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# Promoter hypermethylation of *TIMP3* and *PCQAP 5'* tumour suppressor genes in saliva of oral and oropharyngeal cancer patients: A pilot study in Sri Lanka

C.D.K. Liyanage,[1] K.B.W.M.R.A.I. Wathupola,[1] K.A. Perera,[2] and P.V. Udagama[1]*

[1]*Department of Zoology and Environment Sciences, University of Colombo, Colombo 3,*
[2]*National Cancer Institute, Maharagama*

Epigenetic and genetic alterations play a major role in the development of oral and oropharyngeal cancers (OOPC). Epigenetic alterations, such as DNA methylation events are frequently accompanied by the excessive consumption of tobacco (smoking/chewing) and alcohol, which are strongly associated with an increased risk of OOPC incidence. Previous studies showed that silencing of tumour suppressor genes (TSGs) by DNA promoter hypermethylation is an early event of OOP carcinogenesis. The current study aimed to evaluate the degree of the methylation level in the promoter CpG islands of TIMP3 and PCQAP 5' TSGs in saliva derived DNA. Using a sensitive methylation-specific PCR (MSP) coupled with densitometric analysis, the current study recorded a marked elevation of the promoter methylation level in both TIMP3 and PCQAP 5' TSGs ($p$<0.05 for both TSGs) in saliva collected from OOPC patients (n=60) compared to normal healthy controls (n=60). Aberrant methylation of at least one of the TSGs was detected in 80% (48/60) of OOPC tumours; 86% (52/60) at TIMP3 and 72% (43/60) at PCQAP 5'. Tumour cell specific promoter hypermethylation of both TSGs was presented in all OOPC tumour grades and stages while TIMP3 promoter hypermethylation was significantly higher in poor to undifferentiated ($p$<0.05) than in moderate to well differentiated OOPC ($p$>0.05). The effect of established risk factors on promoter hypermethylation and relative odds ratios (OR) were assessed using a binary logistic regression analysis (IBM-SPSS Inc). Overall, a significant correlation ($p$<0.05) was noted between positive betel quid (with tobacco) chewing history and promoter hypermethylation of both TSGs (TIMP3; OR=3.5, PCQAP 5'; OR=3.0). In conclusion, the study demonstrated that TSG promoter hypermethylation can be detected by the MSP analysis of DNA extracted from saliva collected from OOPC patients. These signature candidates of hypermethylated TSGs may function as potential diagnostic biomarkers for OOPC in Sri Lanka.

**Keywords:** Oral and oropharyngeal cancers, saliva, Epigenetics, Promoter hypermethylation.

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*preethi@zoology.cmb.ac.lk 071-4416050