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Research Article

SEROFREQUENCY OF EPSTEIN -BAR VIRUS AMONG SUDANESE PATIENTS WITH NASOPHARYNGEAL CARCINOMA ADMITTED TO NATIONAL CENTER FOR RADIOTHERAPY AND NUCLEAR MEDICINE KHARTOUM _SUDAN

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ABSTRACT

A total of 51 patients suffering of nasopharyngeal carcinoma who attending national center for radiotherapy and nuclear medicine during November 2013-january 2014 and 26 control were enrolled in this is study, Their age ranges from3 years _60 years with mean (30).The aim of this study was to detect frequency of EBV IgA antibodies, and to evaluate the risk factor associated with EBV and NPC, serum specimens and analyzed by ELISA technique. The results showed that 27(53%), of patients were positive for EBV and 2(7%) of control was positive for EBV.

Statistical analysis showed insignificant relation (P value > 0.05) between EBV seropositivesity among patient and control , age and gender .

Keywords: Epstien-Bar virus – IgA- Nasopharyngeal carcinoma -ELISA-Khartoum

INTRODUCTION

Epstein- Bar- Virus also called human herpes virus (HHV-4). also associated with particular forms of cancer, such as Hodgkin's lymphomas Burkett's lymphoma, Nasopharyngeal carcinoma and conditions associated with (HIV) such as hairy leukoplakia and central nervous system lymphoma[1,2] and also associated with a higher risk of certain autoimmune diseases[3], especially dermatomyositis, Sjorgen's syndrome[4,5] and multiple sclerosis[6] .

Infections with EBV occur by oral transfer of saliva and genital secretions[7]. (EBV was formed to be the major cause of infectious mononucleosis (IM), a usually self limited clinical syndrome[13] only about 5% of adult in western societies remain EBV un infected . thus anti body prevalence reach 95% or higher among eldery individuals[14] EBV infect B cell of the immune system and epithelial cell .

NPC is cancer found in upper respiratory tract most commonly in nasopharynx and in linked to EBV virus it is found predominantly in south China and Africa due to both genetic and inveromantal factors it is much more common in people of Chinese ancestry (genetic), but it also linked to Chinese diet of a high amount of smoked fish which contain nitrosamines, well known carcinogens (environmental). NPC commonly known as nasobharyngeal caner is classified as amlignant neoplasm, or caner, arising from the mucosal epithelium of nasopharynx the world health organization classified NPC into three types:

Type 1 squamous cell carcinoma

Type 2 is keratinizing undifferentiated carcinoma

Type 3 is non keratinizing undifferntiated carcinoma which known as lymphoepithelioma is most common and it is strongly associated with EBV cancerous cells[9].

NPC is caused by combination of factors viral and environmental influences. It is not genetic. The viral influences are associated with infection with Epstein barr virus[10] the EBV is one of the most common virus.

The WHO does not have set preventative measures for these viruses because it is so easily spread and is worldwide.

Very rarely does EBV lead to cancer which suggests variety of influencing factors of .other likely etiological factors include genetic susceptiblity, consumption of food (in particular salted fish[11] containing carcinogenic nitosamines[12] cervical lymphoadenopathy (swelling of lymph node) in neck the initial presentation in many patients, and the diagnosis of

NPC is the often made by lymph node biopsy. symptoms related to the primary tumor include trismus, pain, otitis media, nasal regurgitation due to pariesis of the soft palate, hearing loss and cranial nerve paralysis. larger growths may produce nasal obstruction or bleeding .metastatic spread may result in bone pain or organ dysfunction the WHO does detail the highest incidence of NPC 20 per 100.000 is in the Southern china other region Kuwait , Israel. NPC affects men twice as often as women and most common between ages from 20-50 years[15] in the case of EBV both epithelial cells and human B. lymphocytes are infected. Epithelial cells are infected during the active phase, memory B. Lymphocytes on other hand are where the latent EBV resides.

