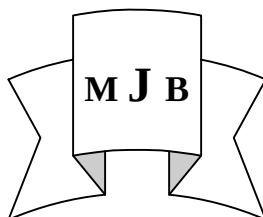


Some Biochemical Changes in Patients with Bladder Cancer

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Abstract

Objective: To estimate some biochemical changes in patients with bladder cancer.

Patients: One hundred patients admitted to Al-Hilla General Teaching Hospital and to the Radiation Oncology and Nuclear Medicine Hospital in Baghdad, and 30 healthy controls were included in this study which lasted from February to June 2011. The patients included 72 males and 28 females, their ages ranged from 41-84 years, with a mean age of 63 years. Those patients were divided into male and female subgroups. As well as, the healthy controls were matched in age and sex with them.

Results: This study demonstrated that the majority of patients (42%) fall in the age group 60-70 years; the male to female ratio was 2.57:1 with 72% of patients being males and 28% being females. Smoking was the most important risk factor for developing bladder cancer accounting for 55% of patients followed by occupational exposure (42%) and environmental exposure (15%). Further; 80% of patients were found to have stage II and III disease accounting for 50% and 30%, respectively, and 95% of patients presented with grade II and III disease, being 64% and 31%, respectively. The most common type of bladder cancer was transitional cell carcinoma accounting for 95% of patients.

The total serum protein, albumin and globulin were found to have a significant decrement; whereas C-reactive protein was significantly increased in both male and female patients compared to the control group. Serum iron and calcium were significantly decreased in both male and female patients in comparison with the control group. Serum zinc was significantly decreased while serum copper showed a significant increment in both male and female patients compared to the control group.

Conclusion: The changes of all previously mentioned markers in bladder cancer patients might be potentially important findings as a biochemical tools for the disease.

بعض التغيرات الكيموحيوية لدى المرضى المصابين بسرطان المثانة

الخلاصة

الهدف: هدفت هذه الدراسة إلى معرفة التغيرات الحاصلة لبعض المعايير الكيموحيوية لدى المرضى المصابين بسرطان المثانة.

المرضى: تضمنت الدراسة مئة مريض مصاب بسرطان المثانة ممن أدخلوا إلى مستشفى الحلة التعليمي العام ومستشفى الإشعاع والطب النووي في بغداد، بالإضافة إلى ثلاثين شخصاً من غير المصابين بالمرض (السيطرة)؛ وقد استمرت من شباط لغاية حزيران ٢٠١١ م. ضمن مجموعة المرضى كان هنالك ٧٢ ذكراً و ٢٨ أنثى تراوحت أعمارهم بين ٤١-٨٤ سنة، وبمعدل عمري هو ٦٣ سنة. يقم المرضى إلى مجاميع ذكور وإناث وتمت مطابقة مجموعة السيطرة معهم من حيث العمر والجنس.

النتائج: ضمت المجموعة العمرية ٦٠-٧٠ سنة غالبية المرضى حيث أن ٤٢% من المرضى كانوا ضمن هذه المجموعة؛ نسبة الذكور للإناث كانت ٢:١ حيث كان ٧٢% من المرضى ذكراً و ٢٨% إناث. أهم عوامل خطورة الإصابة بالمرض كان التدخين حيث أن ٥٥% من المرضى كانوا من المدخنين، تبعه التعرض المهني مسجلاً نسبة ٤٢% من المرضى، فلتعرض البيئي (١٥%). وجد أن لدى ٨٠% من المرضى المرحلة الثانية والثالثة من المرض، مسجلين نسب ٥٠% و ٣٠%، على التوالي؛ وأن لدى ٩٥% من المرضى الدرجة الثانية والثالثة من المرض مسجلين نسب ٦٤% و ٣١%، على التوالي وكان النوع الأكثر شيوعاً لسرطان المثانة هو سرطان الخلية الانتقالي بنسبة ٩٥% من المرضى.

