NASOPHARYNGEAL CARCINOMA: THE ROLE OF IMMUNOHISTOCHEMISTRY AND ELECTRON MICROSCOPY IN DIFFERENTIATION BETWEEN UNDIFFERENTIATED CARCINOMA AND MALIGNANT LYMPHOMA: REPORT OF 10 CASES AND REVIEW OF LITERATURE

F. Ensani and Sh. Karimi

Institute of Cancer, Imam Khomeini Hospital, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract - Undifferentiated nasopharyngeal carcinoma is frequently misdiagnosed as large cell malignant lymphoma. The aim of this study is to evaluate the diagnostic role of immunohistochemistry and electron microscopy in this tumor.

81 cases of nasopharyngeal carcinoma were studied. Patients ranged from 9-90 years in age (mean : 48.5 years). The age incidence curve was bimodal with two peaks in 2-3 and 5-6. Upper cervical mass was the first manifestation of the tumor in the majority (87.6%) of cases. Microscopically most of tumors were undifferentiated nasopharyngeal carcinoma, WHO type III. Differentiation between undifferentiated nasopharyngeal carcinoma and malignant lymphoma, large cases cell type was not possible in 12. Ten of these cases were chosen for immunohistochemical and electron microscopy studies.

In our cases cytoplasmic reactivity for cytokeratin and/or epithelial membrane antigen was present. These tumors possessed desmosomes and tonofilaments in electron microscopy. While in malignant lymphoma (three cases) tumor cells had leukocytic common antigen remaining. they were discohesive and had irregular nuclei. In the cases, the studies were inconclusive. Acta Medica Iranica 38 (1): 55-60; 2000

Key Words: Undifferentiated nasopharyngeal carcinoma, (UNC) Immuno histochemistry in UNC

INTRODUCTION

The majority of nasopharyngeal tumors are malignant. These tumors are classified according to their frequencies as:

- Squamous cell carcinoma
- Malignant lymphoma
- Miscellaneous : (adenocarcinoma, plasmacytoma, cylindroma, rhabdomyosarcoma)

Nonglandular, nonlymphoid malignant tumors of nasopharynx are generally termed NPC. These are the most common tumors in this region. According to WHO, they are classified into three types:

(1) Squamous cell carcinoma, keratinizing, type I.

(2) Squamous cell carcinoma, nonkeratinizing, type II.

(3) Undifferentiated carcinoma, type II.

The most frequent type of NPC is undifferentiated type, also-called lymphoepithelioma. Epstein Barr (EB) viruses play an important role in pathogenesis of this tumor. Undifferentiated nasopharyngeal carcinoma (UNC) is one of the most common tumors in southern provinces of China. Unilateral upper cervical metastasis is usually the most common clinical presentation. Similar tumors have been reported in the stomach, skin lung, thymus, salivary glands, tonsil, and uterine cervix (1-6).

MATERIALS AND METHODS

All cases of NC and metastatic undifferentiated carcinoma to upper cervical lymph nodes reported in the Institute of Cancer during 1990-1994 (5 years, 118 cases) were reviewed.

34 cases were omitted from the study for reasons such as absence of clinical & microscopical features of NPC, poor quality of slides, unavailable paraffin blocks and known primaries.

88 cases (81 patients) including 37 nasopharyngeal and 44 lymph nodes biopsies were chosen. 7 patients had synchronous or metachronous lymph-node and nasopharyngeal biopsies.

Tissue samples were fixed in formalin, embedded in paraffin, cut to thin sections and stained (H & E) for light microscopy.

For immunohistochemical (IHC) study, after

deparaffinization sections were stained by antibodies for leukocytic common antigen (LCA) epithelial membrane antigen (EMA) (monoclonal antibody, Merck) and cytokeratin (DAKO). For electron microscope (EM) study, paraffin blocks were deparaffinized (using Xylen or chloroform), hydrated, cut into 1 mm^2 pieces, washed in buffered solution, processed, embedded and blocked in Epon. From these blocks thick and thin sections were prepared and studied by electron microscope (Philips).

