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Effect of Chromium and Cadmium on Genetic and Oxidative Stress mechanisms in Bladder Cancer Patients

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Abstract: Background: Heavy metals toxicity has been linked to cancer progression. The relation between some metal ions and bladder cancer (BC) has been elucidated in previous studies. But the influence of these metals on bladder carcinogenesis still needs further study.

Methods and results: This study includes25 BC patients. Chromium and cadmium levels, markers of oxidative stress and m-RNA of IL-6 and Bax were detected in tissues obtained from BC patients. Cr and Cd levels were highly expressed in tumor tissues than normal. The concentration of Malondialdehyde (MDA) was highly detected in tumor tissues, while Superoxide dismutase (SOD) level was lower. IL-6 was up-regulated in tumor tissues than in normal tissues while Bax level was down regulated in tumor tissue. In cancer tissue, there were significant correlations between Cd level with Bax (Bcl-2 Associated X-protein) and IL-6 gene expression.

Conclusions: The correlation between Cd and Bax and IL-6 gene expression may indicate the carcinogenic role of Cd in bladder cancer patients

Keywords: Bladder cancer; Chromium; Cadmium; Oxidative stress; Gene expression.

Introduction

Bladder cancer (BC) is considered one of the most abundant urinary tract cancers. Heavy metals are one of important BC risk factors [1].Heavy metals are naturally occurring in the earth's crust. These metals are produced by, gasoline combustion, incinerators, foundries, insecticides, agricultural products, and paints and remain in the environment for many years. Metals can be ingested, inhaled, or absorbed through skin contact. They have the potential to result in intoxication; their severity depends on the kind of accumulated metal, the length of exposure, and the person's genetic vulnerability. They can cause acute intoxication, the severity of which is determined by the type of metal accumulated, the duration of exposure, and the individual's genetic susceptibility. IARC has classified a number of metals as certain or probable carcinogens. The most toxic elements are copper (Cu), cadmium (Cd), nickel (Ni), mercury (Hg), cobalt (Co), arsenic (As), lead (Pb), and chromium (Cr). Cr and Cd are classified as certainly or probably carcinogenic [2].

Cd is documented as a human carcinogen by the United States Environmental Protection Agency (US-EPA). Epidemiological studies have revealed possible links between Cd and BC. Although the process of Cd tumorigenesis is complicated, gene expression instability is essential. [1]. Heavy metal tumorigenesis is primarily associated with the production of reactive oxygen species and the interaction with DNA repair processes via influencing transcription signaling pathway [2].Previous studies reported that low Cr concentrations might induce DNA breakage and prolonged

occupational exposures to Cr at high concentrations can promote carcinogenicity [3].

Table 1: List of primer sequence

Gene	Sequence	product length (bp)	Accession no
Bcl2	F: 5- GTGGAGGAGCTCTTCAGGGA-3	304	XM_047437733.1
	F: 5- AGGCACCCAGGGTGATGCAA-3		
Bax	F: 5- GGCCCACCAGCTCTGAGCAGA-3	527	XM_047439168.1
	F: 5- GCCACGTGGGCGTCCCAAAGT-3		
GABDH	F: 5- GTCTCCTCTGACTTCAACAGCG -3	131	NM_001357943.2
	R: 5- ACCACCCTGTTGCTGTAGCCAA -3		

Inflammation is an interaction in tissues resulted from external or internal affects that cause cellular injury. Cadmium is pro-inflammatory and upregulated interleukin-6 (IL-6) which is a pro-inflammatory cytokine that present during the transitions of acute inflammation to chronic inflammation [4]. DNA damaging agents, including induction by heavy metals, plays a role in activating pro-apoptotic Bax gene to activate the apoptotic program [5].

The link between Cr and Cd toxicity and Bax and IL-6 genes in BC tissues is still not clear. So, in this work we aimed to evaluate the toxic effect of Cr and Cd in BC and their effect on oxidative stress markers and Bax and IL-6 expression.

Patients and Methods:

Patients

The present study encompass a total of 25 BC patients who under radical cystectomy in Urology and Nephrology Center, Mansoura University, Egypt. The research was authorized from Mansoura University, Institutional Review Board of, faculty of Medicine approved with code (MS.21.12.1795). Informed consents were taken from all patients before the beginning of the study. From each patient, two tissue samples were obtained: tumor (from the central part of the tumor) and normal (from the non-cancerous area of the bladder) tissue.

