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RESEARCH ARTICLE

Epstein –Barr Virus VCA- IgG Antibodies in Nasopharyngeal Carcinoma patient and Control group -A laboratory based study.

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Abstract

Epstein Barr virus (EBV) is a member of human herpes virus which is etiologically closely related to nasopharyngeal carcinoma (NPC). NPC is generally seen to be associated with elevated levels of antibodies against antigens in the EBV lytic phase. This study was aimed to assess the serum level IgG antibodies against the viral capsid antigen of Epstein Barr virus as well as to compare the levels in Nasopharyngeal carcinoma patients and healthy controls. The study was carried out as a case-control study in a tertiary hospital of a Northeastern state of India. A total of 90 samples (50 nasopharyngeal carcinoma and 40 normal healthy controls) were tested by Enzyme Linked Immunosorbent Assay by using EBV-VCA IgG ELISA kit. The male to female ratio is 3:1and majority (64%) NPC cases are from Mongoloid groups of people. All the Nasopharyngeal Carcinoma cases (100%) showed positive IgG antibodies with higher titer but majority of randomly selected healthy control groups shows lower titer against the viral capsid antigen of Epstein Barr virus. Thus, elevated antibody level could serve to predict diagnosis of NPC among patients suspected of having NPC on one hand, and on the other hand, it could serve as a risk marker to predict occult tumour patients among apparently healthy subjects

INTRODUCTION

Epstein-Barr virus (EBV) is a ubiquitous human herpes virus with worldwide infection. It is associated with Burkitt's lymphoma in Africa and nasopharyngeal carcinoma (NPC) in Asian countries (Liu and Yeh 1998). NPC is an epithelial neoplasm arising from the fossa of Rosenmuller of the post nasal space (Li et al., 2010). NPC is a disease with a remarkable racial and geographical distribution (Yu, 1991).It is a rare malignancy in most part of the world; but endemic in Southern China, Hongkong, Singapore and some parts of Southeast Asia (Marcus and Tishler, 2010). The incidence of NPC in the Indian subcontinent is not well documented, but it seems to show geographical variation. NPC is generally rare in India as compared to other Southeast Asian countries, with the exception of some north-eastern states, mainly Nagaland (Chelleng et al. 2000). Other populations with elevated rates include the natives of Southeast Asia, the natives of the Arctic region, and the Arabs of North Africa and parts of the Middle East (Yu et al, 2002). Most of the studies revealed male preponderance over the female. In Hongkong incidence rates of NPC among males and females was found in the ratio 3: 1(Hongkong Cancer Registry, 2010). As a very ancient parasite of humans, EBV has established a directly balance relationship with its host that is almost entirely benign under normal conditions. Natural primary infection is usually inapparent, and is always followed by a lifelong, silent, carrier state that becomes manifest as disease- only if the virus-host balance is disturbed (Epstein and Crawford, 1998). An association between EBV and nasopharyngeal carcinoma was first suggested by the observation that patients with this malignancy had elevated IgG and IgA titers to EBV lytic antigens (VCA and EA).

(Dorothy, 2004).An important development arising from EBV's association with NPC is the use of anti-EBV antibody responses as diagnostic and prognostic tools in disease monitoring. A key observation in this context was that most NPC patients have not only elevated IgG but also detectable IgA responses to VCA and EA (D) (Rickinson and Kieff, 2001). IgM antibodies (VCA IgM) usually appear at the time of the onset of the clinical symptoms of acute infection and disappear within a few weeks, however, antibodies against the capsid antigen IgG (VCA IgG) typically appear at the same time as of VCA IgM and remain positive for life (Schillinge et al., 1993). This study thus aimed at studying VCA-IgG level among the NPC cases and healthy controls and to compare the titer values targeting the population in this part of the globe.

MATERIALS AND METHODS

Study population: A total of 50 patients with Nasopharyngeal carcinoma and age-sex matched 40 normal subjects were screened for Epstein- Barr virus IgG antibodies to viral capsid antigen during a period of one year. Samples were collected from the patient admitted as well as attending outdoor patient department of tertiary care hospital, in north east of India. These patients were clinically diagnosed as having nasopharyngeal carcinoma.

Selection of cases: Selection of cases was made among the patients having clinical feature as well as based on histopathological and radiological investigation. Randomly selected normal healthy individuals and patient relatives without relevant history in recent past were taken as controls. Only cases that voluntarily co-operated were included in the study. Consent was taken from all controls before procedures.

Scheme of case taking: The relevant history was recorded in each case on a pre designed proforma. Special interest was given on clinical presentation, race, sex, age, occupation, food habit, family history etc.

Collection of specimen: Under all aseptic care 5 ml of venous blood was collected from the patients. Blood were allowed to clot and serum was separated by centrifuging at 3000 rpm in a centrifuge machine and a code number in the proforma and the samples. The serum samples were stored at -20^0c.

