UNIVERSITY OF MEDICINE AND PHARMACY
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DOCTORATE THESIS
CONTRIBUTIONS TO THE HISTOLOGICAL STUDY OF THE RHINOPHARYNGEAL TUMOR PATHOLOGY
ABSTRACT

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CRAIOVA
2012
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KEY WORDS: Rhinopharyngeal tumors, benign rhinopharyngeal tumors, malignant rhinopharyngeal tumors, histopathology, immunohistochemistry, vimentin, S-100, Ki-67, CD20, 34betaE12, CD44.

Introduction

This thesis is a retrospective study, an analysis of the current state of things regarding the occurrence and evolution of the benign and malignant rhinopharyngeal tumors of the last 10 years in the case of the patients of the ENT Clinic at the County Emergency Hospital in Craiova.

EXISTING INFORMATION

1. The first chapter of this thesis amply describes the anatomy and histophysiology of the rhinopharynx, which is necessary in order to understand the cases occurring at this level. It provides information regarding the anatomical and histological structure of the pharyngeal wall, the anatomical characteristics of the muscular wall and of the subjacent fasciae, the immunological and mechanical defensive mechanisms and the phonatory and resonating functions.

2. The second chapter amply describes the various types of rhinopharyngeal tumors mentioned in the specialized literature.

Most benign tumors are pedunculated ones originating in the choanal frame, the cavum or the lateral walls of the epipharynx. The benign rhinopharyngeal tumors are mostly conjunctive (fibromas, enchondromas, lipomas etc.) and they are rarely of an epithelial nature (papillomas, cysts). Teratomas can also exceptionally occur.

As for the malignant tumors, they develop onto one of the fundamental tissues (epithelium and supporting tissue), as epitheliomas and sarcomas, with different structural aspects.

Among the benign tumors it is worth mentioning the nasopharyngeal fibroma (also known as the bleeding tumor of male puberty) which is a rough, fibrous, very bleeding tumor originating in the rhinopharynx and expanding, leading to major bone destructions if not discovered on time.
While less frequent, about 0.8-1% of the cases of cancer and 2% of the upper aerodigestive tract cancers, the occurrence of the rhinopharyngeal cancer is slightly increasing. Its etiology remains obscure, but it is generally thought that certain environmental factors and genetic susceptibility have an important role, which explains the frequent occurrence of the rhinopharyngeal cancer in southern China, Hong Kong and Singapore (10-50 times) and in the Mediterranean region.

According to the predominant histological aspect, there are 3 histological types of nasopharyngeal carcinomas: conventional keratinized squamous carcinoma, non-keratinized squamous carcinoma, and the undifferentiated carcinoma, the latter being the most frequent.

All three histological types react to cytokeratin. The undifferentiated nasopharyngeal carcinoma can be difficult to histologically differentiate from a non-Hodgkin lymphoma with big cells in the absence of specific immunohistochemical tests.

The specialized literature mentions five types of initial stages of the rhinopharyngeal cancer: a ganglion stage, the auricular stage, the nasal respiratory stage, the neurological stage and the ocular stage.

A diagnosis of the rhinopharyngeal cancer can be based on a clinical or paraclinical examination (lab analysis, X-rays), CT scans or RMN, but the only way to correctly determine it is by means of a histopathological examination of biopsy fragments dipped in paraffin, and of an immunohistochemical examination, when necessary.

PERSONAL CONTRIBUTIONS

3. The third chapter, the first one in the second part of the thesis, dedicated to the personal studies, consists of a clinical and statistical study of the tumor pathology in the respective time frame.

As for the incidence rate, statistics show that rhinopharyngeal neoplasms occur in 0.85-1.15% out of the total number of tumors, or in 2% out of the total number of upper aerodigestive tract cancers (Anghelide R, Sbenghe-Țețu L,1986). Regarding the incidence of nasopharyngeal fibromas, studies show that they occur in 0.5% out of the total number of cases of head and neck tumors, be that an 1:5000 - 1:60000 incidence rate (Ted L. Tewfik, 2011).
3.1. Materials and methods

The clinical and statistical study supporting this thesis was a retrospective epidemiological one, based on the number of cases of neoplasms and benign rhinopharyngeal tumors at the patients in the ENT Clinic at the County Emergency Hospital in Craiova in 10 years’ time, between 2003 and 2012. 22 903 patients were admitted in the Clinic over these years, 2804 of whom for benign and malignant tumor lesions. In order to conduct the study I used the information in the clinical observation sheets, in the surgical protocols and in the histopathological bulletins. The methods I used for the clinical examinations were the general clinical examinations and the specialized (ENT) ones.