RESULTS

Patients ranged in age at diagnosis from 9-90 years. (mean: 48.5). There were 57 males and 24 woman (M/F = 2.3, Fig. 1). age distribution curve was bimodal with two peaks at decades 2-3 and 5-6, (Fig. 2) the most frequent clinical presentation was neck mass (71 cases, 87.6%). The other clinical presentations are listed in (Fig. 3).

Table 1. Lymphatic spread of UNC

	%
* Jugulodigastric	70
* Upper deep cervical	66
* Jugulohyoid	34
* Spinal accessory	28
* Inferior cervical	20

The most common histological type was UNC, WHO type III (69 cases, 78.4%, (Fig. 1). Three cases (3.45%) were nonkeratinizing squamous cell carcinoma, WHO type II (Fig. 2) and four cases (4.5%) were malignant lymphoma.

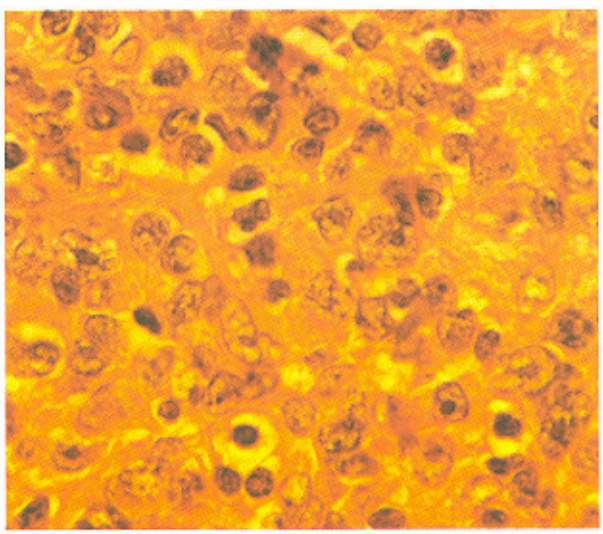

Fig. 1. Undifferentiated nasopharyngeal carcinoma, type III WHO. × 1000

In those cases in which differentiation between malignant lymphoma, large cell type and UNC was not possible stainig for anti LCA were positive in 3 cases (Fig. 3).

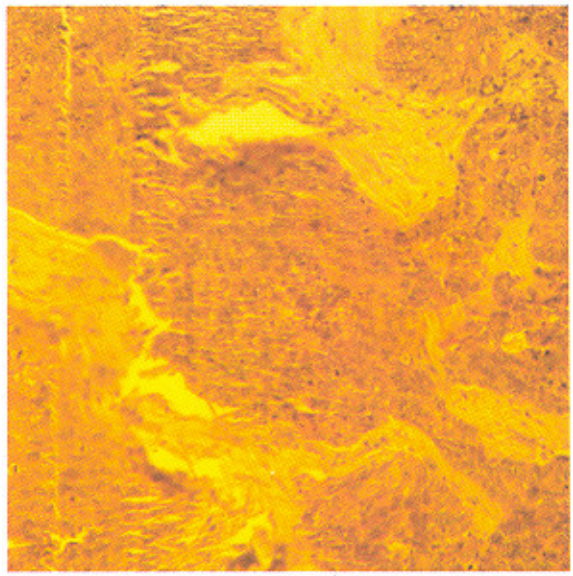

Fig. 2. Undifferentiated nasopharyngeal carcinoma, type II WHO. × 400

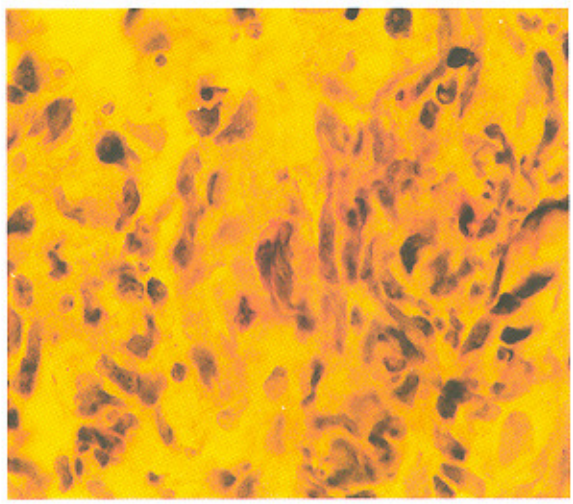

Fig. 3. Immunohistochemical staining for LCA. × 1000

Positive cytoplasmic reactivity for EMA and/or cytokeratin were seen in four cases (Fig. 4,5).

