UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA

MEDICINE FACULTY

DOCTORAT TESIS

ABSTRACT

CLINICAL, IMMUNOHISTOCHEMICAL AND VIRUSOLOGICAL STUDY OF NASOPHARYNGEAL CANCER

STINTIFIC LEADER:

PROF. UNIV DR. ELENA IONIȚĂ

POSTGRADUATE:

CIOROIANU LUMINIȚA

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Introduction

Cancer is a public health problem due to its high frequency in the population and the negative consequences and impact on social and economic level.

Cancer is an entity with great impact on the individual, family and society in general, it is the second leading cause of morbidity and mortality after cardiovascular diseases, dominating the morbid picture of the 21st century.

Cancer must be diagnosed in specialized medical units correlating several diagnosis techniques, making a full assessment of the case to identify the extent of the tumor and allow the identification of more effective therapeutic procedures.

Currently, evidence of the patients suferring from cancer is found in the National Cancer Register (NCR), whose functionality is ensured with great efforts by the National Cancer Programs (NCP). Nasopharyngeal cancer is a rare neoplastic disease in most regions of the world, with an incidence of less than 1 per 100,000 people, making it one of the most confusing, poorly understood, wrong and late diagnosed diseases. In the last decades this disease has attracted worldwide attention due to the complex genetic, viral, environmental interactions and diet, all of which may be associated with the etiology of this disease.

Nasopharyngeal cancer represents 2% of all cancers developed in the head and neck and as cavum is a "mute" area from the functional point of view it is necessary to have an accurate and serious study of etiopathogenesis and to determine and follow certain diagnosis criteria for early diagnosis and appropriate treatment methods. Nasopharyngeal cancer is a major global health problem regarding its discovery in the early stages of the disease by clinical immunohistochemical and serological evaluation especially of the population exposed to risk factors incriminated in the occurrence of this disease and taking into account the fact that this malignancy has a well-defined geographical distribution, most commonly affecting people of Southern China and Southeastern Asia.

In this project I have proposed to conduct a study of nasopharyngeal cancer patients, run within a specified period, trying to determine a correlation between environmental factors, heredity, age and infection with Epstein-Barr (EVB). Determination of anti-EVB antibodies and their use as tumor markers is a concern of researchers in the field, trying to demonstrate the great importance of latent infection with Epstein-Barr virus has in nasopharyngeal cancer onset.

Cavum cancer, whose incidence is steadily increasing in recent years in our country, though known since antiquity as a pathological entity, raises important and interesting issues of diagnosis and

treatment, which my research tried to capture in a clinical and statistical study of the cases admitted in ENT Craiova within a period of 10 years (2003-2012).

Specific problems encountered when establishing diagnosis derived primarily from reduced availability of this anatomic region, the central craniofacial massif; cavum examination is definitely the hardest examination in ENT specialty and also very difficult to interpret. Cavum is a region which is vey rich in lymphatic tissue, often invaded by the neoplastic product of epithelium or of supportive tissue, resulting in shapes that create confusion in the classification of these tumors and their evaluation in terms of histopathology.

In addition to the anatomical peculiarities, structural features and complex ratios, the onset of the disease, the long and mute evolution, combined later with symptoms borrowed from neighboring organs, and on the other hand, the difficulty of a high quality examination often lead to a delayed diagnosis and treatment with a worsening prognosis.

Epidemiologically, there is a clinically and experimentally verified hypothesis of the viral origin of certain forms of this neoplastic location. It is also put into question the finding that in certain populations (peri-mediterranean and belonging to southern China) the frequency of this cancer is higher than in the population living in other geographic regions.

Taking into account this diagnosis, evolutionary and epidemiological features, it is required seriuos efforts to study and discover the clinical aspects of the disease, with multiple ways of its onset, clinical forms, paraclinical investigation possibilities to establish the earliest and best treatment. It was observed that in terms of treatment, in recent years radiotherapeutic intake increased in quality, drawing on modern technology procurement.

Although there was a small number of cases of cavum cancer, this study focusing on etiopathogenic, clinical peculiarities, on developmental prognosis and treatment features, joins other large studies on this pathology; due to the severity of this disease it is necessary to determine and assess rapid diagnosis methods for effective therapeutic intervention, which extend comfort, life quality and survival time of the patient.

Anatomy of nasopharinx

The pharynx is an aero-digestive junction, being shaped of a semi-cylindrical pipe that connects the nasal fossae to the larynx and oral cavity to the esophagus. It extends from the skull base to the top of the sixth cervical vertebra, crossing cephalic and cervical region. It has three overlapping segments: upper, middle and lower (Byron J. Bailey 2006). Examining a vertical and cross section of the pharinx, it appears like an irregular, infundibuliform tube, suspended above the base on the lower side of the skull, and continues down through its top with the esophagus.

Rhynopharinx, nasopharynx or cavum represents the upper segment of the pharynx. It has: an anterior wall formed by choanae, which communicates with the nasal fossae; a rear wall curved in the front side, which corresponds to the anterior arch of the atlas; two side walls, each of them having the pharyngeal opening of the Eustachian tube, around which there is a conglomerate lymph (Gerlach's tonsil) and a depression (fossa of Rosenmüller) in the rear; a top wall, the vault or the ceiling of the cavum, corresponding to the basilar apophysis of the occipital and containing pharyngeal tonsil of Luschka; a lower, virtual wall which becomes real during the swallowing and phonation by lifting and stacking the palate to the posterior pharyngeal wall (in the rest, the palate hangs inertly, like a curtain to buccal-pharingeal) (Obreja S. 1998 Ionita E.2003, Becker W.1999).

Nasopharyngeal mucosa is composed of stratified cylindrical epithelium and ciliated epithelium, which descending towards the oropharynx, tends to become squamous (Poirier J. 1999).