3.2. Results

The study I have conducted for this doctorate thesis covered a period of 10 years, between 2003 and 2012, during which 22903 patients were admitted in the ENT Clinic at the County Emergency Hospital in Craiova. 2804 of them had ENT-related benign and malignant tumors, be that 12,24% of the total number of patients.

The total number of patients with rhinopharyngeal tumors was 179, 72 (40,22%) of whom had benign tumors, and 107 (59,78%) of them had malignant tumors. The patients included in the study were aged between 6 and 85 years old (a 42,64 years old average).

As for the sex distribution, the M:F ratio was of 1,38:1 (104 male patients and 75 female patients). There have been no differences between these two groups in terms of age.

World statistics show that rhinopharyngeal cancer occurs up to 3 or 4 times more frequently in men than in women, while the current study shows a less frequent occurrence (2.34 times) in male patients.

As for the histological variety, we had 56 cases of undifferentiated squamous carcinomas (52,33%), 6 poorly differentiated carcinomas (5,61%), 8 well differentiated carcinomas (7,47%). It has been established that the lymphoepithelioma which Regaud and Schmincke described in 1921 was also a carcinoma because the epithelial cells show signs of malignity and the lymphoid infiltrate (the T lymphocytes) is only passive. The lymphoepithelioma is now listed as a third type in the WHO classification, which is a non-differentiated carcinoma of a nasopharyngeal type.
As for the age distribution of the rhinopharyngeal cancer, the patients between 50 and 59 years old are the most frequently affected group, up to 31.77% of all those who were diagnosed with rhinopharynx neoplasms (34 patients). This percentage confirms the statistical data which place the peak of the incidence of the disease between 40 and 60 years of age.

The prognosis of the treated cavum tumors depends on how early they were discovered, diagnosed and treated, and on the histological type of the tumor. The tumor extension also conditions prognosis. Big tumors have a worse prognosis than small ones. Another aggravating factor is the extension of the tumor towards the ganglions.

Surgical treatment and the multiple techniques that can be used have been left out and tumor cytoreduction has only been used in rare circumstances (complete nasal obstruction). Irradiation is the basic treatment for the tumor. Chemotherapy is useful in two circumstances: 1) in the case of previously treated, but not sterilized tumors; 2) in the case of those adenopathies that are too large to be effectively treated by using surgery, in order to reduce their size so as to make them accessible to irradiation.

In the case of nasopharyngeal fibromas the paralateronasal approach was used, while for the other benign tumors the intrabucal approach was preferred, which is similar to the ablation of adenoid vegetations (most of which are lymphoid leftovers) with or without and endoscopic intra- and postsurgical control of the tumor excision. For the inverted papillomas we used a mixed approach, both transaxillary and endonasal, which was further enhanced by the use of Hopkins needles to perform the endoscopy.

4. The fourth chapter presents the histopathological study of the benign and malignant tumors performed on the 179 patients who were between 6 and 85 years old.

4.1 Materials and methods

In order to perform the histopathological study, the necessary samples were obtained by means of endoscopically controlled or blind biopsies. The operating pieces were fixed in 10% formalin, paraffin-dipped, sectioned at 3-5 μm and coloured with standard hematoxylin and eosin (Bio-Optica kit).

The histological technique of paraffin inclusion consists of the following stages: dehydration, clarification, paraffinizing, inclusion of the paraffin blocks, sectioning of the blocks, sticking the sections to the lamella.
This preparation is followed by the coloration of the blocks with hematoxylin and
eosin, according to a technique which includes deparaffinizing, cleansing, hydrating,
coloring the nucleus with Mayer hematoxylin, differentiating in chlorhydric acid,
colouring with lithium carbonate, eosin coloring of the respective sections, dehydrating,
xylol clearing, applying Canada balsam and drying.

Results of the coloring process: nuclei: violet blue, nucleoli: violet blue, cytoplasm:
red pink, collagen fibres: pale pink.

