

Pro-inflammatory Cytokines for Evaluation of the Diagnostic Performance for the Urinary Bladder Cancer.

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ABSTRACT

Objective: This study designed to evaluate the role of serum cytokines marker in the diagnosis and prognosis of urinary bladder cancer.

Patients and Methods: The study was conducted in Basrah Hospitals, Iraq between September 2011 till July 2012.

Blood samples were collected from 45 patients (mean age 63.52 ± 10.96 years) with urinary bladder cancer and from 45 apparently healthy control groups with (mean 46.41 ± 8.23 years).

ELISA test was used for detection of serum TNF- α , IFN- γ , IL-8 and IL-12.

TNF- α revealed higher serum level in patients (20.8 ± 27.22) $\mu\text{g/ml}$ in comparison with control group (1.189 ± 26.51) $\mu\text{g/ml}$ ($p < 0.5$).

A significantly higher serum IFN- γ of patients ($5.272.41$) $\mu\text{g/ml}$ in comparison with control group (4.01 ± 1.1) $\mu\text{g/ml}$ ($p < 0.5$).

Similar a significantly increase serum level of IL-8 in patients ($51.4938.43$) $\mu\text{g/ml}$ in comparison with control group (36.55 ± 15.28) $\mu\text{g/ml}$ ($p < 0.5$).

IL-12 revealed a significantly higher serum level in patients (4.29 3.95) ng/L than that of the control control group (1.09 ± 2.14) ng/L ($p < 0.5$).

Conclusion: Serum cytokines are important diagnostic and prognostic tools for urinary bladder cancer and good predictor for disease.

INTRODUCTION

Urinary bladder cancer incidence varies widely throughout the world. It is the fourth commonest cancer in Iraq (incidence is 2.8/100 000).¹ About 9 out of 10 people with this cancer are over the age of 55 years old. Men are 3 times more likely to get bladder cancer during their lifetime than women.² There are several risk factors that make a person more likely to develop bladder cancer³ such as smoking, industrial chemicals like aromatic amines which were used in the manufacture of dyes and pigments for textiles, paints, plastics, papers and hair dyes,⁴ radiation^{5,6} and certain cancer chemotherapeutic

Key words: Cytokines, Malignant disease, Urinary bladder Cancer.

agents including cyclophosphamide,^{7,8} hormonal factor as oophorectomy,⁹ white race,¹⁰ older age,¹¹ sex,¹¹ schistosomiasis,¹² family history,¹³ bladder birth defects¹⁴ and low fruit and fluid intake.^{15,16}

In urinary bladder cancer, there are acquired alterations in DNA often lead to either induction of oncogene or negation of tumor suppressor genes resulting in a malignantly transformed cell.¹⁷ The elaboration of cytokines are also important to tumor rejection, especially interferon type gamma (IFN- γ) owing to its activation of other effectors cells such as macrophage that are known to infiltrate the tumor.¹⁸

Cancer often involves inflammatory processes. Recent studies show that immune mediators (cytokines) may serve as biomarkers for bladder cancer.^{19,20} Therefore, the aim of this study is to investigate the role of tumor necrosis factor- α , interferon- γ , interleukin-8 and interleukin-12 in serum of patients with urinary bladder cancer in the diagnosis and prognosis of the disease.

PATIENTS & METHODS

Patients

This study enrolled 45 patients (39 males, 6 females), aged from 29-91 years with the mean age of (63.52 \pm 10.96) years, through the period from September 2011 till July 2012 (23 from oncology center of AL-Sadeer Teaching Hospital, 20 from Basrah General Hospital, 2 from AL-Tahreer Hospital). Most of patients were males n=39 patients (86.7%), while females n=6 (13.3%). Patients were diagnosed by transurethral resection. Also 45 apparently healthy persons were involved in this study as a control group with main age of (46.41 \pm 8.23) years.

Sampling

Blood samples were collected from patients and centrifuged at 5000 rpm for 5 minutes and sera were stored at -20°C. Also blood samples have been collected from 45 healthy persons.

Cytokines immunoassay

All procedures and reagent preparation were done according to instruction of manufacturer included with Enzyme-Linked Immunosorbent Assay (ELISA) kits. IL-8, IL-12 kits were purchased from Ray Biotech, Inc. USA. IFN- γ and TNF- α kits were purchased from Immunotech Sas-France.

Statistical Analysis

Statistical analysis was performed by SPSS version 15. P value of <0.05 was considered to indicate statistical significance. Results are depicted as t-test, df. The general characteristics of the study group include in this study were explored using mean, SD and percentages. In addition to t-test, person test used for determination of correlation between measured parameter.

RESULTS

The general characteristics of the patients:

The general characteristics of the patients included in this study were shown in the Table 1. The histopathological type of the bladder cancer among studied patients was transitional cell carcinoma.

Table (1) The general characteristics of the patients

Characteristics	Number of patients	Percentages %
Smoking	31	68.8%
Occupation	7 farmers	15.5%
	5 military	11.1%
	33 other different jobs	73.4%
Schistosomiasis	7	15.5%

Serum cytokines

The results of ELISA test for serum TNF- α revealed higher level in patients (20.81 ± 27.22) pg/ml in comparison with control group (1.189 ± 26.51) pg/ml ($p < 0.5$) (Table 2).

The results of ELISA test for IFN- γ revealed a significantly higher serum level for patients ($5.272.41$) pg/ml in comparison with control group (4.01 ± 1.1) pg/ml ($p < 0.5$) (Table 2).

The results of ELISA test for IL-8 revealed a significantly increase serum IL-8 of patients ($51.498038.43384$) pg/ml in comparison with control group (36.55 ± 15.28) pg/ml ($p < 0.5$) (Table 2).

