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Epstein – Barr virus antibody titers in non nasopharyngeal head and neck squamous cell carcinoma

[*]Majidi M. MD[1], Rafat panah H. MD[2], Zamanian A. MD[3], [4]Hassanzadeh N. DSS

[1]Assistant professor of Otorhinolaryngology. [2]Assistant professor of Immunology and Allergy.
[3]Assistant of Otorhinolaryngology, [4]Assistant of Orthodontics-
Mashhad University of Medical Sciences

Abstract

Introduction: The object of this study was to compare EBV antibody titers between various non-nasopharyngeal head and neck SCC and healthy control persons.

Materials and Methods: During 2 years period ELISA was used to investigate titer of different classes of antibody against VCA, EA, and EBNA component of EBV in patient and control group.

Results: of 41 patients enrolled in the study 21 were diagnosed by pathological study with pharyngeal and 20 with laryngeal carcinoma. All of different classes of EBV antibody except for EBNA (IgG) in patient group were significantly higher than those in control group. Statistical analysis did not show a significant difference in antibody titers between laryngeal and pharyngeal location of disease as well as between tobacco user and non user groups.

Conclusion: Based on the present study there is an association between high EBV antibody titers and non nasopharyngeal head and neck squamous cell carcinoma. Further experiments on tissue samples could investigate the role of EBV in tumor genesis of laryngopharyngeal carcinoma.

Keywords: Squamous cell carcinoma, Epstein – Barr virus, Antibody titer, Head and neck.

Introduction

The Epstein – Barr virus (EBV) is ubiquitous and antibodies to certain EBV-specific antigens are detectable in a large percentage of the general population. There are the different classes of antibody to EBV compound which elevated during various phases of EBV replication.

Viral capsid antigen (VCA) and early antigen (EA) are expressed during the lytic cycle of EBV infection and are associated with virus replication.

[*]Address: Department of ENT-Ghaem Hospital-Mashhad
Tel: 0511-8413492 Email: MRZ@ mums.ac.ir
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VCA – IgM is detected at the initial stage of EBV primary infection while VCA – IgG indicates EBV seropositivity.

EBV nuclear antigen (EBNA) which generally detected in serum by florescence method has 3 subtypes [1, 2, 3 (A, B, C)]. It has been found during primary infection. The antibody response to EBNA-1 is markedly delayed relative to the response to the other EBNAs. After the course of primary EBV infection latently infected B cells persist in blood and lymphoid cell tissues for a lifetime (1- 3).

Epstein-Barr virus is the cause of infectious mononucleosis. This virus has also been associated with Burkitt's lymphoma.

Initially in 1966 Old et al detected precipitating antibodies to a soluble antigen of EBV in the serum samples of patients who had poorly differentiated or anaplastic nasopharyngeal carcinoma (NPC) (4).
Further investigation revealed that patients with NPC had significantly elevated titers of antibodies of the IgG class to both the early antigen and viral capsid antigen of EBV and it was noted that these antibodies increased with tumor Burden (5- 8).

There are a few published series, however, that demonstrated significantly elevated titers in patients with epidermoid cancer of head and neck when compared with healthy controls (9-11). Thus we decided to perform the study to further delineate whether the elevated EBV specific antibody titers are related only to patients with NPC or if there exist a significant relationship to epidermoid cancer from other sites in head and neck in patients studied from Ghaem general hospital in city of Mashhad.

Materials and Methods

Serum samples were collected from 21 patients with pharyngeal SCC, 20 patients with laryngeal SCC and from 40 random healthy controls Who did not show any sign or symptom attributed to Epstein barr virus or any other viral infection .All serum samples were collected prior to instituting any therapy. The serum samples were routinely stored at (-20˚c) until used and then were evaluated for both IgA and IgG antibody levels to EBNA, EA, and IgM antibody level to VCA using ELISA kit (IBL-Hamburg GmbH) in immunology laboratory of Ghaem hospital.

Results

65% (27) of patients were men. The age range of patients with tumor was from 25 to 78 years, and of controls from 23 to 79 years. (Table 1) list the levels of different classes of EBV antibody in patients and controls the all antibody levels except for EBNA1 (IgG) were significantly higher in patients in comparison with healthy donors with approximately 2 fold difference in antibody levels.

Table 1- Comparison of the antibody levels between patient and control

antibody	Mean titers in control group	SD in control group	Mean titers in Patient group	SD in patient group	P .Value
EBNA-1 (IgA)	3.89	2.01	2.51	3.43	%003
EA (IgA)	2.98	1.30	6.10	3.58	%000
VCA (IgA)	3.57	2.94	7.91	5.33	%000
VCA (IgM)	6.63	2.81	11.20	5.96	%000
VCA (IgG)	47.79	57.85	104.66	72.51	%000
EBNA-1 (IgG)	56.57	41.68	66.45	38.24	%234

(Table 2, 3) compare the levels of anti EBV antibodies between laryngeal and pharyngeal epidermoid carcinoma as well as tobacco user and non user. According to these tables there is not any significant difference between laryngeal and pharyngeal group or tobacco user and non user. It was not possible to compare antibody levels between early and late stages of disease because 95% of patients were in stage III and IV.