4.2. Results

At a microscopic level it has been noticed that on the surface of the polyps there is a
pluristratified non-keratinized squamous epithelium, subjacent a fibrocollagenous stroma
with lax areas with many veins, inflammatory infiltrate with lymphocytes and
mononuclear, necrotic detritus.

The Thornwaldt cyst displayed a fibrocollagenous wall surrounded by a squamous
epithelium. At a sub epithelial level it was noticed the existence of an inflammatory
infiltrate with a string disposition or forming microabcesses.

The adenomatoid hamartoma featured an excessive proliferation of the epithelial
component with cystically dilated glands with mucus and stroma with hyaline areas. The
glands originate in the invagination of the superficial epithelium in its own lamina and,
consequently, they can oftentimes maintain direct continuity with the surface.

Papillomas are, at a microscopic level, tumors which originate in the squamous
epithelium (be it keratinized or not), and they consist of epithelial proliferations
(acanthosis, hyperkeratosis), around a conjunctive and vascular axis. The width of the
epithelium can vary between 5 and 20 layers of cells. The mitosis are rare and always
typical.

The nasopharyngeal angiofibroma is, at a microscopic level, a tumor consisting
of tree-shaped fibroites or starred cells in a conjunctive stroma with a rich vascular
network. The vascular spaces vary in size, as they can be small, and the diameter of the
capillaries can be large, like venules. The veins are surrounded by endothelial cells which
are in direct contact with the stromal ones, but there are no smooth muscle cells between
these two types of cells.
The nasopharyngeal carcinoma is the most frequent malignant tumor of the nasopharynx. There was a total number of 27 cases of keratinized carcinomas (27.55%) and 71 cases of non-keratinized carcinomas (72.45%).

The keratinized squamous carcinoma took many histological forms, such as the in situ carcinoma, which does not go beyond the basal membrane, the microinvasive carcinoma, where groups of tumor cells break the basal membrane and invade the stroma, as well as the three types: the well differentiated, moderately differentiated and the poorly differentiated carcinomas.

The well differentiated carcinoma (G1) displays strings of malignant tumor cells (which are polygonal, similar to the cells of the spinous layer of the epidermis, but which display certain atypical features), of an infiltrative nature (the basal membrane is destroyed). The degree of differentiation of the tumor is determined by the cellular atypias and by the production of keratin by the tumor cells. In the keratinized squamous carcinomas, keratin accumulates in the centre of the tumor areas as concentric lamellae, which are intensely eosinophilic, and which are called keratosic pearls.

The moderately differentiated squamous carcinoma (G2) featured a small number of keratosic pearls, incomplete keratinisation, and persisting nuclei. There are more frequent mitosis and cellular atypias than in the well differentiated carcinoma.

The poorly differentiated squamous carcinoma (G3) showed, at a microscopic level, compact cell islands of irregular sizes and shapes. There has been no evidence of keratinisation, or, if any, it had a unicellular nature, with marked cellular atypia and and a large number of atypical mitosis. It has sometimes been noticed the existence of an abundant fibrocollagenous stroma and the formation of tumor pseudo nodules. There has been a moderate inflammatory infiltrate.

The non-keratinized carcinomas were 72.45% out of the total number of rhinopharynx carcinomas that have been researched. 15 of them were differentiated and 56 of them were undifferentiated tumors. At a microscopic level, the differentiated type of non-keratinized carcinomas showed evidence of highly pleomorphic tumor cells, and multiple development patterns, such as epithelioid, fusiform, transitional, lymphoepithelial, clear-celled, anaplastic. These patterns frequently combine.
Out of all histological types of lymphomas, only the non-Hodgkin’s malignant diffuse large B-cells lymphoma and the folicular lymphoma were diagnosed. 

The folicular lymphoma was characterized at a microscopic level by a nodular proliferation of small lymphocytes with poor cytoplasm, incised nuclei and no nucleoli; among them it has been noticed the presence of rare cells with eosinophilic cytoplasm, 2 vesicular nuclei and 1-2 nucleoli attached to the nuclear membrane. There has been no evidence of germinal centres, macrophages and tingible bodies and no mantle zone.

The diffuse large B-cell lymphoma was characterized at a microscopic level by a monomorphous proliferation of large B-cell lymphocytes with clear eosinophilic cytoplasm and certain cells with basophilic cytoplasm. The nuclei have a pleomorphic aspect and certain cells are multinucleated.

