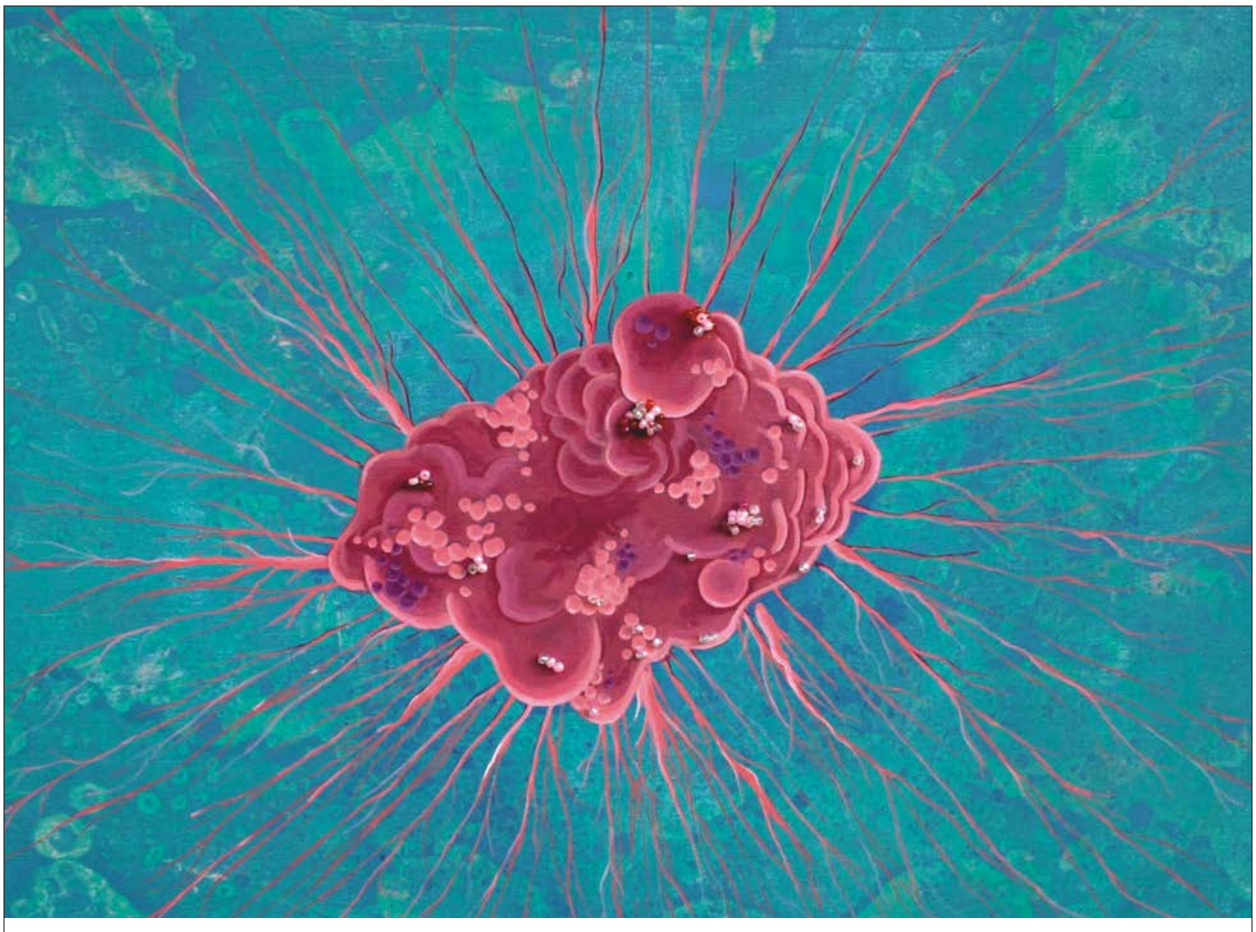

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The Association Between Epstein-Barr Virus with Nasopharyngeal Carcinoma in Patients from Southwestern Region of Iran

