The relationship between smoking and Urokinase gene 3'-UTR T/C expression on occurrence of bladder cancer

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Abstract

Bladder cancer (CA Bladder) is the malignancy that affects the lining of the bladder (which is the most common types of urinary epithelial cancers that associated with the most invasive types of cancers and has the highest incidence of recurrence and infections). This study was designed to investigate the role of cigarette smoking on expression of urokinase gene that has a prominent role in the incidence of CA bladder. The samples were collected from 90 patients after being clinically diagnosed by the specialist surgeon of Imam Hussein Center for Cancer Diseases, Holy Kerbala, Iraq who were heavily smokers (more than one pocket/day). These patients were compared to 90 non-smoker patients with CA bladder and 90 persons who apparently healthy individuals as a control groups, DNA was extracted from all blood samples. The level of gene expression was correlated with cycle threshold value calculated by using real time PCR.

The results showed significant association between smoking and occurrence of CA bladder (by increased expression of urokinase 3'-UTR T/C gene) in comparison to both control and non-smoker patients groups.

Keywords: *CA bladder DNA, cigarette smoking, Urokinase gene 3'-UTR T/C, real time PCR.*

Introduction

The genetic investigations for polymorphisms help to distinguish between the different inherited forms of the gene and their impact on the occurrence of many human diseases include cancer [6]. The extent of the risk factors like smoking and genetic predisposition of many affect the occurrence of many diseases such as cancer, heart disease and diabetes [1] .The urokinase-type plasminogen activator uPA system is a responsible for transforming plasminogen into plasmin, which has different physiological functions [10] .It additionally assumes a key signaling protein in disease intrusion and metastasis both locally and spread away for far destinations [4].

The urokinase gene is located at chromosome 10q24 [9]. Polymorphisms of a C/T transversion at the 3' UTR (+4065 nucleotide) were demonstrated by C/T substitution in exon 6 and a T/C substitution in intron 7 [8]. Among the risk factors that participate in the occurrence of CA bladder, smoking was detected as one of major one [3]. High levels of urokinase have been depicted in bladder tumors especially in those who were heavily smokers [5].

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Methodology

Blood samples were collected from males of age group ranging from 50-60 years old by the Imam Hussein Center for Cancer and Hematology at the Imam Hussain Teaching Hospital in Holy Karbala province, Iraq. The samples were include a 90 heavily smoker patients after the clinically determination of bladder malignancy (transitional cell carcinoma) by authorized specialist, 90 patients with CA bladder with no history of smoking for at least the past 25 years, and 90 apparently healthy non-smoker individuals as a control group. Both patients and control groups were matched regarding age, body weight, past medical history and any associated confounding factors. This study was extended for period from January to August 2018. DNA was extracted from all blood samples by DNA extraction

kit (Bioneer,Korea). Polymerase chain reaction (PCR) was adopted for amplification of gene, The Premieres were Designed by STS Accession No. G27040: forward primer 5'-CCGCAGTCACA- CCAAGGAAGAG-3'and backward primer 5'GCCTGAGGGTAAAGCT-ATTGTCGTGCAC-3'.

The RT-PCR protocol involve the following steps: (a) initial one cycle of denaturation for 5 minutes at 95C^{o}, (b) followed by annealing at 58 C^{o} for 40 seconds, (c) then extension at 72 C^{o} for 40 seconds, (d) with final step of holding the specimen at 8 C^{o} [11] Real time PCR was used for quantitative assessment of urokinase gene 3'-UTR T/C expression by measuring cycle threshold (cT) value (which is inversely proportional to the level of gene expression) [12]

Statistical Analysis

Data were statistically represented as mean±SD and analyzed by detection of variance of significance using ANOVA test by sigma plot software version 12.5.

Results and Discussion

Smoking was thoroughly studied as a predisposing factor for many human disease include cardiovascular disorders, respiratory diseases malignancies, neurological deficits and many other organic dysfunction [14]. In current study, the aim was to demonstrate the

Table (1): the cT value of urokinase gene 3'-UTR T/C (expressed as Mean±SD) estimated by real time PCR.

Group Name	No.	Mean±SD
CA smokers	90	12.584±2.692
CA non smokers	90	23.213±3.569
healthy non smokers	90	24.865±2.861

The difference in cT values were quite significant between smoker patients as compared to both non-smoker patients and non-smoker healthy persons (12.584±2.692 versus 23.213±3.569 at P<0.001 and 12.584±2.692 versus 24.865±2.861 at P<0.001 respectively). On the other hand, no significant difference was detected between non-smoker patients in comparison to non-smoker healthy individuals (23.213±3.569 versus 24.865±2.861). These findings suggested that smoking has potential inducing effect for expression of urokinase gene 3'-UTR T/C which has crucial role in occurrence of CA bladder in heavily smokers. Other finding was noticed that the occurrence of CA bladder in non-smoker patients may be attributed to other sort of predisposing factor or gene.

This association was mentioned in certain study [7] ,which suggested that chronic exposure to cigarette smoking had strong association with development of urothelial carcinoma.

In another study done to evaluate the effect of smoking on soluble 3'-UTR isoforms(regulatory pathway of bronchial epithelial cell function) [13], similar results were founded supporting the potential role of smoking as predisposing factor for CA bladder in our study.

This effect of smoking was explained in certain study as a result of the effect of smoking on p53 pathway that mediate apoptotic signaling that responsible for eradication of cancer cells [2].

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Education for Pure Sciences, University of Kerbala, Kerbala, Iraq and all experiments were carried out in accordance with approved guidelines.

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