Malignant tumors of nasopharyngeal epithelium differ clinically depending on the course of disease and applied therapy. They are presented in regard to the sex, age, smoking habits, alcohol usage and nutrition factors. Various studies already showed various etiological-causal links with Epstein-Barr virus (EBV). This leads to diversity of various morphological and histological types of diseases belonging to various classifications. In this work we presented 60 diagnosed and treated cases with malignant tumor of nasopharyngeal epithelium in the Institute for Otologyngology and maxillofacial surgery of Clinical Center of Serbia. All of them were pathologically examined and 24 of them received serological examination in regard to the concept of association between malignant epithelial tumour and EBV. An important correlation between histopathology and serology was found.

Finally, the terminology used by WHO classification is not optimal for further histological determination of nasopharyngeal malignancy; therefore we recommend the French classification of C. Micheaua.

Key words: malignant, epithelial, tumours, nasopharynx

INTRODUCTION

Morphology of undifferentiated nasopharyngeal carcinoma is characteristic, though there are arguments on grouping the undifferentiated carcinomas showing loose or "compact, fibrous" configuration as compared to the carcinoma groups of syncytial look of rich lymphatic infiltration (UCNT). Other tumours show squamous, glandular or neuroendocrine differentiation. Since there is great vagueness about the term to be used with regard to squamocellular, low differentiated or sometimes unkeratinizing carcinoma, there is also vagueness with regard to the implementation of therapy, which, according to the most recent understanding, differs greatly for these two types of tumour. The morphological tumour characteristics include also the variants of its stroma and cell infiltration variants. Differential diagnosis UCNT includes both benign and malignant lymphoid lesion and any tumour with subtle differentiation. The known histological divisions are in everyday practice in the world, of which the most important are the classifying of epipharynx localization neoplasms, showing new etiopathogenetic entities. The most important classifying are WHO classifying, the French Micheau classifying and the German classifying by Krueger and associates.

The main drawback of the once very modern WHO classifying was the difficulty in distinguishing the type 2 tumour, id est, the unkeratinizing, the so-called NKC from the type 3, id est, the undifferentiated carcinoma of nasopharyngeal type, that is, UCNT. Shanmugaratnam with his associates used this classifying on a large number of NPC in Singapore but failed to find correlation between the histology and distribution of age, sex, HLA antigens, cells immunologic status. Together with his associates, he concluded at that time: "The three subtypes of tumours, according to the WHO classifying, most probably are the variant of a very homogeneous group of neoplasms in Singapore population".

Neel, Pearson and Taylor came to similar conclusions researching correlation of histology and EBV serology on NPC patients.

The clinical value of histological classifying is based upon capability to foresee answers to treatment and possibility to compare the obtained results. There is reliable evidence that squamocellular carcinoma (SCC) should be classified separately because of relative radioresistance and bad prognosis. WHO classifying divides these tumours into unkeratinizing carcinomas NKC (not showing either squamous or glandular differentiation) and undifferentiated carcinoma UCNT. This division of undifferentiated carcinomas seems not to have therapeutic and prognostic significance. The two types of tumour are not final. Some authors pointed to the differences in prognosis between UCNT and compact type, such as WHO classifying...
uneratinizing carcinoma. It seems that better prognosis is with the first tumour type as compared to the second one.

OBJECTIVE

We wanted to clear vagueness concerning the recognition of histological classifying of nasopharyngeal tumours in our surroundings, on the basis of morphological differences that are in accordance with the clinical and serological parameters of our surveyed patients, in compliance with the new cognitions in the literature in the world.

MATERIAL AND METHODS

Pathohistological specimens of 60 patients, with malignant epithelial nasopharyngeal tumour diagnosed and treated during the period 1989-1998 in Belgrade, dyed hematoxylin-eosin (HE) were examined. Simultaneously 24 of them were prospectively chosen for serological and histological examinations in the Institute Gustave Roussy in Paris, where methods of immuno–fluorescent dyeing was applied. Serum of the same number of volunteering blood donors was used as the control group for serological examination. The serum preparations of 24 prospectively followed up patients, the tumour specimen of which had been examined in Paris, were serologically examined for the presence of Epstein-Barr virus (EBV) antibodies. An original method of immuno-fluorescence was used, which positivity means detection of two antigen groups of virus Epstein Barr: EA test discovering antibody presence in the patient’s serum on virus EA (early antigen) as well as test VCA (virus capsid antigen). Test discover, through indirect immuno-fluorescence with different titres of the patient serum, the antibodies specific for structural antigens of the viral capsid EB. The most powerful immuno-fluorescence, obtained in a series of different serum titres of the surveyed patient and behaving as a geometric progression, is taken for statistical processing. Logarithmic values of the serum titres are statistically processed by use of the method of one factor analysis of variance.

