THE PREVALENCE OF HUMAN PAPILLOMAVIRUS 16 AND EPSTEIN-BARR VIRUS IN PATIENTS WITH ORAL SQUAMOUS CELL CARCINOMA

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SUMMARY – It has been suggested that certain viruses such as human papillomaviruses (HPV) and Epstein-Barr virus (EBV) might have a role in oral squamous cell carcinoma (OSCC). However, results of the published studies are controversial and are dependent on the geographic distribution and methods of sampling and sample analysis. The aim of this study was to determine the prevalence of HPV 16 and EBV in OSCC patients. In 24 patients with OSCC (mean age 59.6±8.8) and 30 controls (mean age 49.1±8.3), 5 mL of blood was collected to determine the prevalence of EBV by serologic methods. In addition, swabs were obtained to analyze the presence of HPV 16 and EBV by use of polymerase chain reaction. Statistical analysis was performed by use of Mann-Whitney test, χ^2-test and Spearman correlation test. The results of this study showed that there were no significant differences between OSCC patients and control subjects according to the presence of EBV or HPV 16. Therefore, it can be concluded that the role of the aforementioned viruses is less likely in our population of OSCC patients.

Key words: *Oral squamous cell carcinoma; Papillomaviruses; Epstein-Barr virus*

Introduction

Syrjänen and Syrjänen[1] were the first to report that human papillomaviruses (HPV) might have a role in oral and oropharyngeal carcinomas. Although certain HPV subtypes might be present in oral tissues, it seems that HPV 16 is the one most frequently found in oral carcinomas[1].

It seems that there is geographic difference in HPV prevalence. Additional problems are different sample collection procedures (biopsy specimens, swabs, etc.) followed by different methods of evidencing viral presence (polymerase chain reaction, *in situ* hybridization, immunohistochemistry). On top of that, it is quite usual that in literature reports, tonsillar, laryngeal and pharyngeal carcinomas are called oral carcinomas[2].

A meta-analysis of the literature published from 1982 until 1997 has shown that together with the increase in the degree of dysplasia, the likelihood of HPV presence increases as well. In 4680 specimens from 94 studies, HPV was identified in 10% of normal healthy tissues, while its prevalence in oral squamous cell carcinoma (OSCC) was 46.5%. Thirty percent of all OSCC were positive for HPV and most frequently HPV 16 and 18 were identified from these lesions[3].

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A substantial number of studies confirm the etiologic role of Epstein-Barr virus (EBV) in nasopharyngeal carcinoma as well as in other malignancies such as Hodgkin's disease, nasal T cell lymphoma, cervical uterine carcinoma, tonsillar carcinoma, etc.[4].

The prevalence of EBV in clinically healthy oral mucosa is quite variable, probably due to the fact that EBV DNA is present in the saliva. EBV prevalence is highly dependent upon the method of sample collection, i.e. oral swabs are known to show positive results at a rate of 20%-90% when compared to biopsy specimens[5]. Mao and Smith[5] report that exfoliated oral mucosa cells might contain EBV DNA, while at the same time saliva samples are negative for EBV DNA. Sand *et al*.[6] report that EBV prevalence in healthy oral mucosa is 7.3%. Results of a study where polymerase chain reaction was employed in biopsy specimens showed that there were no differences in EBV prevalence between OSCC patients and control group[7-9]. Also, it has been reported that EBV prevalence varies from 0%-100% in OSCC lesions[10].

However, its role in the strictly oral carcinoma which is confined to the oral cavity is not clear. Furthermore, previous studies have reported that certain viruses might have synergistic role in the development of some carcinomas, i.e. EBV and HPV in nasopharyngeal carcinoma as well as herpes simplex virus and HPV in oral and laryngeal carcinoma.

Therefore, the aim of the present study was to determine the presence of HPV 16 and EBV in oral swabs and the presence of EBV in sera of the same patients, and finally to compare these results with those recorded in control subjects.