In EM study two of the three LCA-positive cases were losely cohesive and had irregular nuclei (Fig. 6), while three of the four cases which were EMA- and/or cytokeratin-positive, showed tonofilament and/or desmosomes (Fig. 7,8). These procedures were inconclusive in five cases, (Tab. 2).

Table 2. Components of prognosis score in nasopharyngeal carcinoma*

	Score	
Risk factor	IF yes	IF no
Score to death		
Seven or more symptoms	+1.14	0
Nodes positive in lower neck or supraclavicular region +	+1.10	0
WHO type I tumor	+1.04	0
Extensive tumor in nasopharynx	+0.53	0
Symptoms for < 2 months	-0.63	0
Score to death in cluding ADCC		
ADCC titer < 1:960	+1.36	0
Seven or more symptoms	+1.28	0
Nodes positive in lower neck or supraclavicular region	+1.19	0
WHO type I tumor	+1.14	
Extensive tumor in nasopharynx	+0.56	0
ADCC titer > 1: 15.360	-0.86	0
Symptoms for < 2 months	-1.05	

* Prognosis score is equal to sum of scores.

Regression weights: negative value reflects better survival (lower score)

Nodes below skin crease extending laterally and backward from the laryngeal eminence and including the supraclavicular fossa (Ho's line)

Abbreviations : ADCC: Antibody-dependent cellular cytotoxicity ; WHO: World Health Organization