أظهر بروتين المصل الكلي وكذلك بروتينات الألبومين والكلوبولين الموجودة في مصل الدم انخفاضاً معنوياً؛ بينما سجل بروتين C التفاعلي زيادة معنوية لدى المرضى بالمقارنة مع مجموعة السيطرة. فيما يخص الحديد والكالسيوم في مصل الدم فقد سجل انخفاضاً معنوياً لدى مرضى سرطان المثانة وكذلك أظهر الخارصين هذا الانخفاض؛ في حين سجل النحاس زيادة معنوية لدى مرضى سرطان المثانة مقارنة بمجموعة السيطرة.

الاستنتاج: إن جميع ما ذكر من متغيرات لدى مرضى سرطان المثانة قد تكون نتائج مهمة كمعايير كيموحيوية لهذا المرض.

Introduction

Bladder cancer is one of the most commonly diagnosed malignancies worldwide. It is the most common urological cancer; it comprises a significant part of urologists' work [1]. The term bladder cancer is used to describe collectively tumors of urinary bladder urothelial origin which exhibit diverse biological behavior, ranging from relatively benign to highly malignant. Thus, bladder cancer can be without serious clinical consequences for the patient who has an isolated superficial bladder tumor, or it can be a lethal disease. Eighty per cent of bladder cancers are superficial at presentation in that they have not invaded into the muscle. The remaining 20% are muscle-invasive, which carry a much worse prognosis [2].

Two main types of bladder cancer are identified: the transitional cell carcinomas (TCC), related to cigarette smoking and most prevalent in Western and industrialized countries, and the squamous cell carcinomas (SCC),

which are more frequently seen in some Middle Eastern and African countries, where urinary schistosomiasis is an endemic disease. Rare types of bladder cancer include small cell carcinoma, carcinosarcoma, primary lymphoma, and sarcoma [3].

Bladder cancer occurs after the fifth decade and more frequently in men than in women (sex ratio 3:1). The incidence of bladder cancer is highest in industrialized countries. It has been associated mainly with smoking, but also with occupational exposure to carcinogens from aniline dyes, paints, rubber, and chronic irritation of the bladder mucosa due to bladder stones, or schistosomiasis [4].

The classic presentation of bladder cancer is painless gross hematuria, which is seen in approximately 80-90% of patients. Physical examination results are often unremarkable. Cystoscopy, cytology, and biopsy when necessary are the principal diagnostic tests [5].

Bladder cancer has the highest recurrence rate of any malignancy. Although most patients with bladder cancer can be treated with organ-sparing therapy, most experience either recurrence or progression, creating a great need for accurate and careful surveillance [6].

The aim of this study was the estimation of some changes in patients with bladder cancer because this will help the medical staff for proper management with less morbidity and mortality.

Patients and Methods

One hundred patients admitted to Al-Hilla General Teaching Hospital and to the Radiation Oncology and Nuclear Medicine Hospital in Baghdad, and 30 healthy controls were included in this study which lasted from February to June 2011. The patients included 72 males and 28 females, their ages ranged from 41-84 years, with a mean age of 63 years. Those patients were divided into male and female subgroups. As well, the healthy controls were matched in age and sex with them.

All patients underwent a case history questionnaire and were sent for the biochemical investigations. Blood sample was drawn from both patients and controls for the estimation of these investigations.

The total serum protein (TSP) was measured photometrically at 546 nm wave length, according to the procedure recommended by the total serum

protein kit from Human company, Germany [7]. Serum albumin was estimated using spectrophotometer at 630 nm wave length, according to the procedure recommended by albumin kit from Human company, Germany [7]. Serum globulin was determined by subtracting albumin from total serum protein and the result represents the value of serum globulin [8].

Estimation of C reactive protein (CRP) was done using CRP-latex slide agglutination test, according to the procedure recommended by CRP kit from Spinreact company, Spain [9].

Serum iron estimation was done photometrically at 562 nm wave length, According to the procedure recommended by the iron kit from BioMeghreb company, Tunis [10]; and serum calcium also was estimated using spectrophotometer at 570 nm wave length, according to the procedure recommended by the total calcium kit from Human company, Germany [7].

