Evaluation of macrophage migration inhibitory factor in bladder cancer

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Background
Bladder cancer is the sixth most common malignancy in men worldwide. Smoking is the main responsible factor. Urothelial carcinoma is the most common type of common bladder cancer. Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine secreted by a variety of cells. MIF is expressed in human urothelial cells, released into the bladder lumen. Released MIF binds to MIF receptors to mediate bladder inflammation.

Aim
The aim of the study was to (a) evaluate macrophage MIF as a novel marker for bladder cancer; (b) investigate correlations between MIF in both bladder diseases and urinary bladder cancer; and (c) to investigate correlations of MIF in urinary bladder cancer stage and size.

Patients and methods
This study was conducted on 80 patients, which included 40 bladder cancer patients (stage T1 and T2) and 40 bladder disease patients (urinary polyp, uninary tract infection, and bladder stone). Ten healthy individuals served as the control group. Serum macrophage MIF was measured by the enzyme-linked immunosorbent assay technique using the human MIF enzyme-linked immunosorbent assay kit.

Results
The level of MIF was significantly higher in patients with bladder cancer in comparison to those with bladder diseases and the control group.

Conclusion
Serum MIF was significantly increased in both urinary bladder diseases and urinary bladder cancer groups, while it was significantly increased in Urinary bladder cancer (UBC) compared with Urinary bladder disease (UBD). There was significant positive correlations between MIF and both UBD and UBC groups. MIF could be used as a diagnostic tool in bladder cancer.

Keywords:
MIF, bladder, cancer, disease

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Introduction

Cancer of the bladder is the world's 10th most prevalent cancer. It is becoming more common in wealthy countries. Men are four times as likely than women to be diagnosed with bladder cancer. This disparity can be due to tobacco use; men still have a higher relative risk of bladder cancer death than women who smoke [1].

The most common compliant of bladder cancer is hematuria. Macroscopic hematuria is associated with advanced stage of bladder cancer [2].

The most prevalent type of bladder cancer is urothelial carcinoma, which arises from the epithelium. Squamous cell carcinoma, small-cell carcinoma, and adenocarcinoma are examples of bladder malignancies with different histologies (10–25% of cases) [3].

Muscle-invasive bladder cancer (MIBC) refers to tumors that infiltrate the detrusor muscle and are more prone to spread to lymph nodes or other organs. Non-MIBC accounts for about 75% of newly diagnosed patients, while MIBC or metastatic disease accounts for 25% [4].

There are three FDA-approved diagnostic assays for bladder cancer. These are nuclear mitotic protein 22, bladder tumor antigen, and fibrinogen degradation product. Unfortunately, none of these assays has sufficient diagnostic accuracy to replace cystoscopy [5].

Migration inhibitory factor (MIF) is a proinflammatory cytokine, which has chemotactic and growth-promoting activities. In addition, MIF is involved in both innate and adaptive immune responses, as well as having pathologic implications in a number of diseases [6].

MIF is a cytokine that plays an important function in inflammation. MIF secreted from immunological and epithelial cells binds to its receptor, CD74, and increases the production of cytokines such as interleukin 8 and tumor necrosis factor alpha [7].

MIF has the ability to regulate both normal and abnormal physiological and pathological states.

MIF stimulates inflammation, cell proliferation, and apoptosis inhibition as a result of its pleiotropic activity. All these factors create a microenvironment favorable to the development of cancer [8].

Patients and methods

Eighty participants were enrolled in this trial, which included 40 bladder cancer patients (stage T1 and T2) and 40 bladder disease patients (urinary polyp, uninary tract infection, and bladder stone). The patients were selected from the Urology Department, Assiut University Hospital between October 2018 and June 2019. Ten healthy individuals were included in this as a control group. Their age ranged from 39 to 59 years. Formal consent was obtained from patients and controls.

The following investigations were done for all participants:

(1) Kidney function, liver functions, and random blood glucose were done on Dimension (RxL Max, Xpand Plus), Siemens, Germany.
(2) Prothrombin time and concentration were measured on Sysmex CA-1500, Siemens by photo-optical method.
(3) Complete blood count was done on an automated blood cell counter CELL DYN (1700) and ADVIA (2120) Siemens by light scattering technology.
(4) Complete urine analysis.
(5) Serum macrophage MIF determination:

Serum macrophage MIF was measured by the enzyme-linked immunosorbent assay (ELISA) technique using Human MIF ELISA Kit, Catalog No: SG-10672, purchased from SinoGeneClon Biotech Co., China.

