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

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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].

Table 1: Serological results in both groups.

Groups	EBNA		EA	
	Positive	Negative e	Positive	Negative
SCC (n=53)	3.8% (n=2)	96.2% (n=51)	3.8% (n=2)	96.2% (n=51)
NPC (n=34)	20.6% (n=7)	79.4% (n=27)	29.4% (n=10)	70.6% (n=24)
All (n=87)	10.3% (n=9)	89.7% (n=78)	13.8% (n=12)	86.2% (n=75)

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.

Patients and Methods

This cross sectional study was performed between 2003 and 2007 on 87 patients with NPC and Laryngeal SCC. We collected paraffin wax embedded blocks of tissue from 34 patients with NPC and 53 patients with laryngeal SCC operated in Al-Zahra and Kashani hospitals in Isfahan, Iran, from our archives in the department of pathology in the same medical centres. The specimens were categorized upon gender and age. Then, according to the patients' information available in the department of pathology, we found their addresses and 5cc of blood was taken from each case. Dead patients and those who had changed their addresses were excluded from the study. Sera were stored in -20°c for a maximum period of one week.

The first and last sections (5µm) of the paraffin embedded tissues were stained with hematoxylin and eosin and histologically examined for confirmation of previous diagnosis.

The serum level of circulating EA-IgG and EBNA-IgG were measured by means of Enzyme-linked Immunosorbent Assay (ELISA) method using monoclonal antibodies to EA-IgG and EBNA-IgG, according to the manufacturer's protocol. Normal serum level of both anti EBV antibodies was up to 10 IU/ml, according to the manufacturer's protocol.

Data was stored on a computer database and was analyzed using SPSS statistical package version 12 for windows (SPSS Inc, Chicago .IL, USA). The statistical analysis of the EBV status in relation to clinical and histopathological data was performed by the Chi-square test and Fisher's exact test .The t test was performed to compare means of continuous variables. All P values were two-sided and considered significant at $P<0.05$.

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

Table 2: Association of age and presence of anti-EBV antibodies in both groups.

Groups		Results		Mean age	P value
	SCC	Positive	3.8% (n=2)	22.5±0.7	**0.03**
		Negative	96.2% (n=51)	51.2±18.03	
	NPC	Positive	20.6% (n=7)	59±11.3	0.56
		Negative	79.4% (n=27)	55±17.3	
	SCC	Positive	3.8% (n=2)	22.5±0.7	**0.03**
		Negative	96.2% (n=51)	51.2±18.03	
	NPC	Positive	29.4% (n=10)	56.5±16.7	0.67
		Negative	70.6% (n=24)	54±15.3	

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 PNC 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 all showed that other than the increase of EBNA-antibody in NPC, serum levels of acute-phase proteins (haptoglobuline, a1 acid glycoprotein, and a1antitrypsin) 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

1.Sham JS, Choy D, Wei WI, et al. Detection of subclinical nasopharyngeal carcinoma by fibreoptic endoscopy and multiple biopsy. Lancet. 1990; 335: 371-74.

2.Krishna SM, James S, Kattor J, et al.Serum EBV DNA as a biomarker in primary nasopharyngeal carcinoma of Indian origin.Jpn J Clin Oncol.2004;34(6):307-11

3.Bar-Sela G, Kuten A, Minkov I, et al. Prevalence and relevance of EBV latency in nasopharyngeal carcinoma in Israel.J Clin Pathol.2004;57(3):290-3

4.Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J, eds. Cancer incidence in five continents, vol 7. IARC. 1997; 143: 814-15.

5.Mutirangura A, Tanunyutthawongese C, Pornthanakarem W, et al. Genomic alterations in nasopharyngeal carcinoma: loss of heterozygosity and Epstein–Barr virus infection. Brit J Cancer. 1997;76:770–6.

6.Le Roux F, Joab I. Epstein-Barr virus and nasopharyngeal carcinoma. Epstein- Barr Virus Report 1998;5:53–7.

7.Nielsen NH, Mikkelsen F, Hansen JP. Nasopharyngeal cancer in Greenland: the incidence in an Arctic Eskimo population. Acta Pathol Microbiol Scand. 1977; 85: 850-58.

8.Dickson RI. Nasopharyngeal carcinoma: an evaluation of 200 patients. Laryngoscope.1999;91:333

9.Shanmugaratnam K, Sobin LH. Histological typing of tumors of the upper respiratory tract and ear. In: Shanmugaratnam K, Sobin LH, eds. International histological classification of tumors: no 19. Geneva. WHO. 1991: 32-3.

10.Pathmanathan R, Prasad U, Chandrika G, et al. Undifferentiated, nonkeratinizing and squamous cell carcinoma of the nasopharynx. Variants of Epstein-Barr virus-infected neoplasia. Am J Pathol. 1995;146:1355–67.