Methods:

Assay of heavy metals concentration

Tissue samples were digested by adding 200 mg of tissue samples in the digestion vessels that contained 2 ml of hydrogen peroxide and 4 ml of nitric acid then, incubated for15 min. The digestion cycles were performed by Speed wave four, Berghof Products, Germany, as following: 15-min ramp; 1600 W (100%); at 200 °C temperature. A ten ml of distilled water was added to digested samples [6].The Agilent technologies 720 ICP-OES Series; Santa Clara, CA, USA was utilized to detect Cr and Cd concentrations.

Detection of MDA level and SOD activity

Markers of oxidative stress (MDA) and antioxidant (SOD) were estimated in bladder tissue samples according to the protocol using Biodiagnostics, Cairo, Egypt commercially kits [7].

Detection of Bax and IL-6 relative gene expression

Trizol reagent was utilized to retrieve total RNA from bladder tissue samples then, reverse transcribed cDNA by Thermo Fisher Scientific, high capacity C-DNA, Waltham, MA, USA. Step one plus real-time from Applied Biosystems was used to detect Bax and IL-6 relative gene expression. Thestudied primer sequence for Bax and IL-6are listed in Table 1. The gene expression was detected according to $RQ = 2^{-\Delta\Delta CT}$ equation [8].

Statistical analysis

All data were analyzed by SPSS-PC version 20. The normally distributed data were expressed as mean standard deviation (SD) and the independent-Sample t Test was carried out to determine their significance. While, categorical data were expressed as percentages and contrasted using Chi-square. The correlation coefficients (r) were detected. The data were significant if $p \leq 0.05$.

Results

Twenty five BC patients were included in the study with mean age 60.84 ± 1.09 years.21 (84.6%) patients were males and the rest 4 (16.4%) patients were females.

Chromium and cadmium analysis

Table 2 revealed the concentrations of heavy metals in cancerous and non-cancerous bladder tissues. The level of Cr and Cd was up-regulated in tumor tissues than normal ($p<$ 0.001).

Table 2: Heavy metals between tumor and normal tissue

	Cancer tissue	**Non-cancerous tissue**	***p* value**
Cr (µg L^{-1}**)** Median, (Range)	39.39 (13.76- 344.34)	25.47 (8.53 – 46.32)	< 0.001
Cd (µg L^{-1}**)** Median, (Range)	5.1 (1.35 – 12.16)	1.76 (0.1 – 3.78)	< 0.001

Level of MDA and SOD activity

Tumor tissue showed a significant up and down regulation in MDA and SOD concentrations, respectively (p< 0.001) compared with normal tissue (Table 3).There was no correlation between MDA and SOD and the level of Cd and Cr in both tumor and normal tissues (Table 4)

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Table 3: MDA level and SOD activity in Tumor and normal tissue

	Tumor tissue	**Normal tissue**	***p* value**
MDA (nmol ml^{-1}**)** Median, (Range)	526.2 (172 - 1582)	100 (59.1 – 156.25)	< 0.001
SOD(U/gm) Median, (Range)	1900 (190 – 3714)	3195 (1710 - 5221)	< 0.001

Table 4: Correlation coefficient (*r*) between blood levels of heavy metals with oxidative stress markers in tumor tissue and normal tissues. *r:* Correlation coefficient

	Tumor tissue				**Normal tissue**			
	MDA (nmol ml^{-1})		**SOD(U/gm)**		**MDA(nmol ml^{-1})**		**SOD(U/gm)**	
	r	***P***	***r***	***p***	***r***	***p***	***r***	***p***
Cr(µg L^{-1})	0.097	0.64	0.16	0.43	0.16	0.25	0.06	0.64
Cd(µg L^{-1})	0.09	0.64	0.19	0.36	0.23	0.10	0.02	0.87

Relative expression of IL-6 and Bax genes

Table 5 represented the expression level of Bax and IL-6. The Bax gene was down-regulated in tumor tissues in compare with normal tissues **(*p*< 0**.001). While, IL-6 showed significant increase in tumor tissues in compare to normal tissues (p< 0.001) (Table 6). There was an association among Cd concentration and the expression of Bax and IL-6 (p< 0.001) (Table 6)