Principle of the test

Sandwich ELISA was used for quantitative determination of human MIF concentrations in the sample. By comparing the optical density of the samples to the standard curve, the concentration of MIF in the samples may be calculated, calculate the sample concentration, multiplied by the dilution factor the result is the sample actual concentration.

Statistical analysis

SPSS was used to collect and analyze the data (Statistical Package for the Social Sciences, version 20; IBM, Armonk, New York, USA). Continuous data were expressed as mean, SD, or median (range), whereas nominal data was expressed as frequency (percentage).

The χ^2-test was used to compare nominal data between groups, whereas the *t* test was used to compare continuous data between groups, followed by post-hoc analysis. Diagnostic accuracy of MIF in the diagnosis of bladder cancer was assessed by the receiver-operating characteristic (ROC) curve.

Pearson correlation was used to analyze MIF's relationship with other factors.

Because the level of confidence was held at 95%, a *P* value of 0.05 was considered significant.

Ethical consideration

The study was approved by the Ethics Committee of Faculty of Medicine, Assiut University, IRB#17101545.

Results

The study enrolled 90 participants classified as follows:

Group 1: 10 healthy individuals.

Group 2: 40 patients with urinary bladder diseases were further subgrouped according to the cause into:

2A: Bladder stone: 26 patients.

2B: Uninary tract infection: 12 patients.

2C: Urinary polyp: two patients.

Group 3: 40 patients with urinary bladder cancer were further subgrouped according to the tumor stage and size as follows:

3A: Tumor stage:

1-TI: 22 patients.

2-TII: 18 patients.

3B: Tumor size:

I: Group 3I (<3 cm): 10 patients.

II: Group 3-II (3–6 cm): 18 patients.

III- Group 3-III (> 6 cm): 12 patients.

Table 1 shows that the mean age of patients with bladder cancer was 61.75 ± 8.73 years and the majority (82.5%) of them were males, while the mean age of patients with urinary bladder was 52.32 ± 8.43 years and majority (77.5%) of them were males.

It was noticed that patients with bladder cancer had a significantly higher age in comparison to other groups, with insignificant differences between the studied groups as regards sex distribution.

Patients with bladder cancer had significantly higher frequency of diabetes mellitus (42.5 vs. 7.5) and hypertension (40 vs. 5%) in comparison to those with bladder diseases, but both groups had insignificant differences as regards smoking (62.5 vs. 55%).

Table 2 shows that the level of MIF was significantly higher in patients with bladder cancer in comparison to those with bladder diseases and the control group. Also, patients with bladder diseases had significantly higher MIF in comparison to the control group.

Table 3 shows that the level of MIF was insignificantly higher in the case of stage I bladder cancer in comparison to those with stage II cancer.

Table 4 shows the different subgroups of patients with bladder cancer based on tumor size who had insignificant differences as regards MIF.

Table 5 shows that the level of MIF in group 2A ranged from 223.5 to 1036 pg/ml with mean ± SD (448.65 ± 187.34), while in group 2B it ranged from 238.5 to 949 pg/ml with mean ± SD (444.62 ± 187.40). It was noticed that the level of MIF was insignificantly lower in the case of urinary tract infection in comparison to those with bladder stones (P = 0.95).

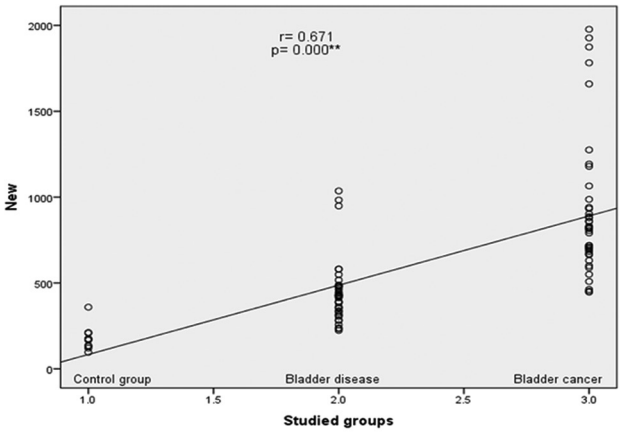
Fig. 1 shows that there was a significant positive correlation between MIF and studied groups (r = 0.671) (P = 0.00).

ROC curve plotted to show diagnostic accuracy of macrophage MIF in the prediction of bladder cancer.

It was noticed that at a cutoff point of 581.5 pg/ml, MIF had 87.5% sensitivity and 94% specificity with an overall accuracy of 91.11% for the diagnosis of bladder cancer.

The area under the ROC curve of 0.82 (Fig. 2).

Figure 1



Correlation of macrophage migration inhibitory factor and studied groups.