The esthesioneuroblastoma showed, at a microscopic level, tumor cells with a vaguely lobular pattern or nests which were disposed in a neurofibrillar matrix and which occasionally formed rosettes (Homer-Wright), separated by a highly vascularised stroma. The tumor cells are small, round, with a low amount of cytoplasm, dispersed nuclear chromatin, with a “salt and pepper” aspect.

The malignant peripheral nerve tumor (MPNTS) consists of dense cellular fasciae which alternate with myxoid areas. The fusiform cells areas alternate with those of round, epithelioid cells, necroses areas and cellular pleomorphism.

The fibrosarcoma is characterized at a microscopic level by the proliferation of fibroblasts which are disposed in short fasciae, with large cells (sometimes multinucleated ones) with pleomorphic nuclei, with dense collagen stroma.

5. The fifth chapter presents information about the immunohistochemical study of the rhinopharynx tumors. It was absolutely necessary to use immunohistochemical markers in the study of the malignant rhinopharyngeal tumors for diagnose purposes, for an accurate identification of the exact origin of undifferentiated tumors, for assessing the prognosis and for scientific purposes.
5.1. Materials and methods

Immunohistochemical studies have been performed on 46 cases. 40 of them were carcinomas and 6 of them were dysplasias.

The studies consisted of having 4μm serial sections applied on polylysine coated lamellae and put inside a thermostat at 37ºC for 6 hours or at room temperature for 12 hours. The sections which were thus acquired were subjected to the work protocol, which varied according to the antibody.

Each of the antibodies that were used was positively controlled (normal or pathological tissue sections which contain the investigated antigen, the normal ones being checked to see the efficiency of the reactive agents and the correctness of the technique) and negatively controlled (tissue sections originating in the same paraffin blocks as the investigated tumors, the primary antibody being replaced with immunoglobulin that belongs to the same type as the primary antibody, with the same dilution). A positive internal control in the diagnosis tissue sections was also among the objectives of the study (the immunocoloration of the normal tissue structures adjacent to the lesions, known to normally contain the respective antigen).

The interpretation of the immunohistochemical reactions aimed at emphasizing the existence of the chromogen at the level of the antigenic targets. The assessment of the immunohistochemical expression of the antibodies was the following:

- score 1 for the quantitative assessment of these colorations
- score 2 for the qualitative assessment of the intensity of these colorations
- score 3 for the interpretation of the Her2/neu marking, according to the criteria of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) for the breast carcinoma.

The clinical, histopathological and immunohistochemical data was recorded in an electronic database which used the Microsoft Access 2003 platform and helped establish the lot of patients to be statistically analyzed.

5.2. Results

Out of the 89 rhinopharyngeal carcinomas in study, 40 tumors were investigated from an immunohistochemical point of view, including 12 cases of keratinized carcinomas and 28 non-keratinized ones, with various degrees of differentiation. 6 dysplastic
papillomas of different gravity degrees were also compared from an immunohistochemical point of view.

It is worth mentioning the fact that every non-differentiated nasopharyngeal carcinoma (the lymphoepithelial subtype) was confirmed from an immunohistochemical point of view, by immunomarking with AE1-AE3 and 34beta E12 cytokeratin cocktails, along with immunocoloring for EBV (the Ebstein Barr virus).

For the immunohistochemical study of the 40 cases of nasopharyngeal carcinoma and the 6 cases of dysplasia certain markers were used, which are involved in the adjustment of the cellular cycle in order to- at least partly- understand the pathology of the tumor and to identify the specific markers which are involved in assessing the prognosis. To this purpose the two lesion categories were assessed according to the following immunoexpressions: p53, Ki67, Cyclin D1, EGFR and Her2/neu.

THE P53 IMMUNOEXPRESSION

The study of the p53 immunoexpression for the 40 nasopharyngeal carcinomas indicated a positivisation of the immunoreaction in 35 cases, that is in 87.5% of them. Dysplasias were positive for p53 in 3 cases (50%). It was noticed the fact that the immunocoloration pattern had no connection to the architectural one or to the degree of differentiation of the tumors that was identified during usual colorations.

