



Histopathological, Histochemical and Immunohistochemical Study on the Association of Epstein Barr Virus with Nasopharyngeal Carcinoma

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ABSTRACT:

This study aimed to investigate the association between Epstein Bar virus (EBV) and nasopharyngeal carcinoma using Immunohistochemical technique and Macchiavell's stain. One hundred biopsies were randomly collected from patients who attended the radiation and isotopes center in Khartoum (RICK) and presented with nasopharyngeal tumors. Sections were prepared and stained using three different methods: Hematoxylin and eosin (H&E) for histopathology, Macchiavello's stain for demonstration of EBV inclusion bodies and immunohistochemistry using monoclonal mouse antigen (DAKO) for detection of EBV latent membrane protein. The patient ages ranged between 16 and 88 years old; 55% of them were males and 45% were females. Histopathologically, 70 (70%) of the cases were diagnosed as nasopharyngeal carcinoma and 30(30%) as benign tumor. EBV antigen was detected by immunohistochemistry in 52(74.3%) of the carcinoma and in 17 (56.7%) of the benign tumor. Intranuclear inclusions (suggesting viral infection) were demonstrated by Macchiavello's stain in 34 (48.6%) of the carcinoma and 14 (46.7%) of the benign tumor sections. This study indicated that infection with Epstein Bar virus may play an important role in the development of nasopharyngeal carcinoma. No association between family history, age, and sex and nasopharyngeal carcinoma was observed.

اجرى هذا البحث لدراسة العلاقة بين فيروس ابستين و السرطان الانفبلعومى لدى مرضى السرطان البلعومى المترددى على مركز الاشعاع و النظائر المشعه بالخرطوم. اخذت مائة عينة من مرضى يشكون من تورم فى الانف و البلعوم ثم فحصت باستخدام صبغة الهيماتوكسيلين و الايوسين و صبغة ميكافيللى و الكيمياء المناعية وذلك لفحص امراض النسيج و اثبات وجود اجسام مضاده لفيروس ابستين و الكشف عن البروتين الغشائى للفيروس على التوالى. تراوحت اعمار المصابين من 16 الى 80 سنة. 55% منهم كانوا ذكور و 45% منهم كانوا اناث. اوضحت النتائج ان 70% من المرضى مصابون بسرطان الانف البلعومى و 30% لديهم اورام حميدة. تم اكتشاف المستضاد للفيروس فى 74.3 % من حالات السرطان و 56.7% من حالات الاورام الحميدة. اظهرت صبغة ميكافيللى اجساما مضادة داخل 6 و 48% من حالات السرطان و 14% من بين الاورام الحميدة. اثبتت الدراسة ان الاصابة بفيروس ابستين لها دورا كبيرا فى تطور سرطان الانف البلعومى. وانه لا توجد علاقة ربط بين تاريخ المرض الاسرى و العمر و النوع من جهه و سرطان الانف البلعومى من جهة اخرى.

keywords: pharyngeal carcinoma, EBV, cancer, immunohistochemistry.

INTRODUCTION:

Nasopharyngeal carcinoma (NPC) is a cancer originating in the nasopharynx, the uppermost region of the pharynx or "throat", where the nasal passages and auditory tubes join the remainder of the upper respiratory tract. NPC differs significantly from other cancers of the head and neck in its occurrence, causes, clinical behavior, and treatment. It is most common in certain regions of East Asia and Africa⁽¹⁻³⁾, in addition to southern China and Tiawan⁽⁴⁻⁶⁾. The etiology is multifactorial with viral, dietary and genetic factors been implicated^(1,2,7). In Asia, NPS is seen primarily in middle aged persons while a high proportion of African cases appear in children. The undifferentiated type is the most common and is strongly associated with EBV infections^(3,4). There is increased risk for NPC in EBV endemic regions but the reason is not entirely clear⁽⁸⁾. Beside NPC and EBV were reported to be tightly associated with other lumen cancers such as Burkett's lymphoma and Hodgkins lymphoma^(9,10). NPC is uncommon in the United States and most other nations, but is extremely common in southern

regions of China and Tiawan^(5,6). This could be due to the South East Asian diet which typically includes consumption of salted vegetables, fish and meat⁽⁷⁾. While NPC is seen primarily in middle-aged persons in Asia, a high proportion of African cases appear in children. The cause of increased risk for NPC in the EBV endemic regions is not entirely clear⁽⁸⁾. Epstein Barr Virus is tightly associated with different human cancer, and several malignancies as Burkett's lymphoma, Hodgkins lymphoma and nasopharyngeal carcinoma^(9,10). Several studies have recently hinted at a possible role for EBV in the pathogenesis of breast carcinoma, which represents the most common carcinoma of female in the western world^(11,12). Diagnosing of nasopharyngeal carcinoma usually begins with a general examination which include observation of early signs and symptoms of nasopharyngeal carcinoma. It may take months of investigations before a definitive diagnosis is made. Taking biopsies for histopathology is essential for diagnosing and describing the type of NPC⁽¹³⁾.

MATERIALS and METHODS:

Ethical consideration and samples collection:

Each participant was well informed about the study and asked to sign a written ethical consent form before taking the sample. One hundred biopsies were randomly collected from patients presenting with nasopharyngeal tumors by special physicians teams at the RICK under high quality control measures which were adopted during sample collection and processing.