Material and Methods

Prior to the study, an informed consent was obtained from all participants and appropriate approval for the study protocol from the Ethics Committee of the School of Dental Medicine, Zagreb. Study group included 24 patients (3 female and 21 male) with OSCC. Control group consisted of 30 healthy subjects (6 female and 24 male). The mean age of all study participants was 53.9±10.1 (study group 59.6±8.8 and control group 49.1±8.3) years.

Each participant filled in a questionnaire that was specially designed for this study, where data on smoking habit, alcohol use, number of sexual partners and orogenital sex practice were entered.

Decayed, missing and filled teeth (DMFT) were recorded according to the World Health Organization[11] and plaque index according to Löe[12].

In each participant, 5 mL of venous blood was collected from cubital vein to assess the presence of EBV. Serologic diagnosis was based on the VIDAS EBV VCA IgM, VIDAS EBV VCA/EA IgG and VIDAS EBV EBNA IgG kits, according to the manufacturer's instructions.

In each participant, oral mucosal swab was obtained (from carcinoma tissue in the study group and from healthy oral mucosa in the control group) to assess the presence of EBV or HPV 16. HPV analysis was performed using QIA amp Mini Elute Virus Spin kit (Quiagen) Digene HPV Genotyping RH test, according to the manufacturer's instructions.

Results

Table 1 shows carcinoma localization as well as tumor, nodes and metastases (TNM) classification and presence of HPV and EBV in the study sample. There were no significant differences between the presence of HPV and EBV viruses regarding carcinoma localization and TNM classification. There were no significant differences between the study and control groups according to smoking habit, cigarette packs *per* year and duration of smoking habit ($p>0.05$). Significantly more alcohol units *per* day were consumed by control subjects as compared with study group ($p<0.05$). There were no significant differences between the study and control groups according to the duration of alcohol consumption (Mann-Whitney test, $p>0.05$). Mann-Whitney test yielded no significant between-group differences according to the number of sexual partners ($p>0.05$). However, significant between-group difference was found according to orogenital sex practice (χ^2-test, $p<0.05$) (Table 2).

Oral hygiene scores measured by DMFT and plaque index were significantly increased in study group as compared to control group (Mann-Whitney test, $p<0.05$) (data not shown). There were no significant differences between the study and control groups according to the prevalence of EBV antibodies in sera and prevalence of EBV and HPV in oral swabs ($p>0.05$) (Table 3).

Table 1. Carcinoma localization, TNM and presence of HPV and EBV in study sample

	Age	Sex	TNM	Localization	HPV PCR	EBV PCR
P1	43	m	T4N2aM0	Sublingual area	pos	neg
P2	55	f	T3N1M0	Mandibular gingiva	pos	neg
P3	59	m	T4N1M0	Tongue base	pos	neg
P4	69	m	T2N2bM0	Sublingual area	neg	neg
P5	50	m	T1N0M0	Sublingual area	neg	pos
P6	72	f	T4N0M0	Mandibular gingiva	neg	neg
P7	57	m	T1N0M0	Tongue border	neg	neg
P8	58	m	T1N0M0	Sublingual area	pos	neg
P9	58	m	T3N0M0	Palatine tonsil	pos	neg
P10	46	m	T1N0M0	Tongue border	neg	neg
P11	48	m	T2N0M0	Uvula	pos	pos
P12	58	m	T2N0M0	Tongue base	neg	neg
P13	69	m	T2N1M0	Sublingual area	neg	neg
P14	58	m	T2N1M0	Tongue base	pos	neg
P15	57	m	T2N0M0	Sublingual area	pos	neg
P16	74	m	T2N0M0	Palatine tonsil	neg	neg
P17	67	f	T1N2bM0	Tongue base	pos	neg
P18	44	m	T4N1M0	Tongue base	neg	neg
P19	53	m	T2N0M0	Tongue border	neg	neg
P20	65	m	T2N0M0	Maxillary gingiva	pos	neg
P21	56	m	T2N2bM0	Tongue base	neg	neg
P22	66	m	T4N2bM0	Soft palate	neg	neg
P23	56	m	T3N2bM0	Tongue border	pos	neg
P24	76	m	T3N2bM0	Tongue base	neg	neg

