Humoral immune factor changes in group of patients with Non-muscle invasive bladder cancer treated with intravesical therapy.

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Abstract:

Background: Bladder cancer (BC) one of the most common urologic cancer characterized by the highest recurrence rate, many types belong to BC, but most common of them worldwide are transitional cell carcinoma(TCC) which constitute about 90-95% cases, squamous cell carcinoma (SCC) and adenocarcinomas

Objective: This study was designed to evaluate parameters of humoral immunity in Non-muscle invasive (superficial or early) bladder cancer patients in Iraq that may provide a new insight into the future of immunotherapies development and BC management.

Materials and methods: Fifty-nine volunteer's patients ranged from 24 to 83years old, and 30 control individuals ranged from 51-80 years old, who attended and admitted to Hospital of Gazi AL-Harery in medical city of Baghdad, and Al-Emamain Al-Khadhemain Teaching hospital was recruited in this investigation. The sera of study groups were subjected to serological test to estimate the levels of (IgA, IgG, IgM and C3) by Single radial immunodiffusion (sRID) assay and Enzyme-Linked immunosorbent assay (ELISA) was used to estimate the serum levels of IL-10.

Results: The ages ranged from 24 to 83years (females: 26 to 72years, males: 38 to 83years) with male to female ratio 4.9:1 and ages of controls ranged from 51 to 80years (females: 54 to 70years, males: 51 to 80years) with male to female ratio 2.75:1, the mean ages of cases and controls groups were (61.65±11.04) , current study showed significance dropping in C3 levels in patients control and Mitomycin C groups compared with apparently healthy, levels of IgM showed significant elevation in BCG group compared with apparently healthy group while patients control and mitomycin C groups showed insignificant elevation of IgM. Levels of IgG showed significant elevation in patients control and mitomycin C groups compared with apparently healthy group while in BCG group showed no differences. Serum levels of IL-10 showed no differences between apparently healthy group and each of patient's groups, also showed no differences within patient's groups

Conclusions: Bladder cancer is a common urologic malignancy in male than female patients enrolled in this study. Intravesical BCG or Mitomycin C leading to effective anti-bladder cancer immunity in the majority of Patients

Keywords: Keywords: humoral immunity, BC, MMC, BCG, IL-10, C3, IgA, IgM, IgG.

Fac Med Baghdad 2017; Vol.59, No.4
Received: July 2017
Accepted: Dec., 2017

Introduction:

Bladder cancer (BC) one of the most common urologic cancer characterized by the highest recurrence rate of other malignancy, many types belong to BC, but most common of them worldwide are squamous cell carcinoma (SCC), adenocarcinomas and transitional cell carcinoma (TCC) which constitute about 90-95% cases (1). Painless gross hematuria the classic presentation of this disease, which is seen in approximately 80-90% of patients. the principal diagnostic tests included cystoscopy, cytology, and biopsy while physical examination results are often unremarkable (2) Tumor microenvironment contains many cells and the most prominent innate immune cells are macrophages, natural killer cells and dendritic cells. At the same time, adaptive immune cells such as T-cells and B- cells play an important role in the initiation of immune response against cancer cells and their surrounding stroma (3). These Immune cells can control tumor growth either by direct contact or by producing of cytokines such as IL-10 and chemokines which lead to activate various immune modulators and activator as well as activation of complement system which has been recognized as a central part of the innate immune response and important factor for immunosurveillance against cancer cells (4) Intravesical immuno and/ or chemotherapy therapy used as adjuvant treatment to prevent recurrence and progression of the disease after Transurethral Resection of Bladder Tumor and is also the treatment of choice for Carcinoma In Situ (CIS) (4). Mycobacterium bovis bacillus Calmette-Gu´erin (BCG) immunotherapy is the most well-known and studied of these adjunctive treatments in non hematuric patients. which induces a cell-mediated immune response, thus triggering an attack against malignant cells (5). It is known to induce a local