Serum zinc and copper were determined using spectrophotometer at 578 nm and 580 nm wave length, respectively; according to the procedures recommended by the zinc and copper kits from LTA company, Italy [11,12].

Results

Age distribution: The age of patients was classified into five groups as follow: 40-50 years, 50-60 years, 60-70 years, 70-80 years and ≥ 80 years, as shown in Figure (1).

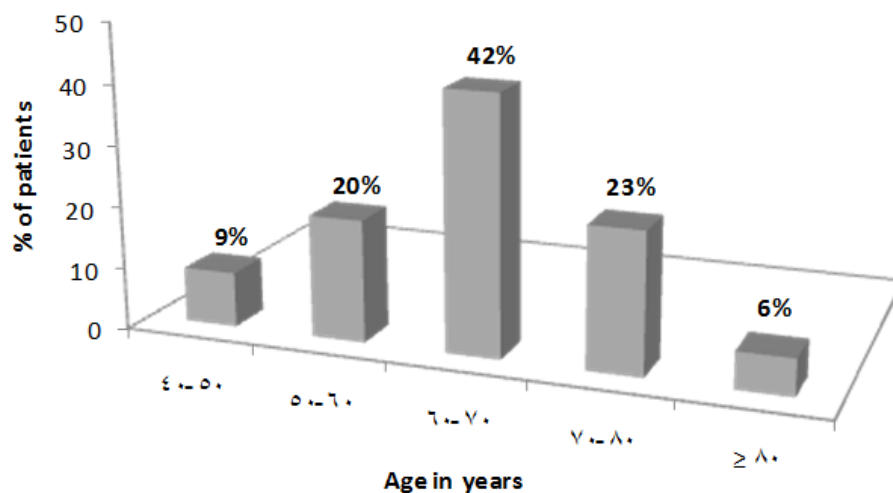


Figure 1 Frequency distribution of patients with bladder cancer by age groups.

Sex distribution: The male to female ratio was 2.57:1, 72% of patients were males and 28% were females as revealed in Figure (2).

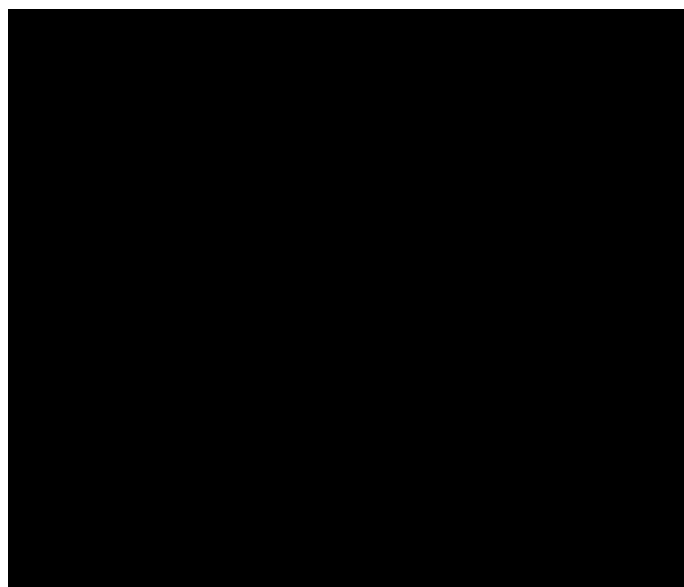


Figure 2 Frequency distribution of males and females among bladder cancer

Risk factors: The frequency distribution of risk factors among bladder cancer patients was shown in Figure (3).