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Abstract: The aimed to evaluate the plasma level of Viral Capsid Antigen (VCA)-IgA and IgG in family members of Nasopharyngeal Carcinoma (NPC) patient in comparison to healthy controls in Southwestern of Iran. Total 60 NPC patients were compared with 60 sex, age and ethnically matched healthy controls. The obtained serum samples participants were tested for VCA-IgA, VCA-IgG, Early Antigen (EA), IgG, EBNA-IgG by ELIZA. There was no significant difference in all EBV antibodies between patients and control groups ($p>0.05$). The serological of three IgG antibody meant that 66.6% of two groups had the past infection; of NPC families 6.6% and from controls 1.6% were susceptible to infection with EBV. 3.3% in members of NPC families had reactivation infection. The sex of the patients in case group had positive correlation with VCA-IgA, EBNA-IgG, EA-IgG and negative correlation with VCA-IgG. The age of the patients also showed positive correlation with EBNA-IgG, EA-IgG, VCA-IgG and negative correlation with VCA-IgA. None of the EBV antibodies had significant correlation with age and sex of the patients. Because of no statistical difference between VCA-IgA mean titr from members of NPC families and controls, there is the not higher risk for members of NPC families to controls for NPC. The cause that had positive VCA-IgA also had positive EBNA-IgG and VCA-IgG. Anti-EBV antibodies can be used as diagnostic markers of NPC in Southwestern region of Iran. The combined use of two or more markers marginally improved the discriminating power but that has to take into consideration the higher costs.

Key words: Nasopharyngeal carcinoma, viral capsid antigen-IgA, Early Antigen (EA), Epstein-Barr Virus (EBV), EBV Nuclear Antigen 1(EBNA1)

INTRODUCTION

The effect of positive familial history of cancer has been investigated previously (Makarian *et al*., 2007). Nasopharyngeal Carcinoma (NPC) is an epithelial neoplasm arising from the fossa of Rosenmuller of the postnasal space (Li *et al*., 2010). This is a rare tumor in

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most part of the world; but it occurs endemically in Southern China, Hong Kong, Singapore and some other part of Southeast Asia with high incidence (Marcus and Tishler, 2010). Epstein-Barr Virus (EBV) has long been implicated as an important etiological factor of NPC with histological evidence, indicating the consistent presence of the viral DNA and proteins in malignant tissue (Valentine *et al*., 2010). The EBV is clonally present in virtually 100% of undifferentiated NPC cases (Zhan *et al*., 2009).

Carcinoma-associated EBV activity in NPC patient may be reflected in the circulation by a typical anti-EBV serological profile and increased viral DNA level. In comparison to healthy member carriers, NPC patients generally show strong IgG and especially IgA reactivity to EBV early antigens, viral capsid antigens and EBV Nuclear Antigen 1 (EBNA1) (Zhan *et al*., 2009). The association between specific serologic responses to EBV and NPC has been exploited to develop serologic tumor markers for this cancer.

The anti viral capsid antigen IgA antibody (IgA-VCA) measured by Immunofluorescence (IF) or ELIZA, is one of the most widely used antibody markers used for assisting in diagnosis and for screening (Ainbinder *et al*., 2009). The antibody titer by ELIZA was 12 times higher than that by IF (Luo *et al*., 2009; Low *et al*., 2000). Its sensitivity in the diagnosis of types II and III (WHO classification) of the NPC in endemics and non endemics areas has been generally reported to be 85-90%, although, the sensitivity for VCA-IgA and EBV DNA were 81 and 95%, respectively (Tsang *et al*., 2004).

The specificities were 96% for VCA-IgA and 98% for EBV DNA. The selective application of EBV DNA in an VCA-IgA-based screening protocol could improve screening accuracy with only moderate increases in cost (Tsang *et al*., 2004). Today the detection of elevated IgA against VCA and other EBV proteins are routinely used for serological diagnosis and screening of NPC in South China (Feng *et al*., 2007).

Early detection is the best way to improve survival for NPC. It continued to follow-up to surveillance of healthy individuals with increased VCA-IgA specially in the family member group, is worthwhile to exclude the emergence of cancer (Tsang *et al*., 2004). These individuals may also serve as a target group for tissue sampling by noninvasive methods, such as nasopharyngeal brushing for EBV DNA, which has been reported to have a high accuracy in diagnosis of NPC.

The aim of this study is to evaluate the plasma level of VCA-IgA and IgG in family members of NPC patient in comparison to healthy controls in Southwestern of Iran.

