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HPV infection in relation to OSCC histological grading and TNM stage. Evaluation by traditional statistics and fuzzy logic model

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Summary We aimed to evaluate if in oral squamous cell carcinoma (OSCC) there is a relationship between histological grading (HG), TNM clinical stage and HPV infection; and to study the performance of fuzzy logic compared to traditional statistics, in the analysis of HPV status and correlates of OSCC. In cross-sectional analysis, the study group comprised 63 patients (mean age 68.89 years (SD ± 11.78), range (32—93); males 28 (44.4%), females 35 (55.6%)) with OSCC histologically diagnosed. HPV-DNA was studied in exfoliated oral epithelial cells by *nested* PCR (MY09/MY11 and GP5+/GP6+ primers). Data were analysed in parallel by traditional statistics with multivariate analysis and a fuzzy logic (FL) technique (membership functions as input, the ANFIS methodology, and the Sugeno's model of first order). HPV infection was detected in 24/63 (38.1%) of OSCC, as being HPV+ve 14/36 (38.9%) in G1, 7/18 (38.9%) in G2, and 3/9 (33.3%) in G3; HPV+ve 8/33 (24.2%) in Stage I, 9/12 (75.0%) in Stage II, 6/11(54.5%) in Stage III, and 1/7 (14.3%) in Stage IV. In both methods of analysis, no significantly increased risk of HPV infection was found for any HG score; whereas, TNM stage II was significantly associated to HPV infection ($p = 0.004$; OR = 9.375 (95% CI = 2.030:43.30); OR′ = 11.148 (95% CI = 1.951:43.30)), and, in particular, to primary tumour size T2 ($p = 0.0036$; OR = 7.812 (95%

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CI = 1.914:31.890); OR′ = 9.414 (95% CI = 1.846:48.013)); FL (% of prevision: 79.8; Root Mean-Square Error (RMSE): 0.29). No association was found between HPV infection and any demographical variable. Our findings show an association between HPV infection with TNM (stage II − T2), but not with histological grading of OSCC. Also, FL seems to be an additional effective tool in analysing the relationship of HPV infection with correlates of OSCC.

Introduction

Nowadays there is increasing evidence for the role of high risk (HR) human papillomavirus (HPV) in the pathogenesis of oral squamous cell carcinoma (OSCC),[1–7] till to consider HPV-positive (HPV+ve) OSCC as a distinct clinicopathological entity with different outcome.[3,5,8–12] HR HPV have been found to be risk factors for head and neck cancer, independently from alcohol and tobacco use.[5,13–17] Furthermore, it has been observed that oral cavity is exposed to nutritional and environmental mutagenesis[18,19] and that multiple risk factors (e.g. cigarette smoking, alcohol, chronic irritants, oral mechanical trauma) could co-operate with the transforming ability of HPV in inducing malignant oral lesions.[20]

Several studies have evaluated correlates of OSCC (e.g. histological and clinical parameters) in relation to HPV infection, and contradictory findings have been reported.[5,21–26] A possible explanation is that parameters evaluated are also conditioned by additional concurrent risk factors, and they require complex multistep statistical models. Hence, besides the traditional multivariate analysis, artificial neural network (ANN) analysis could be a powerful tool for accurately detecting causal relationships. The fuzzy neural network (FNN), useful in setting fuzzy logic (FL) systems, is one of the most advanced ANN models, and its most attractive feature is that relationships between input and output variables can be described accurately from the available data.

The aim of the present study was to evaluate the risk of HPV infection in OSCC in relation to histological grading (HG) and TNM clinical stage, also to some social-demographical variables (i.e. age, gender, smoking and alcohol drinking habits); a secondary aim was to verify the use of a FL system in modeling the significance of any variable, compared to traditional statistics.

Subjects and methods

Study design

In this cross-sectional hospital-based study, the study population was composed of 63 patients (mean age 68.89 years (SD ± 11.78), range (32–93); males 28 (44.4%), females 35 (55.6%)) with OSCC histologically diagnosed: 36 (57.1%) in G1 (grading 1: well differentiated), 18 (28.6%) OSCC in G2 (grading 2: moderately differentiated), and 9 (14.3%) in G3 (grading 3: poorly differentiated); 33 (52.4%) in Stage I, 12 (19.0%) in Stage II, 11 (17.5%) in Stage III, and 7 (11.1%) Stage IV. Out of 63 OSCCs, 28 were localised on the tongue (lateral border), 14 on buccal mucosa, 12 on alveolar ridge mucosa, 6 on the palate, and 3 on the lower lip. Lesions were initially identified based solely on clinical features; thereafter, lesions were histologically evaluated and only those confirmed as OSCC were included in the present investigation. Informed consent was obtained from all participants.

Histological analysis

Microscopic evaluation was performed by one oral pathologist (E.M.), who confirmed OSCC diagnosis and also determined the score of HG. The World Health Organization criteria were used as follows: grade 1 (G1), well differentiated; grade 2 (G2), moderately differentiated; and grade 3 (G3), poorly differentiated. Grade is dependent on the degree of prickle formation, keratinization, and overall resemblance of carcinoma to normal squamous epithelium.

Anamnestic and clinical evaluation

Historical and clinical data of each subject was recorded on a clinical report form and collected by means of a data entry program for PC. Information regarding age, smoking and alcohol use was obtained by personal interviews. Patients were divided into three smoking categories, namely non-smoker, current smoker and ex-smoker. Subjects were included in the last case if they had not smoked within the last 3 years. For alcohol consumption, subjects were assigned to one of two drinking categories: non-drinker and current drinker.

Virological evaluation

HPV-DNA presence was researched by nested (MY/GP primers) PCR assay (*n*PCR) in oral mucosa brushed cells, and HPV genotype was determined by direct sequencing of PCR fragments.

Sample collection and processing

Oral cytological specimens were obtained from the site of the OSCC, by means of a cytobrush (RAM, Mirandola, MO, Italy).