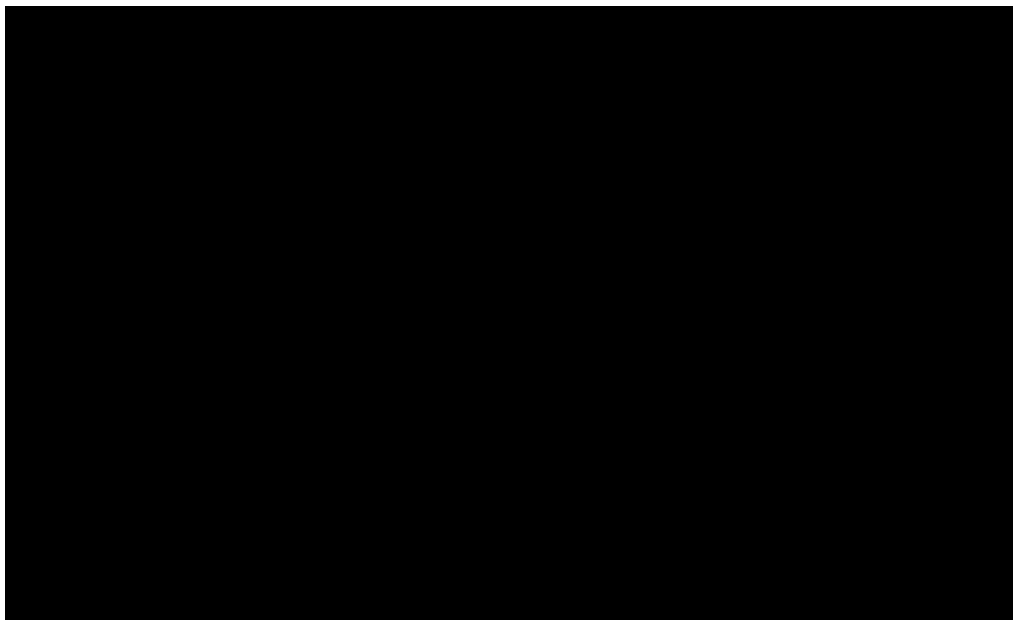


Figure 3 Frequency distribution of risk factors among bladder cancer patients.

Cancer stage: Figure (4) showed the frequency distribution of bladder cancer stage among the patients. Stage I (Tumor invasion of subepithelial connective tissue) accounted for 15% of cases, stage II (Tumor invasion of muscle) accounted for 50%, stage III (Tumor invasion of perivesical tissue) accounted for 30% and stage IV (Tumor invasion of prostate, uterus, vagina, pelvic wall and abdominal wall) accounted for 5% of cases.



Figure 4 Frequency distribution of bladder cancer stage among the patients.

Cancer grade: Figure (5) illustrated the frequency distribution of bladder cancer grade among the patients. Grade I (Well differentiated tumor) accounted for 5% of cases. grade II

(Moderately differentiated tumor) accounted for 64% and grade III (Poorly differentiated tumor) accounted for 31% of cases.



Figure 5 Frequency distribution of bladder cancer grade among the patients.

Type of bladder cancer: The frequency distribution of the type of bladder cancer among the patients was demonstrated in Figure (6). Transitional cell carcinoma

accounted for 95% of cases followed by squamous cell carcinoma which accounted for 4% and lastly the adenocarcinoma which accounted for 1% of cases.