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inflammation within the bladder mucosa following instillation, characterized by influx of inflammatory cells and production of inflammatory cytokines. (6) the most commonly used intravesical chemotherapy for bladder cancer is mitomycin C (MMC) which is an antitumor antibiotic that is made from a soil fungus called *Streptomyces caespitosus*. Mitomycin inhibits DNA synthesis by producing DNA cross-links which halt cell replication and eventually cause cell death. Indeed, cancer cells are divided faster and with less error correcting than healthy cells, consequently they are more sensitive to this damage. This cell damage slows or stops the growth of cancer cells (7). As a result of these different forms of inflammation, the tumor microenvironment contains both of innate immune cells (including macrophages, neutrophils, mast cells, myeloid derived suppressor cells, dendritic cells, and natural killer cells) and adaptive immune cells (T and B lymphocytes) in addition to the cancer cells and their surrounding stroma. These diverse cells communicate with each other by means of direct contact or cytokine and chemokine production and act in autocrine and paracrine manners to control and shape tumor growth. Proof that B-cell stimulation is associated with cancer progression comes from the presence of antibodies that recognize tumor antigens in cancer patients. Most confirmation proposes that, once the tumor is built up, B cells presumably negatively affect defensive antitumor reactions and may even encourage tumor advancement (8). This study was designed to evaluate some of humoral immunity in Iraqi BC patients that may provide a new insight into the future of immunotherapies development and BC management.

Patients and Methods:

Over nine months from December 2013 to August 2014 blood samples obtained from histopathological confirmed Non muscle invasive (superficial or early) bladder cancer . Fifty-nine volunteer's (49 males, 10 females) patients ranged from 24 to 83years old, who attended and admitted to Hospital of Gazi AL-Harery in medical city of Baghdad, and Al-Emamain Al-Khadhemain Teaching hospital was recruited in this investigation.

According to type of intravesical chemotherapy or immunotherapy, patients were divided into three groups, 20 subjects newly diagnosed untreated patients, they were considered as patients control (PC), 22 subjects were treated patients with Mitomycin C chemotherapy (MMC) and 17 subjects were treated with immunotherapy (BCG). Family unrelated, apparently healthy (AH) control 30 (males 22, females 8) individuals ranged from 51-80 years old, collected from Al-Emamain Al-Khadhemain Teaching hospital and College of Medicine/ Al-Nahrain University were selected to represent as control group. Five ml of venous blood wre collected in plan tube from each participant; blood sera were obtained by centrifugation and stored at -20 °C until used. The sera of study groups were subjected to serological test to estimate the levels of (IgA, IgG, IgM and C3) by Single radial immunodiffusion (sRID) assay (IMMUCHEM , BELGIUM) and Enzyme-Linked immunosorbent assay (ELISA) was used to estimate the serum levels of IL-10 (USBiological, USA). Each test was conducted according to manufactured instructions. This study approved by Research Ethical Committee (REC) in the College of Medicine /AL-Nahrain University. This study was conducted in the Medical Research Unit and Microbiology Department/ College of Medicine-Al-Nahrain University.

Statistical analysis: The Statistical Package for the Social sciences (SPSS, version 14) was used for statistical analysis. Continuous variables were expressed in mean ± standard error (SE). The Pearson's Chi-square test or Fisher exact test was used for comparing the categorical variable. A *p*-value < 0.05 was considered statistically significant.

Results:

Demographic information of the patients.

This study involved 89 Iraqi patients with bladder cancer; The ages ranged from 24 to 83years (females: 26 to 72years, males: 38 to 83years) with male to female ratio 4.9:1 and ages of controls ranged from 51 to 80years (females: 54 to 70years, males: 51 to 80years) with male to female ratio 2.75:1, the mean ages of cases and controls groups were (61.65±11.04) table (1).

Table (1): Ages means± SD of studied groups.