Table 1 Baseline data of enrolled groups

	Group 3 (n=40)	Group 2 (n=40)	Group 1 (n=10)	P_1	P_2	P_3
Age (years)	61.75±8.73	52.32±8.43	45.30±6.39	<0.001	<0.001	0.02
Range	40-80	40-70	39-59			
Sex [n (%)]						
Male	33 (82.5)	31 (77.5)	6 (60)	0.30	0.33	0.16
Female	7 (17.5)	9 (22.5)	4 (40)			
Smoking	25 (62.5)	22 (55)	0	0.23	<0.001	<0.001
Diabetes mellitus	17 (42.5)	3 (7.5)	0	<0.001	<0.001	0.21
Hypertension	16 (40)	3 (7.5)	0	<0.001	<0.001	0.21

No history of Bilharziasis in the studied groups.

Table 2 Level of macrophage migration inhibitory factor among the studied groups: *P*1 compares between bladder cancer group and bladder diseases group, *P*2 compares between bladder cancer group and control group, *P*3 compares between bladder diseases group and control group; *P* value was significant at <0.05

MIF (pg/ml) groups	Range	Mean±SD	P
Group 1: control group (n=10)	96.80-359.20	178.02±73.65	$P1$<0.001*** $P2$<0.001*** $P3$<0.001***
Group 2: other urinary bladder diseases (n=40)	223.50-1036	440.20±182.86	
Group 3: urinary bladder cancer (n=40)	447-1977	914.09±405.42	

MIF, migration inhibitory factor. ***P value is significant if p value <0.05

Table 3 Level of macrophage migration inhibitory factor in the cancer subgroup according to the tumor stage *P* value was significant at <0.05

MIF (pg/ml) subgroup	Range	Mean±SD	*P*
Group 3-1 (*n*=22) Stage TI	447-1977	998.58±450.84	*P*=0.14
Group 3-2 (*n*=18) Stage TII	452-1927	810.22±324.74	

MIF, migration inhibitory factor.

Table 4 Level of macrophage migration inhibitory factor in the cancer subgroup according to tumor size: *P*1 compares between group 3-I and group 3-II, *P*2 compares between group 3-I and group 3-III, *P*3 compares between group 3-II and group 3-III; *P* was significant at <0.05

MIF (pg/ml) subgroup	Range	Mean±SD	*P*
3- I (<3 cm) (*n*=10)	447-1874	978.40±495.91	
3-II (3-6 cm) (*n*=18)	452-1977	921.71±415.48	*P*1=0.73 *P*2=0.46 *P*3=0.64
3-III (>6 cm) (*n*=12)	462-1659	849.07±326.02	

MIF, migration inhibitory factor.

Table 5 Level of macrophage migration inhibitory factor according to bladder disease; *P* value was significant at <0.05

MIF (pg/ml) group	Range	Mean±SD	*P*
Group 2A (*n*=26) bladder stone	223.5-1036	448.65±187.34	*P*=0.95
Group 2B (*n*=12) UTI	238.50-949	444.62±187.40	

MIF, migration inhibitory factor; UTI, uninary tract infection.

Discussion

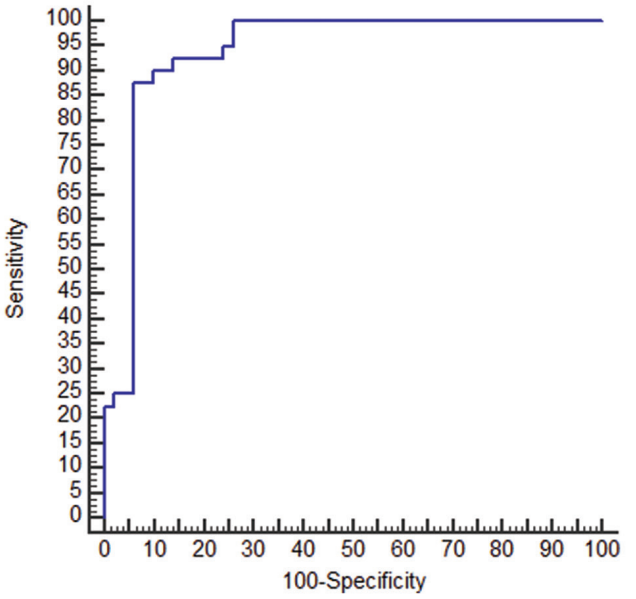
In this study, we investigated the expression of macrophage MIF as a marker for bladder cancer.

