Prevalence Of Anti-EBV Antibodies in Adult Patients with Nasopharyngeal Carcinoma During 2003-2007 In Isfahan, Iran

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Abstract

Background: Nasopharyngeal carcinoma (NPC) is a nonlymphomatous squamous cell carcinoma (SCC) that occurs in the epithelial lining of the nasopharynx. Viral, geographic, and ethnic factors are responsible for its multifactorial futures. Previous studies have showed the role of Epstein-Barr virus (EBV) in the pathogenesis of NPC but no study has been conducted on the Iranian population to assess the etiology of NPC and to investigate the role of EBV in carcinogenesis of nasopharyngeal carcinoma.

Methods: We collected 87 paraffin wax embedded blocks of NPC (n=34) and Laryngeal SCC patients (n=53) operated in Isfahan Hospitals during 2003-2007 from the archives of the department of pathology and then sera of patients were provided. We measured the titers of early antigen (anti-EA) and Epstein-Barr virus nuclear antigen (anti-EBNA) antibodies by means of ELISA method in sera of patients.

Results: Our data showed a significant association between elevated titer of these antibodies and the presence of NPC (P value =0.016 for anti-EBNA and 0.001 for anti-EA antibodies); however, we did not find such a relationship about Laryngeal SCC.

Conclusion: The prevalence of EBV infection in patient with NPC is significantly higher than the control group. Further studies should investigate the value of serum markers of EBV infection in the follow up or early diagnosis of NPC in high risk patients.

Keywords: nasopharyngeal carcinoma, Epstein-Barr virus, squamous cell carcinoma

Introduction

The epithelial lining of the nasopharynx may be affected by a non-lymphomatous squamous cell carcinoma named Nasopharyngeal Carcinoma (NPC). Frequently, this tumor occurs in pharyngeal recess (Rosenmuller’s fossa) posteromedial to the medial crura of the Eustachian tube opening in the nasopharynx [1]. This cancer is a unique type of head and neck squamous cell carcinoma (HNSCC) [2] and has a multifactorial etiology. The role of viruses, environment and genetic have been previously proved [3]. Incidence of this cancer has showed a geographic variation. Although NPC is a rare cancer worldwide (one per 100 000)[4], it is one of the most common cancers in southeast Asia specially southern China, where it has an incidence of 80 per 100 000 individuals each year [5-6]. Other high risk areas are northern Africa, Alaska, Hong Kong, Italy, Greece and Turkey [7-8].

In 1978, WHO categorized NPCs into three groups: squamous cell carcinoma with marked keratinization similar to those found in the rest of upper aerodigestive tract (type I), non-keratinizing squamous cell carcinoma (type II), and undifferentiated carcinoma (type III) [9]. Tumors of type II or III are definitely associated with Epstein-Barr virus, whereas the role of viruses in NPC type I still remains controversial [10].

It has been reported that NPC is greatly linked to smoked foods consumption; some studies demonstrated the association between NPC and consumption of smoked and preserved food, exposure to soot and dust and occupational contacts to formaldehyde and various herbal oils containing EBV activating compounds [11].
Epstein, Achong, and Barr discovered EBV by electron microscopy of cells cultured from Burkitt's lymphoma tissues 42 years ago [12]. Six years later, in 1970, in tissues from patients with NPC, EBV DNA was detected [13].

A serological test for EBV-associated antibodies was suggested as a screening test in NPC [14]. Quantitative EBV DNA analysis, detection of tumor markers in samples collected directly from nasopharynx via a non-invasive procedure [15-16], and recently serum cell-free EBV DNA assay are some laboratory procedures employed for NPC screening [17-19].

Antibodies to Epstein-Barr virus nuclear antigen (anti EBNA) and early antigen (anti EA) are two antibodies synthesized against EBV and for patients affected by NPC are of diagnostic and prognostic importance [20]; however, there is no evidence on the involvement of EBV in the carcinogenesis of laryngeal squamous cell carcinoma, so subjects with laryngeal SCC are the qualified control group in the present study.

So far, no study has been conducted on the Iranian population to assess the etiology of NPC and to investigate the role of EBV in the carcinogenesis of nasopharyngeal carcinoma.