Laboratory methods: According to the instruction, the Serum samples were tested for IgG antibodies against the viral capsid antigen (VCA) of Epstein Barr virus by Enzyme Linked Immunosorbent Assay by using EBV-VCA IgG ELISA kit produced by Demeditec Diagnostics GmbH, GERMANY. For a quantitative evaluation, the absorbance of the standards and controls are graphically drawn against their concentrations. The calculated absorbance for the patient sera are compared with the value for the cut-off standard. From the resulting reference curve the concentration values for each sample are extracted in relation to their absorbance.

Statistical analysis: All the data collected were entered and analysed using SPSS 16.0 version.

RESULTS

Among the 50 NPC cases, highest numbers 28(56%) are in the age group of 40-59 yrs as shown in table 1. The mean age of the cases and the controls are 50.08+12.836 years and 48.28+13.148 years respectively, calculated by independent T-test. The study group consists of 38(76%) males and 12 (24%) females (table1). The male to female ratio is almost 3:1 (3.2: 1). Majority 32(64%) NPC cases are from Mongoloid groups of people (table2). All the Nasopharyngeal Carcinoma cases (100%) showed positive IgG antibodies against the viral capsid antigen of Epstein Barr virus as shown in table 3. While in randomly selected healthy control group, only 25(62.5%) shows positive ELISA. All the NPC cases Shows Higher titer of more than 100. Highest titer (>200 U/mL) is seen among 14 NPC cases. On the other hand, majority of randomly selected healthy control groups are with a lower titer less than 100 and 5 (12.5%) controls which are patient's relative showed significantly higher titer of more than 100.Mean VCA-IgG titer of NPC cases is 174.28+25.122 and that of control group is 39.32+39.601. Chi-square test for patients results showed significant difference ($p<0.05$) among the variables like sex, race, presence of clinical conditions, VCA-IgG titer values and Age category whereas no significant difference in smoked and dried food eating habits ($p>0.05$).Among the control group significant differences are observed in race, VCA-IgG titer values and age category (table-4).

Table 1: Age and sex distribution among NPC cases and healthy controls.

Age in years	NPC Cases(50)		Control group(40)	
	Male	Female	Male	Female
0-19	1	0	1	1
20-39	6	2	3	2
40-59	20	8	15	10
60 and above	11	2	5	3

Table 2: Race distributions among NPC cases and healthy controls

NPC CASES		CONTROL	
MONGOLOID	OTHERS	MONGOLOID	OTHERS
32(64%)	18(36%)	32(80%)	8(20%)
TOTAL= 50		TOTAL= 40	

Table 3: Different titer of IgG antibodies against viral capsid antigen among the NPC cases and control group.

Titers	NPC cases	Control groups
<10	0	15
10-50	0	19
50-100	0	1
100-150	8 (16%)	5(patient relatives)
150-200	28(56%)	0
>200	14(28%)	0

Table 4: Significance analysis of different variables among NPC cases and control group.

Variables	NPC cases **Chi-Square, df, (p-value)**	Control groups **Chi-Square, df, (p-value)**
Sex	13.520,1, **(0.000)**	1.600,1, (0.206)
Race	3.920,1,**(0.048)**	14.400,1, **(0.000)**
Clinical Conditions	31.694,5, **(0.000)**	-
Food habits	0.320,1, (0.572)	0.400,1, (0.527)
VCA-IgG Category	23.120,1, **(0.000)**	2.500,1, (0.114)
Age Category	31.440,3, **(0.000)**	31.800,3, **(0.000)**
IgG-VCA Titre	65.200,19, **(0.000)**	84.950,16, **(0.000)**

DISCUSSION

Our study confirmed the age old recognised pattern of age and sex distribution of NPC varies in different parts of the world. Among the 50 NPC cases in the study, highest numbers28 (56%) of NPC had seen in the 40-59 yrs of age group. Similar age distribution of NPC cases are also found by Commun et al (1974); Balakrisnan et al (1976); Hirayama (1978) and Karry et al (2005). Kumar et al (1996) also found no specific bimodal age peaks from NE region of India. Our study group consists of 38(76%) males and 12 (24%) females. The male to female ratio is almost 3:1 (3.2: 1).Which is similar to study by Hidayatalla A et al (1983);Zinyu et al(1990); and Nwaorgu et al(2004).

There is a variation of incidence of nasopharyngeal carcinoma among different ethnic groups in countries with multiracial populations. In the present study 32(64%) cases of NPC are from Mongoloid group of people. Kumar and Mahanta (1998) and Yu and Yuan (2002) also reported relatively higher NPC among the mongoloid group of the people in the North Eastern Region of India as compared to other parts of the country. Thus it can be inferred that NPC is relatively higher among the Mongoloid group of people in the NE region of India compared to Caucasian population residing in other parts of the country.