EBV is often found in human saliva this can be during an active disease state or most commonly during a periodic reactivation . due to this most infections are due to direct contact with infected saliva, human beings are the primary reservoir for EBV the EBV will stay latent in person for their life time and reactivation . Nasopharyngeal carcinoma (NPC) occurs with high incidence in certain regions, such as South-East Asia Two major histological types of NPC are recognized, non-keratinizing carcinoma and squamous cell carcinoma. Non-keratinizing NPCs associated with Epstein–Barr virus (EBV) infection, regardless of the geographical or ethnic origin of the patients. squamous cell NPCs have been collated from an area where NPC is endemic, Hong Kong, and from two regions where NPC occurs with a lower incidence, Chengdu, PR China, and Birmingham, United Kingdom. In situ hybridization for the detection of the small EBV-encoded nuclear RNAs (EBERs) demonstrated that all 22 cases from Hong Kong were EBV-positive. By contrast, EBV was detectable in 7 of 19 cases from central China, and in 3 of 7 cases from the U.K. Expression of the virus-encoded latent membrane protein 1 (LMP1) was detected in 3 of 32 EBV-positive squamous cell NPCs. These results indicate that the association of squamous cell NPCs with EBV shows geographical variability[20].

MATERIALS AND METHODS

This was descriptive- cross sectional study which had been conducted in Khartoum state during period from November 2013 to January 2014, 51 patients of NPC and 28 healthy individual as control were enrolled Data was collected by using direct interviewing questionnaire; ethical clearance was obtained for research ethical committee of faculty of graduate studies and ministry of Health Khartoum state, written consent also was obtained from NPC patients.

Experimental work

Samples collection:

blood samples were collected from 51 nasopharyngyeal carcinomas patient and 26 healthy individual as control, under direct medical supervision by medical vein puncture using 5 ml syringe into plain tube to obtain serum by centrifugation at 5000 rpm for 10 mm. serums was kept in -20°C till serological study was performed.

Specimens were processed by Enzyme linked immune sorbent assay (ELISA) (3rd generation ELISA) (Weka- China) for detection 1gA of EBV.

Enzyme linked immune sorbent assay for detection anti EBV IgA

All reagents and samples were allowed to reach room temperature for 15minutes before use washing buffer was prepared 1:10 from buffer concentrate with distilled water.

Hundrad ul of the calibrator, positive, negative controls and diluted patients samples were pipptted into the micro plate wells :

- The plate was Incubated 30 min at room temperature.
- Then washed 3 times.
- We're Left the wash buffer in each well for 60 seconds per washing cycle then empty the wells.
- Then tapped micro plate on absorbent paper .
- 100 ul of enzyme conjugate was added into each well. incubated for 30 min at room temperature .
- Then Washed (empty the wells).
- 100 UL of chromogen/substrate solution was added into each wells and incubated for 15 min at room temperature.
- 100 UL of stop solution was added into each of the microplate wells in the same order as the Chromogen/substrate. measuring the absorbanc:
- Photometric measurement of the color intensity was made at a wave length of 450 nm.
- The results were calculated by relating each sample optical density (O.D) value to the cut off value of plate
- Ratio = exiction of the control or patient samples /exicion of calibrator
- Ratio <0.8 = negative
- Ratio ≥ 0.8 +o<1.1= border line
- Ratio ≥ 1.1 positive.

Measuring the absorbance:

Absorbance was read at 450nm. The results were calculated by relating each sample optical density (OD) value to the Cut off value of plate. Calculation of Cut off (C.O) value.

Interpretation of Results:

Negative results: samples giving absorbance less than Cut-off value are negative for this assay. Positive result: sample giving absorbance equal to or greater than Cut-off considered initially reactive. Borderline: sample with absorbance to Cut-off value are considered borderline and retesting of these samples in duplicate is recommended. Data analysis: Data was analyzed by SPSS (Statistical Package of Social Science) software program version 16.