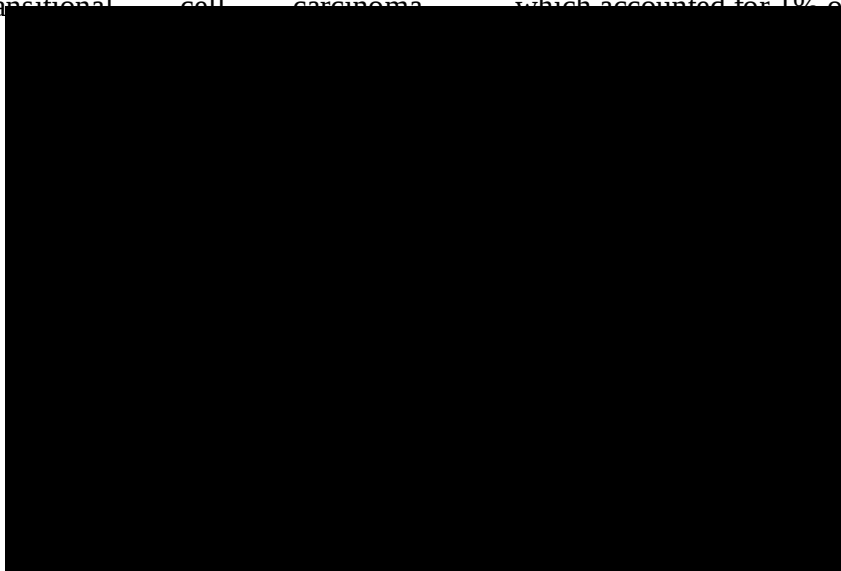


Figure 6 Frequency distribution of the type of bladder cancer among the patients

Total serum protein, albumin, globulin and C reactive protein:

Tables (1) and (2) showed the values of total serum protein (TSP), albumin, globulin and C reactive protein (CRP) for male and female bladder cancer patients and control group. All the values of TSP,

albumin and globulin showed an extremely significant decrement ($P<0.001$); while CRP values showed an extremely significant increment ($P<0.001$) in both male and female patients compared to the control group.

Table 1 Means and standard errors (SE) of total serum protein (TSP) and albumin for bladder cancer patients and control group.

Sex	TSP g/dl		Serum albumin g/dl	
	Patients	Controls	Patients	Controls
Male	***		***	
	4.122±0.061	6.896±0.118	2.125±0.063	4.042±0.090
Female	***		***	
	4.111±0.109	6.786±0.212	2.271±0.105	3.966±0.130

- Values are mean ± SE.

- *** ($P<0.001$).

Table 2 Means and standard errors (SE) of serum globulin and C-reactive protein (CRP) for bladder cancer patients and control group.

Sex	Serum globulin g/dl		C reactive protein mg/L	
	Patients	Controls	Patients	Controls
Male	***		***	
	1.997±0.058	2.966±0.106	24.81±3.898	4.52±0.148
Female	***		***	
	1.839±0.131	2.822±0.186	36.82±7.938	4.00±0.289

- Values are mean ± SE.

- *** ($P<0.001$).

Serum iron and calcium: Table (3) showed the values of serum iron and calcium for male and female bladder cancer patients and control group. Serum iron values were significantly

decreased ($P<0.05$); whereas serum calcium values showed an extremely significant decrement ($P<0.001$) in both male and female patients in comparison with the control group.

Table 3 Means and standard errors (SE) of serum iron and calcium for bladder cancer patients and control group.

Sex	Serum iron ?g/dl		Serum calcium mg/dl	
	Patients	Controls	Patients	Controls
	*		***	
Male	82.74±4.002	98.76±3.487	6.724±0.077	8.471±0.135
	*		***	
Female	73.71±10.823	84.00±5.713	6.626±0.117	8.800±0.291

- Values are mean ± SE.

- *** ($P<0.001$).

- * ($P<0.05$).

Serum zinc and copper: Table (4) showed the values of serum zinc and copper for male and female bladder cancer patients and control group. The values of serum zinc were

significantly decreased ($P<0.05$); but serum copper values showed a significant increment ($P>0.05$) in both male and female patients compared to the control group.

Table 4 Means and standard errors (SE) of serum zinc and copper for bladder cancer patients and control group.

Sex	Serum zinc ?g/dl		Serum copper ?g/dl	
	Patients	Controls	Patients	Controls
	*		*	
Male	80.34±1.398	87.05±3.025	97.29±2.009	90.62±4.644
	*		*	
Female	79.39±2.285	85.00±3.232	92.42±2.652	86.78±6.374

- Values are mean ± SE.

- * ($P<0.05$).

Discussion

The age of bladder cancer patients was classified into five groups (Figure 1), which showed that the disease is more common between the ages 50-80 years, especially in the age group 60-70 years, which accounted for 42% of patients. This result was consistent with that of Jemal *et al.* [13], who stated that the incidence of bladder cancer increases with age, with the median age at diagnosis being 62 years.

