Abstract: Epstein-Barr virus (EBV) and human papillomavirus (HPV) are known to exhibit oncogenic potential. Target cells for both viruses include oropharyngeal elements. The present study investigated whether EBV or HPV infection are associated with palatine tonsil carcinoma (PTC) and/or tongue carcinoma (TC). The study included 28 adult patients with oropharyngeal squamous cell carcinoma, including 14 patients with PTC and 14 patients with TC. The control group included 20 healthy adult volunteers. Sera of all patients and controls were tested for IgG anti-EA antibodies, IgG anti-VCA antibodies, and IgG anti-EBNA antibodies. DNA extracted from the tumors was tested for the presence of EBV DNA and HPV DNA using PCR-ELISA. In parallel, the presence of EBV DNA was tested in the peripheral blood in all healthy individuals and patients. In addition, attempts were made to detect HPV 16 and HPV 18 using other PCR amplification techniques. Serum anti-EBV antibodies were detected in 24 patients (12 patients with PTC and 12 patients with TC). The frequency of detection of the antibodies did not significantly differ between the groups of patients and the control individuals. Most positive patients and controls demonstrated a serological pattern typical for past EBV infection. EBV DNA was identified in 12 cases of PTC and in 12 cases of TC (altogether 86% cases). In 10 PTC patients, 8 TC patients, and only 2 healthy individuals EBV DNA was detected in peripheral blood. HPV DNA was detected in only 3 cases (1 sample of PTC and 2 samples of TC). These results suggest that the etiopathogenesis of oropharyngeal cancers may be associated with EBV infection much more frequently than with HPV infection.

Key words: Epstein-Barr virus • Human papillomavirus • Anti-EBV antibodies • Palatine tonsil carcinoma • Tongue carcinoma

Introduction

Both human papillomavirus (HPV) and Epstein-Barr virus (EBV) infections seem to be associated with the development of epithelioid malignancies. To date, more than 100 HPV genotypes have been recognized, of which more than 20 sexually transmitted types (in particular HPV 16 and HPV 18) have been found in a worldwide epidemiological survey to represent the principal etiological factor leading to invasive carcinoma of the uterine cervix [1–3]. Nevertheless, association of the viruses with oropharyngeal squamous cell carcinomas (SCC) still remains obscure. Another virus, the infectious mononucleosis-inducing EBV, may play a significant role in carcinogenesis. Such a role for EBV has been documented in the etiopathogenesis of endemically manifested nasopharyngeal carcinoma, and recent studies point to its association with some cases of gastric carcinoma [4, 5]. This suggests EBV infection might be involved in the devel-
Detection of HPV 16 and HPV 18

HPV 16-specific (160-bp) and HPV 18-specific (240-bp) sequences of DNA were detected by PCR amplification using primers that were homologous to the E6 region (TIB Molbiol). Detection was carried out in 2% agarose gels using ethidium bromide (Sigma).

Detection of serum anti-EBV antibodies

Anti-EBV antibodies were quantified by an ELISA. Sera were tested using kits to detect early antigen complex, IgG anti-EA antibodies (ETI-EA-G, DiaSorin), antibodies to viral capsid antigen, IgG anti-VCA (ETI-VCA-G, DiaSorin), and antibodies to Epstein-Barr nuclear antigen, IgG anti-EBNA (ETI-EBNA-G, DiaSorin). Absorbance values were recorded at 450/630 nm using the Behring Microstrip Reader. The results were expressed in arbitrary units (AU) per milliliter, where AU is related to a reference antibody preparation. ETI-EA-G sensitivity, defined as the apparent concentration of analyte that can be distinguished from a negative sample, was 2.8 AU/ml at 95% confidence limit. The corresponding sensitivities of ETI-VCA-G and ETI-EBNA-G were 3.5 AU/ml and 2.5 AU/ml, respectively. Values equal or higher than 20 AU/ml in the tests were taken as positive.

Statistical analysis

Differences in frequencies of positive results were compared with chi-squared test and were considered significant at \( P < 0.05 \).

Results

Results of detection of viral DNAs are summarized in Tables 1 and 2. EBV DNA in tumor specimens was detected in 12 patients with PTC and 12 patients with TC. Samples of peripheral blood were found to contain EBV DNA in 18 patients (10 with PTC, 8 with TC) and in 2 healthy individuals. In only 3 cases (11%, 1 PTC sample and 2 TC samples) was HPV DNA detected, in each case in parallel with detec-