DNA was detected in two patients but none was found in control subjects. The relation between demographic and risk factor variables was not statistically significant.
**Conclusions:** HPV infection has significant impact on HNSCC. Despite HPV-16 possesses the stronger impotent, HPV-18 is more probable to cause malignant degeneration in such cancers amongst some communities. It is a necessity to introduce and conducting immunization program in health care system in order to some extent safeguards such communities.

**8523** POSTER

**Up-regulation of Neutrophil Gelatinase-associated Lipocalin in Oral Squamous Cell Carcinoma − Relation to Cell Differentiation**

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**Background:** Neutrophil gelatinase-associated lipocalin (NGAL; also known as Lipocalin2, LCN2) is a secreted glycoprotein and elevated expression has been observed in solid tumours. The expression and function of oral cancer, however, is unclear. We investigated the expression of NGAL in oral cancer tissues and oral cancer cell lines.
**Material and Methods:** Surgical specimens were obtained from 40 patients, including 5 cases of normal mucosa, 5 cases of leukoplakia of the tongue, and 30 cases of tongue cancer (tumour tissue). Tumour tissue were stained H-E for histological examination. Immunohistochemical examination were performed by the ABC staining methods. Moreover, Eight oral carcinoma cell lines (SCCKN, HSC-2, HSC-3, OSC-19, OSC-20, HOC-313, SCC-25, TSU) were lysed in lysis buffer and were performed for Western blot analysis. Gelatin zymography, that were detected for MMP-2 and MMP-9, were performed for condition medium of each cell line.
**Results:** By immunohistochemical examinations, NGAL expression was strongly up-regulated in well-differentiated OSCC tissues and moderately to weakly in moderately to poorly differentiated OSCC tissues. In contrast, NGAL expression was weak or very weak in the normal mucosa and leukoplakia. By Western blot analysis, NGAL expression levels positively correlated with cell morphology pattern and loss of E-cadherin. In addition, the enzymatic activity of the NGAL/MMP-9 complex significantly correlation with the results obtained by zymographic analysis.
**Conclusion:** NGAL is highly expressed in well-differentiated cancer, suggesting that NGAL may be a possible role as diagnostic marker of tumour-cell differentiation.

**8524** POSTER

**A Functional Analysis of Zyxin in Epithelial-mesenchymal Transition of Oral Squamous Cell Carcinoma**

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**Background:** Epithelial-mesenchymal transition (EMT) confers destabilization of cell-cell adhesion, cytomorphologic change and cell motility required for cancer invasion and metastasis. Zyxin, one of the focal adhesion-associated LIM domain proteins, is essential for actin reorganization for cell migration. The involvement of Zyxin in EMT was investigated.
**Material and Methods:** According to the classification of mode of invasion in squamous cell carcinoma of the oral cavity (OSCC) by Yamamoto and Kohama (1984), eight OSCC cell lines were divided: SCCKN, HSC-2, OSC-20 in grade 3, HSC-3, SCC-25, OSC-19 in grade 4C (cord-like type) and HOC-313, TSU in grade 4D (diffuse type). Expressions of E-cadherin, N-cadherin and Zyxin as EMT marker were examined by histocytochemistry and western blot. Cell growth was examined by MTT assay and cell motility was examined by scratch assay and invasion assay. Expression of Zyxin was knocked down using siRNA.
**Results:** Decreased expression of E-cadherin and increased expression of N-cadherin and Zyxin were found in parallel with mode of invasion from grade 3 to grade 4. Zyxin was markedly expressed in HOC313 and TSU. Treatment of HOC-313 with si-Zyxin resulted in cell morphologic change from spindle to polygonal shape. The si-Zyxin treatment of HOC313 inhibited cell growth and invasion significantly as compared to si-control treatment. When Rho family proteins such as RhoA, Rac1 and Cdc42 were examined, there was no significant difference in expression of RhoA and Cdc42, but expression of Rac1 was weaker in si-Zyxin treatment than in si-control treatment. In addition, expression of Zyxin in HOC-313 was inhibited by Rac-1 inhibitor.
**Conclusions:** These results indicated that Zyxin may become a possible EMT marker and overexpression of zyxin promotes cell growth and invasion of HOC-313 cells via up-regulation Rac-1.