Typical of epithelial malignancies, the risk of bladder cancer increases with age, with an average age at diagnosis of 65 years. The incidence peaks in patients who are 80 to 84 years, suggesting that the incidence of this common cancer may substantially increase as the population ages [14].

Age is a strong and independent risk factor for the development of bladder cancer. Various demographic studies have shown that individuals aged more than 65 years have 11 times the incidence of cancer in general and a 15 times greater cancer mortality rate than individuals aged less than 65 years [15].

The male to female ratio among bladder cancer patients was 2.57:1 (Figure 2); this result agreed with that of Jemal *et al.* [13], who stated that bladder cancer is about three times higher in men than in women.

However; while the disease affects men more than women, the prognosis of men with bladder cancer is better than that of women, and women present with more advanced disease. In fact, although men are nearly three to four times more likely to develop the disease than women, they are only twice as likely to die from bladder cancer, comprising 3% of all cancer deaths in men and 1.5% in women in the USA.

Thus the ratio of bladder cancer-specific mortality to incidence is lower for men than for women [16].

Among the patients of this study, 55% were found to be smokers (Figure 3); hence, smoking was considered to be an important risk factor for developing bladder cancer in this study. This result was similar to that of van der Vaart *et al.* [17], who stated that cigarette smoking is a risk factor for bladder cancer and may cause oxidative stress. In addition, this result showed an agreement with that of Aveyard *et al.* [18], who stated that a prominent risk factor for bladder cancer is cigarette smoking, which triples the risk of developing the disease. Smoking leads to higher mortality from bladder cancer during long-term follow up. Compared with nonsmokers, smokers have a 2-6 times increased risk of developing bladder carcinoma.

The supposed carcinogenic constituents of tobacco smoke include arylamines, particularly the potent carcinogen 4-aminobiphenyl (4-ABP), polycyclic aromatic hydrocarbons (PAHs), N-nitroso compounds, heterocyclic amines and various epoxides [19].

The results of this study showed that 42% of the patients had a history of occupational exposure (Figure 3), the majority of them were farmers and only two of them were textile workers; therefore, this risk factor was also considered to be a predisposing factor for developing bladder cancer in this study.

The probable and potential route of chemical contact for those patients was from exposure for long duration to work chemicals such as insecticides for farmers and dyes for textile works.

The above study results were consistent with those of Koutros *et al.* [20], who stated that excess risk of bladder cancer has also been reported among farmers and agricultural workers in regard for the duration of employment; probably due to exposures that are common in their work environments.

There was also an agreement to the study results by Dryson *et al.* [21], who demonstrated that exposure to specific chemical carcinogens is another well-established risk factor for bladder cancer. Exposure to aromatic amines is associated with bladder cancer. An excess risk of bladder cancer was also reported for dyers in textile industries, painters, varnishers and hairdressers.

Among the patients in this study, 15% were found to be exposed to environmental factors from living in areas that were vulnerable to excessive explosions and thus to warfare chemicals (Figure 3), and this was considered to be a probable risk factor for developing bladder cancer in those patients. This result was agreed with that of the National Research Council [22], who stated that recent reviews of studies of bomb survivors have documented elevated risks of bladder cancer associated with ionizing radiation.

The use of depleted uranium (DU) ammunition and bombs on Iraqi territory never stopped since 1991 [23]. During the 2003 attack on Iraq, and according to UK Ministry of Defense, 1.9 tones of depleted uranium were fired by UK forces during the invasion [24]. Researchers at the Armed Forces Radiobiology Research Institute (AFRRI) in Bethesda have found that uranium causes mutations in DNA and uranium exposure can result in increased

chromosomal aberrations. It is a widely accepted principle in molecular biology that agents which cause mutations or DNA damage can cause cancer [25].