RESULTS

Undifferentiated carcinoma of nasopharyngeal type has reticular architecture (structure), stratified arrangement of syncytial luminous cells with vesicular nucleus. Usually present is, more or less shown, lympho-plasmocyte infiltration.

Tumour more frequently has a reticular than syncytial look. The walls of the cells are difficult to differentiate (Figure 1). Tumour cells are arranged in irregular and differently defined masses and bands, which could be seen in the same tumour. The cells themselves do not produce mucin. Pin-like tumour cells with hyperchromatic nucleuses dominate (Figure 2). When the lymphoid stroma is so thick partially to hide carcinoma cells, there are differential diagnostic difficulties as compared to other malignant tumours, primarily the lymphomas, this being the reason in the past to utilize most frequently for this tumour the term lymphoepithelioma. Dependent on the density of lymphocyte infiltrates, lymphoepitheliomas could

FIG. 1. PRIMARY NPC. TUMOUR CELLS ARE ARRANGED IN IRREGULAR AND DIFFERENTLY DEFINED MASSES AND BONDS WHICH COULD BE SEEN IN THE SAME TUMOUR. THE CELLS THEMSELVES NOT PRODUCE MUCIN. PINK-LIKE TUMOUR CELLS WITH HYPERCHROMATIC NUCLEUSES DOMINAT. HE, x80.

FIG. 2. DOMINEERING CARCINOMA FIELD MADE OF SEEMINGLY SYNCTIAL LUMINOUS CELLS WITH VESICULAR NUCLEUS, UCNT. BORDER TOWARDS LYMPHO-PLASMCYTE STROMA RATHER VAGUE. HE, 260.
be divided into two subtypes: "Regaud" and "Schminke". Type "Rigaud" possesses little islands of undifferentiated cells of typical morphologic cohesive groups surrounded by lymphocyte infiltrates. Type "Schminke" shows explicit lymphocyte infiltration and could be considered a lymphoma (Figure 3).

Important diagnostic feature in HE specimen is the identification of cytoplasmatic ties between tumour cells (Figure 4). In prognostic sense, there is no practical significance of making distinction between the two undifferentiated carcinomas. The term lymphoepithelium generally is accepted as inadequate as well as that the lymphocyte infiltration is reactive and not neoplastic. (Table 1)

Titre anti-IgA VCA of antibody considerably is higher in the patient’s serum with UCNT and amounts to 143.9 as compared to the patients with squamocellular tumour type, which could be confused on a light microscope for undifferentiated tumour type, and amounts to 8.71. Statistically is highly important that the titre anti EA antibody from the class IgA of a patient with UCNT is higher as compared to the titre of a patient with SCC lower differentiated tumour form. (Table 2)

Correlation of EBV serology with the appearance of NPC and non-NPC, is shown in the Table 2. While the IgA anti–VCA antibodies were found with 97.3% of NPC patients, it was found as well with 32.8% of other, non-NPC surveyed patients. They belonged in most cases to non-neoplastic conditions.

Specificity of serologic testing EBV grows with titre increase, while its sensitivity is reduced with titre decrease. Since sensitivity of IgA anti–EA is smaller, and specificity of the same antibodies greater as compared to IgA anti–VCA, we obtain in less than 3% of non–NPC patient cases a detectable titre IgA anti–EA. Test efficacy is calculated within the term of confident capability correctly to diagnose NPC. Maximum test efficacy is 0.88 for IgA anti–VCA at titre of 40 and amounts to 0.94 for IgA anti–EA at titre of 5.

**DISCUSSION**

Pathohistological specimens of 60 patients with malignant epithelial nasopharyngeal tumour were examined simultaneously and 24 of them were prospectively chosen for serological and histological examinations in the Institute Gustave Roussy in Paris, where methods of immunofluorescent dyeing was applied.

After examination of 24 pathohistological specimens of NPC patients from different parts of southeastern Europe, it is confirmed that two main groups of malignant epithelial tumours exist in nasopharynx: SCC and UCNT. Patients with squamous cell carcinoma (SCC) had low anti EBV titres, etiopathogenetically are associated with consumption of alcohol and tobacco, react much worse to radio and chemotherapy, have lower rates of five year life span. The same correlations were obtained in research made by the authors Anderson–Anvret, Saltiel–Berdah and Resta when using Micheau’s classifying on two basic tumour types SCC and UCNT. According to present day understanding, numerous tumours are connected with EBV. Encompassed here are lymphomas such as Burkitt...
lymphoma, Hodgkin’s decease\textsuperscript{18}, some T cell lymphomas and B cell proliferations joined with immunodeficiency syndromes (typical of acquired immunodeficiency syndrome). Tumours of epithelial nature related to EBV are nasopharyngeal carcinomas (UCNT), undifferentiated carcinoma of salivary glands (malignant lymphoepithelial lesion)\textsuperscript{19} and tymmoms (both benign and malignant)\textsuperscript{20}. Isolated cases with undifferentiated lung carcinoma related to EBV were published, though they refer only to the eastern peoples\textsuperscript{22-26}.