**8525** POSTER

**The Prevalence of Microsatellite Instability and Loss of Heterozygosity in Bulgarian Patients With Laryngeal Carcinoma**

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**Background:** A significant proportion of tumours develop through the microsatellite instability (MSI) pathway as a result of impaired DNA mismatch repair. However, data about the prevalence of MSI in head and neck carcinomas are still controversial − some authors have found MSI in more than 40% of the cases, while others claimed that not MSI but loss of heterozygosity (LOH) is often observed in these cancers. In a previous study, high level of epigenetic silencing of *MLH1* gene was found in laryngeal tumours of Bulgarian patients, suggesting involvement of MSI in their development. Thus, we aimed to examine MSI at five loci in patients with carcinoma of the larynx.
**Materials and Methods:** Forty-eight patients with laryngeal carcinoma were included in the present study. It was approved by the ethical committee of Medical University − Sofia, and informed consent was obtained from all patients. DNA was extracted from fresh frozen tumour tissues and blood samples of the selected patients with laryngeal cancer. Automatic fragment analysis was performed after PCR amplification of the selected microsatellites: D2S123, D5S346, D18S35, FGA and Bat26.
**Results:** MSI was observed only in one marker − D18S35, in one patient, who had methylated *MLH1* promoter. While MSI was a rare event in the studied tumours, loss of heterozygosity (LOH) was found to be a common feature − 50% of the carcinomas showed LOH in at least one of the five microsatellites. However, no correlation with *MLH1* methylation status was observed. Statistically significant association was found between LOH and the age of patients − LOH occurred frequently in patients over 60 ($p < 0.05$). Also, LOH was observed mainly in heavy drinkers ($p = 0.001$). No MSI or LOH was detected in the group of non-smokers.
**Conclusions:** Our results show that not MSI, but LOH is a common feature of laryngeal carcinomas in Bulgarian patients. LOH is associated with the age and the impact of external risk factors like alcohol consumption and tobacco smoking.

**8526** POSTER

**Fibronectin Induces Matrix Metalloproteinase-9 (MMP-9) in Human Laryngeal Carcinoma Cells by Involving Multiple Signaling Pathways**

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**Background:** Cell adhesion to extracellular matrix initiates intracellular signaling cascade regulated by integrin family of receptors. Evidences show that cultured cells in presence of extracellular matrix adhesion molecule Fibronectin (FN) stimulates secretion of matrixmetalloproteinases (MMPs), facilitating cancer cell invasion. Amongst all MMPs, MMP-9 is often reported to play crucial role in tumour cell growth and metastasis.
The present study aims at examining the effects of FN on MMP-9 in laryngeal carcinoma cell line, HEp-2, and understand the molecular mechanism(s) involved.
**Materials and Methods:** The methods used were gelatin zymography, western blot, semi quantitative and quantitative real-time RTPCR, ELISA, Immunocytochemistry, siRNA studies, MTT assay and inhibitor studies and EMSA.
**Result:** The study reports that FN induces the activity, mRNA and protein expression of MMP-9 in HEp-2 cells. This effect is mediated mainly by integrin receptor a5b1, since, the blockade of a5 abrogated FN mediated stimulatory responses. siRNA, inhibitor studies and immunoblots suggested involvement of Focal adhesion kinase (FAK), Phosphatidyl-inositol-3-kinase (PI-3K), Extracellular regulated kinase (ERK) and nuclear factor-kappa-B (NF-kB) in FN-mediated MMP-9 induction. Immunocytochemical analysis demonstrated the nuclear localization of ERK, PI-3K and NF-kB. FN-induced transactivation of MMP-9 gene by enhanced DNA-binding activity of transcription factors NF-kB, Activator protein-1 (AP-1) and Specificity protein-1 (Sp1) to the MMP-9 promoter.
**Conclusion:** This study suggests that extracellular matrix protein FN induces MMP-9 in HEp-2 cells mainly by involving integrin receptor a5b1 and involves activation of multiple signaling pathways which independently or in "cross-talk" to each other finally leads to the transactivation of the MMP-9 gene.