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A STUDY OF THE EFFECT OF SOME FACTORS ON BIOCHEMICAL VARIABLES IN BLADDER CANCER PATIENTS

Mohammed Hussin Mousa Aljomaely[1]
Mohammed Bahry Hasan Al-Saadoon[2]
Department of Chemistry, College of Science, Mosul University, Iraq
E1, mohammed.20scp139@student.uomosul.edu.iq
E2, mohammedsadoon77@uomosul.edu.iq, orcid: 0000-0003-1120-3981

Abstract

The study was conducted in Mosul city during the period from 1/3/2022 to 1/6/2023. It included 75 patients with bladder cancer, 68 males and 7 females, with ages ranging from 50 to 80 years. The levels of biochemical variables were estimated for them: albumin, bilirubin, iron, total protein, uric acid, zinc, cyclooxygenase-2 enzyme, malondialdehyde, vitamin C, glutathione, tumor necrosis factor, and interleukin-6. In addition, the study investigated the effect of smoking, age, and gender on these variables in bladder cancer patients. The study was conducted in the laboratories of the College of Science/University of Mosul. The results were as follows: Age showed a significant effect (P≤0.05) on the concentrations of bilirubin, uric acid, cyclooxygenase-2 enzyme, malondialdehyde, vitamin C, and tumor necrosis factor, which increased significantly to 0.29, 5.19 µmol/L, 162.26 U/L, 1.42, 0.116 µmol/L, and 356.47 pg/ml, respectively. The concentrations of iron and zinc decreased to 57.24 µmol/L and 88.08 mg/L. It was found that the effect of smoking was statistically significant in the averages of cyclooxygenase-2 enzyme and malondialdehyde only. The smoker category recorded the highest averages for these two variables, reaching 131.54 U/L and 1.308 µmol/L, respectively. The gender factor had a significant effect at a probability level (P≤0.01) in the concentration of cyclooxygenase-2 and malondialdehyde, and at a probability level (P≤0.05) in the concentration of bilirubin and iron. The male category recorded the highest averages for the concentrations of bilirubin, iron, and malondialdehyde, which reached 0.203, 53.75, and 1.021 µmol/L, respectively. The female category recorded the highest average for the concentration of cyclooxygenase-2, which reached 132.81 U/L.

Keyword: Bladder cancer, Cyclooxygenase-2 enzyme, Age, Smoking.

Introduction

Cancer is a genetic disorder that occurs in the natural processes of cell division, involving several molecular changes to induce normal cells to form cancerous cells.

Cancer is a misnomer used to describe an estimated 200 different malignant tumors, characterized by uncontrolled growth and spread of abnormal cells. When the control over division is lost in some cells, they begin to divide randomly to form a mass of cells. This new growth of abnormal cells is called a tumor or neoplasm which may be benign or malignant (Zaahkouk et al., 2015).

Bladder cancer is the ninth most common type of cancer worldwide and the sixth most common in men worldwide (Antoni et al., 2017). It is one of the most expensive malignant tumors to treat due to high rates of recurrence and lack of improved treatment options over the past several decades, with a cost approaching \$150,000 per patient (Siegel et al., 2018). Three-quarters of new cases occur in men (higher in some regions reflecting occupational, smoking, and access to healthcare differences), but women have a higher rate of disease-specific mortality (Ferlay, 2012). Bladder cancer arises in 90% of all cases from the epithelial lining of the bladder known as urothelium. This type of bladder cancer is known as transitional cell carcinoma or urothelial carcinoma (Dobruch et al., 2016).

Most cases of bladder cancer arise from exposure to external carcinogens through the respiratory tract, digestive tract, or by skin contact. The most common risk factors for bladder cancer are tobacco smoke, occupational and environmental carcinogens (Cumberbatch et al., 2015). Tobacco smoke accounts for 50% of bladder cancer, but the attributable risk varies by sex, smoking history, and type of tobacco consumed (light and dark tobacco, which are processed through flue-curing and air-curing, respectively). Dark tobacco is more carcinogenic due to its increased concentration of nitrosamines, dioxins, and aromatic amines (Samanic et al., 2006). In a meta-analysis of seventeen studies, opium smoking was found to confer increased risks for bladder cancer (Afshari et al., 2017). Marijuana has also been linked to bladder cancer through a large population-based study in the United States (Thomas et al., 2015). However, tobacco is often the most influential in such studies, and legalization of recreational cannabis in some countries may lead to an increased burden from this mode of smoking (Cumberbatch and Noon, 2019). In addition, polycyclic aromatic hydrocarbons associated with environmental pollution, dietary habits, and drinking water containing high levels of chlorine are other risk factors. Furthermore, genetic factors play an important role in the development of bladder cancer (Babjuk et al., 2017).