Lymphatic system - the lymphatic vessels of rhinopharynx open to retropharyngeal lymph nodes (which are very important in infancy) and hence higher in the superior group of internal jugular lymph nodes chain and posterior spinal. Lymphatic vessels are crossed, which explains the precociuos onset of bilateral adenopathies. Retropharyngeal lymph nodes are situated on the lateral side of the atlas and right inside the carotid group. The most frequently invaded and pathognomonic ganglia in rhinopharyngeal cancer is located in the deep retromandibular side, underneath the upper part of the sternocleidomastoid muscle, near the mastoid. In terms of frequency - spinal chain, posterior cervical triangle, cervical group or middle jugular or supraclavicular. Rarely, there may be involved submandibular glands, submental and preauricular (Anghelide R., 1986, Licitra L. 2004).

Motor innervation is provided by the glossopharyngeal nerve (IX) for upper constrictor, pharyngo-stafilin and pharingolaryngeal muscle and vagus nerve (X) for other constrictor (Ballenger JJ 2008).

Sensory innervation is provided by branches of the glossopharyngeal, vagus and cervical sympathetic performing pharyngeal plexus (Papilian V. 1993).

Physiology of rhinopharinx

The functions the pharynx performs are as following: deglutition, breathing, phonation, hearing and defense. Numerous tissues and organs, whose coordination and implementation is run by the nervous system, perform these complex functions.

Deglutition is the main function of the pharynx – the food bolus formed in the mouth is pushed into the esophagus, and from there on into the stomach. Deglutition has three steps: buccal, pharyngeal and esophageal.

Respiration. During respiration the rhinopharynx communicates with bucco-pharynx, the soft palate hanging owing to the relaxation of the entire pharynx so that the air from nasal fossae passes into

the larynx. The air is heated by the pharyngeal mucosa which is richly vascularized, humidified and purified by mucous gland secretion and cylindrical ciliated epithelium of the nasopharynx.

Phonation is one of the most complex functions that work together with a series of organs such as: lips, tongue, pharynx, larynx, lungs and face sinuses. This harmonious cooperation is possible with the coordinating role of the cerebral cortex. There is a fundamental sound, the so-called "chord tone" caused by vocal cord vibration in expired air flow, which gains intensity, height and timbre in the pharyngeal cavity, nasal cavity and buccal cavity, thus forming the spoken and sung voice. And there are harmonic sounds resulting from air vibration in resonant cavity - the pharynx – which join this fundamental sound (Călăraşu 2002).

Audition. Swallowing, which is the second step of deglutition, is accompanied by the opening of the Eustachian tube by contraction of muscles; the air that enters the middle ear allowing a pressure equal to atmospheric pressure, thus ensuring a normal audition (Sarafoleanu D.2000).

Defense. The sensitivity and motility of the pharynx make possible the expel of any hot, caustic or disagreeable liquid, or any foreign object, taking part in the the vomit reflex by exerting the tongue base and posterior wall (pharyngeal reflex).

Pharyngeal lymphoid system (Waldeyer ring) occurs by Luschka's tonsil and tubal tonsils in pharyngeal physiology with its own action of general lymphatic system (Mogo L. 2004).

Due to its reticulo-histocytic origin, pharyngeal lymphoid tissue has primarily a defensive microbial role in the development of immunological processes (Raica M. 2002). This tissue is included in the tonsils (belonging to the lymphatic ring of Waldayer) and produces timodependent lymphocytes (lyT) involved in cellular immunity and timoindependent lymphocyte (lyB) involved in humoral immunity (the production of immunoglobulins G, A, M, D , E).

Epidemiology and pathogenesis of nasopharyngeal cancer

Nasopharyngeal cancer is a major global health problem regarding its discovery in the early stages of the disease by clinical immunohistochemical and serological evaluation especially of the population exposed to risk factors incriminated in the occurrence of this disease and taking into account the fact that this malignancy has a well-defined geographical distribution, most commonly affecting people of Southern China and Southeastern Asia.

Its **frequency** is variable. . On average, it is assumed that nasopharyngeal neoplasm represents generally 0.8-1% of cancers and 2% of upper aerodigestive cancers. However, this relatively low percentage should not hide the number of cases, which is not negligible, met in large populations. Statistics can not cover all cases. The incidence ranges from 0.19 to 53 cases per 100,000 people, depending on geographic areas.

The average **age** is, in all statistics, about 45. Extreme ages are summarized in the specialty literature, from 3 months to 93 years. However, it remains clear from the statistics that the highest incidence is between 45 and 60 years.

Gender. Recorded data differ. Although both sexes can be dependent, however, there is a predominance of cases in men.

Geographical distribution is a newer finding, which is given special importance in the last decade. It was found that the frequency is very high in the population of southern China, unlike the inhabitants of northern China, where the incidence is insignificant. Also,it was found that the Chinese from southern China emigrated to the United States developed cavum cancer, unlike the Chinese born in the United States, but progenities of parents emigrated from southern China

Histologically, epithelial cancers represents 85%, the lymphoid 7-9%, and the conjunctive (limphosarcomas, reticulosarcomas, fibrosarcomas, mixo-and liposarcomas) 2%. The rest of them are of different nature. Of epithelial cancers, epidermoid carcinomas are in the first place and of these, limphoepithelioma is a particular form in terms of nasopharyngeal location. A second place is taken by adenocarcinomas developed by seromucous glands or salivary accesory glands of cavum mucosa (Luiz Carlos Junqueira 2005).

The hypothesis of viral origin hypothesis of nasopharyngeal cancers, namely forms of undifferentiated epitheliom. It was found that in many cases there is an association between cavum cancer and Epstein-Barr (EB). This virus infects only B lymphocytes and causes a latent infection both in vitro and in vivo. In both cases, cells contain Epstein-Barr virus genome and express viral nuclear antigen that causes precocious and structural antigens. Cells that synthesize these antigens disappear. Epstein-Barr virus is found in humans in two forms of cancer: Burkitt's lymphoma and nasopharynx cancer. At an international symposium held in Kyoto researchers determined the following findings: in Burkitt's lymphoma, cancer disease reaches B lymphocyte genome occurring EB, while in cavum cancer the neoplastic process affects the epithelial cells of cavum mucosa and genome EB is found only in neoplastic epithelial cells and not in lymphocytes. There are several hypotheses to explain the role of EB virus in the malignant process: 1) the virus E.B. enters the normal epithelial cell in the nasopharynx, which can destroy the virus and break apart sometimes or even turn into cancer cell, 2) the virus does not enter the normal epithelial cell; the epithelial cells may become neoplastic only if they are in a precancer condition but initially, it is not compulsory that the carcinogen be a virus, 3) normal epithelial cells does not allow the virus. This allowed two profound interactions between epithelial cells and infected B lymphocytes: oncogene viral information goes in epithelial cells either by fusion of the two cells and the birth of new hybrid, neoplastic cells or by direct passage of viral DNA in epithelial cells. Nasopharynx cancers

containing Epstein-Barr virus are undifferentiated or poorly differentiated squamous epitheliomas. Well differentiated tumors do not contain EB virus information. The highest cancer risk was found in the Chinese province of Guangdong and that is why some authors labeled this neoplasia - "Chinese Cantons disease."