MATERIALS AND METHODS

Study Design and Population

This research is a case-control study design in 60 adults (32 male, 28 female) that conducted from July 2008 to Dec 2009 in Imam Khomeini and Apadana hospitals, Ahwaz, Iran. The mean age of case and control groups was 29.8 and 30.86 years, respectively. Serum samples were obtained from 60 histologically confirmed NPC patients as a case group. These patients were referred to Radiotherapy department in Golestan hospital, Ahvas, Iran (The only Radiotherapy center in Khuzestan). Control sera (60) from sex, age and ethnically matched individuals were collected from non-NPC patients of the same Hospital. Informed consent was obtained from all the individuals from whom blood was collected. The study was approved by the University Hospital and Ahwaz Jondishapour University of Medical Sciences Ethics Committees and all subjects and their guardians in case of children, granted informed consent to participate.

Sample Collection

To perform this survey, 5 mL bloods were taken then serum was collected and divided in two equal fractions. One of them stored at 20°C and another stored at -70°C. Finally, sera from 90 individuals were tested by a quantitative-qualitative ELIZA for EBV antibodies: VCA-IgA, VCA-IgG, EA-IgG and EBNA-IgG as described by Lin *et al.* (1985). Each antibody titer compared with standard concentration in its kit. Then the standard curve was designed and exact titer was read from it. For VCA-IgA, EA-IgG and EBNA-IgG titers <8 μ mL^{-1} was taken as the negative, titer between 8 and 12 μ mL^{-1} was taken equivocal and titer >12 μ mL^{-1} was taken as positive. For VCA-IgG titres <5 μ mL^{-1} was taken as negative and titer >5 μ mL^{-1} was taken as positive.

Statistical Analysis

All statistical analyses were carried out using the SPSS version 13.0. The mean of antibody titer from case and controls were analyzed using one sample t-test. Person correlation was used in determining the correlation between antibody titer and sex or age. Before sampling, all participants were informed electively about the study.

RESULTS

Out of 60 individuals in case group, one had VCA-IgA positive titer (23 μ mL^{-1}). She was 18-year-old and was a daughter of one NPC patient. Another person had an equivocal titer (10 μ mL^{-1}) and also was a daughter of another NPC patient. In control group, one had an equivocal titer, 10 μ mL^{-1} (65-year-old, female). All of three cases had VCA-IgG and EBNA-IgG positive titer that means they had the past infection with EBV. The mean titer of VCA-IgA in case group was 2.59±3.24 and in controls was 2.11±2.12 (Table 1). There was no significant difference between them (p = 0.459). The result include: 53 positive titer in case and 25 in controls. The mean titer of VCA-IgG in the first group was 41.48±3.97 and in controls was 39.93±3.11 (p = 0.861) (Table 1).

The EBNA-IgG were seen in 38 positive titer patients in case and 19 positive titers in control (Table 1). The mean titer of EBNA-IgG in case group was 44.76±4.91 and in control was 37.77±3.61. There was no significant difference (p = 0.438). In case of EA-IgG, two persons of case group had the positive titer. No controls had the positive titer. The mean titer of EA-IgG in the first group was 4.97±2.61 and in controls was 3.37±1.88 (Table 1). There was no significant difference (p = 0.321). To interpretation 3 IgG antibody simultaneously, 67.7% of total had the past infection (Positive VCA-IgG and EBNA-IgG), 10% were susceptible (negative IgG) to viral infection and 2.2% had reactivation infection (Positive IgG). As shown in Table 2 the sex of the patients in case group had positive correlation with VCA-IgA, EBNA-IgG, EA-IgG and negative correlation with VCA-IgG. The age of the patients also show positive correlation with EBNA-IgG, EA-IgG, VCA-IgG and negative correlation with VCA-IgA. None of the EBV antibodies had significant correlation with age and sex of the patients (Table 2).