In this study, 60% of bladder cancer patient were males and the mean age was 45.30 ± 6.39 years. It was noticed that patients with bladder cancer had significantly higher age in comparison to other groups with insignificant differences between the studied groups as regards sex distribution [9]. It has been reported that bladder cancer is many times more common in men than it is in women [10] According to the survey, 90% of bladder cancer cases are identified in those over the age of 50 years.

Our study reveal that the patients with bladder cancer had significantly higher frequency of diabetes mellitus and hypertension in comparison to those with bladder diseases. The same was reported by Xu *et al*. [11]. Diabetic patients had a risk of developing bladder cancer or cancer mortality; hyperglycemia in these patients might lead to the misbalance of energy level, which affects metabolism and decrease the immune system [12]. These events are associated with increased risks of cancer at different organs. Diabetes mellitus is associated with a, increasing risk of urinary tract infections, which may lead to bladder cancer [13].

In our study, we noticed that out of the studied patients the level of MIF was significantly higher in patients with bladder cancer in comparison to those with

Figure 2



Diagnostic accuracy of MIF in the diagnosis of bladder cancer. MIF, migration inhibitory factor.

bladder diseases and the control group. The same was reported by Salih *et al*. [14] that the average level of serum MIF in UBC patients was more than the level observed in UBD patients and healthy controls. Also Alchalabi [15] it has been reported that the mean level of serum MIF in UBC patients was greater than that observed in UBD patients and healthy controls.

Bucala and Donnelly [16] reported that the level of MIF is greater in bladder cancer than in normal bladder tissue especially in the case of MIBC. AlChalabi *et al*. [17] reported that the MIF level was high in UBC, than UBD patients and healthy controls.

To explain the role of MIF in bladder cancer Bach *et al*. [18] reported that MIF acts on tumor suppressor gene p53 and inhibits its effect to downregulate the transcriptional activity of p21. The cells that has no MIF lead to the activation of p53 and induce apoptosis.

Inhibition of p53 by high amounts of MIF generated by tumor cells or surrounding inflammatory cells leads to cell proliferation, increased lifetime, decreased cell response to gene damage, and an increase in the level of oncogenic mutations in tumor tissue [14].

MIF's increasing macrophage activity and viability, connected with its depression effects on T cytotoxic cells which has antitumor activity, and MIF overexpression in growing malignancies is thought to play a key role in facilitating enhanced tumor growth, implying a link between inflammation and cancer due to its proinflammatory activity [19].

In our study, we noticed that the level of MIF was insignificantly higher in the case of stage I bladder cancer in comparison to those with stage II cancer. The same is reported by Guo *et al*. [20] that the MIF levels were observed mostly in tumor cells and were inversely associated to tumor stage, while Alchalabi [15] reported that the serum mean level of MIF was increasing according to the tumor stages of UBC patients. Also Salih *et al*. [14], the relationship between the serum mean level of MIF and the tumor stages of UBC patients was reported to reveal that the greatest amount was found in the sera of UBC patients in stage T3, followed by T4, T2, and T1 [14]. It has been reported that the MIF plays an important role in tumor cell growth, angiogenesis, or depression of tumor cell immune controlling method when MIF is coupled with CD74, initiate survival effect and cell proliferation which lead to a high level in invasive stages than in noninvasive tumors.

Our study reveal that the different groups of patients with bladder cancer based on tumor size had insignificant differences as regards MIF. Also Guo *et al*. [20] reported that MIF inversely correlated with tumor size.

To explain the effect of MIF on tumor size Oda *et al*. [21] reported that angiogenesis, which is dependent on several biological components such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), hypoxia-inducible factor 1 (HIF-1), and angiopoietin, is said to influence tumor size. Hypoxia produces a rise in HIF-1, which leads to an increase in VEGF, bFGF, and angiopoietin levels. Also, HIF-1 promote the production of MIF, which, in turn, has an important function in tumor angiogenesis. Also, Veillat *et al*. [22] it has been reported that MIF induces dose-dependent secretion of bFGF, VEGF, and interleukin 8.

In our research, we discovered that the amount of MIF was somewhat lower in people who had a urinary tract infection compared with those who had bladder stones. Others demonstrate that the concentration of MIF in UBC patients was higher than that observed in UBD patients and healthy controls but has not demonstrated the difference in urinary bladder diseases [14].

Finally, our study demonstrate that at a cutoff point of 581.5 pg/ml, MIF had 87.5% sensitivity and 94% specificity with an overall accuracy of 91.11% for the diagnosis of bladder cancer, which is better than the urine cytology with a sensitivity of 54% and a specificity of 93.9% used for the early prediction of bladder cancer [23].

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Nil.

Conflicts of interest
There are no conflicts of interest.

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