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Sero-detection of Epstein- Barr Virus among Patients with Nasopharyngeal Carcinoma Attending National Cancer Institute, University of Gezira - Sudan

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Abstract: Background: *Epstein-Barr virus (EBV) infects around 95% of the world's population. This infection has been linked with lymphoid and epithelial cancers; persistence of infection in lymphoid or epithelial cells may result in virus-associated B-cell tumors or nasopharyngeal carcinomas (NPC).* ***Objective****: The study aimed to detect Epstein-Barr virus antibodies (IgG) against viral capsid antigen (VCA) among patients with nasopharyngeal carcinoma.* ***Methodology****: Descriptive laboratory, hospital based study was conducted in National Cancer Institute, University of Gezira, Sudan during March to October 2017. A total of 61 patients with NPC were included; structured questionnaire was designed to collect the data. Blood samples were collected into lithium heparin anticoagulant and plasma was tested for EBV antibodies (IgG) against VCA by quantitative enzyme linked immune sorbent assay (ELISA).* ***Results****: From a total of 61 cases there were 62.3% (38/61) males and 37.7% (23/61) females with ratio 2:1 (Male:Female), the age ranged between 4 to 70 years, with their means of (39.7 ± 17.2). Squamous cell carcinoma was the most common histopathological subtype and accounted of 91.8% (56/61). Most of NPC patients were within WHO typing; type III was 90.2% followed by type II. VCA antibodies (IgG) of EBV was detected with 93.4% (57/61) of studies cases. There was no significant differences between age, sex, WHO typing and NPC stage. However there was a significant correlation (P=0.05) between histopathology and the present of EBV.* ***Conclusion:*** *VCA antibodies (IgG) of EBV was significantly detected among patients with nasopharyngeal carcinoma especially, squamous cell carcinoma and WHO type III.*

Keywords: EBV, Nasopharyngeal carcinoma, University of Gezira, Sudan, ELISA.

1. INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a common challengeable cancer in developing countries, and it may has a complex interaction of many risk factors such as genetic, environmental, dietary and viral infections. A report from East Asia and Africa, with viral, dietary and hereditary elements as causation of NPC were published[1]. According to the WHO, NPC is classified in to keratinized squamous cell carcinoma and non-keratinizing carcinoma, and the later classified more into differentiated and non-differentiated[2]. The undifferentiated form is the most common, and strongly associated with Epstein-Barr Virus (EBV) infections of the cancerous cells[3,4]. The number of nasopharyngeal carcinoma cases increased in Sudan due to increase in exposure to carcinogenic factors. In line, smoking and other Tobacco usage associated with about 75 % of NPC cases in Sudan[5]. In addition, NPC is more prevalent in certain parts of Asia and North Africa, particularly in Southeast China, and reported among recent Chinese immigrants in the United States of America[6]. Abu Idris in 2008 indicated that, infection by Epstein-Barr Virus is associated with several human carcinomas, especially NPC[5]. The strong association between NPC and EBV infection was confirmed with 100% genes detection rate[7]. The association between specific serologic responses to EBV and NPC, and differences in susceptibility to EBV-related tumors were also reported[8,9]. The link between NPC and EBV is well established, as the sera of patients with this malignancy were found to contain precipitating antibodies against the virus, moreover, virus genome was also detected in biopsy materials from most NPC patients. The objective of this study was to detect the IgG antibodies level of viral capsid antigen (VCA) of EBV among nasopharyngeal carcinoma patients.

2. MATERIALS AND METHODS

Sixty-one consecutive cases of NPC were enrolled during March to October 2017, cases were diagnosed at National Cancer Institute, University of Gezira, Wad Madani, central Sudan. Data were collected via hospital medical records and patient self-reporting. Data collected from medical records included patients' demographics, medical history, histological results and, tumor stages and grades. Self-

reporting was carried out using questionnaire specifically designed to investigate nasopharyngeal carcinoma. Blood samples were collected into lithium heparin anticoagulant; plasma was tested for anti EBV; VCA (IgG) by ELSIA product by EUROIMMUN Medizinische Labordiagnostika, AG-Germany, and Solid phase enzyme-linked immunosorbent assay (ELISA) based on sandwich reaction and standard curve.

3. Data Analysis

The data were analyzed for presence of EBV in esophageal cancer tissues. Relative risks (RRs) and 95% confidence intervals (95% CIs) were calculated. Data management was done using Statistical Package for Social Sciences (SPSS version 16). SPSS was used for analysis and to perform Pearson Chi-square test for statistical significance (P value). The 95% confidence level and confidence intervals were used.

Results:

There was 62.3% (38/61) males and 37.7% (23/61) females patients with ratio of 2:1, the age ranged between (4 to 70 years) with their mean of 39.7 ± 17.2 years. The peak age group was between 18 - 40 and 41-75 years and showed 44.3% (27/61) of participants. Smoking behavior was recorded with 18% (11/61) while positive family history for cancer was observed with 13.1% (8/61) of patients Table (1).

Table (1): Demographic data of study subjects

		Frequency	Percent
Gender	Male	38	62.3
	Female	23	37.7
	Total	61	100
Age groups	0 - 16 years	7	11.4
	18 - 40 years	27	44.3
	41 - 75 years	27	44.3
	Total	61	100
Smoking	No	50	82
	Yes	11	18
	Total	61	100
Family history of Cancer	No	53	86.9
	Yes	8	13.1
	Total	61	100

The dominant histopathological features were WHO type III and type II with percentage of 90.2% (55/61) and 9.8% (6/61) respectively, noted that, 70.5% of the cases were diagnosed at late stages IV (a, b and c) Table (2).