Histopathological methods: Trimmed biopsy samples were processed in an automated tissue processor. Paraffin sections, 3-5µm, were prepared (3 sets). One set was stained with hematoxylin and eosin (H&E) for histopathological examination⁽²⁾.

Histochemical and immunohistochemical methods:

The 2nd set of paraffin sections was stained with Macchiavello’s stain for demonstration of EBV inclusion bodies in the cells. The remaining paraffin sections were used to detect EBV latent membrane protein (LMP) using monoclonal mouse antibodies⁽⁶⁾.

Data analysis

Brown nuclear staining is considered positive reaction in infected tissue. Data was analyzed using SPSS computer program. Frequencies, cross tabulation and chi-square were calculated.

RESULTS:

Patients age and gender:

Fifty five percent of patients investigated were males and 45% were females. Their ages ranged from 16 to 80 years and 45% of them were below the age of 35 years.

Histopathological results:

Seventy percent of the biopsy samples were diagnosed as nasopharyngeal carcinoma and 30% as benign tumors. The frequency of of the tumor with respect to gender and age group of patients can be seen in table 1. The frequency of both tumors was high in males than in females.

Age group	Benign tumor				Nasopharyngeal carcinoma			
	Male		Female		Male		Female	
	No	%	No	%	No	%	No	%
<35	10	22.2	7	15.6	16	35.5	12	26.7
36-45	4	20.0	2	10.0	7	35.0	7	35.0
46-55	1	6.6	0	0.0	6	40.0	8	53.4
56-65	2	12.5	2	12.5	7	43.8	5	31.2
>66	1	25.0	1	25.0	1	25.0	1	25.0
Total	18	18.0	12	12.0	37	37.0	33	33.0

Table (1): Distribution of study population by age, sex and type of nasopharyngeal tumor.

Histochemical and immune-histochemical results:

Using Macchiavello's stain, intranuclear inclusions (inclusion of the viral infection) were seen in 34 sections (48.6%) of the NPC and 14

(46.7) of the benign tumor biopsies. EBV latent membrane protein was detected by immunohistochemistry in sections of 52 (74.3%) and 17 (58.7%) of the NPC and benign tumor cases respectively (table 2).

Table (2): Relation between nasopharyngeal tumors and the presence of EBV indicated by immunohistochemistry and Macchiavello's stain.

Type of nasopharyngeal tumor	Immunohistochemistry results				Macchiavello's stain results			
	+ ve		- ve		+ ve		- ve	
	N	%	N	%	N	%	N	%
Nasopharyngeal carcinoma	52	74.3	18	25.7	34	48.6	36	51.4
Benign tumor	17	56.7	13	43.3	14	46.7	16	53.3
Total	69	69.0	31	31.0	48	48.0	52	52.0

N is the number of patients.

DISCUSSION:

Epstein-Barr virus (EBV) is a herpes virus that infects a vast majority of the world's adult population^(14,15). Oncogenic viruses can contribute to different steps of the carcinogenic process and the association of virus with a given cancer amounts to 15% up to 100%⁽¹⁶⁾. The

present results indicate positive association between EBV and NPC. The EBV virus was considered as a risk factor for this type of cancer. The detection of nuclear antigen associated with EBV and viral DNA in NPC type revealed that EBV can infect epithelial cells and is involved with their transformation. The etiology of NPC seems to follow a multi-step process, in which EBV, ethnic background, and environmental carcinogens all seem to play an important role⁽¹⁷⁾.

The present results support the former postulation that EBV is closely related to NPC and plays an important role in its origin^(18,19). Moreover in this study, males constituted 55% of the total studied patients. This observation is in consistence with the former reports which pointed out that, frequency rate of EBV infection was higher in males than in females⁽²⁰⁾. On the other hand, this result contradicts with the results of Mounir and his co-workers who reported that the immunohistochemical

expression of EBV in nasopharyngeal carcinoma was not related with patient's sex⁽²¹⁾. The group of patients below 35 years old (45% of cases of both genders) showed a relatively high frequency of nasopharyngeal tumor among the samples investigated in our work. Such age group was formerly found to have high frequency of EBV infections among different age groups examined⁽²²⁾. The frequency of EBV infection in different age groups in this study were 45 (45%), 20 (20%), 15 (15%), 16 (16%) and 4 (4%) in the age groups of less than 35 years, 36 – 45 years, 46-55 years, 56-65 years and above 66 years respectively. However, this type of distribution of EBV infection among different age groups is in agreement with the manner of distribution described in an earlier work of Sudha and his co-workers⁽²³⁾. The overall positive infection of EBV in this research was 69%. The frequency of EBV in the present work was similar to that reported in England⁽²⁴⁾. This rate of EBV infection is also comparable to the infection rate reported recently in China and some other parts of Asia^(25,26). Up to our knowledge, this is the first study conducted in the Sudan to stain EBV inclusion bodies using Macchiavello's stain to stress on the role of EBV in nasopharyngeal

carcinoma and benign tumor of nasopharyngeal region.

CONCLUSIONS:

This study showed that infection with Epstein Bar Virus plays an important role in the development of nasopharyngeal carcinoma. In this study, there is no association between family history, age, and sex and nasopharyngeal carcinoma and EBV infection.

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