The results of ELISA test for IL-12 revealed a significantly higher serum IL-12 of patients ($4.233.9$) ng/L in comparison with control group (1.098 ± 2.14) ng/L ($p < 0.5$) (Table 2).

The range of the correlation coefficient is from -1 to +1. The closer correlation coefficient is to +1, the stronger the evidence of *positive* association between the immunological parameter and the bladder cancer.

The closer correlation coefficient is to -1, the stronger the evidence of *negative* association between the two variables. If correlation coefficient is close to 0, there is evidence of no linear relation between the immunological parameter and the bladder cancer. Because the linear correlation coefficient is a measure of strength of linear relation, correlation coefficient close to 0 does not imply no relation, just no linear relation (Table 3).

DISCUSSION

Bladder cancer continues to be one of the most common malignancies. Those who have been already diagnosed are at high risk for recurrence. This study provided a minimal-invasive means of diagnosing the disease. There is a role for chemical carcinogen in the etiology of urinary bladder cancer due to metabolized chemicals, the excretion and delivery of the substances in the urinary system may cause malignancies. In this study the cigarette smoking patients were 31 patients (68.8%) while 7 patients give a history of schistosomiasis related to their jobs as farmers.

It is well known that TNF is pro inflammatory cytokine. The major source of the TNF is macrophage and T lymphocytes and proliferating B cell. It is established

Table (2): The mean serum levels of cytokines for patients and control groups by ELISA test.

Cytokine Pg/ml	Patients(n=45) Mean \pm S.D	Control (n=45) Mean \pm S.D	P value	Level of sig- nificance
TNF- α	20.81 \pm 27.22	1.189 \pm 26.5	<0.05	0.001
IFN- γ	5.27 \pm 2.41	4.01 \pm 1.1	<0.05	0.007
IL-8	51.49 \pm 38.43	36 \pm 15.28	<0.05	0.019
IL-12	4.29 \pm 3.95	1.09 \pm 2.14	<0.05	0.000

(Table 3): Correlation and level of significance of the immunological parameter to the urinary bladder cancer.

Parameters	Correlations	Level of significance
TNF	0.088	0.001
IFN	-0.3	0.007
IL-8	0.011	0.019
IL-12	0.22	0

in pathogenesis of chronic inflammatory disease. TNF has been found to have a pro cancerous effect it can act as a carcinogen by increase DNA damage and inhibit DNA repair enzyme so it cause and increase genetic mutation.²¹ TNF destroys of the tumor vessels and block the blood flow to the tumor and cause necrosis .The increase production of the TNF in the serum is related to bad prognosis specially in patient with urinary bladder cancer and prostatic cancer.²² TNF- α is one of the major mediators of inflammation and has been linked to all steps involved in tumor genesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis.²³

IFN- γ , or type II interferon, is a cytokine that is important for innate and adaptive immunity against intracellular bacterial infections and for tumor control. Interferon is expressed rapidly following exposure to a wide variety of infectious agents and plays a key role in innate control of virus replication.²⁴ IFN- γ is produced predominantly by natural killer (NK) cells as a part of the innate immune response, and by CD4 Th-1 and CD8 cytotoxic T lymphocyte. IFN- γ is a pro-inflammatory cytokine that promotes and enhance lymphocyte function, stimulating adhesion molecules, and up-regulating MHC class-I and MHC class-II expression. In certain modern studies have demonstrated that IFN is a cytokine that is able to suppress the proliferation of malignant cells, and sometimes is causing their direct lysis of tumor cells ; it causes suppression of angiogenesis in tumor tissue and stimulates specific immune response and nonspecific antitumor resistance ; IFN possesses antimutagenic activity, promotes apoptosis in tumor cells induced by different agents; it suppresses spread of tumor cells and expression of oncogenes; IFN influences the mechanisms of tumor cell differentiation.²⁵

Recently, it has been shown that IL-8 plays a critical role in cancer invasion, angiogenesis and metastasis.²⁶ It is a chemotactic factor for T cells, neutrophils, and basophils besides its pro-inflammatory role, IL-8 has been evaluated as a pro-oncogenic effector in various types of human cancers, including leukemia, astrocytoma, melanoma, breast cancer, ovarian cancer, lung cancer, prostate cancer, colon cancer, urinary system cancer , gastric cancer and pancreatic cancer.²⁷

The present study agrees with Hikmet, et al.²⁰ results who revealed a high IL-8 levels which is related to invasive bladder cancer patients and not to individuals with superficial bladder cancer with 71% assay sensitivity and specificity. So IL-8 is not a good marker for early

diagnosis of bladder cancer, but may serve as a predictor of prognosis.²⁰

This study shows significant elevation in serum level of IL-12 in comparison to the control group. IL-12 is mainly produced by monocytes, macrophages, and dendritic cells in response to bacterial products such as lipopolysaccharides or tumor cells. IL-12 is especially important because its expression during infection regulates innate responses and determines the type and duration of adaptive immune response.²⁸. Interleukin 12 (IL-12) is an important regulatory cytokine that has a function central to the initiation and regulation of cellular immune responses. It has the capacity to regulate the differentiation of naive T cells into TH1 cells, which is effective in determining resistance and the type of response that will be occurred in response to a particular pathogen. It stimulates the growth and function of T cells and alters the normal cycle of apoptotic cell death. The Normal Function of IL-12 is induction of a cell-mediated immune response to a specific antigen by the release of cytokines. IL-12 is responsible primarily for the subsequent production of IFN- γ and TNF- α from both NK cells and helper T cells.

In conclusion, IFN- γ , TNF- α , IL-8 and IL-12 can be used as a diagnostic biomarkers for urinary bladder cancer. In addition, those markers might serve as a predictor for prognosis.

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