The fact that the disease has a bimodal distribution by age, cited in recent studies, the incidence peaking between 15-24 years and 45-54 years, urges us to a thorough study of the close relationship between risk factors, geographical distribution, asymptomatic, silent evolution of the disease in time to discover the disease in early stages, thereby providing better survival rate.

Epidemiological characteristics of nasopharyngeal cancer (a tumor mainly prevalent in Chinese Canton wherever they live in the world) and the association that exists between this malignancy and Epstein-Barr herpesvirus type, make it a unique cancer in which we can investigate all specific risk factors involved, either genetic or environmental (viruses and / or chemical carcinogens) (Majid Ezzati 2005). Arguments supporting the existence of this association are numerous, epidemiological and pathogenic.

Studies in the literature often have a different interpretation concerning the involvement of genetic and environmental factors incriminated in the etiology of nasopharyngeal cancer, almost all authors agree on the role of viral etiology in onset of the disease. It can not be disputed Epstein-Barr virus involvement in the etiology of nasopharyngeal cancer.

Epstein-Barr virus (EBV) is a ubiquitous lymphotropic herpesvirus, infecting approximately 95% of the population to adulthood. It represents the etiologic agent of infectious mononucleosis and is also involved in Burkitt's lymphoma, nasopharyngeal carcinoma, X-linked lymphoproliferative syndrome and chronic fatigue syndrome.

Virus transmission is primarily through contact with infected oropharyngeal secretions. EBV replication occurs in the oropharyngeal epithelium, resulting in the release of virions from infected lymphocytes and their excretion in saliva. In children the infection is often asymptomatic. Infectious mononucleosis occurs most often in young adults who had no prior exposure to the virus. After primary infection, EBV remains in the body throughout life in a latent state. In immunocompetent patients B lymphocytes latently infected and immortalized are controlled by T cells, that is why most infections remain subclinical reactivated.

Confirmation of the diagnosis of acute EBV infection is usually determined by demonstrating the presence of heterophile antibodies in serum. However, diagnosis difficulties may occur in situations in which heterophile antibodies are absent and clinical manifestations are atypical. Heterophile antibodies are absent in 10-20% of cases of infectious mononucleosis in adults, the percentage is higher in children with this developed infection. In such cases, confirmation of the diagnosis is based on detection of

specific antibodies against EBV proteins: viral capsid antigen (VCA) and early antigen-diffuse (EAD) (Ling W. 2009).

Nasopharynx neoplasm is subject to very thorough epidemiological research due to etiological theory consisting of a combination of infectious factors (EBV), certain genetic, immunological and environmental factors (macroclimate or microclimate).

These studies have led to the above-mentioned evident etiopathogenic factors, which creates a broader base to understand the etiology of cancer disease in general. They added new aspects regarding the possibility of an early diagnosis and accurate knowledge of histogenesis and natural history of these cancers, as well as improving the healing rate by introducing new therapeutic methods such asr high-energy radiation and concomitant chemotherapy.

Arguments supporting the existence of this association are numerous and they are epidemiological and pathogenic. Recent studies conducted in Hong Kong brought new elements to assess the role of Epstein-Barr virus in nasopharyngeal cancer development highlighting and demonstrating the infection of normal epithelial cells of rhinopharynx with Epstein-Barr virus in patients with nasopharyngitis. These studies have reported potentiation of Epstein-Barr virus carcinogenicity by dietary factors (Cantonese salted fish) compared with their separate action as etiological factors in cancer of the nasopharynx (Mc Coy GD 2001 W. Ling 2009).

Henle and colleagues described for the first time that serum IgA antibodies to viral capsid antigen (VCA) and early antigen (early antigen) are associated with nasopharyngeal cancer, these antibodies were detected by indirect imunofluorescent tests in the patients` serum. Although the causative role of Epstein-Barr virus is not yet fully understood these imunofluorescent tests allow early detection of disease in geographical areas with a high incidence, differential diagnosis in areas with low incidence and mass screening of the population at risk as well.

Additionally, in the serum of patients there are detected antibodies against membrane antigen complex induced by Epstein-Barr virus while testing cell antibody- dependent cytotoxicity test. This test is highly predictive in the evolution of patients with nasopharyngeal cancer (Angela Lo Kwok Fung 2006).

Based on these tests, a prospective study in North America showed that 73% of the tested patients were positive to IgG anti- "early antigen" antibodies, 68% were positive to IgA anti-antigen viral capsid antibodies. This study demonstrated the very close link between antibody titre and histological type of neoplasia.

By the time an accurate analysis of existing epidemiological data is performed, studies should be enhanced in three areas: development of an experimental model for nasopharynx cancer, development of

a better understanding of the changes caused by Epstein-Barr virus at cellular level and further evaluation of the importance of certain reactivity of antibodies in disease development.

Involvement of genetic and environmental factors incriminated in the etiology of nasopharyngeal cancer raises doubts among researchers and, thus, studies in the literature often have a different interpretation. However, almost all authors agree on the role of viral etiology and onset of the disease. Epstein-Barr virus involvement in the etiology of nasopharyngeal cancer can not be disputed.

Among the determining factors in the epidemiology of cavum cancer we can include diet rich in meat and preserved salted fish, viral agents and genetic susceptibility (Mc Coy GD 2003). Also, the preparation of these products release volatile nitrosamines which are absorbed in the nasopharynx mucosa and are taken by the inspired air. Evident epidemiological possibiliy incriminated in the development of cavum cancer is Epstein-Barr virus (EBV). Old and his collaborators first demonstrated the presence of anti-virus antibodies Epstein-Barr in the serum of patients with nasopharyngeal cancer.

