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8-HYDROXYDEOXYGUANOSINE IN TRANSITIONAL BLADDER CARCINOMA

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ABSTRACT

Objectives: As oxidative stress is known to be associated with the development of cancer, we investigated whether the tissue expression of 8-hydroxydeoxyguanosine (8-OHdG) which is a marker of DNA damage were increased in patients with transitional bladder carcinoma (TBC).

Design and Methods: Fifty patients with transitional bladder carcinoma (TBC) and 18 age-matched cancer –free patients were involved in this study. 8-hydroxydeoxyguanosine (8-OHdG) in tissue were examined by immunohistochemistry (IHC).

Results: Expression of 8-OHdG in transitional bladder carcinoma (TBC) tissues were higher than in control tissue. Significant differences were shown in 8-OHdG levels in the cancerous and cancer-free tissues between high grade and low grade TBC patients ($P < 0.05$)

Conclusions: 8-OHdG reflects oxidative stress in transitional bladder carcinoma tissue together with staging of cancer. Because of many factors that could influence the oxidative modification of DNA bases, its role as a diagnostic and/or prognostic factor in transitional bladder carcinoma seems to be limited.

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INTRODUCTION

Cancer is the leading cause of death worldwide. Despite the significant research effort, underlying mechanisms of carcinogenic processes are still poorly understood. In recent decades, a group of extremely reactive oxygen metabolites, reactive oxygen species (ROS), have been linked closely to carcinogenesis (Brieger *et al*., 2012). Enhanced ROS production and/or disturbed antioxidant function can lead to a state of oxidative stress, where ROS escape the control of antioxidants and are free to cause damaging reactions with cellular macromolecules such asproteins, lipids and DNA (Valko *et al*., 2006). DNA and nucleotides are at high risk of being oxidized by reactive oxygen species (ROS), which are generated as byproducts of oxygen respiration or molecular executors in host defence (Nakabeppu *et al*., 2006) or through environmental exposure (Hallberg *et al*., 2012).

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Among bases, guanine is the most susceptible DNA target for oxidation reactions (Cadet *et al*., 1999). 8-hydroxydeoxyguanosine (8-OHdG), one of the major DNA base-modified products, may be induced by hydroxyl radicals, singlet oxygen, or photodynamic action (Anna Płachetka *et al*., 2013). This compound is known to be mutagenic by mispairing with adenine; thus, during the next round of replication, faultily paired adenine will pair with thymidine and transversion G:C→T:A arises (Nakabeppu *et al*, 2006). Increased oxidative stress is generally thought to be associated with tumorigenesis. Many observations suggest the role of oxidative stress in colon cancer pathogenesis. Chang *et al*. reported that the content of 8-OHdG in serum can act as a sensitive biomarker for colorectal carcinoma (Chang *et al*., 2008). Moreover, Dincer *et al*. concluded that low plasma levels of 8-OHdG (together with altered antioxidant activity) may implicate the defective repair of oxidative DNA damage in patients with colon and gastric cancer (Dincer *et al*., 2007). It is difficult to exclude all factors that could affect the status of

oxidative DNA damage and contribute to the increasing level of 8-OHdG in the entire human body. The direct measurement of 8-OHdG in the appropriate tissues can give a more precise picture of what is happening directly in the tissue. Therefore, we decided to determine the 8-OHdG expression directly in tumor and non-tumor tissue for comparison.

MATERIAL AND METHODS

Study group

The patients included in the study underwent Transurethral resection (TUR) at AL-Shaheed Gazi AL-Hariri Hospital for Specialist Surgery/Baghdad/ Medical City, Iraq during March 2013 to February 2014. The study group consisted of 50 patients (40 males, 10 females) diagnosed with transitional bladder carcinoma. The patients further subdivided into two subgroups according to WHO/ISUP grading system (MacLennan *et al*., 2007):

A- 28 of them were patients with high grade transitional cell carcinomas of bladder (20 males, 8 females) with age range from (37-85) years.

B- 22 patients with low grade transitional cell carcinomas (20 males, 2 females) with age range from (31-80) years.

With 18 age-matched cancer–free patients were selected as tissue control. The study was approved by Institutional Ethical Committee. Informed consent was obtained from all patients who participated in this study.

Tissue samples

Formalin –fixed paraffin –embedded blocks of each biopsy were subjected to cut as serial thin sections of (4µm) thickness and were sticked on charged slides for immunohistochemistry (IHC).

