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Epstein-Barr virus antibodies in patients with laryngeal and hypopharyngeal cancer

Epstein-Barr virus (EBV) is a member of the herpesvirus family. The EBV genome consists of a linear DNA molecular that encoded nearly 100 viral proteins (6). EBV is one of the most successful viruses, infecting over 95 percent of the adult population of the world (6). EBV infection usually occurs after contact with oral secretions. The virus replicates in epithelial cells in the oropharynx, and nearly all seropositive persons actively shed virus in the saliva (15). Although earlier studies indicated that the virus replicated in epithelial cells in the oropharynx (13) and that B cells were subsequently infected after contact with these cells (1), other studies suggested that B cells in the oropharynx may be the primary site of infection (2, 9).

In recent years, the EBV has been implicated in the aetiology of an increasing number of different malignancies. The virus is well known for its association with Burkitt's lymphoma, nasopharyngeal cancer and Hodgkin's disease. IgA antibodies concentration in sera of patients with nasopharyngeal cancer is a marker of disease control (7, 10,17). Recent studies suggested a role of EBV infection in the pathogenesis and development of the laryngeal cancer and hypopharyngeal cancer (8, 11).

The aim of the present study was to determinate the correlation of Epstein-Barr antibodies presence in sera with clinical features of tumour in patients with laryngeal and hypopharyngeal cancer. The presence of different groups of antibodies in patients with cancer was compared with those of a healthy control group.

MATERIAL AND METHODS

Serum samples were collected from 30 patients with laryngeal squamous cell carcinoma and from10 patients with hypopharyngeal squamous cell carcinoma. Serum from 21 healthy subjects served as age and sex matched control group. Serum samples were obtained at the time of admission, before initiation of treatment and stored at -20^0 C until further analysis. The group included 7 females and 53 males, at the age of 35 to 78 years (mean 52.6). The tumour and nodal status was classified according to UICC TNM classification (14). None of the patients had had primary radiotherapy or chemotherapy.

The serum samples were analyzed for Epstein-Barr IgM, IgG and IgA antibodies to viral capsid antigen (VCA), IgG and IgA nuclear antigen (EBNA), and IgA antibodies to viral early antigen (IgA/EA) by enzyme linked immunosorbent assay (ELISA) technique using commercially available kits (Vironostica , Organon Teknika Inc.).The immunoglobulin presence in patients with hypopharyngeal and laryngeal cancer were compared with that of controle group using χ^2 test. The relationship between TNM classification and serum EBV antigens incidence was also analyzed.

RESULTS

Positive and negative results of IgG, IgM and IgA EBV antibodies in cancer patients and in the control group are shown in Table 1. Only 5 (12,8 %) of 39 patients with laryngeal or hypopharyngeal cancer were positive for anti IgM/VCA and all the control group showed negative results. Thirty six (92.3 %) of 39 patients with cancer and 17 (94.4 %) of 18 of the control group were positive for anti IgG/VCA. The presence of anti IgG/EBNA was detected in 27 (65.8 %) of 41 cases with cancer and in 11 (61.1 %) of 18 from the control group. The anti IgA/VCA antigen was detected in 7 (17.9 %) of 39 patients with cancer and all the subjects from the control group showed negative result. None of the patients from the control group had early antigen for anti IgA Epstein-Barr and only one patient (2.6 %) from the group with laryngeal and hypopharyngeal cancer had this antibody. Three (7.7 %) of 39 patients with cancer, and two (11.1 %) of 18 from the control group had anti IgA Epstein-Barr nuclear antigen.

Table 1. Incidence of Epstein-Bar virus antibodies in laryngeal and hypopharyngeal cancer and control group

EBV antibody panel	EBV VCA IgG		EBNA IgG		EBVCA IgM		EBVEA IgA		EBVVC IgA		EBVNA IgA	
	+	-	+	-	+	-	+	-	+	-	+	-
Control group (n=20)	17	1	11	7	0	18	0	19	0	19	2	17
%	94.4	5.6	61.1	38.9	0	100	0	100	0	100	10.5	89.5
Patients with cancer (n=41)	36	3	27	14	5	34	1	38	7	32	3	36
%	92.3	7.7	65.9	34.1	12.8	87.2	2.6	97.4	18	82	7.7	92.3
P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Total (n=61)	53	4	38	21	5	52	1	57	7	51	5	53

NS – not significant

The differences in presence of the evaluated antibodies between the control group and the study group were not significant. No significant difference in EBV examined antibodies incidence was found between T1+T2 and T3+T4 laryngeal and hypopharyngeal cancer patients. No significant difference in EBV examined antibodies presence was found between N0 and N1-3 cancer patients.

DISCUSSION

Yao et al. showed that the EB virus replicates in epithelial cells of the oropharynx, and nearly all seropositive subjects actively shed virus in the saliva (15). Other studies suggest that B cells in the oropharynx may be the primary site of infection (2,9).

In our study the IgM/VCA antibodies were not detected in the control group and they were present in 8.7% of subjects with squamous cell carcinoma. The IgM/VCA are known to decline within few weeks after primary EBV infection. In contrast the IgG/VCA and IgG/EBNA antibodies are present many months after the infection and they can by detected through a lifetime (5).