Table 1: Serological results in both groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>EBNA</th>
<th>EA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>SCC (n=53)</td>
<td>3.8% (n=2)</td>
<td>96.2% (n=51)</td>
</tr>
<tr>
<td>NPC (n=34)</td>
<td>20.6% (n=7)</td>
<td>79.4% (n=27)</td>
</tr>
<tr>
<td>All (n=87)</td>
<td>10.3% (n=9)</td>
<td>89.7% (n=78)</td>
</tr>
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</table>

Results

Our participants included 34 patients with Nasopharyngeal Carcinoma (NPC) and 53 patients with Laryngeal Squamous Cell Carcinoma (SCC) of both genders. SCC group contained 50 males and 3 females and NPC group consisted of 23 men and 11 women. The characteristics of the subjects are depicted in Table-1.

We measured two major anti Epstein Barr Virus (EBV) antibodies (EBNA and EA) in both groups, data were evaluated based on gender and age. 20.6% of the cases in NPC group (n=7) and 3.8% of cases in SCC group (n=2) were positive for anti EBNA. The prevalence of anti EA in NPC and SCC groups were obtained 29.4% (n=10) and 3.8% (n=2), respectively.

Fisher’s exact test showed significantly elevated EBNA in NPC (p value = 0.016) but we found no such relation between EBNA and SCC. We found the...
same association between EA antibody and NPC: this antibody was positive in 29.4% of NPC cases against 3.8% in SCC cases (p value = 0.001).

Positive EBNA and EA antibodies were not correlated with gender in SCC and NPC groups (p value = 0.89 for SCC and 0.4 for NPC). We demonstrated a significant correlation between age and the presence of anti EBV antibodies in the SCC group: EBNA and EA antibodies were predominantly negative in older patients rather than younger participants (p value = 0.03) but no such relationship was detected in the NPC group (Table-2).

**Discussion**

Previous studies have revealed that the diagnosis of nasopharyngeal carcinomas are difficult because of their non-specific clinical manifestations and restricted inspection of nasopharynx [21]. A large number of lesions can be detected after metastasis to deep cervical lymph nodes [19], so early diagnosis and definite cure of this cancer improves the patient’s survival, reduces morbidity, and prevents metastasis.

Almost all humans carry EBV and 90% of adults are seropositive. The genome of this virus is composed of 100 genes (172kb). EBNA1 (the most important component of EBNAs) is required for replication and maintenance of the viral genome during cell division [22]. Some studies have showed that EBV infection is dominantly associated with WHO II and III subtypes of NPC [23-25] so only in these subtypes of NPC the serum level of IgG and IgA to EA and EBNA1 were significantly elevated than control group [26, 2].

We found that the serum level of anti-EA and anti-EBNA is significantly elevated in NPC patients in comparison to SCC patients as control group. The observed correlation in our subjects is in line with another study [27] in which they proved that anti-EBNA in NPC is 10 times higher than Hodgkin’s Disease and non-Hodgkin lymphoma as control groups. Also, Cevenini et al. demonstrated such a relationship for anti-EA antibody in NPC patients [28]; however, Makuch et al showed that other than the increase of EBNA-antibody in NPC, serum levels of acute-phase proteins (haptoglobin, α1 acid glycoprotein, and α1antitrypsin) are significantly elevated [29]. The correlation between anti-EA and treated or untreated NPC patients reported by Yang Cs et al [30].

Our data showed a significant correlation between age and anti-EBV antibodies in the SCC group. Because EBV infection mostly occurs in the young population, serum markers of EBV infection are significantly higher in young patients with SCC. On the other hand, age of onset of SCC is less than NPC and the chance of EBV infection in young patients with SCC is equal to the normal population.

Parallel to the measurement of serum anti-EBNA and anti-EA antibodies, some articles have also suggested that serum level of anti Virus Capsid Antigen antibody increases in nasopharyngeal carcinoma compared to the control group [28, 30, 31].

In our study, the mean age of NPC was 55.8 years old; however, in another study, the mean age of NPC is reported to be 51 years and it seems that such a difference may be due to the fact that this cancer can be easily overlooked by patients and/or physicians because the clinical futures of NPC is non-specific. So we suggest that evaluating anti EBV antibodies might also serve as a screening test for individuals who are at high risk for developing NPC [32].

**Conclusion**

There is a significant association between anti-EBV positivity and NPC. Therefore, further investigations should highlight the correlation.
between serum levels of anti-EBV antibodies with different stages of NPC for:
1. Preventing the progression of NPC to higher stages.
2. Screening of high risk cases.

Also we suggest that other authors perform some new studies on the role of serial measurements of anti-EBV antibodies in follow up of the patients and on the effect of tumor resection on serum titers of anti-EBV antibodies.

References