All 50 NPC cases showed positive IgG antibodies against EBV by ELISA. Similar findings are reported by Fachiroh et al (2006); and Zeng et al (1986). Ringborg et al (1983) also reported 100% positivity against EBV specific Anti-VCA IgG among 17 NPC patients. Mun-Hon et al (2006) found 100% seroposivity among non-keratinizing NPC patients. Liu, et al (1997) also stated ELISA as the most sensitive method for detection of NPC. The serum positive rate of NPC patients was 100% by ELISA in a study by Wan et al (1998). Because of its high sensitivity, specificity and rapid performance, the enzyme-immunoassay is a useful tool for large scale epidemiological studies for the early diagnosis and monitoring of patients with nasopharyngeal carcinoma as reported by Dolken et al (1984). On the contrary, Roy et al (1994) found 6 positive out of 9 nasopharyngeal carcinoma cases. Fones-Tan A et al (1994) also found 81 positive out of 100 NPC cases by IgG assay. Rathaur et al (1999) also detected EBV in 28 cases out of 40 (70%) nasopharyngeal carcinomas in a study carried out in India.

Titer value of more than 100 by ELISA is seen among all 50 NPC cases in the study,with mean titer 173.72+24.523 and that of control group is 39.32+39.601. Highest titer of more than 200 is seen among 14 NPC cases. Similar reports of higher titer are also documented by Lin et al(1975); Sako et al(1975); Kottaridis et al (1977);Micheau et al (1980); Cai et al (1983);Gurtsevitch et al (1986); Lin et al(1986);Linde et al(1987); Mendoza et al(1992); Yip et al (1994); Dardari et al(2001); Tiwawech et al(2003); and Wong et al(2005). Kumar, Wairagkar and Mahanta (2001) also demonstrate higher level of EBV IgG VCA antibody in the sera of patients with nasopharyngeal carcinoma in North-eastern region of India.

Among 40 healthy control in the study, 25(62.5%) were tested positive and 15(37.5%) were found negative for IgG antibodies against EBV by ELISA. Lin et al (1975); Sako et al (1975) and Kumar et al (2001) also found lower EBV positivity among the control group. But Linde (1996) found 98% EBV seropositivity among Swedish blood donors. This might be explained by the fact that EBV is a ubiquitous human herpes virus that persists for life following childhood infection.

From the study it is seen that, majority of the control groups are with a lower titer of less than 100 except five healthy controls of patient relative. Similar findings of lower titer are also found by Lin et al(1975);Sako et al(1975);Gong et al(1978);Micheau et al (1980);Harder et al (1986);Kumar et al(2001);Dardari et al(2001) and Wong et al(2005). But in our 40 healthy control group, 5 relative of patients shows higher titer of 100. Similar findings are also reported by Ho (1971), where he found significantly higher frequency of NPC in close blood relatives of NPC patient. Kumar and Mahanta, (1998) also suggested that genetic susceptibility act as a constant etiological factor for NPC. The familial risk of nasopharyngeal carcinoma is among the highest of any malignancy as reported by Friborg et al (2005). Mun-Hon et al (2006) also found higher titer among close blood relatives of NPC patient.

From this discussion it is seen that serum antibody titers to EBV antigens can be used to confirm the diagnosis of NPC and to monitor the progress of the disease. In seroepidemiological studies the presence or absence of IgG antibodies to VCA is generally used to screen sera, since these antibodies arise early in primary infection and thereafter persist for life. The approach takes advantage of a common feature of NPC that serum levels of EBV antibodies are generally elevated for an average period of 3 year before symptomatic onset and patients sustained high levels of a broad spectrum of EBV antibodies thereafter as disease further progresses. Therefore, elevated antibody level could serve to predict diagnosis of NPC among patients suspected of having NPC on the one hand, and on the other hand, it could serve as a risk marker to predict occult tumour patients among apparently healthy subjects as described by Mun-Hon et al (2006).

The present study reveals that antibodies to certain EBV antigens can be detected in a large percentage of the general population. This might be explained by the fact that EBV is a ubiquitous human herpes virus that persists

for life following childhood infection. Results of this study demonstrate the importance of EBV serology to identify high risk individual and diagnosis of NPC in areas of low incidence of nasopharyngeal carcinoma. Serological assay are to be utilized in the mass screening of high risk populations for the early detection and control of nasopharyngeal carcinoma.

CONCLUSION

In conclusion, we found evidence that EBV-VCA IgG ELISA can serve as a good serological tool for the easy diagnosis of nasopharyngeal carcinoma in high risk population. There is statistical difference between VCA-IgG mean titer among NPC patients and healthy controls suggestive of a better diagnostic tool for NPC.A larger prospective study is needed to validate the performance of this serological test so as to enable it to distinguish accurately between the cases from their relatives as controls.

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