RESULTS

A total of 51 (66%) patients and 26 (33.8%) control were participated in this study. serofrequency of EBV among patients and control was 27 (53%) , 2 (7%) respectively (table 1)

According to the studied group 24 (31.2%) were males had EBV and 5 (6.5%) were females had EBV table (3).

Regarding age distribution of studied group most of seropostivesity results of EBV observed among most than 41 years age range table (4).

Statistical analysis showed insignificant relation (P value > 0.05) between EBV seropositivesity among patient and control, age and gender table (2, (3), (4).

Table 1: Serofrequency of EBV among patients and control group

	NPC	Control	Total
Positive for EBV	27 (53%)	2 (7%)	29
Negative	24 (47%)	24	48
Total	51 (100%)	26 (100)%)	77

Table 2: Relation between seropositivesity of EBV among NPC patient and control group

Crosstab					
			Group		Total
			NPC	Control	
Result of EBV IgA	+ve	Count	27	2	29
		% of total	35.1%	2.6%	27.7%
	-Ve	count	24	24	48
		% of total	31.2%	31.2%	62.3%
Total		count	51	26	77
		% of total	66.2%	33.8%	100.0%

p- value = 0.386 (p-value >0.05 Not significant)

Table 3: Serofrequency of EBV in relation to gender

Crosstab					
			Gender		Total
			Male	Female	
Result of EBV IgA	+ve	Count	24	5	29
		% of total	31.2%	6.5%	37.7%
	-Ve	count	37	11	48
		% of total	48.1%	14.3%	62.3%
Total		count	61	16	77
		% of total	79.2%	20.8%	100.0%

p- value = 0.386 (p-value >0.05 Not significant)

Table 4: Serofrequency of EBV in relation to age

Crosstab						
			Age			Total
			3-16	17-40	41+	
Result of EBV IgA	+ve	Count	2	13	14	29
		% of total	2.6%	16.9%	18.2%	37.7%
	-Ve	Count	5	25	18	48
		% of total	6.5%	32.5%	23.4%	62.3%
Total		Count	7	38	32	77
		% of total	9.1%	49.4%	41.6%	100.0%

p- value = 0.624 (p-value >0.05 Not significant)

DISCUSSION

The EBV is consistently detected in patients with nasopharyngeal carcinoma to determine whether EBV infection is an early initiating even event in development of this malignant tumor. This study presented the most recent data on the serofrequency of EBV in NPC patients in term of age group to detect antibodies against EBV and to evaluate the risk factor associated with EBV and NPC.

The present study revealed that 27 (53%) from patients were positive for EBV and 2 (7%) of control was positive for EBV, this finding similar to that obtained by krishnasm and others who reported EBV in NPC India patients serum 58% and EBV DNA in 69% of biopsies[16] .

But when compared with other finding regarding it is lower than which obtained by Adam et al. Khartoum Sudan. 2014. who reported all NPC cells are clearly EBV infected that (100%) were positive for Epstien _Bar virus Encoded RNA1 (EBER1)[17].

in other study obtained by Jansevan Rensburg *et al* 2000 in south Africa patients the result Showed that EBV could detected in 82% of the patients of NPC that strong association was found between EBV and NPCs[18].

Also in study done by Ahmed Hidatalla and his colleague in 2004 in patient with NPC in Sudan cancer registry and radiation Isotope center of Khartoum they analyzed 374 – 512 patients, NPC formed 5.8% of all cancer cases in Sudan cancer registry and 7.2% at Radiation isotope center of khartoum(Rick) and they found in Rick male/ female ratio 3:1 NPC, male at Rick (21.1%) . the result showed that EBV might be an existing factor in NPC[19].

Other study done by Eduardo et al June 2010, Portugal ENT institute. The results indicate a higher frequency of positive EBV cases among patients with Nasopharyngyeal carcinoma[21].

CONCLUSION

EBV infection was found in most cases of NPC which confirms the etiological role of EBV in NPC. Further research must be conducted with large sample size to confirm finding

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