The latest progress in molecular biology has brought more data on carcinogenic properties of this herpesvirus, including the identification of EBV-related peptides capable of inducing malignant transformation in vitro limphoblastomas. An increased incidence of the disease has been demonstrated in people carrying the antigen B17 (Ling W. 2009). Henderson and colleagues suggest that an environmental factor may be smoke from wood stoves used for cooking, particularly in southern China.

Studies on virological and serological association between Epstein-Barr virus and nasopharyngeal cancer lead to three hypotheses about the etiological relationship between them:

- Epstein-Barr virus is a parasite passed into the tumor, being induced by environmental factors (possibly associated with Chinese lifestyle), based on a certain Chinese genetic susceptibility to such chemical carcinogens (polycyclic hydrocarbons, nitrosamines, etc.).

- Epstein-Barr virus associated to nasopharyngeal cancer is a necessary and sufficient factor in tumor development, explained by the assumption that all patients with nasopharyngeal cancer of Chinese, African or Caucasian origin have very strong and similar reactivity against this virus.

- The virus is the first etiological factors involved in a multiphase transformation in which the virus acts in relation to internal factors (which are genetically controlled) and external factors (influenced by the environment); this hypothesis assumes carcinogenesis as a multifactorial phenomenon in which chemical and biological carcinogens initiate malignancy of epithelial cell of nasopharyngeal mucosa (Edward Gershburg, Joseph S. Pagano 2005).

Although many of the studies related to pathogenesis of nasopharyngeal cancer take into account the geographical distribution of the disease, it was demonstrated by accurate immunological and biochemical investigations that Epstein-Barr virus is the etiologic factor for certain cancers of the nasopharynx regardless of geographical area (Bailey B.2006).

Epstein-Barr virus involvement in the pathogenesis of nasopharyngeal cancer and some of its features (viral markers) are of clinical importance in raising suspicion of a nasopharyngeal cancerand having a positive diagnosis of this cancer; monitoring patients with this disease as well as Epstein-Barr virus serology is valuable to detect disease relapses. Study of immunohistochemical characteristics of Epstein-Barr virus can lead to the preparation of certain screening programs, the development of new therapies for nasopharyngeal cancer and specific methods to prevent this disease. Epstein-Barr virus was ranked in the 1st group of carcinogens by the International Association of Cancer due to its association with nasopharyngeal cancer.

Epidemiological picture is complicated by the presence of anti-virus antibodies Epstein-Barr in the serum of cavum cancer patients but not in the serum of those with other cancers of the head and neck; proving that the cavum cells contain Epstein-Barr virus genome.
In Romania the peak frequency is between the age of 50-60 years, but there are many cases encountered at a younger age (25 years). Cavum cancer incidence in the U.S. is 0.8 to 100,000 inhabitants for men and 0.3 to 100,000 women (Lalwani A. 2007).

The incidence ratio in males and women is 2 to 1, and the peak incidence corresponds to the age group 50-59 years, although the statistics of Mallinckrodt Institute of Radiology mentions 30% of the patients with cavum cancer who were under 50 years.
Gender - regardless of frequency, in all countries there was a predominance in males, the ratio male / female ranging between 2:1 and 3:1.

Histopathology of nasopharyngeal cancer

Pathological stage of nasopharyngeal cancer includes the following: the starting point of the tumor, connections to pharyngeal walls, macro and microscopic appearance of the tumor and its evolution.

Nasopharyngeal tumors originate in the tissues of this cavity, most often in lymph tissue in the pharynx vault and rarely originate in lateral, posterior and anterior walls. (N. Costinescu 1989).

HISTOPATHOLOGICAL CLASSIFICATION OF CAVUM MALIGNACIES

A.1. Epitheliomas:

- undifferentiated;
- poorly differentiated;
- differentiated.

A.2. Lymphoepitheliomas

A.3. Cylindromas

B.1. Sarcomas

B.2. Lymphosarcoma

B.3. Reticulosarcoma:

- undifferentiated;
- differentiated.

C. Plasmacytoma

D. Dysembryoplastic malignacies

D.1. Cordom

D.2. Craniopharyngioma

D.3. Extraselar pituitary adenoma

D.4. Rhabdomyosarcoma

CLASSIFICATION AND STAGING OF NASOPHARYNGEAL CANCER

TNM Classification (UICC 1997, Edge S.B 2010):

T1 - tumor does not exceed nasopharynx

T2 - tumor spreads to the soft tissues of oropharynx and nasal fossae

T3 - tumor spreads to bone structures and / or paranasal sinuses

T4 - intracranial extension, invasion of cranial nerves and / or invasion of infratemporal fossa, hypopharynx or orbit

Nx - adenopathy has not been studied

N0 - without lymphadenopathy

N1 - single ipsilateral node <or equal to 6 cm above the supraclavicular fossa

N2 - bilateral nodes <or equal to 6 cm above the supraclavicular fossa

N3 - lymphadenopathy> 6 cm or extension to supraclavicular fossa extension

M0 - without metastases

M1 - presence of metastasis

TNM Staging

- *Stage 0* $\mathbf{T_{is}\ N_0\ M_0}$
- *Stage I* $\mathbf{T_1\ N_0\ M_0}$
- *Stage II* $\mathbf{T_2\ N_0\ M_0}$
  $\mathbf{T_2\ N_1\ M_0}$
  $\mathbf{T_1\ N_1\ M_0}$
- *Stage III* $\mathbf{T_3\ N_0\ M_0}$

T_1 N_2 M_0

T_2 N_2 M_0

T_3 N_1 M_0

T_3 N_2 M_0

- *Stgel IV* **T_4 any N M_0**

  any T N_3 M_0

  any T any N M_1

TNM STAGING (AJCC 1988, AJCC 2010):

It is used now worldwide to determine clinically the malignacy stage of the tumors. It refers to three aspects:

T - appreciates the size of the primary tumor;

N – estimates the lymph node status (derived from the English word "lymph nodes");

M - indicates distant metastases.

