



**Ministry of Higher Education
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University of Baghdad
College of Dentistry**



THE EFFECT OF SMOKING AND (DNMT1) GENE POLYMORPHISIM ON THE METHYLATION LEVEL OF (MLH1) GENE IN ORAL MUCOSA

A thesis

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ABSTRACT

Introduction: Exposure to tobacco smoke is considered to be the most important etiological factors for the development of squamous cell carcinoma of the head and neck (SCCHN). Hypermethylation functions as gene silencing and can be observed at a high frequency in squamous cell carcinoma as well as in tissues adjacent to tumors and dysplastic tissues, the most commonly methylated genes are DNA repair genes, MutL homolog 1 (MLH1) gene. Also DNA-methyltransferase 1 (DNMT1) is a major enzyme that determines genomic methylation patterns. The DNA-methyltransferases that are responsible for regulating the methylation status of other genes are associated with increased risk of cancer. This study aimed to investigate methylation status of MutL homolog 1 (MLH1) gene and polymorphism of DNA-methyltransferase 1 (DNMT1) gene in chronic smokers and non-smokers, with detection of cotinine concentration in serum and saliva, an established marker of smoking exposure.

Materials and methods: The study sample consisted of 100 males, age between (20-40) years 58 subjects were smokers as a study groups and 42 subject who were non-smokers as a control group. This study was conducted at the Specialized Blood Bank of Babylon health center. The inclusion criteria for study group were the following: no history of malignant neoplasia, absence of visible alterations in the normal oral mucosa. Serum and saliva cotinine concentration was assessed using Enzyme-linked Immunosorbent Assay (ELISA). DNA methylation (MLH1 repair gene) and polymorphism of (DNMT1 gene) analysis was performed using specific PCR technique (MSPCR) and (RFLIP) respectively.

Results: The results of this study showed a significant effect of tobacco smoking in methylation of MLH1 gene promoter in comparison to non-smoker group, ($P < 0.05$), with Odds ratio = 4.957 C/I (2.1-11.7). Genotyping of DNMT1 SNP shows significant correlation between subject with GG genotype and methylation status. Cotinine can be used widely in future compared to other diagnostic tools because of its higher sensitivity, specificity, long half-life. Moreover, it is the best indicator for distinguishing the tobacco smokers from non-smokers, saliva cotinine cut-offs in this study that can be used to distribute smokers and non-smokers with impartially high sensitivity and specificity.

Conclusions: Chronic smoking has direct effect on oral mucosa leads to the methylation of repair gene (MLH1). The DNMT1 polymorphism may constitute an inheritable risk factor for chronic smokers (association with methylation status). Cotinine can be used widely in compared to other diagnostic tools because of its higher sensitivity, specificity, Moreover, saliva cotinine cut-offs in this study that can be used to distribute smokers and non-smokers with impartially high sensitivity and specificity.



جمهورية العراق
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آثار التدخين وتعدد الاشكال لجين (DNMT1) على مستوى الميثيل في محفز (MLH1) الجين في الغشاء المخاطي للفم

رسالة مقدمة الى كلية طب الاسنان – جامعة بغداد كجزء من متطلبات نيل درجة الدكتوراه في
طب الفم

من قبل

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