Subjects	Age (Mean±SD) years		Gender		P value
	Females	Males	females	males	
Cases (59)	61.56±11.04		10	49	0.822
	55.80±13.07	62.73±10.34			
Controls (30)	61.03±8.88		8	22	
	62.38±8.45	60.55±9.18			

Distribution of patients according to treatment and type of intravesical chemotherapy or immunotherapy compared with apparently healthy were shown in table (2)

Table (2) Distribution of patients according to treatment

Subjects=89	Cases groups	Females	Males
Patients=59		10	49
	untreated =20	3	17
	mitomycin C =22	4	18
	BCG=17	3	14
Control =30		8	22

Regarding C3 levels statistical analysis showed significance dropping in patients control and Mitomycin C groups compared with apparently healthy, present study showed significance elevation in the level of C3 in BCG group patients compared with another groups, C3 within patients' groups showed no differences in Mitomycin C and significant dropping in BCG group compared with apparently healthy group. Also showed significant

elevation in BCG group compared with Mitomycin C group Table (3).

Table (3): Means of C3 serum levels in studied groups.

Groups	Mean ± SE(mg/dl)	P value		
		PC	MMC	PCG
AH	160.08 ± 4.76	0.01	0.05	0.01
PC	143.73 ± 6.28		0.57	0.00
MMC	140.73 ± 4.57			0.00
BCG	186.33 ± 14.17			

AH: Apparently healthy, PC: patients control, MMC: mitomycin C, BCG: Bacillus Calmette-Guerin.

Serum levels of IgA showed insignificant differences in patient's groups compared with apparently healthy group, also showed no differences within patient's groups table (4).

Table (4): Means of IgA serum levels in studied groups.

Groups	Mean ± SE (mg/dl)	P value		
		PC	MMC	BCG
AH	243.09 ± 64.31	0.78	0.17	0.83
PC	384.15 ± 36.90		0.32	0.66
MMC	539.96 ± 109.88			0.16
BCG	310.68 ± 38.76			

AH: Apparently healthy, PC: patients control, MMC: mitomycin C, BCG: Bacillus Calmette-Guerin.

Levels of IgM showed significant elevation in BCG group compared with apparently healthy group while patients control and mitomycin C groups showed insignificant elevation of IgM. Table (5).

Table (5): Means of IgM serum levels in studied groups.

Groups	Mean ± SE (mg/dl)	P value		
		PC	MMC	BCG
AH	155.44 ± 12.99	0.20	0.13	0.05
PC	180.22 ± 15.11		0.87	0.50
MMC	183.65 ± 11.43			0.60
BCG	194.94 ± 17.15			

AH: Apparently healthy, PC: patients control, MMC: mitomycin C, BCG: Bacillus Calmette-Guerin.

Levels of IgG showed significant elevation in patients control and mitomycin C groups compared with apparently healthy group while in BCG group showed no differences. IgG level within patients group, showed significant differences in BCG group compared with mitomycin C group, while mitomycin C and BCG groups showed no differences compared with patients control group table (6).

Table (6): Means of IgG serum levels in studied groups.

Groups	Mean± SE(mg/dl)	P value		
		PC	MMC	BCG
AH	1216.16 ± 53.24	0.05	0.001	0.79
PC	1409.10 ± 384.30		0.18	0.15
MMC	1553.05 ± 72.22			0.007
BCG	1243.70 ± 96.43			

AH: Apparently healthy, PC: patients control, MMC: mitomycin C, BCG: Bacillus Calmette-Guerin.

Serum levels of IL-10 showed no differences between apparently healthy group and each of patient's groups, also showed no differences within patient's groups table (7)

Table (7): Means of IL-10 serum levels in studied groups.

Groups	Mean ± SE (pg/ml)	P value		
		PC	MMC	BCG
AH	808.43 ± 32.93	0.47	0.50	0.46
PC	845.16 ± 39.93		0.95	0.19
MMC	841.58 ± 32.91			0.20
BCG	768.80 ± 45.25			

AH: Apparently healthy, PC: patients control, MMC: mitomycin C, BCG: Bacillus Calmette-Guerin.