The results of this study (Figure 4) showed that stage I accounted for 15% of bladder cancer patients, stage II for 50% of patients, stage III accounted for 30% of patients and stage IV which was accounted for 5%, there were no patients with carcinoma in situ.

These findings were approximately similar to those of Al-Bazzaz [26], whose study results showed that at the time of diagnosis, 48 of study patients (66.6%) had superficial lesions (Ta, or T1), 22 (30.6%) had muscle invasive lesions (T2/T3), and two patients (2.8%) had a locally metastasized tumor. There were no patients with carcinoma in situ, which is probably due not to taking random bladder biopsies.

On the other hand, the results were inconsistent with those of Herr [27], who reported a high incidence of superficial stages and a low percentage of muscle invasive lesions in the United States of America, which reflects a proper screening program for their population, early detection and early appropriate management.

Factors that result in diagnosis being made at a more advanced stage of the disease include a possible under-reporting of superficial cancers, delayed diagnosis, and/or more frequent occurrence of more aggressive variants of transitional cell carcinoma in some people [28].

Figure (5) revealed that grade I accounted for 5% of bladder cancer patients, grade II for 64% and grade III accounted for 31% of patients.

These results were consistent with those of Rafique and Javed [29], who revealed a high incidence of moderately and poorly differentiated tumors (86.8%).

In comparison, the study results were inconsistent with those of Al-Bazzaz ⁽²⁶⁾, who revealed that the majority of the study patients (32/72, 44.4%) had well differentiated tumor (G1). Moderately differentiated tumor (G2) was found in 38.9% (28/72), while poorly differentiated tumor (G3) was found in 16.7% (12/72) of the study patients.

In Figure (6), it was shown obviously that transitional cell carcinoma accounted for 95% of bladder cancer patients in this study, while squamous cell carcinoma accounted for 4% of the patients, and adenocarcinoma for 1% of them. These results were agreed with that of Sternberg and Swanson [30], who stated that the majority of bladder carcinomas are transitional cell carcinoma, while squamous cell carcinoma and adenocarcinomas account for 5% and 2% respectively.

Transitional cell carcinomas arise anywhere in the urothelial lining of the bladder, adenocarcinomas may arise in the dome of bladder from a primary site in the urachus, but most often occur in the trigone. Squamous cell carcinomas are often associated with chronic irritation or infection [31].

All the values of TSP, albumin and globulin, as were illustrated in Tables (1) and (2), were found to have a significant decrement in both male and female patients compared to the control group.

These results showed a consistency with those of Ali *et al.* [32],

who found a significant reduction in total serum protein content in bladder cancer patients compared to the control healthy group of their study.

The probable explanation is that bladder cancer, like any other malignancy, can lead to a considerable reduction in serum proteins. This proposal was agreed by Alexandrakis *et al.* [33], who found that malignant neoplasms and cirrhosis can cause a significant reduction in serum protein.

Rossi *et al.* [34] reported that whole body protein breakdown has been demonstrated to increase in cancer patients and correlated with gluconeogenesis in malnourished cancer patients.

In contrary to the above study result; Metwally *et al.* [35] stated that their study showed a slight increase in total protein concentration in serum of bilharzial bladder cancerous patients compared to healthy ones while, there was non-significant decrease in total serum protein of non-bilharzial bladder cancer patients when compared to healthy ones.

The reduction in serum albumin was in harmony with the statement of McMillan *et al.* [36], who mentioned that as a part of the systemic inflammatory response to the tumor, proinflammatory cytokines and growth factors are released and have a profound catabolic effect on host metabolism. Interleukin-6, produced by the tumor or surrounding cells, stimulates liver production of acute-phase reaction proteins (such as C-reactive protein). The lower serum albumin concentration may be due to the production of cytokines such as IL-6, which modulate the production of albumin by hepatocytes. Alternatively, tumor

necrosis factor may increase the permeability of the microvasculature, thus allowing an increased transcapillary passage of albumin.