11.Yu MC, Yuan JM. Epidemiology of nasopharyngeal carcinoma. Semin Cancer Biol. 2002;12:421–9.

12.Epstein MA, Achong BC, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. Lancet. 1964;1:702–3.

13.Zur Hausen H, Schulte-Holthausen H, Klein G, et al. EBV DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. Nature. 1970;228:1056–8.

14.Lo YM, Chan LY, Lo KW, et al. Quantitative analysis of cell-free Epstein–Barr virus DNA in plasma of patients with nasopharyngeal cancer. Cancer Res. 1999;59:1188–91.

15.Lin SY, Tsang NM, Kao SC, et al. Presence of Epstein–Barr virus latent membrane protein-1 gene in nasopharyngeal swabs from patients with nasopharyngeal cancer. Head Neck. 2001;23:194–200.

16.Teo P, Yu P, Lee WY, et al. Significant prognosticators after primary radiotherapy in 903 nondisseminated nasopharyngeal carcinoma evaluated by computer tomography. Int J Radiat Oncol Biol Phys. 1996;36:291–304.

17.Mutirangura A, Pornthanakanem W, Theamboonlers A, et al. Epstein–Barr viral DNA in serum of patients with nasopharyngeal carcinoma. Clin Cancer Res. 1998;4:665–9.

18.Lo YM, Chan LY, Chan AT, et al. Quantitative and temporal correlation between circulating cell-free Epstein–Barr virus DNA and tumor recurrence in nasopharyngeal carcinoma. Cancer Res. 1999;59:5452–5.

19.Feinmesser R, Miyazaki I, Cheung R, et al. Diagnosis of nasopharyngeal carcinoma by DNA amplification of tissue obtained by fine-needle aspiration. N Eng J Med. 1992;326:7–21.

20.Lohr GW.Detection of IgA antibodies to Epstein-Barr virus-associated antigens by ELISA. J immunol methods . 1984;68(1):331-9.

21.Zong YS, Sham JS, Ng MH, et al. Immunoglobulin A against viral capsid antigen of Epstein–Barr virus and indirect mirror examination of the nasopharynx in the detection of asymptomatic nasopharyngeal carcinoma. Cancer. 1992;69:3–7.

22.Raab-Traub N. Epstein–Barr virus in the pathogenesis of NPC. Semin Cancer Biol. 2002;12:431–41

23.Chan MK, McGuire LJ, Lee JC. Fine needle aspiration cytodiagnosis of nasopharyngeal carcinoma in cervical lymph nodes: a study of 40 cases. Acta Cytol. 1989;33:344–50.

24.zur Hausen H, Schulte-Holthausen H, Klein G, et al. Epstein–Barr viral DNA in biopsies of Burkitt tumors and anaplastic carcinomas of the nasopharynx. Nature. 1970;228:1056–8.

25.Busson P, Ganem G, Flores F, et al. Establishment and charecterization of three transplantable EBV-containing nasopharyngeal carcinomas. Int J Cancer. 1988;42:599–606.

26.Young LS, Dawson CW, Clark D, et al. Epstein–Barr virus gene expression in nasopharyngeal carcinoma. J Gen Virol. 1998;69:1051–65.

27.Lennette ET,Rymo L,Yadav M, et al.Disease-related differences in antibody patterns against EBV-encoded nuclear antigens EBNA1, EBNA2 and EBNA6.Eur J Cancer.1993;29(11):1584-9.

28.Cevenini R, Donati M, Caliceti U, et al. Evaluation of antibodies to Epstein_barr virus in Italian patients with nasopharyngeal carcinoma. J Infect. 1986;122:127-31.

29.Baskies AM, Chretien PB, Yang CS, et al. Serum glycoprotein and immunoglobulins in nasopharyngeal carcinoma: Correlation with Epstein-Barr virus associated antibodies and clinical tumor stage. Am J Surg.1979;138(4):478-88.

30.Yang CS, Yang H, Yeh YS, et al.Epstein-Barr virus associated antibodies in IgG and IgA of nasopharyngeal carcinoma patients.Comp Immunol Microbiol Infect Dis. 1979;2(2):167-75.

31.Molnar Z, Biberfeld p, Klein G. Immuno-fluorescence and immuno-electron microscopy on EBV-associated membrane antigen of a nasopharyngeal carcinoma derived lymphoblastoid cell line (LY-28). Eur J Cancer.1974;10(10):633-6.

32.Tiong TS, Selva KS. Clinical presentation of nasopharyngeal carcinoma in Sarawak Malaysia. Med J Malaysia. 2005;60(5):624-8.