TNM = tumor-nodes-metastasis classification; HPV PCR = human papillomavirus polymerase chain reaction; EBV PCR = Epstein-Barr virus polymerase chain reaction

Table 2. Demographic data of oral squamous cell carcinoma patients and control subjects

	Patients	Controls	p
Sex:			
male	21	24	0.041*
female	3	6	
Age (median ± SE)	58±1.8	47±1.5	0.0009*
Smoking, n (%)			
active	20 (83.3%)	25 (86.2%)	0.534
nonsmokers	4 (16.7%)	4 (13.8%)	
Packs *per* year (median ± SE)	40±4.1	37.5±5	0.473
Alcohol units *per* day	7.5±1.6	15±1.1	0.0001*
Number of sexual partners	6.5±1	5±1	0.274
Oral sex practice, n (%)	15 (62.5%)	9 (31%)	0.022*

*significant difference ($p<0.05$)

Table 3. EBV prevalence in sera together with EBV and HPV prevalence in swabs obtained from patients with oral squamous cell carcinoma and controls

	Patients, n (%)		Controls, n (%)		p
	Positive	Negative	Positive	Negative	
EBV IgG	24 (100%)			30 (100%)	
EBV IgM		24 (100%)		30 (100%)	
EBV EBNA		24 (100%)			
EBV PCR	11 (45.8 %)	13 (54.2%)	14 (46.7%)	16 (53.3%)	0.580
HPV PCR		24 (100%)		30 (100%)	

EBV IgG = Epstein-Barr virus IgG; EBV IgM = Epstein-Barr virus IgM; EBV EBNA = Epstein-Barr virus-associated nuclear antigen; EBV PCR = Epstein-Barr virus polymerase chain reaction; HPV PCR = human papillomavirus polymerase chain reaction

Discussion

One of the largest multicenter studies on oral head and neck carcinomas was performed in nine countries (Italy, Spain, Ireland, Poland, India, Cuba, Canada, Australia and Sudan). In 1670 patients with carcinoma (1415 with oral and 255 with oropharyngeal carcinoma) and 1732 controls, HPV DNA was proved in 3.9% of biopsy specimens of oral carcinoma and in 18.3% of biopsy specimens of oropharyngeal carcinoma. Most frequently viral DNA HPV 16 was isolated but this finding was not in correlation with the risk of carcinoma development or with the detection of HPV DNA in biopsy specimens[13]. Koskinen *et al.*[14] report that 37 of 61 (61%) head and neck carcinoma samples were HPV positive, whereas HPV 16 was most frequently found. Blomberg *et al.*[15] proved significant influence of HPV (especially HPV 16) on the epidemiology of head and neck carcinomas in Denmark.

The lowest HPV prevalence is reported from Africa. Van Rensburg *et al.*[8] and Boy *et al.*[16] report on HPV prevalence to range from 0% to 11.9% in OSCC patients, while Ibrahim *et al.*[17] could not detect HPV DNA in oral carcinoma samples.

However, we were unable to confirm the findings reported by the authors mentioned above, as we recorded positive HPV 16 finding in only two OSCC patients.

Miller *et al.*[18] found no EBV viruses in 30 biopsy specimens of healthy tongue mucosa, while Anwar *et al.*[19] could not confirm the role of EBV in the process of carcinogenesis of oral and esophageal carcinomas by use of *in situ* hybridization. This was also confirmed by Shimakage *et al.*[20], Talacko *et al.*[21], and Shaam *et al.*[22].

Sand *et al.*[6] found no significant differences in EBV prevalence between patients with oral lichen planus and OSCC. Furthermore, the same authors conclude that smoking, alcohol use, and age do not seem to be risk factors for EBV infection.