Table 1: EBV antibodies in study groups

	Case (n = 60)			Control (n = 60)			
Assay	P	N	Titer (Mean±SD)	P	N	Titer (Mean±SD)	p-value
VCA-IgA	3	57	2.59±1.24	2	58	2.11±1.12	0.459
VCA-IgG	53	7	41.48±3.97	25	35	39.93±3.11	0.861
EBNA-IgG	38	22	44.76±4.91	19	41	37.77±3.61	0.438
EA-IgG	2	58	4.97±2.61	0	60	3.37±1.88	0.321

P: Positive; N: Negative

Table 2: Correlations between EBV antibodies with age and sex of the patients in case group (n = 60)

Correlations	Correlation coefficient	p-value	Description
Sex and VCA-IgA	0.088	0.411	PC
Sex and VCA-IgG	-0.008	0.945	NC
Sex and EBNA-IgG	0.019	0.857	PC
Sex and EA-IgG	0.083	0.437	PC
Age and VCA-IgA	0.046	0.664	PC
Age and VCA-IgG	0.140	0.214	PC
Age and EBNA-IgG	-0.107	0.316	NC
Age and EA-IgG	0.028	0.794	PC

PC: Positive correlation; NC: Negative correlation

DISCUSSION

The anti-VCA IgA antibody was chosen as the reference marker for this study because of high sensitivity for diagnosis of NPC and population screening. The present study showed that 1.6% from NPC patients had elevated VCA IgA titer. Tang *et al.* (2007) evaluated sera from healthy members of NPC family and compared them with healthy controls. They found that VCA IgA and EBNA IgA in healthy members of NPC family was 5-6 times higher than in controls ($p<0.01$). Present finding was in agreement with Tang *et al.* (2007) study. Yi *et al.* (2009) screened 395 persons from high incidence area in China for VCA-IgA antibody in their serum. They found 6.7% (26 case) with elevated IgA. After 2 years follow up 0.09% (4 cases) presented with NPC.

Present research findings (1.6%) is close to their result (6.7%), if compare the sample size of two studies. Previous studies have shown that VCA-IgG in NPC patients are higher than controls. In Asian patient, it is more significant. All NPC patients had abnormal anti-EBV Igv diversity patterns as determined by immuno-blot analysis. On the other hand/healthy EBV carriers with positive EBV IgA ELIZA result showed normal IgA diversity patterns. By using EBV IgA immuno-blot diversity as a confirmation assay for EBV IgA ELIZA-positive samples/the sensitivity and specificity for NPC diagnosis increased to 98 and 99/2%. Respectively/in the Indonesian NPC samples (Valentine *et al.*, 2010). In the present study, the cases that had elevated IgA had the IgA antibody titer that similar to controls and agree with these studies (Zhan *et al.*, 2009).

Karray and coworkers showed that young NPC patients had significantly been more restricted anti-EBV IgA and IgA antibody responses with aberrant IgC VCA/EA levels in 78% in comparison to 91-7% in elder patients (Englert *et al.*, 2004). The finding of present study agrees with their results that showed that IgA VCA/EA levels in young cases were 79% in comparison to 91% in elder cases. In Taiwania study that evaluated healthy members of NPC families the VCA-IgA and EBNA-IgA antibody titer was higher among older individuals and among female. In our study, no correlation was found between age and sex to antibodies titer but 3 cases that had the elevated VCA-IgA titer were female also the Pearson coefficient had the negative sign it correlation between the age and antibody titer that means the IgA titer decrease by age increasing (Lester and Thompson, 2007).

The follow up studies that were taken long-time after initial screening to follow healthy individual with increased IgA-VCA titer showed that there is a variable time between elevation of IgA and cause symptomatic NPC, In honk kong it was 18 months (Li *et al.*, 2010). If individuals had entered this window, the serological screening was positive and if they had not, the result of screening was negative (Wang *et al.*, 2009). In the present study, the cases were not follow these rules except one case.

CONCLUSION

Anti-EBV antibodies can be used as diagnostic markers of NPC in Southwestern region of Iran. The combined use of two or more markers marginally improved the discriminating power but that has to take into consideration the higher costs. The sex of the patients in case group had positive correlation with VCA-IgA, EBNA-IgG, EA-IgG and negative correlation with VCA-IgG. The age of the patients also show positive correlation with EBNA-IgG, EA-IgG, VCA-IgG and negative correlation with VCA-IgA. None of the EBV antibodies had significant correlation with age and sex of the patients. Because of no statistical difference between VCA-IgA mean titr from members of NPC families and controls, there is the not higher risk for members of NPC families to controls for NPC.

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