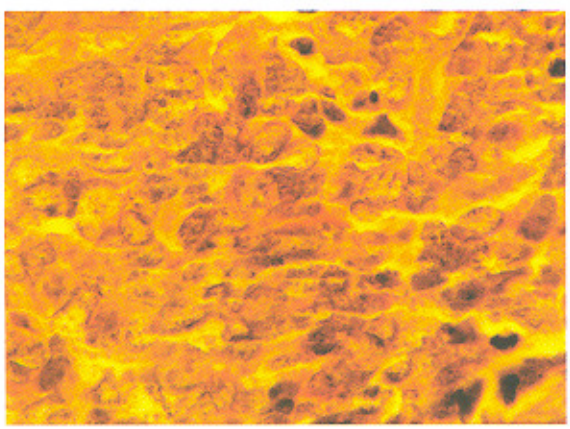

Fig. 4. Immunohistochemical staining for EMA. × 1000

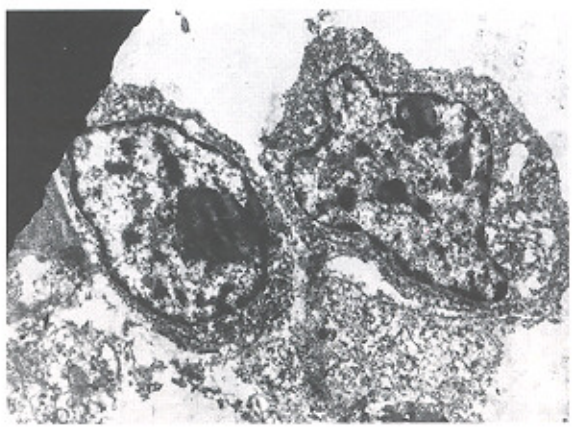

Fig. 6. Malignant lymphoma, note the dischohesive tumoral cells. × 8775

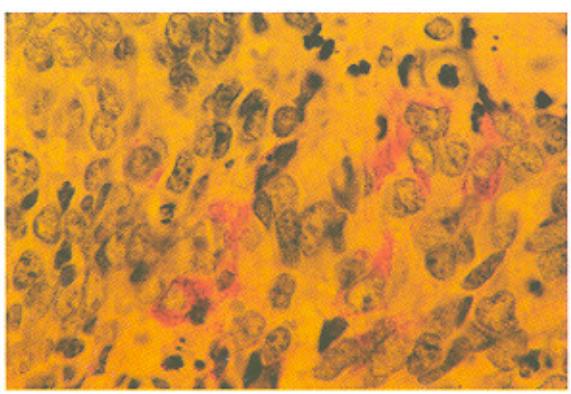

Fig. 5. Immunohistochemical staining for cytokeratin. × 1000

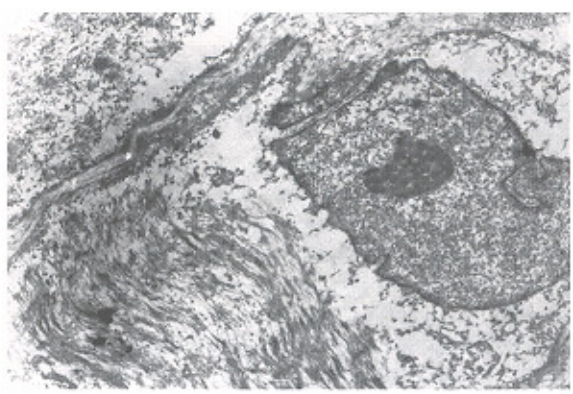

Fig. 7. UNC Intracellular tonofilament. × 9750

Table 2.Results of EM and IHC study

No	Cytokeratin	EMA	LCA	Desmosome	Tonofilament
1	+	+	-	+	+
2	-	-	+	-	+
3	-	-	*	+	-
4	-	-	+	-	-
5	-	+	-	-	+
6	-	-	-	*	*
7	-	-	+	*	*
8	+	-	-	-	*
9	-	-	+	-	-
10	+	+	-	+	-

+: positive -: Negative *: inconclusive

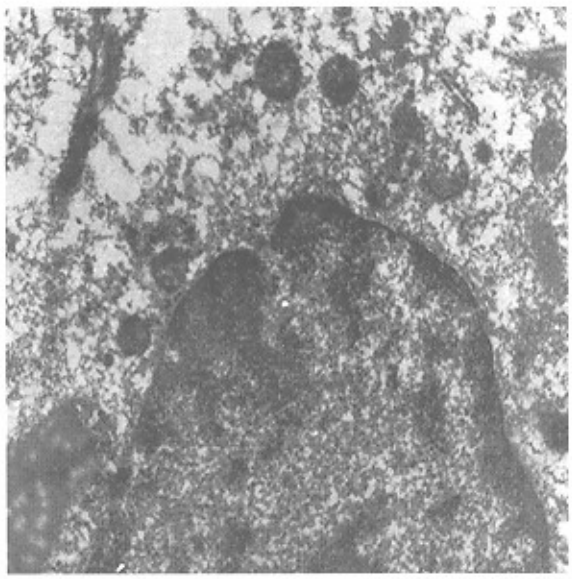

Fig. 8. UNC desmosome. × 27250

DISCUSSION

Nasopharyngeal carcinoma accounts for 5% of malignant tumors of head and neck (7). Its distribution varied in different parts of the world. It is one of the most common malignant tumors in southern provinces of China (8).

Age incidence curve is bimodal with peaks at second and sixth decades (9). In this study patients are 9-95 years old (mean = 48).

NPC has a close relationship to viral infection. Oncogenic potential of the EB virus in Burkitt's lymphoma and NPC is well-known (10,11,12,13, 14). Direct and indirect effects of this infection is only seen in undifferentiated nasopharyngeal carcinoma, WHO type III. Serological tests like antibody titer to early antigene (Ag), virus caspid Ag, and antibody dependent cellular cytotoxicity (ADCC) are reliable tests not only in follow up of patients but also in epidemiologtic studies. There is a reverse relationship between ADCC and antibody to viral caspid Ag. HLA A2, BW 46, B16, smoking vitamin A and vitamin C deficiencies are other persumptive etliologic factors. Neither of these factors are investigated in the present study.