Tsuhako *et al.*[23] found higher EBV prevalence in squamous cell carcinoma by use of polymerase chain reaction, while they detected no EBV prevalence by use of *in situ* hybridization.

Goldenberg *et al.*[24] did not confirm the presence of EBV in oral carcinoma, which is consistent with the findings reported by Jang *et al.*[25], who concluded that the presence of some viruses in oral carcinomas might be a coincidence rather than etiological factor.

Talacko *et al.*[21] used *in situ* hybridization and were unable to detect EBV DNA in neoplastic cells within oral cavity. Therefore, they conclude that EBV does not play an important role in malignant transformation of oral mucosa in immunocompetent individuals.

It has been reported that the prevalence of EBV varies between 0% and 100% in patients with OSCC. Results of the studies where polymerase chain reaction was employed to determine EBV prevalence showed no differences between OSCC patients and controls[7-9]. This finding is consistent with the results of our study, as there were no significant differences between the study and control groups according to the prevalence of EBV either in sera or in oral swabs.

Sand *et al.*[6] found EBV prevalence to be 32.7% in patients with oral lesions. The same authors conclude that it is a consequence of immunity disturbance, local or systemic. We can confirm the finding reported

by Sand *et al.*[6], as there were no differences between the study and control groups according to EBV prevalence. Our control group consisted of individuals who were alcoholics and therefore known as being immunosuppressed.

McLemore *et al.*[26] report that there were no significant correlations between HPV positive tumors and co-infections with other viruses such as EBV, HSV-1, HSV-2 and HHV-8. This is in concordance with our finding, as there was no correlation between HPV and EBV either in the study group or in the control group.

There were no significant between-group differences according to the number of sexual partners, which is in contrast with Schwartz *et al.*[27] and D'Souza *et al.*[28], who found that HPV within OSCC was more frequently found in persons who had more than 26 partners. However, significant between-group differences were found according to orogenital sex practice, as study group patients practiced more orogenital sex than controls, which is consistent with Dahlstrom *et al.*[29] and Tachezy *et al.*[30].

Oral hygiene scores measured by DMFT and plaque index were significantly increased in the study group as compared with control group, therefore their dental status and oral hygiene were compromised in comparison to the control group. This finding is in concordance with many authors who studied oral and dental status in OSCC patients[31-33].

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Sažetak

UČESTALOST HUMANOG PAPILOMAVIRUSA 16 I EPSTEIN-BARROVA VIRUSA KOD BOLESNIKA S ORALNIM KARCINOMOM PLOČASTIH STANICA

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Čini se kako određeni virusi poput humanih papilomavirusa (HPV) i Epstein-Barrova virusa (EBV) mogu imati ulogu u nastanku karcinoma pločastih stanica u usnoj šupljini (OPCK). Ipak, rezultati objavljenih istraživanja na tu temu su kontroverzni i ovise o geografskoj distribuciji i metodama uzimanja uzoraka odnosno raščlambi uzoraka. Cilj ovoga istraživanja je bio odrediti učestalost HPV 16 i EBV u osoba s OPCK. U 24 osobe s OPCK (srednje dobi 59,6±8,8) i u 30 kontrolnih ispitanika (srednje dobi 49,1±8,3) je uzeto 5 mL krvi kako bi se odredio EBV serološkim metodama. Uz to su uzeti i brisevi kako bi se odredila prisutnost HPV 16 i EBV uz pomoć reakcije lančane polimeraze. Statistička analiza je napravljena pomoću Mann-Whitneyeva testa, χ^2-testa i Spearmanova korelacijskog testa. Rezultati ovoga istraživanja pokazuju kako nije bilo znakovitih razlika između osoba s OPCK i kontrolnih ispitanika s obzirom na prisutnost HPV 16 ili EBV. Može se zaključiti kako ovi virusi u našoj populaciji oboljelih od OPCK vjerojatno nemaju veliku ulogu.

Ključne riječi: *Karcinom pločastih stanica usne šupljine; Papilomavirusi; Epstein-Barrov virus*