IgA/VCA has been shown in many reports to be a valuable marker for nasopharyngeal carcinoma (NPC). Applying indirect immunofluorescence staining Shimakage et al. (12) reported 16 NPC cases with positive anti-IgA/VCA antibody titer. Other authors claimed that IgA/VCA titer showed neither correlation with the clinical stage and histopatology of NPC nor any change in relation to the clinical course and effect of therapy (3.16). In our report only 17.9% of subjects with laryngeal and hypopharyngeal cancer showed positive anti-IgA/VCA antibody titer and all the subjects from the control group were negative.

The patients with laryngeal and hypopharyngeal carcinoma had only 2.6% IgA/ EA and 7.9% IgA /EBNA positive reaction. In contrast, the NPC patients showed 19.16% positive detection (3). A multicenter study found that after long-term remission, the IgA/EA serology test was critical for the detection of relapse 6 to 22 months before any observable clinical symptoms (4).

EBV IgG/VCA antibodies was detected in 94.4% of the control group and 92.3% of our patients with laryngeal and hypopharyngeal squamous cell carcinoma. EBV antibodies were in 80–100% of adult population of the world (6). Roy et al. (11) measured IgG antibody of EBV-VCA in sera of Indian patients with respiratory tract carcinomas, and found 60% of patients with laryngeal and hypopharyngeal cancer to be positive, while none of them was positive for the IgM antibody to viral capsid antigen (VCA).

In the present study EBV antibodies presence did not depend significantly on tumour (T) and lymph nodes (N) status of laryngeal and hypopharyngeal cancer. When evaluating IgG/VCA and IgG/EBNA antibodies titer (8), we found the antibodies titer in N1-3 larynx cancer patients to be higher than that of N0 patients.

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SUMMARY

Epstein-Barr virus (EBV) is a member of the herpesvirus family. The EBV genome consists of a linear DNA molecular that encoded nearly 100 viral proteins. EBV is one of the most successful viruses, infecting over 95 percent of the adult population of the world. Sera were collected from 61 patients (21 healthy patients without neoplasms, 30 cases with laryngeal squamous cell carcinoma and 10 patients with hypopharyngeal squamous cell carcinoma). The sera were titrated for EBV IgM/VCA, IgG/VCA, IgG/EBNA, IgA/VCA, IgA/EBNA and IgA/EA antibodies by enzyme linked immunosorbent assay (ELISA) technique. Only 5 (12.8 %) of 39 patients with cancer were positive for anti IgM/VCA and all the controls showed negative results. 36 (92.3 %) of 39 patients with cancer and 17 (94.4 %) of 18 of the control group were positive for anti IgG/VCA. Seropositive anti IgG/EBNA were detected in 27 (65.8 %) of 41 cases of cancer and in 11 (61.1 %) of 18 healthy control group. The positive anti IgA Epstein-Barr viral capsid antigen was detected in 7 (17.9 %) of 39 patients with cancer and all the control group showed negative result. No subject of the control group was positive for anti IgA Epstein-Barr early antigen and only one patient (2.56 %) was positive from the group with laryngeal and hypopharyngeal cancer. 3 (7.69 %) of 39 patients with cancer and 2 (11.1 %) of 18 controls were positive for anti IgA Epstein-Barr nuclear antigen. The differences were not significant.

Przeciwciała wirusa Epstein-Barr u chorych z rakiem krtani i gardła dolnego

Wirus Epstein-Barr (EBV) należy do rodziny herpesvirus. Genom EBV składa się z linijnego cząsteczkowego DNA, kodującego około 100 białek wirusa. EBV jest jednym z najbardziej upowszechnionych wirusów i zakaża około 95 % dorosłych osób na świecie. Celem tej pracy było

badanie występowania przeciwciał klasy IgG, IgA i IgM przeciwko antygenowi kapsydowemu (EBVCA), jądrowemu (EBNA) i wczesnemu (EA) wirusa Epstein-Barr w surowicy krwi chorych na raka krtani i gardła dolnego. Badania przeprowadzono w grupie 61 pacjentów, w tym 30 chorych z rakiem krtani, 10 chorych z rakiem gardła dolnego leczonych operacyjnie, a grupę kontrolną stanowiło 21 osób zdrowych. Obecność przeciwciał klasy IgM/VCA w grupie badanej stwierdzono u 5/39 (12,8%), natomiast w grupie kontrolnej nie stwierdzono obecności przeciwciał. Przeciwciała klasy IgG/VCA miało 17 z 18 (94,4%) osób w grupie kontrolnej oraz 36 z 39 (92,3%) chorych z rakiem krtani i gardła dolnego. Występowanie przeciwciał klasy IgG/EBNA wykazano u 11 z 18 (61,1%) osób w grupie kontrolnej oraz u 27 z 41 chorych z rakiem krtani i gardła dolnego. Obecność przeciwciał klasy IgA/VCA w grupie badanej stwierdzono u 7 z 39 (17,9%), natomiast w grupie kontrolnej nie znaleziono tych przeciwciał. Nie znaleziono przeciwciał klasy IgA/EA w grupie kontrolnej, a w grupie badanej stwierdzono je u jednego z 39 (2,56%) chorych. Przeciwciała IgA/EBNA stwierdzono u 2 z 18 (11,1%) badanych w grupie kontrolnej i u 3 z 39 (7,69%) pacjentów grupy badanej. Różnice pomiędzy chorymi na raka a grupę kontrolną nie były istotne statystycznie.