Table 2- comparison of the antibody levels between laryngeal and pharyngeal epidermoeid carcinoma

antibody	Mean titers in pharyngeal group	SD in pharyngeal group	Mean titers in laryngeal group	SD in laryngeal group	P .Value
EBNA-1(IgA)	6.43	3.64	5.67	3.24	%514
EA (IgA)	5.46	3.19	6.78	3.90	%171
VCA (IgA)	9.10	6.7	6.66	3.07	%361
VCA (IgM)	11.04	5.16	11.37	6.84	%896
VCA (IgG)	111.27	73.28	97.73	72.92	%764
EBNA-1 (IgG)	64.41	35.61	68.6	41.64	%835

Table 3- comparison of the antibody levels between tobacco user and nonuser

antibody	Mean titers in tobacco user group	SD in tobacco user group	Mean titers in Tobacco non user group	SD in Tobacco non user group	P .Value
EBNA-1 (IgA)	6.24	3.14	5.84	3.82	%346
EA (IgA)	5.90	3.26	6.34	3.09	%917
VCA (IgA)	6.92	3.12	9.05	7.02	%556
VCA (IgM)	11.59	6.56	10.74	5.28	%744
VCA (IgG)	106.43	74.82	102.62	71.74	%804
EBNA-1 gG)	75.17	43.45	56.35	29.10	%097

Discussion

In our study the serum antibody levels against VCA (IgA) and EA (IgA) were significantly elevated in patients with SCC when compared with controls. Morshed et al indicated that only 5 (12.8%) of 38 patients with head and neck SCC were positive for anti VCA (IgM) and all the controls showed negative results (12).

Callaghan et al reported that the incidence of elevated IgA anti VCA titers was greater than 50% in patients with epidermoid carcinoma of the larynx, oral cavity and nasopharynx (9).

IgG anti VCA was the least specific serologic parameter. It was elevated to high levels in the majority of patients who had epidermoid carcinoma of head and neck. The incidence ranged between 62–100% depending on the site of the lesion this titer elevation may represent an activation of a latent EBV infection occurring after a state of immunosuppression develops in the host from the effects of the tumor (9, 13).

In our study, anti VCA (IgG) titers in patient group were significantly higher than healthy controls (P=o.ooo). This disagrees with Morshed et al who reported there is no significant difference in anti–VCA titers between patient and control groups.

Henderson et al reported significantly elevated IgG anti EA and anti–VCA antibody titers in patients with oropharyngeal epidermoid carcinoma when compared with controls (14).

EBNA1 encodes a DNA binding protein that is essential for replication of the virus within infected cells and required for maintenance of tumor cells the classification of EBNA to three subtypes is generally belie-ved to reflect the antigenicity of tumor cells against cytoxict T cells (15, 16).

In our study, the IgA antibody against EBNA1 in patient group was significantly higher when compared with controls (P=o.oo3) but EBNA IgG levels was not significantly higher in patients in compare-ison with controls. In two studies accomp-lished by Morshed no significant differences in anti – EBNA (IgG) were reported between patient and control group (10,12).Chen et al analyzed antibody titers to different EBV components (EBNA,VCA,LMP2A) in sera of patients with EBV associated malignancies (including epidermoid carcinoma).

They reported a high antibody levels to EBNA,VCA antibody (in comparison with anti LMP2A antibody) in majority of cases (17).We didn't observe any differences in antibody titers between various sites of head and neck epidermoid carcinoma. This agrees with Callaghan et al study who reported no differences in antibody titers between various sites of hood and neck epidermoid carcinoma (9).

In our study, no significant difference in antibody titers was found between tobacco users and non users. This finding follows the observation made by Jenson et al who evaluated the effect of smokeless tobacco purified proudest on latent Epstein barr virus (18).

Conclusion

Based on the present study there is an association between high EBV antibody titers and non nasopharyngeal head and neck sqamous cell carcinoma further experiments on tissue samples could investigate the role

of EBV in tumor genesis of laryngopharyngeal carcinoma.

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خلاصه

بررسی تیتر آنتی بادی ها بر علیه ویروس اپشتاین بار در بیماران مبتلا به کارسینوم سلول سنگفرشی غیر نازوفارنژیال سر و گردن

دکتر محمد رضا مجیدی، دکتر هوشنگ رفعت پناه، دکتر احمد زمانیان، دکتر نادیا حسن زاده

مقدمه : هدف از این مطالعه مقایسه تیترهای آنتی بادی بر علیه ویروس EBV در بیماران مبتلا به کارسینوم سلول سنگفرشی در مناطق سر گردن (به جز نازوفارنکس) و افراد نرمال می باشد.

مواد و روش کار: تیترهای سرمی آنتی بادی بر علیه اجزاء VCA, EBNA, EA ویروس اپشتاین باردربیماران مبتلا به کارسینوم فوق و گروه کنترل مقایسه شد.

نتایج: از ۴۱ بیمار پذیرفته شده در این مطالعه۲۱ بیمار مبتلا به کارسینوم فارنژال و ۲۰ بیمار مبتلا به کارسینوم لارنژال بودند. تمامی کلاس های آنتی بادی های ضد EBV به جز EBNA (IgG) در بیماران مبتلا به کارسینوم سلول سنگفرشی به صورت معنی داری بیشتر از افراد کنترل بود. مطالعات آماری اختلاف معنی داری را در تیترهای آنتی بادی بین بیماران مبتلا به کارسینوم لارنژال و فارنژال و یا بین بیماران مصرف کننده یا عدم مصرف کننده تنباکو نشان نداد.

نتیجه گیری: بر مبنای این مطالعه ارتباطی میان تیترهای آنتی بادی های ضدEBV و کارسینوم سلول سنگفرشی در مناطق سر وگردن (به جز نازوفارنکس) وجود دارد. مطالعات بیشتر بر روی نمونه های بافتی می تواند نقش EBV را در تشکیل کارسینوم غیر نازوفارنژال مشخص نماید.

واژه های کلیدی: ویروس اپشتاین بار، تیترآنتی بادی،کارسینوم سلول سنگفرشی، سرو گردن