There have been some studies that have evaluated whether the stage at diagnosis of bladder cancer differs by occupational exposure burden to carcinogens, for example, Noon et al. (2018) showed that "diverse construction workers" and "male chemical workers" were more likely to undergo localized intervention for bladder cancer. The

limitations of this study were the inability to control for smoking and to identify treatment information. Furthermore, data on the type of handling of chemicals and the protective clothing used are not always available.

Bladder cancer also increases with age and is more common in countries with good resources, which partly reflects tobacco smoking and the spread of carcinogens in the environment (Greiman et al., 2017). The age-standardized incidence rate worldwide (per 100,000 people/year) is 9.6 for men and 2.4 for women (Antoni et al., 2017). In Europe, the overall age-standardized incidence rate is 20.2 for men and 4.3 for women. Approximately 550,000 new cases of bladder cancer were diagnosed worldwide in 2018, with 200,000 deaths (Antoni et al., 2017). The incidence rate of bladder cancer has also increased in many European countries, although mortality rates have decreased in more advanced regions. With population aging and growth, the absolute incidence rate of bladder cancer may continue to increase in European countries (Ferlay, 2012).

The current study aims to evaluate the effect of age, smoking, and gender on some biochemical variables in bladder cancer patients.

Materials and Methods

1. Samples used: Venous blood was drawn from a vein in a volume of (3.5 ml). The blood samples were collected in dry, clean tubes and placed in a water bath at (37 °C) for (15) minutes, after which the clotted part was separated from the clear solution using a centrifuge at 3000 revolutions. / minute for a quarter of an hour, as the clear solution represents blood serum, and it was kept in a gel tube type test tube for conducting clinical examinations and kept at a temperature (-10 °C) until it is used to measure the variables specified in the research (Brake, 2019).

1.1- Collection and preservation of serum samples: Venous blood was drawn in a volume of 3.5 ml. The samples were placed in clean, dry tubes and then incubated in a water bath at 37 °C for 15 minutes. The serum was separated from the clot by centrifugation at 3000 rpm for 15 minutes. The serum was stored in a gel tube at -10 °C for future analysis (Brake, 2019).

2. Estimation of biochemical variables in serum:

2.1- Estimation of albumin concentration in serum: The albumin concentration in serum was determined using a standard assay from BIOLABO France. The assay is based on the binding of albumin to a dye called bromocresol green. The absorbance of the complex was measured at a wavelength of 630 nm (Tietz, 1982).

2.2- Estimation of bilirubin concentration in serum: The bilirubin concentration in serum was determined using a Bilirubin meter from EMCLAB Germany. The bilirubin concentration was measured according to the manufacturer's instructions.

2.3- Estimation of total iron concentration in serum: The total iron concentration in serum was determined using a colorimetric assay from BIOLABO France. The assay is based on the separation of ferric ions from transferrin in an acidic environment. The ferrous ions then form a colored complex with bathophenanthroline disulfonate. The absorbance of the complex was measured at a wavelength of 535 nm (Dreux, 1977).

2.4- Protein quantification: The total protein concentration in serum was determined using a standard assay from Biolabo France. The assay is based on the reaction of peptide bonds in proteins with copper ions in a basic environment. The absorbance of the complex was measured at a wavelength of 550 nm (Burtis and Ashwood, 1999).

2.5- Determination of uric acid concentration in Blood serum: Uric acid concentration in serum was determined using a standard assay from BIOLABO France. The assay is based on the reaction of uric acid with the enzyme uricase to produce a colorless and soluble compound called allantoin, hydrogen peroxide, and carbon dioxide. Uric acid concentration was estimated based on the concentration of hydrogen peroxide produced, which is affected by the presence of the peroxidase enzyme with the chromogenic group (amino antipyrine and dichloro-hydroxyl benzene sulfonate) to form a red complex called quinoneimine, and the absorbance was read at a wavelength of 520 nm (Tinder, 1969).

2.6- Determination of zinc in blood serum: Zinc concentration in serum was determined using an atomic absorption spectrophotometer at a wavelength of 213.86 nm. The standard curve of a zinc sulfate solution ($ZnSO_4$) was used as a standard with concentrations ranging from 5 to 25 mg/L.