The criteria used in TNM classification have undergone some changes over the years; currently it is used TNM classification proposed by UIC, AJCC 1988. This classification, unlike the next one, determines the stage of lymph node metastasis in terms of the lymph size and not the mobility (a clinical aspect which depends on the examiner`s subjectivity).

classification TNM classification in nasopharynx cancer:

Primary tumor

T1 - tumor confined to a rhinopharyngeal region;

T2 - tumour invades more than one rhinopharyngeal region;

T3 - tumor invades the nasal fossae and / or oropharynx;

T4 - tumor invading the skull and / or cranial nerves.

Lymph nodes metastases

Nx - regional lymph node metastases can not be assessed;

N0 - without regional lymph node metastases;

N1 - single lymph omolateral node with diameter less than or equal to 3 cm;

N2 - unique omolateral lymphadenopathy with diameter greater than 3cm, but less than 6 cm in diameter; multiple omolateral lymph nodes with diameter less than 5cm, bilateral or controlateral lymph nodes less than 6 cm;

N2a - unique omolateral lymphadenopathy with diameter between 3 and 6cm;

N2b - multiple omolateral lymph nodes with diameter less than 6 cm

N2c - bilateral or controlateral lymph nodes less than 6 cm

N3 - lymphadenopathy with diameter greater than 6cm.

Distant metastases

Mx - presence of distant metastases cannot be assessed

M0 - without distant metastases;

M1 - without distant metastases.

CLINICAL AND THERAPEUTIC STAGING:

Stage I - T1, N 0, M 0

Stage II - T2, N 0, M 0

Stage III - T3,No, M 0

- TI orT2 or T3, N 1, M 0

Stage IV - T4, N 0 or NI,MO

- Any T,N2 or N3,M0

- Any T, any N,M1.

HO STAGING (HONG KONG) OF NASOPHARYNX CANCER

T

T1: tumor confined to nasopharynx;

T2: extension to the nasal cavity, oropharynx or adjacent muscles or nerves situated below the base of the skull;

T3: tumor exceeds T2 limits;

T3a: bone invasion below the base of the skull, including the floor of sphenoid sinus;

T3b: skull base invasion;

T3c: invasion of cranial nerves;

T3d: invasion of orbit, pharyno-larynx or infratemporal fossa;

N

N0: without palpable cervical lymph nodes;

N1: one or more lymph nodes exclusively in upper cervical region bounded in the inferior side by extended skin fold and in the rear side in the thyroid notch;

N2: one or more palpable nodes between the skin fold and supraclavicular fossa, its upper limit is a line connecting the upper edge of the collarbone with the tip end of the sternal angle formed by the side surface of the neck and the upper edge of the trapezoid;

N3: one or more palpable nodes in supraclavicular fossa and / or the skin invasion in the form of neoplastic infiltration (armor), or in the form of node satellites above the clavicles.

M

M0: without marrow metastases

M1: marrow metastases and / or lymph node metastases below the clavicle.

CLASSIFICATION ON CLINICAL THERAPEUTIC STAGES (Sobin L.H. et Wittekind C.H. 2003, Joseph Wee 2008):

- **I.** T1 No M0
- **II.** T2 and /or N1
- **III.** T3 and/or N2
- **IV.** N3(any)
- **V.** M1

DIAGNOSIS AND TREATMENT OF NASOPHARYNX CANCER

Positive diagnosis and staging is based on: history, ENT clinical examination with panendoscopy, neurological examination, ophthalmic examination, radiology and imaging, ultrasound, scintigraphy examination, common laboratory tests, tumor biopsy, lymph node excision biopsy / total neck dissection, pathological examination and, in some cases, cytological examination.

Accurate diagnosis is given by nasopharynx biopsy performed either nasally or with posterior rhinoscopy mirror or Yankauer speculum. To accurately determine tumor extension it is necessary to have a computed tomography to reveal bone erosion of middle cranial fossa floor, magnetic resonance imaging offering a better and clearer image of soft tissue expansion. Laboratory tests reveal the presence of anti-virus Epstein-Barr antibodies

CLINICAL AND STATISTICAL STUDY

The next clinico-statistical study of the pathology of cavum cancer is a retrospective study conducted in the past 10 years, namely within 2003 and 2012, on a total number of 106 patients (aged 16 to 83 years) hospitalized in ENT Emergency County Hospital Craiova, requiring biopsy for positive and differential diagnosis.

To complete this study I examined the patients clinical records, urgency records, admission records by ambulatory, surgery protocols and pathological examinations .Diagnosis algorithm included the careful examination of the patient`s history, clinical examination, laboratory investigations - laboratory tests, imaging and histological investigations on biopsy taken from the lesion fragment and histopathology of surgical excision.

I systematized data on age, gender, area of origin, risk factors (smoking, alcohol, other pollutants), ways of disease onset, period of time since the disease onset till the patient received expert advice, period of time covering consultation to diagnosis, conventional exploration, the macrolezional appearance, macroscopic extension, biopsy and histopathological findings (histological types, tumor

grading for squamous cell carcinoma), TNM staging, comorbidities and therapeutic conduct. In our study nasopharyngeal tumors occurred mainly in males, the sex ratio being about 3/1.