Table (2): Histopathology, NPC WHO Types and Stages between Study Subjects

		Frequency	Percent
Histopathology	Non-squamous cell carcinoma	5	8.2
	Squamous cell carcinoma	56	91.8
	Total	61	100
NPC WHO types	Type II	6	9.8
	Type III	55	90.2
	Total	61	100
NPC Stage	Stage 1	1	1.6

	Stage 2	4	6.6
	Stage 3	13	21.3
	Stage 4a	21	34.4
	Stage 4b	20	32.8
	Stage 4c	2	3.3
	Total	61	100

VCA antibodies (IgG) of EBV was detected in 93.4% of overall patients Table (3). There was no significant correlation between infection with EBV and sex, smoking, NPC WHO types and NPC stages, but there was strong correlation with histopathology and family history. Also there was low odd ratio property in smoking, NPC stages and family history and high odd ratio property in sex, histopathology and WHO types Table (4).

Table(3): Distribution of EBV in study subjects

EBV	Frequency	Percent
Negative	4	6.6
Positive	57	93.4
Total	61	100

Table (4): Distribution of EBV VCA antibodies(IgG) in study subject according to risk factors

Risk Factors	Negative	Positive	Odd ratio	95 % Cl		P. value
Male (n= 38)	75% (3/4)	61.4% (35/57)	1.9	0.184	19.28	0.513
Female (n= 23)	25% (1/4)	39.6% (22/57)				
Non-smoke (n= 50)	75% (3/4)	82.5% (47/57)	0.638	0.06	6.78	0.559
Smoker (n= 11)	25% (1/4)	17.6% (10/57)				
Non-squamous cell carcinoma	25% (1/4)	7% (4/57)	4.42	0.370	52.78	0.05
Squamous cell carcinoma	75% (3/4)	93% (53/57)				
WHO Type II	25% (1/4)	8.8% (5/57)	3.47	0.302	39.85	0.346
WHO Type III	75% (3/4)	91.2% (52/57)				
Stage 1 - 3	1(25%)	17(30%)	0.784	0.076	8.08	0.662
Stage 4	3(75%)	40(70%)				
No family history	100% (4/4)	86% (49/57)	0.925	0.856	0.898	0.04
Yes family history	0% (0/4)	14% (8/57)				

4. DISCUSSION:

Cancer continues to be the most serious and fatal disease, especially in developing countries due to late diagnosis and lacking of sophisticated equipments for diagnosis and treatment[10]. NPC is the most common cancer originating in mucosal lining epithelium of the nasopharynx. World Health Organization classified nasopharyngeal carcinoma in three types. Type I (squamous cell carcinoma), Type II (keratinizing undifferentiated carcinoma) and Type III (non-keratinizing undifferentiated carcinoma)[11]. 93% (57/61) of studies patients showed positive for IgG VCA of EBV, the majority of them were in age group (21-40 years) with means (178.7 ±26.3). This finding is comparable with other results reported, for intense, ratio of 2:1 infection among males and females were reported in Middle East[12] and Wad Medani-Sudan[5], Other studies gave a conclusion which differ from this study , like that study done in Khartoum , Sudan[13], Saudi Arabia[9] and Indonesia[14]. In our study, we noticed that the bulk of patients were from Gezira state and this is because study was conducted in NCI Gezira lying in the center of this state, NCI is the second major cancer center in Sudan after RICK- Khartoum. The vast majority of patients in our study presented at an advanced stage, as 97% of patients were diagnosed at stage III and IV, the reason for that all patient were discovered at last stages. This finding in line with previous studies done in Sudan[13] and[5], also recently in Egypt, a study found 86% at stage III and IV[15], and in study done in Saudi Arabia[9]. In our study most of patients were with squamous cell carcinoma pathological types (92%), other subtypes were anaplastic and lymphoepithelioma with percentage 6% and 2% respectively, this finding were in line with study done in Egypt[15]. Most of the patients in our study presented with NPC WHO type III which constituted about 90% of all histological type. This came close to the studies in done in Sudan[16], China[17] and Egypt[15], in which about 70.5% and 76.6% of patients presented with NPC WHO type III respectively. Prevalence of EBV in Nasopharyngeal Carcinoma in Patients were 93% this nearly to study done in Iran[18]. This study found no correlation between sex and EBV , this in agree with study done in Iran also[17]. In our study, though, high percentages of NPC positive EBV were reported among age group 21-40, no significant correlation between age and the prevalence of EBV, this finding was strongly supported by a study done in Iran[17]. Comparing the viral indices across different TNM stages, nasopharyngeal patients at advanced stages had high frequency of EBV without significant correlation, this finding disagree with study done in China whom find significant correlation[19]. In this study the EBV was remarkably higher among those patient has family history to cancer when compared with those without family history (p=0.04) This finding in line with previously study which reported in Iran[18]. The study recommended that Epstein-Barr Virus should be assayed for patients with nasopharyngeal carcinomas.

5. CONCLUSION

There was high prevalence of Epstein-Barr virus antibodies among nasopharyngeal carcinoma patients, there was significant correlation between histopathology subtypes and Epstein- Barr Virus antibodies and there was significant correlation between family history of cancer and Epstein-Barr virus antibodies. There was no significant correlation between age, sex, WHO type and stage.

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