2.7- Determination of cyclooxygenase-2 activity: Cyclooxygenase-2 activity was determined using a colorimetric method that measures the enzyme's ability to oxidize N,N,N,N-tetramethyl phenylenediamine (TMPD) by reducing hydrogen peroxide to produce a blue-colored compound. The absorbance was measured at a wavelength of 610 nm as a function of cyclooxygenase-2 activity, which depends on the estimation of the enzyme's activity that catalyzes the hydrogen peroxide reduction reaction, called peroxidase activity (Locklear, 2008).

2.8- Determination of lipid Malondialdehyde (MDA) in Blood serum: The level of malondialdehyde (MDA) concentration in blood serum was estimated by the reaction between lipid peroxides (mainly MDA) and thiobarbituric acid (TBA). The reaction

occurs in an acidic medium and produces a colored product. The absorbance of the product was measured at a wavelength of 532 nm (Mulish et al., 2002).

2.9- Determination of vitamin C in Blood serum: Ascorbic acid is oxidized by copper to form dehydroascorbic acid and diketogulonic acid. These products then react with 2,4-dinitrophenylhydrazine (DNPH) to form a bis-2,4-DNPH hydrazine derivative. The addition of concentrated sulfuric acid causes rearrangement of the latter compound to form a product that absorbs at a wavelength of 520 nm. The reaction above occurs in the presence of thiourea, which provides a mild reducing environment that prevents interference from other substances (Colowick and Kaplan, 1979).

2.10- Determination of glutathione concentration in Blood serum: The modified method followed by Rotruck et al. (1984) was used to estimate the concentration of glutathione. This method uses a solution of Ellman's reagent, 5,5-dithio bis (2-nitrobenzoic acid) (DTNB), which reacts rapidly with glutathione and is reduced by the glutathione's thiol (SH) group to form a colored product. The absorbance of the product was measured at 412 nm. The intensity of the color formed depends on the concentration of glutathione present in the serum.

2-11- Determination concentration of (TNF-α) in Blood serum: A sandwich ELISA assay was used to estimate the concentration of tumor necrosis factor-alpha (TNF-α) in blood serum. The assay plate wells were pre-coated with a specific antibody against TNF-α. Standard and sample solutions were added to the appropriate wells and incubated with the specific antibody. Then, a horseradish peroxidase (HRP)-conjugated antibody to TNF-α was added to each well and incubated. Excess unbound components were washed away, and then a TMB substrate solution was added to each well. Only the wells that contained TNF-α and the HRP-conjugated antibody to TNF-α turned blue, then yellow after the addition of a stop solution. The optical density (OD) was measured at a wavelength of 450 nm. The OD value was proportional to the concentration of TNF-α. The concentration of TNF-α in the samples was calculated by comparing the OD of the sample to the standard curve.

2-12- Determination concentration of interleukin-6 in Blood serum: A sandwich ELISA assay was used to estimate the concentration of interleukin-6 (IL-6) in blood serum. The assay plate wells were pre-coated with a specific antibody against IL-6. Standard and sample solutions were added to the appropriate wells and incubated with the specific antibody. Then, an HRP-conjugated antibody to IL-6 was added to each well and incubated. Excess unbound components were washed away, and then a TMB substrate solution was added to each well. Only the wells that contained IL-6 and the HRP-conjugated antibody to IL-6 turned blue, then yellow after the addition of a stop solution. The optical density (OD) was measured at a wavelength of 450 nm.

The OD value was proportional to the concentration of IL-6. The concentration of IL-6 in the samples was calculated by comparing the OD of the sample to the standard curve.

3- Statistical analysis: After collecting the required data using Microsoft Office Excel, the results of the clinical tests were analyzed using the SAS V0.9 statistical analysis program to find standard statistical methods to determine the mean (Mean) and standard deviation (SD). This was done using the T-test for two-sample variables (gender and smoking), and the least significant difference test for three-sample variables (age) (Al-Zubaidi and Al-Jubouri, 2022).

Results and Discussion

1- Effect of age

Bladder cancer and prostate cancer are strongly associated with aging, and oxidative damage is associated with the aging process (Toprak et al., 2019). Age is a strong and independent risk factor for bladder cancer. Many demographic studies have shown that individuals over the age of 65 have a 11-fold increased risk of developing cancer overall and a 15-fold higher cancer death rate than those under the age of 65 (Messing, 2008).