NPC is frequently located in the lateral nasopharyngeal wall, around the fossa of Rosenmuller. Considering the numerous lymphatic channels in the submucosa of this region, asymptomatic tumors are apt to metastatize to regional lymph nodes. The first metastatic nodes are prevertebral groups which are not palpable. The most common palpable metastatic nodes are the superior jugular group. Contralateral lymph-node metastasis is not unusual. Lymph-node metastases in order of frequency are listed in table 1.

Local manifestations vary in accordance with tumor location. Tumors located at the Rosenmuller fossa may cause otitis media and hearing loss. If a tumor occludes the posterior nasal ostia, it may cause stuffing, and epistaxis. By invasion of carcinoma via foramen lacerum, paralysis of cranial nerves VI, III, IV, V develop. In extensively invasive tumors paralysis of cranial nerves IX, X may also be seen. Proptosis, Horner's syndrome and rarely paraneoplastic syndromes (15) are other clinical presentations.

The most frequent clinical presentation in our study was cervical mass (71 patients, 87.6%). Pain, nasal stuffing, hoarseness, headache and cranial nerves paralysis are the other clinical manifestations (Figure 3).

Survival is affected by the patient's age and staging. There are different staging systems for UNC including system of the American Joint Committee for Cancer, system of International Union Against Cancer, and the WHO system. Each of these systems have advantages and disadvantages. It seems that WHO system has a better correlation well with prognosis (27). On the bases of a prospective study Brayan Neel proposed for a working formulation for staging as presented in, table 2 (28, 29,30).

Radiation therapy is the treatment of choice for UNC. Adjuvant chemotherapy reduces local and

regional recurrence rate (31,32).

In summery, differentiation of UNC from malignant lymphoma is sometimes difficult and even impossible by light microscopy. In these cases immunohistochemical study for cytokeratin, EMA and LCA as well as EM study are of great diagnostic significance.

Grossly, tumor may be difficult to detect in most of cases. When visible it may be nodular, ulcerated ot exophytic.

Microsopically NPC is of three types: keratinizing squamous cell carcinoma, WHO type I (this tumor like other squamous cell carcinomas of aerodigestive tract has definite squamous differentiation and does not show association with EBV infection), nonkeratinizing squamous cell carcinoma WHO type II, and undifferentiated carcinoma WHO type III. A considerable proportion of types II and III (so-called lymphoepithelioma) are accompanied by a prominent inflammatory infiltrate rich in lymphocytes. In as much as the lumphocytic population is not neoplastic the term "lymphoepithelioma" is a misnomer.

Neoplastic cells are large and have oval vesicular nuclei with a smooth outline and single large cosinophilic nucleoli. Two patterns of growth may be seen. One which is referred to as Regaud's type, consists of well bordered islands of epithelial cells embedded in fibrotic stroma and lymphoid cells. In the other type, which is referred to as Schminke, neoplastic cells have diffuse growth pattern. This type is usually confused with malignant lymphoma, large cell type (16). Careful examination of nuclei of neoplastic cells usually establish the diagnosis. The nuclei of malignant lymphoma are more irregular in shape, containing coarser nuclei and nucleoli which are smaller and basophilic.

The most common histologic type in our study was UNC, WHO type III (69 cases, 78.4%). The histological type of the remaining cases were nonkeratinizing squamous cell carcinoma (3 cases, 3.4%) and malignant lymphoma, large cell type (4 cases, 4.5%). Differentiation between NC and malignant lymphoma was not possible in 12 cases.

Immunohistochemically, UNC shows cytoplasmic reactivity for cytokeratin and EMA (17, 18, 19, 20, 21, 22). A population of S100 protein positive dendritic cells may also be present (23, 24). In our study immunoreactivity for cytokeratin and / or EMA were seen in four cases. Three cases showed cytoplasmic and membranous reaction with antibody to LCA, three cases were nonreactive , because of denaturation of antigenic proteins in fixation and/or processing.

Ultrastructurally, UNC contains tonofilaments and desmosomes (25, 26). In present study these structures were seen in three out of four cases which were positive for cytokeratin and/or EMA. Two of three cases which were LCA-positive were losely cohesive and had irregular nuclear membranes. In five cases, EM study was inconclusive owing to profound processing artefact and inadequate sampling.

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