunomarking for the two markers specific to lymphocyte proliferations (CD20cy and CD45 Ro) indicated positivity in all the cases, with the predominance of lymphocytes B immunomarked specifically with CD20cy, both on the stromal level and also in relation with the supraiacent epithelia.
The analysis of the immunomarking for D2-40 showed positivity on the level of sinus vessels situated sub-capsular, and also in some dendrite cells situated in the lymphoid stroma or in the structure of lymphoid follicle. The tumoral epithelial component is infiltrated with lymphocytes that are a population of polyclonal cells, what makes this tumor not to be a neoplasm. Because of these reasons against a real neoplasia of this entity, several authors tried to identify the real origin of the tumor. The quantification of the tumoral angiogenesis and lymph-angiogenesis represents an important step in understanding the biology of these tumors.

**CHAPTER VIII. CONCLUSIONS**

The study of the 42 cases of Warthin’s tumor selected over 5 years (2007-2011) allowed the following observations:

- For the period of time considered in the study, the distribution of the cases was random, the maximum of prevalence being in 2010.
- To be noticed: the tendency of these tumors to develop to older people especially in their 70s.
- The people more affected by this tumor were mainly males (76,19%, M/F=3,2/1).
- The Warthin’s tumor was located most frequently at the parotid gland, the most affected being the left parotid (57,14%), with one exception when the tumor was located on the left sub-mandible gland.
- Regarding life and work factors of the patients that developed Warthin’s tumor, we could establish an etio-pathogenic correlation, as a greater incidence of these tumors in smokers (85,71%) than in non-smokers.
- In the cases of Warthin’s tumor of our study, the tumors were solitary nodular formations, mobile on the supra and sub-iacent levels, with the medium dimension of 2,67 cm, the aspect of the surface of the section being multi-cystic, with cysts of various dimensions, their content presenting a clear, mucous or brownish aspect.
- The symptomatology was not specific, the most frequent reason for admitting the patients in the hospital being the tumefaction of the affected parotid gland.
- The local clinical examination and the imagery investigations could not establish modifications that are characteristic to this type of tumor.
The treatment for Warthin’s tumors was exclusively surgical, represented most of the times by the removal of a segment of the parotid, preserving the facial nerve, the ratio of the recurrence being zero.

The epithelial component was represented in all of the cases by double layered epithelia, of columnar, oncocytary and basaloïd type that cover the papillary projections in macro and micro cysts.

According to the growth pattern, the tumors presented 3 aspects: in 8 cases (19%) there was a prevalent cystic pattern, in 5 cases (11,9%) there was a prevalent papillary pattern and in 29 cases (69%) there was a mixed pattern – cystic and papillary.

The metaplasia modifications observed in 16 cases (38%) were squamous (7%), mucous (19,1%) or oncocytoma type (11,9%).

The stroma component of the tumors was the lymphoma type, with formation of lymphoid follicles with germinate centers in 24 tumors (57,1%) ; besides the lymphocytes, I have noticed the presence of plasmocytes, mastocytes and macrophage cells in the lymphoma stroma of the tumors.

The analysis of the 42 cases diagnosed with Warthin’s tumor revealed the fact that the epithelial and the lymphoma components of the tumor lead to three different architectural patterns: 285 cases of typical form (59,6%), 14 cases of epithelial prevalence (33,3%) and 3 cases of stroma prevalence (7,1%).

The immunohistochemical analysis of the dual layered epithelia in Warthin’s tumor indicated different immunostainings for the two types of epithelia, the columnar oncocytary and basaloïd.

I have noticed positive results for CK7 and CK19 in all the investigated cases, both on the level of luminal columnar cells and on the level of basal cells, with a stronger staining and better revealed at the level of the epithelial luminal component.

The investigation of the expression CK5/6 also indicated positive results in all the cases of the study, but it was limited to the level of basal cells, with decreased staining.

The epithelia luminal cells similar immunohistochemical characteristics as the cells from
the striate duct, while the basal cells had similar characteristics with the basal cells of the excretory channels, suggesting the presence of the differentiation of two different cellular type of the epithelial component in Warthin’s tumor.

- The immunoexpression for the investigated citokeratins (CK7, CK 9 and CK5/6) was similar to one of the normal salivary glands, but with marked heterogeneity regarding the proportion and the intensity of the staining for the two types of epithelia – columnar and basal.
- The differences that appear in the citokeratins profiles of the luminal and basal cells in Warthin’s tumor can be compared to those of the normal gland and this proves the fact that the tumor may develop at any level of the system of ducts of the salivary gland.
- The fact that p63 is expressed only in the basal cells of benign tumors of the salivary glads suggest the role of p63 in the oncogenesis of these tumors.
- The immunohistochemical identification of CD117 may be useful in making the luminal cellular component of the Warthins’s tumor evident, while p63 may help making the basal cellular component evident.
- The focal expression of CEA suggest that the Warthin’s tumor is formed of a lymphatic reticular tissue similar to the normal one, quite inactive, and of an epithelial component, formed of cells, most of them being well differentiated.
- The stromal component was highly immunoreactive for the markers specific to lymphocyte proliferations, the stroma having a lymphomateus character with the high prevalence of lymphocytes B(CD 20+); this immunostaining accounts for the etiopathogenic theory of the stroma particular to Warthin’s tumor, that turns into an nonreactive prevalence, as a response to the epithelial proliferation that is also modeling.
- The positivity of S100 protein in cells similar to Langerhans cells leads to the conclusion that the delayed hypersensitivity may be the pathogen factor involved in the development of the tumors.
- The presence of D2-40 expression in the great majority of sub-capsular vessels of the tumor, similar to the structure of lymphatic ganglions, confirms the opinion that tumors originate in local lymphatic ganglions.