Table 5: Gene expression in tumor and normal tissue

	Tumor tissue	**Normal tissue**	***p* value**
Bax Mean ± SD	0.51 ± 0.21	0.99 ± 0.081	< 0.001
IL-6 Mean± SD	3.44 ± 1.15	1.0 ± 0.05	< 0.001

Table 6: Correlation coefficient (*r*) between blood levels of heavy metals with gene expression

	Cancer tissue				**Non-cancerous tissue**			
	Bax		**IL-6**		**Bax**		**IL-6**	
	R	***p***	***r***	***p***	***r***	***p***	***r***	***p***
Cr(µg L^{-1})	-0.33	0.1	0.09	0.64	-0.05	0.7	0.28	0.051
Cd(µg L^{-1})	-0.46	0.019	0.43	0.02	0.07	0.6	0.09	0.52

Discussion

Bladder cancer (BC) is becoming more common as a result of increase occupational exposure. Smoking and occupational exposure have both been linked to an increased risk of BC [9].Heavy metals like Cr and Cd are responsible cell metal toxicity. The correlation between the level of Cr and Cd are still lacking in the literature.

The study included 25 BC patients with different grades and stages. The levels of Cr and Cd were measured in tumor and normal

tissue samples. The levels of Cr and Cd were significantly higher in tumor tissues than in normal tissues. Previous research confirmed Cr and CD's findings [10–11].

Some metal ions, such as Cr and Cd, cause oxidative stress, which causes cellular damage

and changes in DNA and protein functions [12].The MDA is an oxidative stress marker while SOD represents a vital antioxidant marker and protects the cells from oxidative stress. Studies demonstrated that MDA level in patients with BC was significantly higher than controls [13-14].MDA level was significantly higher in tumour tissues of BC patients than in benign bladder tissue of the same patients [15] while SOD activity was lower in bladder tumour tissues compared to normal bladder tissues. Moreover, Jeon et al.[16] reported that tumor tissues expressed less catalase and SOD than non-cancerous tissues in BC patients. In consistent to the previous results, our study showed that significant up-regulation in MDA levels and a significant down regulation in SOD in cancer tissues than non-cancerous tissue.

Cancer is a genetic variation that disrupts normal gene functions and results in malfunctioning proteins [17]. In response to DNA damage, apoptosis plays a critical role in the cancer pathway. B-cell lymphoma-2 proteins regulate the signaling of mitochondrial apoptosis through two proteins: pro-apoptotic proteins (Bax) and anti-apoptotic proteins (Bcl-2). The Bax protein triggers mitochondrial dysfunction, which leads to apoptosis [5]. A previous study reported that, Bcl-2 overexpression is linked to the progression and aggressiveness of bladder cancer [18]. Another study reported that Bax overexpression could be used as a predictor for overall survival [19]. In the present study, the expression of the Bcl2 gene was significantly up-regulated in cancer tissues. Our results were consistent with those of Gazzaniga et al. [20]. Cadmium toxicity is associated with oxidative stress, apoptosis and interferes with Bax expression [21]. In our study, a positive correlation was observed between increased expressions of Bax and high concentrations of Cd in cancer tissues.

Previous research has found a link between inflammation and cancer. Interleukin-6 (IL-6) is a type of cytokine that plays a role in immune responses and inflammation. IL-6 regulates metabolism, invasiveness, proliferation, survival, metastasis, apoptosis, and angiogenesis; it is overexpressed in all tumor types [22-23]. Chen et al. [24] found that IL-6 expression was up-regulated in BC tumor tissue than normal tissue. Chen et al. [24] discovered that IL-6 expression in BC tumor tissue was higher than in normal tissue. Previous research has linked exposure to metals such as Cd to IL-6 expression [25]. Our findings revealed that IL-6 was significantly more expressed in tumor tissues from BC than in normal tissues. Furthermore, we showed a relationship between Cd level and IL-6 gene expression in bladder cancer tissue.

Conclusions:

The study concluded that Cr and Cd could have a vital role in the incidence of bladder cancer through the disturbance of Bax and IL-6 expression. The levels of chromium and cadmium didn’t have an effect on the MDA and SOD level. More research is needed to determine the impact of heavy metal levels on

the molecular pathways involved in bladder cancer progression.

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