Discussion:

This study revealed that aging is a risk factor for development bladder cancer, results in current study accordance with those obtained by Breban *et al*. who found that more than 90% of BC cases occur in those older than 55years and 50% in those older than 73years (9), the possible explanation for this results that capability of the p53 response was reduced significantly in old aged compared with young and age group, and this reduction is mostly due to decreased transcriptional efficiency and p53 dependent apoptosis (10) furthermore, Epigenetic alterations elevated with aging, hypermethylation of promoters of tumor suppressor genes were investigated by huge number of studies. Normally, DNA methylation contributes to gene silencing, as regards tumor suppressor genes, leads to unchecked cellular multiplication. Methylation of cytosine-guanine linear nucleotide (CpG) island has been shown to have important role in the BC pathogenesis (11). This study revealed that males were significantly susceptible to develop BC than females that might be due to differences in societal perceptions in male's gender thus refers not only to the physiological differences between sexes but also to the variety of behaviors, expectations, and roles that exist within a social, economic, and cultural context (12). In fact, there is no constant theory to elucidate the differential presentation and behavior of bladder cancer between both genders. Redundant environmental exposure to carcinogens such as industrial chemicals and tobacco in men has been suggested as an explanation. The prognosis of men with bladder cancer is better than that of women. Although men are nearly 3 to 4 times more possible to develop bladder cancer than women, they are only twice as probable to die from bladder cancer comprising 3% of all cancer mortality in men and 1.5% in women. (13). One of the most important mechanisms in body's immunosurveillance against cancer are Complement activation and many cells cancer such as bladder cancer developed inhibitory mechanisms of complement activation as escape method from complement-mediated elimination (14) In current study, significant elevation in C3 level in BCG group compared with apparently healthy group and patients control. The activation of complement system is arbitrated by more than fifty cell surface-

bound proteins which prompt trigger one of three complement activation pathway. The three complement pathways contrast in their methods of target recognition however meet in the activation of the central component C3. Regarding that complement is expected for the identification of non-self-elements, it's well-known that alteration in the composition of tumor cell membranes make these cells an objective for complement realization. reproducible with this presumption, various clinical studies have detailed that increased in the stimulation of complement in tumor patients as a result to elevation of C3 or C5a complement proteins (15) (16). Other aberrant affirmation of complement activation by tumor cells originates from the expanded complement activity found in biological liquid from tumor patients treated with immunotherapy (17) (18) .In contrast, the results of current study showed significant dropping of C3 level in mitomycin group compared with apparently healthy and patients control, the possible explanation for such result may be due to the side effect of chemotherapy on immune system which can lead to inactivation of complement system and may be suppose as early predictive markers of frailer response to chemotherapy.(19) In addition to that, present study revealed differences in IgA, IgG and IgM levels. Results range from a slight rise in levels of these antibodies or decreases in it. This results quite with studies done by keiichi ito *et al* ,2004 (20) and Pavoni *et al* 2007(21) Who found that disseminating antibodies that distinguish antigens explicit by tumor cells have been found in most patients with solid cancer most of these antigens are ubiquitous cytoplasmic proteins for example DNA polymerases, heat-shock proteins actin and cytokeratin. They are not tumor-specific and would be mainly saved from circulating antibodies by their predominantly intracellular location, although such antigens can be externalized throughout inflammatory and apoptotic processes that come with cancer growth. Furthermore, BCG therapy can shift immune response from Th2 toward Th1. (22) Present study showed no differences in IL-10 levels between all studied groups. Many studies revealed that to improve immunotherapy and chemotherapy in bladder cancer, it has been documented that IL-10 plays an inhibitory role in both bladder cancer immunosurveillance and BCG therapeutic efficacy (23) (24) from this study, we concluded that Bladder cancer is a common urologic malignancy in male than female patients enrolled in this study. Intravesical BCG leading to effective anti-bladder cancer immunity in the majority of patients.

Author's Contributions:

(1): Samples and patient's selections, design, acquisition of data and statistical analysis.

(2): Drafting the article and revising it critically for important intellectual content. Analysis and interpretation of results

(3): Conventional methods diagnosis and material supplementation.

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