Table 3-2 shows the effect of age on the biochemical variables studied in bladder cancer patients. The patient group was divided into three age groups: less than 60 years, 60-70 years, and over 70 years. The statistical comparison between the age groups studied was performed using the least significant difference (LSD) method. The results showed that advancing age has a significant effect on the concentrations of most of the biochemical variables studied in bladder cancer patients, except for albumin, total protein, and glutathione, whose levels decreased with age but without statistical significance. Interleukin-6 concentration increased with advancing age but without a significant difference between the mean levels of the age groups. Bilirubin concentrations differed significantly within the age groups studied for bladder cancer patients, with the over-70 age group recording the highest mean of 0.29 µmol/L. For iron concentration in the serum of bladder cancer patients, the under-60 age group had the highest mean of 57.24 µmol/L, and advancing age led to a decrease in iron levels in the serum of patients. Uric acid concentration increased significantly with advancing age, with the over-70 age group recording the highest mean of 5.19 µmol/L. Conversely, zinc levels decreased significantly with advancing age, with a mean zinc of 88.08 units in patients under the age of 60. The over-70 age group recorded the highest mean for the concentrations of cyclooxygenase-2 enzyme, malondialdehyde,

vitamin C, and tumor necrosis factor, which were 162.26 U/L, 1.42, 0.116 µmol/L, and 356.47 pg/ml, respectively.

Table 3-2: Comparison of levels of some biochemical variables in the serum of bladder cancer patients in the age groups (less than 60), (60-69), and (over 70) years.

Age categories (years) Biochemical variables	less than 60 ±SD	60-69± SD	over 70 ±SD	LSD
Albumin (mg/dL)	3.94 a ± 0.24	3.74 a ± 0.28	3.65a ± 0.26	0.297
Bilirubin (µmol/L)	0.15 b ± 0.05	0.18 ab ± 0.19	0.29 a ± 0.07	0.140
Iron (µmol/L)	57.24 a ± 10.22	49.9 ab ± 5.25	44.01 b ± 5.59	8.201
Total protein (g/dL)	5.55 a ± 0.2	5.48 a ± 0.37	5.41 a ± 0.31	0.339
Uric acid (µmol/L)	4.54 b ± 0.59	4.73 ab ± 0.44	5.19 a ± 0.41	0.546
Zinc (mg/L)	88.08 a ± 4.94	82.34 ab ± 6.40	77.63 b ± 5.41	6.244
Cyclooxygenase-2 (U/L)	103.27 b ± 3.17	115.30 b ± 6.66	162.26 a ± 27.27	18.134
Malondialdehyde (µmol/L)	0.89 b ± 0.11	0.93 b ± 0.17	1.42 a ± 0.22	0.195
Vitamin C (µmol/L)	0.098 b ± 0.02	0.105 ab ± 0.01	0.116 a ± 0.01	0.012
Glutathione (µmol/L)	8.764 a ± 0.64	8.711 a ± 0.57	8.216 a ± 0.29	0.587
Tumor necrosis factor (pg/ml)	315.43 b ± 7.47	345.19 ab ± 37.04	356.47 a ± 36.63	33.779
Interleukin-6 (pg/ml)	176.22 a ± 5.16	182.80 a ± 4.52	192.07 a ± 23.91	15.968

-Values followed by the same letter are not significantly different from each other according to the LSD test (P≤0.05).

This result is consistent with the findings of Jemal et al. (2009), who reported that the incidence of bladder cancer increases with age, with a median age at diagnosis of 62 years. It is also consistent with the findings of Messing (2008), who stated that age is now widely recognized as the single greatest risk factor for bladder cancer; while bladder cancer can occur at any age, it is generally a disease of older adults, with a median age at diagnosis of approximately 68 years. Bladder cancer in younger patients was less advanced in grade, stage, and size than in older patients. The recurrence rate was higher in older patients (Toma, 2022).

This age-related trend of increasing urinary MDA concentrations may reflect increased oxidative stress. The decrease in albumin concentration is likely due to a combination of factors, including decreased protein synthesis, increased protein breakdown, and decreased dietary intake, which are all expected with aging. This has been discussed in detail previously (Egea et al., 2017) in a collaborative effort by many researchers studying oxidative stress and health-related outcomes as well as the underlying biochemical mechanisms. Aging has been found to be associated with a functional impairment of protease-mediated degradation of oxidized proteins, which is a critical factor in maintaining protein homeostasis. However, it has been shown that enhanced protease regulation successfully delays aging progression by promoting

oxidative stress resistance in genetically modified animals. Thus, the increases in MDA levels with aging may reflect increased oxidative stress through the progressive functional impairment of the protective proteasomal pathway (Ruano, 2021).

2- Effect of smoking

Smoking causes the accumulation of toxins and increases the production of reactive oxygen species (ROS), which leads to oxidative stress (Goel et al., 2017). Some studies have found that bladder cancer in smokers is often more advanced than in nonsmokers. Sturgeon et al. (1994) reported that the risk of each stage of bladder cancer increases with the number of cigarettes smoked per day, and the relative risk increases with the stage of the disease.