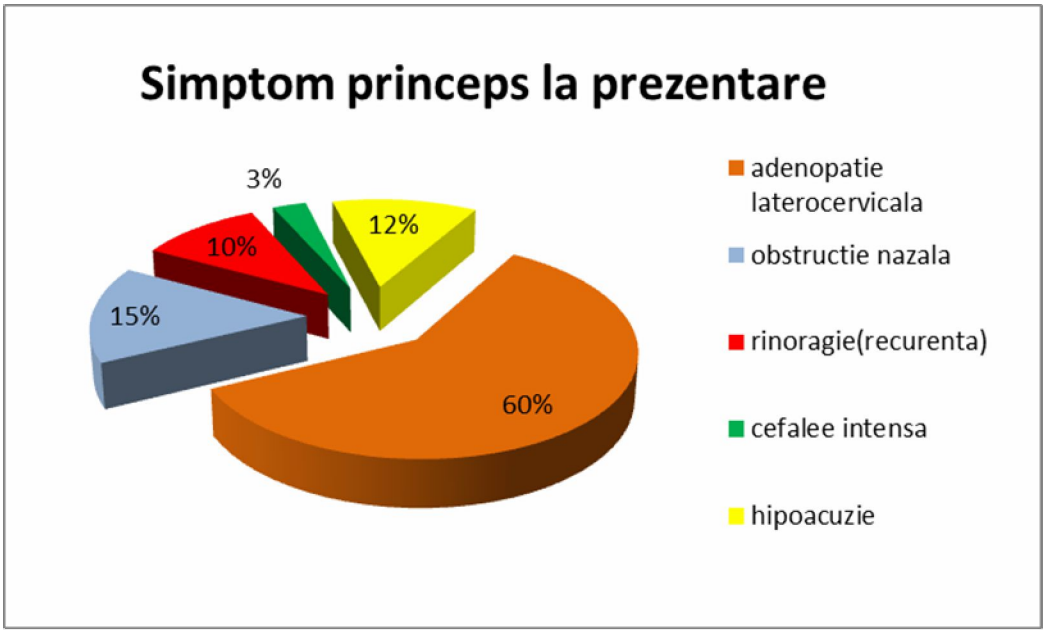
I consider that nasopharyngeal cancer in males is due both to the involvement of infectious Epstein Baar and the combination of exposure to risk factors such as smoking, alcohol consumption, occupational hazards and dietary habits (eating salted and smoked meat and fish, spicy food products).

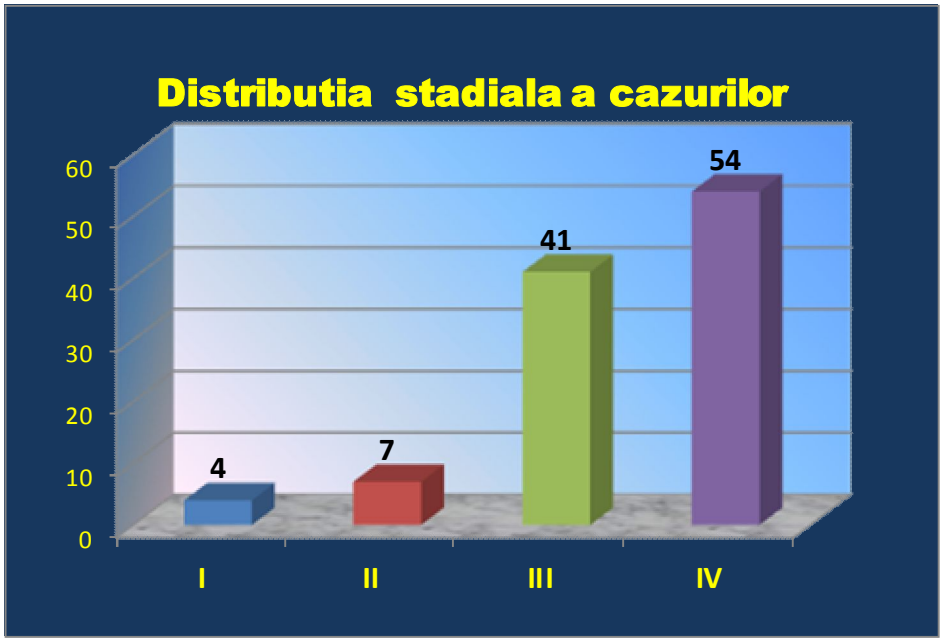
Regarding the distribution in terms of geographical origin of cavum cancerous lesions in our study group, we could not notice significant differences, due to the presence of risk factors equally in both urban and rural areas.

In all cases with tumor lymphoid hypertrophy or not, to have a correct clinical and therapeutic staging there was performed ultrasound examination, which indicated the presence and location of adenopathy, lymph size, their appearance (presence or absence of nodal necrosis), relations with the neurovascular bundle of the neck, mobility in the deep plans.

In our study, nasopharyngeal neoplasms had a higher incidence over the age of 20 years, with peak values around the age of 50 years. Regarding gender distribution, males (78%) were more affected by the disease than women (22%), while the geographical distribution – urban/rural areas - recorded relatively similar percents.

- Pathologically, 62% of the cases were found to be undifferentiated epidermoid carcinomas (lymphoepithelioamas) or poorly differentiated, 27% of them belonged to the moderately differentiated type, 9% were well differentiated carcinoma, and in the remaining 2% other types of cancer such as lymphomas, sarcomas, plasmocytomas, adenocarcinomas or rhabdomyosarcomas are ranked. Of epithelial cancers, epidermoid carcinomas are in the first place and of these, lymphoepithelioma is a particular form for nasopharyngeal location. In the second place there come adenocarcinomas caused by seromucous glands or salivary accessory glands of cavum mucosa.