Immunohistochemical determination of 8-Hydroxydeoxyguanosine (8-OHdG) Expression

Tissue sections were incubated with anti 8-Hydroxydeoxyguanosine (8-OHdG) monoclonal antibody (Northwest, USA, Code No.1C3) at a dilution of (1:50) for 6 hours in a humidity chamber at 4° C. After complete wash, the tissue incubated with secondary polyclonal antibody using envision detection system (Dako, Denmark, Code No. k5007) for 30 minutes. A brown color was developed with 3, 3-diaminobenzidine tetra hydrochloride (DAB) for 5 minutes, washed in distilled water, and counterstained with Mayer's haematoxylin for 1 minute and mounted in aqueous media. A semiquntitative analysis for counting the expression 8-OHdG if present was done using a modified imunoreactivity scoring system from that of German scoring system (Remmele and Schicketanz 1993). The data were analyzed by the SPSS software (version 20). Descriptive results were reported as mean (standard deviation). Independent sample t-tests was used to compare the results among groups. P value of <0.05 was considered as statistically significant.

RESULTS

The 8-OHdG level in the cancer tissues was significantly higher than in control (Table 1). That table and figure (1) detected a highly significant difference (p< 0.0001) in the mean of Immune Reactivity Score (IRS) of 8-OHdG between TBC patients and that of controls with significant difference between high grade and low grade TBC patients (P< 0.05).

Table 1. Distribution of IRS of 8-OHdG in cancer and control tissue in patients with transitional bladder carcinoma expressed as Mean±SD

Group of patients	N	Tissue	IRS of 8-OHdG Score (Mean±SD)	P-value
TBC	50	Cancer	7.740±4.014	
		Tissue Controls	1.278±0.461	0.0001*
High grade TBC	28	Cancer	10.036±3.405[a]	
		Tissue Controls	1.278±0.461	0.0001*
Low grade TBC	22	Cancer	4.818±2.594	
		Tissue Controls	1.278±0.461	0.0001*

[a]Significant difference between high & low grade groups (Student t- test P< 0.05)

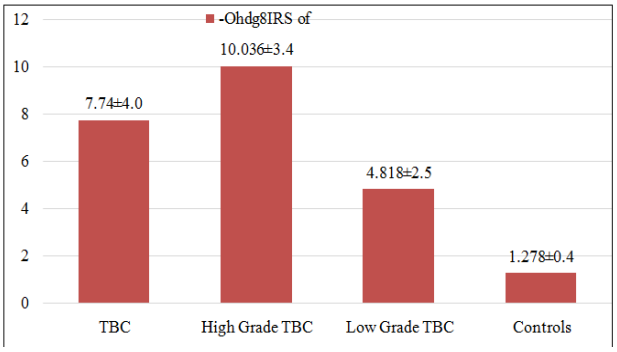

Figure 1. Mean distribution of IRS of 8-OHdG in bladder tissue of studied groups

DISCUSSION

In recent studies, 8-OHdG has been widely used, not only as a biomarker indicating the level of endogenous oxidative DNA damage, but also as a risk factor for many diseases, including cancer (Kondo *et al*., 2000). Generally, we found that 8-OHdG levels were significantly higher in cancer tissue than control. The expression of 8-OHdG was also associated with high grade with significant difference (p<0.05) between the mean of immunoreactivity score of 8-OHdG in high grade and low grade TBC patients when compared with that of controls. The study also found that 8-OHdG levels was tumor grade dependent, that could be connected with a molecular mechanism within tumorous cells. Growing evidence suggests that cancer cells exhibit increased intrinsic ROS stress, due in part to oncogenic stimulation, increased metabolic activity, and mitochondrial malfunction. Since the mitochondrial respiratory chain (electron transport complexes) is a major source of ROS generation in the cells, the

vulnerability of the DNA to ROS-mediated damage appears to be a mechanism to amplify ROS stress in cancer cells. The escalated ROS generation in cancer cells serves as an endogenous source of DNA-damaging agents that promote genetic instability (Helene *et al*., 2004). In DNA (nuclear and mitochondrial), 8-OHdG is one of the predominant agents of free-radical-induced oxidative lesions. This is the reason why 8-OHdG has been used in many studies as a biomarker for the measurement of endogenous oxidative DNA damage, and as a risk factor for many diseases such as cancer or degenerative diseases (Ock *et al*., 2012). The idea of using oxidative DNA damage, like 8-OHdG, as a biomarker of oxidative stress, chronic inflammation, and susceptibility to cancer, gives a new perspective. There are many documented cases of higher level DNA damage in malignant cells and tissues compared to non-malignant controls, but reactive oxygen species, produced either directly by tumors or indirectly via inflammatory responses, can cause DNA damage in healthy neighboring cells as well as distant sites (Krystona *et al*., 2011). Therefore, 8-OHdG as a marker of oxidative DNA damage and/or imbalance in antioxidant processes could be a useful parameter, but its role as a diagnostic and prognostic factor in transitional bladder carcinoma seems to be limited.

Conclusions

8-OHdG reflects the local oxidative stress in transitional bladder carcinoma tissue together with staging of cancer. Because many factors could have an effect on oxidative modification of DNA bases, role of as a diagnostic and/or prognostic factor in transitional bladder carcinoma seems to be limited.

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