THE Ki67 IMMUNOEXPRESSION

The analysis of the Ki67 immunoexpression indicated positivity in all cases, with an average percentage index (PI) that differed for the three lesion categories. The study of the immunomarking for Ki67 in all the 12 cases of keratinized carcinomas showed positivity in all cases, occurring in restricted areas with random distribution, or in the cells in the vicinity of the neoplastic islands. The average immunocoloration was 17%. The examination of the Ki67 immunomarking indicated higher values of the Ki67 index, as compared to the keratinized forms.

THE CYCLIN D1 IMMUNOEXPRESSION

The study of the Cyclin D1 immunoexpression for the 40 cases of nasopharyngeal carcinomas indicated a positivisation of the immunoreaction in 17 cases, that is in 42.5% of them. The positivisation of the reaction also occurred in all cases of papillomas, regardless of their degree of dysplasia. It was noticed that the immunocoloration pattern
had no connection to the architectural one. The highest number of positive cases and the highest scores occurred in the case of keratinized carcinomas and dysplasias.

**THE EGFR IMMUNOEXPRESSION**

The analysis of the EGFR expression showed a positivisation of the reaction in 36 cases, that is in 90% of the cases. 5 of them (12.5%) were keratinized carcinomas, 28 of them (70%) were non-keratinized carcinomas and 3 of them (7.5%) were dysplasias. In the EGFR positive cases it was noticed the existence of a heterogeneous immunomarking, with different scores, and its highest values were in the case of non-keratinized carcinomas.

The non-keratinized carcinomas showed EGFR positivity in all cases, with a 2+ or 3+ score. It was noticed the fact that the lymphoepithelial tumors (non-differentiated carcinomas) had a 3+ score. 3 dysplasias were EGFR positive, one with a 1+ and two with a 2+ score, with no connection between the severity of the dysplasia and the score of the immunomarking.

**THE HER2 IMMUNOEXPRESSION**

The analysis of the HER2 expression showed a reaction in 18 cases, that is in 45% of the cases. Most of them were non-keratinized carcinomas. No case of dysplastic papilloma turned positive for this marker.

**Chapter V – Final conclusions**

The histopathological study, which consisted of 179 tumors with nasopharyngeal locations enabled the following conclusions to be reached:

- 72 of the cases were pseudotumoral lesions and benign tumors in patients of 6 to 85 years of age (an average of 42.64 years of age), with a M:F ratio of 1.38:1 (104 male and 75 female patients)
  - from a histopathological point of view the pseudotumors and the benign tumors belonged to various types and subtypes of lesions, such as lymphoid debris, cysts, polyps, angiofibromas, papillomas, neurilemmomas, etc;
  - 107 cases were malignant rhinopharyngeal neoplasies, at a peak between 50 and 59 years of age (31.77% of the cases) and high incidence in male patients (75 men, 32 women);
  - from a histopathological point of view there were 98 cases of carcinomas, 6 malignant non Hodgkins lymphomas and 3 cases of tumors located in soft parts.
The immunohistochemical study was performed on a number of 46 cases of carcinomas and dysplasias, including 12 cases of keratinized carcinomas and 28 cases of non-keratinized carcinomas, as well as 6 cases of dysplastic papillomas;

- 84.78% of the cases showed p53 immunocoloration. 87.5% of the total cases of carcinomas and in 50% of the total cases of dysplasias turned positive;
- the increasing number of positive cases from dysplasias to carcinomas and of the score of more aggressive lesions imply the presence of the p53 oncoprotein in the nasopharyngeal carcinogenesis from the early stages, as well as a connection to tumor aggressiveness.

- a comparative analysis of the p53 and PI Ki67 expressions indicated higher values of the proliferation index in the case of non-keratinized carcinomas, when the p53 expression as well had the highest score and the most intense expression.
- the correlation between the p53 expression and the proliferative activity in nasopharyngeal carcinomas suggest the use of p53 and MIB-1 in the assessment of the pre-therapeutic aggressiveness of the tumors.
- the fact that all cases of dysplasia and a large number of carcinomas turned positive to Cyclin D1 suggests the precocious intervention of this marker in the nasopharyngeal carcinogenesis.
- the high incidence rate of EGFR positivity emphasizes the potential of using specific medication of monoclonal antibodies to treat the nasopharyngeal carcinomas;
- the mechanism of the overexpression of the HER2 oncoprotein and its biological significance in the development of nasopharyngeal carcinomas is yet to be elucidated, but the HER2 overexpression can prove very useful in identifying a subgroup of patients suitable for new treatment strategies.

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