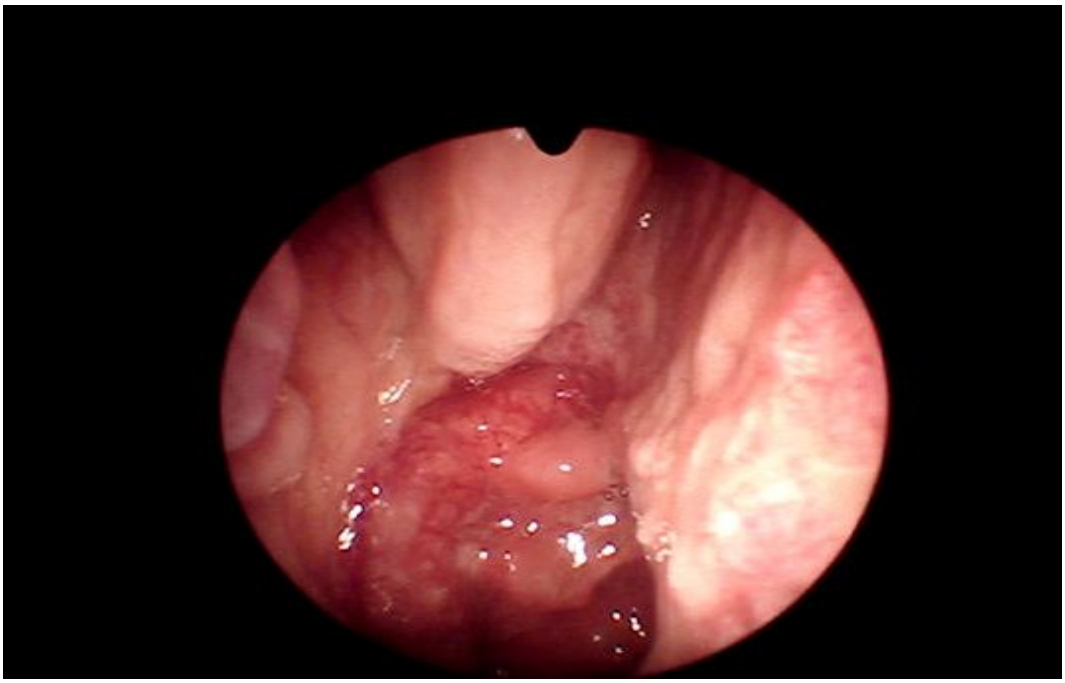




- From the macroscopic point of view, in our study endoscopy images showed most often the vegetable tumor as an anatomoclinical form and secondly, an ulcerative infiltrative tumor type. This

aspect is argued by the percentage difference between princeps symptoms at the disease onset, namely the fact that vegetant exofitic tumors of the cavum primarily produce nasal obstruction (15% of cases),

whereas ulcerative infiltrative tumors of the nasopharynx initially manifest rhynorrhea (10% of cases), then hearing loss (12% of cases) or headaches as the tumor invades the skull base.



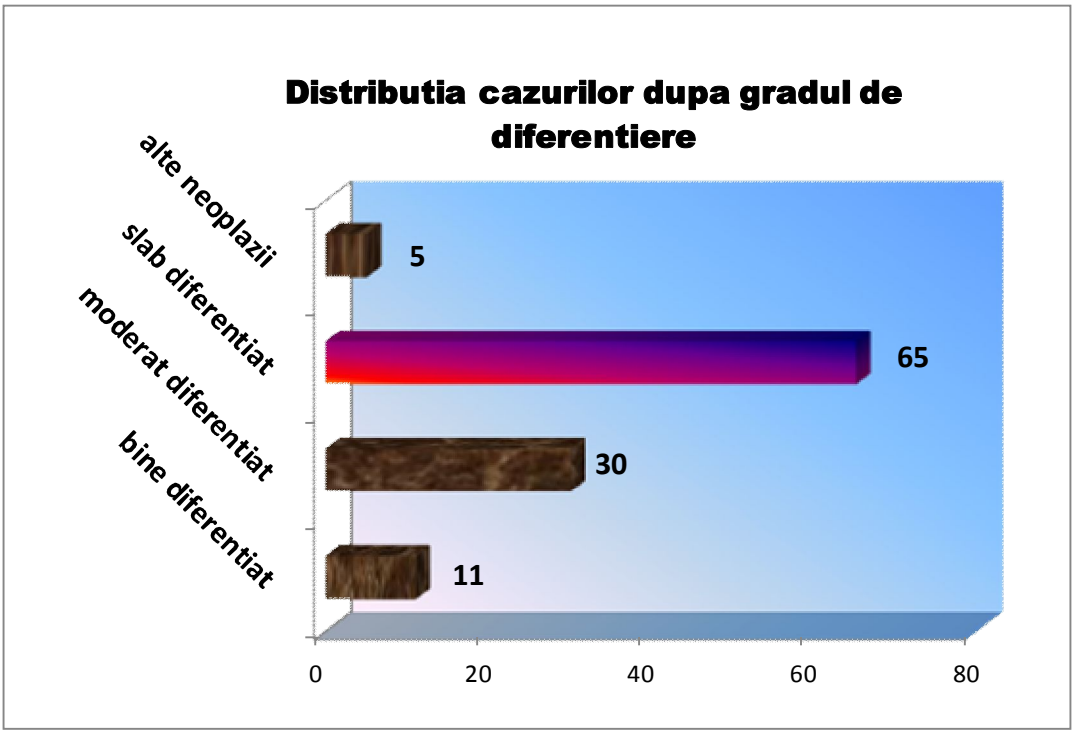
Fi**g. 1-** *Endoscopic image of poorly differentiated squamous cell carcinoma in the rhinopharynx*

HISTOPATHOLOGICAL STUDY OF NASOPHARYNGEAL CANCER

The histological material studied in this thesis consisted of 163 fragments of tumor lesions present in the rhinopharynx obtained during surgical treatment performed for diagnosis / curative reason on a number of 106 patients diagnosed with cavum cancer, aged 16 to 83 years, hospitalized in the ENT clinic Emergency Hospital of Craiova within 2003-2012. Generally, there were collected at least two fragments per patient from the biopsy tumor tissue and peritumoral tissue, hoping to highlight the transition from a premalignant to malignant lesion, but some histopathological material from patients hospitalized in 2003 -2012 were not kept in proper condition and could not be processed for further histological and immunohistochemical studies.

Of the 106 cases of carcinoma, 11 cases (10%) were well differentiated carcinomas, 30 cases (28.30%) moderately differentiated, and 65 cases (61.32%) were poorly differentiated. Epidermoid carcinomas occurring in rhinopharynx developed by malignant proliferation of malpighian epitheliums and of epitheliums in malpighian metaplasia areas.

Mild dysplasia or 1st degree dysplasia is characterized by increasing number of mitosis in the basal layer of the epithelium, resulting in increased number of dysplastic cells. In moderate dysplasia or the 2nd degree dysplasia dysplastic cells affects both basal and intermediate layer of the covering epithelium. In other words, they cover 2/3 of the depth side of the epithelium. 3rd degree dysplasia or severe dysplasia affects the whole thickness of the epithelium, dysplastic cells reaching the surface of the covering epithelium. These severe dysplasias are called carcinoma "in situ" and are lesions with clear evolutionary malignant potential. Dysplastic lesions are accompanied neither by large nuclear changes nor cellular pleomorphism but, similarly to "carcinoma in situ", severe dysplastic lesions undergo a sharp deterioration in the overall architecture of the covering epithelium, which does no longer reflect the normal structure.