In response to reduced serum albumin, the albumin to globulin ratio (A/G) ratio is lowered due to an increase in globular proteins mainly immunoglobulins synthesized by lymphocytes to compensate for the reduced albumin. The failure of these cells to raise the globulins to levels that are high enough to compensate for the reduced albumin may occur in some situations, such as in some cancers, where protein synthesis is reduced and/or protein catabolism has accelerated. This implies that the reduction in serum albumin is further aggravated by the failure of the globulin to compensate [37].

Table (2) demonstrated that the values of C reactive protein showed a significant increment in both male and female patients compared to the control group.

This result was consistent with those of Hilmy *et al.* [38]; who reported a highly elevated C-reactive protein concentration in their studies. He also reported that the elevated C-reactive protein was associated with poor cancer-specific survival in patients with bladder cancer independent of tumor stage and grade.

Systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein, has been shown to be a stage independent prognostic factor in a variety of tumors [39].

Furthermore, because C-reactive protein concentration is independent of tumor stage and grade, the presence or absence of a systemic inflammatory

response, might form the basis of a new prognostic score that reflects not only the tumor but also the host response [40].

Table (3) showed that serum iron values were significantly decreased in both male and female patients compared to the control group.

This result showed an agreement with that of Mazdak *et al.* [41], who stated that the performed analysis of their study indicates that patients having bladder cancer have a lower iron level than controls.

Iron is an essential trace element that is crucial to normal cell functioning and its deficiency or excess is associated with several disease states [42].

Anemia as a result of chronic blood loss and chronic hematuria is one of the mechanisms that may cause a lower iron level in the bladder cancer group [43].

The values of serum calcium showed in Table (3) were found to have a significant decrement in both male and female bladder cancer patients compared to the control group.

The possible explanation to that result is the presence of hypoproteinemia, especially hypoalbuminemia, in the study patients. Hypoalbuminemia will probably lead to hypocalcemia.

This suggestion was agreed by Zivin *et al.* [44], who pointed out that when protein concentrations (particularly albumin) fluctuate substantially, total calcium levels may vary, whereas the ionized calcium, whose level is hormonally regulated, remains relatively stable. Thus, in chronic illness, malnutrition or nephrotic syndrome (where serum protein can be reduced), total serum calcium

concentrations may not accurately reflect the physiologically important ionized calcium concentration.

On the other hand; the absence of hypercalcemia in the study group could possibly reflect the absence of bone metastasis of bladder cancer because that the majority of patients have no advanced stage of the disease (Figure 4) and thus lack bone metastasis which is a potential cause of hypercalcemia.

This statement was conflicting with that of Volkmer *et al.* [45]; who stated that a characteristic feature of many cancer types is their ability to metastasize to the skeleton. This is of clinical importance as metastatic bone disease is associated with increased morbidity and excess mortality. It has been estimated that 12-35% of patients with bladder cancer develop bone metastasis during the course of disease which is, sometimes, associated with hypercalcemia.

Table (4) pointed out that the values of serum zinc were significantly decreased and those of serum copper had showed a significant increment in both male and female patients compared to the control group.

Mazdak *et al.* [41] was in agreement with these results by stating that in their study, a statistically significant decrease in the serum Zn level and a remarkable elevation in serum Cu value was observed in patients with bladder cancer in comparison to the control group.

Zinc is a trace element that occurs in various oxidative stress states and may play an important role in cancer etiology. Zinc plays an anticarcinogenic role by stabilizing the structure of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and ribosome.

Zinc protects cells from free-radical injury. Therefore, a low level may enhance bladder cancer risk due to decrease antioxidants and protective effect of this element [46].

It has been known that Cu participates in the reductive activation of hydrogen peroxide (H_2O_2), causing damage to cellular DNA, proteins and lipids. The interaction of H_2O_2 with Cu generates more reactive oxygen species, such as hydroxyl radicals. These reactive oxygen species have been considered as being responsible for the process of carcinogenesis and that oxidative processes are one of the mechanisms involved in bladder cancer [47].

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