Some typical UCNT appear in other parts of the world. Thanks to our first researches, published in 1986 in USA, we showed that in the region of southeast Europe there are tumours related to EBV\textsuperscript{6,27,28}. EBV serology can usefully decrease errors in diagnostics with patients in initial UCNT phase, particularly those with erroneous negative initial biopsy. In some instances, according to submucous spread of UCNT, the nasopharyngeal endoscopy might have a normal look, in spite of positive biopsy to malignity. In one of our previous studies, this was the case in 20\% of biopsies. According to our own experience, biopsy was negative in 10 of 85 UCNT cases. Practically, we repeated biopsy with every fourth patient in order to diagnose UCNT, and now this is happening in every ninth case and even more rare.

Serology warns the clinic expert against the possibility of failure biopsy, suggesting rebiopsy in order to confirm diagnosis. When the antibodies titre is sufficiently high, it is detectable and has a great importance when related to the clinical assessments and histological diagnosis\textsuperscript{29,30}. EBV serology, specifically IgA anti–EA, could be used as the relapse indicator with patients of confirmed clinical remission within one year. There are several studies engaged in EBV serology NPC in endemic regions\textsuperscript{12,18} and scarce number of data on the same problem from the southeast Europe\textsuperscript{31,32}.

According to our opinion, the above–described histological phenomena, related to EBV, suggest more than a coincidental relationship between different functioning conditions of lymphoid tissue and immunity organs such as mucous membrane of respiratory tract.

We have cleared up, through our team research of this problem, some of the vagueness concerning the modern WHO classifying of nasopharyngeal carcinoma referring to the appearance of these tumours with us, and as it seems, everywhere in the world as well (Sto\textsc{i}-Divjak and associates, 1998,\textsuperscript{17}). Several reasons for the recognition of classifying were summarized as follows:

1. Squamous differentiation focuses are frequently present both in UCNT and in the so-called unkeratinizing carcinoma type, the so-called NKC. Cytologic characteristics of cells UCNT are distinctly separable from NKC and SCC cells.

2. The difference is not clear between the mean and low differentiated SCC and unkeratinizing carcinoma, the so-called NKC. Indicative appearance of squamous differentiation, such as presence of intercellular bridges and formations of squamous beads, could be seen in cases of NKC. Terminology used by WHO is restrictive in the definition of squamous cell carcinoma and names it keratinizing carcinoma. The Committee includes SCC grading in well, mean and low differentiated SCC without specificities. We believe that the SCC qualification as the "SCC keratinizing" should possibly be eliminated.

3. There is prognosis difference with regard to the low differentiated SCC and UCNT. It is to a point dependent on the level of the present squamous differentiation. When we used the WHO classifying, we had approximately three to four times higher number of low differentiated carcinomas under different names in relation to keratinizing or well differentiated SCC. The above mentioned should be a starting point for further research. All the mentioned categories failed to take into consideration the lymphoid infiltration level; in literature, there are many data on its importance. The German classifying

| TABLE 1 |
| SIGNIFICANCE OF DIFFERENCE MEAN GEOMETRIC TITRES IN RELATION TO TWO DIFFERENT HISTOLOGICAL TYPES OF TUMOURS UCNT AND SCC LOW DIFFERENT TYPE |
| Patients | Mean geometric titre of IgA VCA |
| UCNT | 143.90 |
| SCC low different type | 8.71 |

| Patients | Mean geometric titre of IgA EA |
| UCNT | 31.09 |
| SCC low different type | 5.36 |

| TABLE 2 |
| CORRELATION EBV SEROLOGY AND HISTOPATHOLOGY |
| Titar | IgA anti-VCA | IgA anti-EA |
| UCNT (%) | Non-UCNT (%) | UCNT (%) | Non-UCNT (%) |
| >5 | 97.3 | 32.8 | 78.8 | 2.7 |
| >20 | 68.1 | 8.7 | 41/0 | 0.8 |
| >40 | 59.0 | 5.7 | 27.1 | 0.4 |
| >80 | 28.7 | 2.0 | 8.0 | 0.1 |
| >160 | 22.3 | 1.1 | 0.0 | 0.0 |
| >320 | 4.3 | 0.2 | 0.0 | 0.0 |
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REFERENCES