Parenchyma of epidermoid squamous carcinomas seemed to consist of carcinoma cells shaped as islands or cords of highly variable shape and size, with numerous atypical forms and sizes, giving a pleomorphic character.

Nuclei were of variable sizes, the most frequently ones were much higher than normal epithelial cell nuclei, causing reversal ratio nucleus / cytoplasm for the core.

Tinctoriality of nuclei was varied, there appeared cells with hyperchrome or hypochromic nuclei, with irregular outline, with invagination, lobular or nuclear budding with uneven chromatin prepared either as nuclear membrane or as piles. Other times multiple nuclei appeared or nuclei with a huge, monstrous aspect. Nucleoli of carcinoma cells appeared bulky, multiple, with clear aspect, arranged in the centre or near the nuclear membrane.

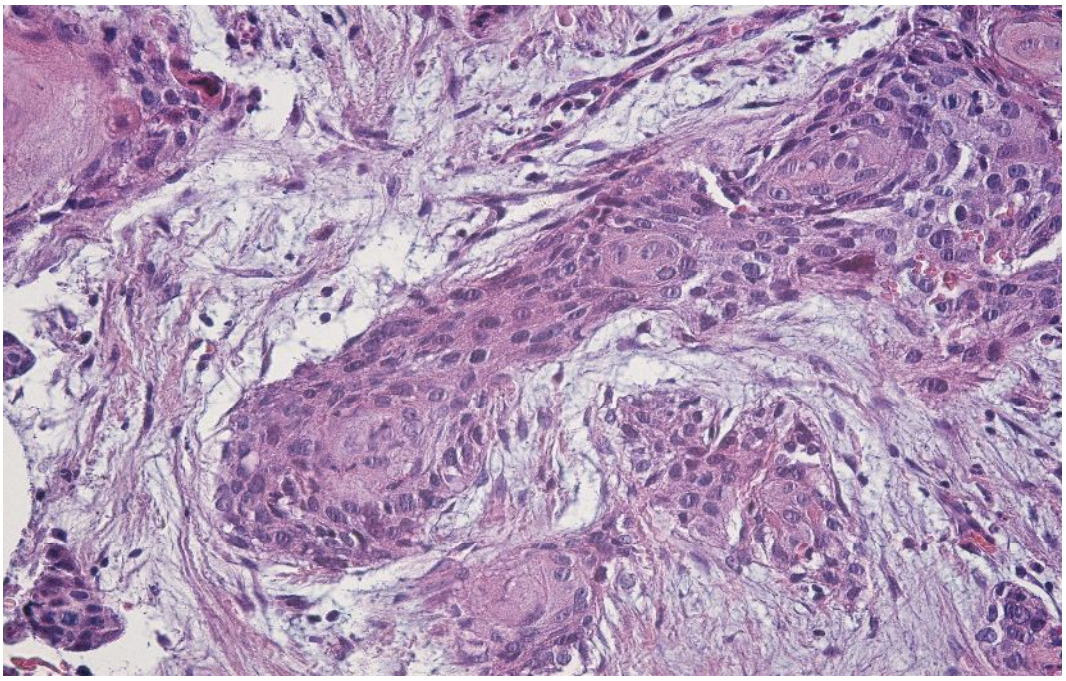


Fig. 1- *Cords of tumor cells in a case of poorly differentiated epidermoid carcinoma associated with fibroid stroma. Hematoxylin - eosin col. X 200*

Tumor stroma showed histological changes varying in terms of carcinoma differentiation degree. Well differentiated carcinomas showed a conjunctive stroma rich in collagen fibers with numerous blood vessels and an inflammatory infiltrate lacking cells. Stroma of poorly differentiated carcinomas was rich in inflammatory cells, particularly lymphocytes and plasma cells.

Immunohistochemical study was performed on a number of 106 patients aged between 16 and 83 years, admitted to the ENT Clinic Emergency Hospital of Craiova within 2003-2012. I intentionally chose tumor and peritumoral epipharyngeal fragments in order to capture and highlight the transition from a premalignant to malignant lesion in the cavum. Immunohistochemical study was really beneficial in determining a positive and differential diagnosis of these types of lesions. In premalignant lesions of the rhinopharynx, represented by lymphoid hyperplasia, leukoplakia and dysplasia, I noted an increase in the intensity of immunohistochemical reaction of cellular proliferation markers (PCNA, p35 and Ki-67), the

positivity of an increased number of nuclei in the basal and suprabasal layer, but without altering the architecture of the covering epithelium. In cavum carcinomas, the intensity of immunohistochemical reaction in cell proliferation markers was maximum, over 90-95% of tumor epithelial nuclei. Cell proliferation markers have been extremely useful to determine the differential diagnosis and in the diagnosis of high-grade dysplasia carcinoma "in situ", tumor cells having reactive nuclei p35, PCNA and Ki-67. In addition, markers of cell proliferation of tumor cells were positive in metastasis. Using CD31 antibody it was possible to visualize the vascular network of the tumor stroma.

If in premalignant lesions there were not observed changes in blood vasculature, tumor stroma was heavily vascularized in carcinomas, vascular microdensity increased, new capillaries characteristic of angiogenesis appeared and they were of normal size or with a large lumen, generally demonstrating the importance of blood vascularity in the process of tumor proliferation.

VIROLOGICAL STUDY OF CAVUM CANCER

The presumed close relationship between Epstein Barr virus and nasopharyngeal neoplasms allowed the development of numerous techniques to reveal the virus or its components useful both in early diagnosis, despite the fact that the cavum biopsy is negative, and in the postoperative records. Virological study revealed the involvement of Epstein-Barr virus in the pathogenesis of nasopharyngeal carcinoma by determination of IgA antibodies directed against VCA, considered specific for this type of cancer.

The study showed that the titration of this antibody correlates with clinical stage of the disease and may become a valuable diagnosis tool in the early stages, a positive tool even under the condition of a clinical suspicion which was not confirmed by other tests.

The involvement of EBV in the pathogenesis of nasopharyngeal cancer, although obvious, is far from being understood. Immunohistochemical study, on the one hand, and virological, clinical and genetic research on the other hand, lead to clarification of certain apects regarding the ways and moment the epithelial cells got infected and developed subsequently in neoplasia.

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