The initial data of this study indicated that 70% of bladder cancer patients were smokers. The results shown in Table 3-3 indicate that the mean values of most of the biochemical variables studied in bladder cancer patients were not significantly affected by smoking. Only the mean values of cyclooxygenase-2 enzyme and malondialdehyde were significantly affected by smoking, with the smoking group recording the highest mean values for these two variables at 131.54 U/L and 1.308 µmol/L, respectively. Albumin, uric acid, vitamin C, and glutathione had the highest mean values in the nonsmoker group, while bilirubin, iron, total protein, zinc, tumor necrosis factor, and interleukin-6 had the highest mean values in the smoking group of bladder cancer patients.

Table 3-3: Levels of some biochemical variables in the serum of bladder cancer patients in smokers and nonsmokers.

smoking categories (years) Biochemical variables	Smokers Mean±SD	Nonsmokers Mean±SD	Probability
Albumin (mg/dL)	3.759 ± 0.302	3.814 ± 0.313	0.6897^{NS}
Bilirubin (µmol/L)	0.237 ± 0.048	0.197 ± 0.113	0.3742^{NS}
Iron (µmol/L)	51.518 ± 4.379	47.629 ± 3.536	0.0495*
Total protein (g/dL)	5.488 ± 0.331	5.457 ± 0.305	0.8329^{NS}
Uric acid (µmol/L)	4.657 ± 0.310	4.882 ± 0.498	0.2817^{NS}
Zinc (mg/L)	82.1 ± 4.016	82.9 ± 5.579	0.7488^{NS}
Cyclooxygenase-2 (U/L)	131.54 ± 7.669	115.78 ± 4.403	<.0001**
Malondialdehyde (µmol/L)	1.308 ± 0.347	0.987 ± 0.237	0.01**
Vitamin C (µmol/L)	0.106 ± 0.014	0.108 ± 0.014	0.8242^{NS}
Glutathione (µmol/L)	8.5 ± 0.5	8.6 ± 0.6	0.8747^{NS}
Tumor necrosis factor (pg/ml)	340.2 ± 34.936	336.25 ± 40.313	0.8126^{NS}
Interleukin-6 (pg/ml)	184.67 ± 4.922	181.33 ± 6.775	0.1899^{NS}

-** and * are significant at a probability level of 0.01 and 0.05, respectively.

-NS - No significant difference.

Based on the large proportion of smokers among cancer patients in this study, which was 70%, smoking can be considered an important risk factor for bladder cancer. Toma (2022) reported that the proportion of smokers among bladder cancer patients in his study was 79%, while it was about 55% in the study of Al-Ka'abi (2011). This is in agreement with what Van Der Vaart et al. (2004) found, who stated that smoking is a risk factor for bladder cancer and may cause oxidative stress. It is also consistent with Aveyard et al. (2002), who stated that one of the prominent risk factors for bladder cancer is smoking, which triples the risk of developing the disease.

A study by Jørgensen et al. (2008) indicated the possibility of predicting cancer through albumin levels, especially its ratio to creatinine. It was significantly associated with bladder and lung cancer, and explained that smoking was a potential cause of albumin concentration disturbance, as it is a widely known indicator of the same cancer sites that the albumin to creatinine ratio predicted, as well as its association with changes in this ratio.

Cigarette smoke contains various reactive oxygen species, toxins, and free radicals, including nicotine, carbon monoxide, nitric oxide, nitrogen dioxide, and peroxynitrite, which later lead to the generation of oxidative stress and disruption of the immune system (Arnson et al., 2010). Uric acid has dual physiological properties as an oxidant and antioxidant (Sautin et al., 2007). Uric acid is a strong physiological scavenger and antioxidant for ROS and free radicals in the laboratory. The decrease in antioxidants such as uric acid, vitamin C, and glutathione in the blood during smoking may be due to the antioxidant effect of ROS and free radicals produced by smoking. These results are in agreement with what Kim and Choe (2019) found, as they noted a similar consistency with the decrease in concentrations of other antioxidants such as ascorbic acid, nitrate, and beta-cryptoxanthin in smoking compared to non-smokers, while the concentrations of glutathione peroxidase, catalase, xanthine oxidase, and malondialdehyde (MDA) increased significantly in smokers. Iron, which increased in concentration in smokers, is capable of stimulating the formation of free radicals, increasing protein and DNA oxidation, promoting lipid peroxidation, decreasing the level of cytochrome c oxidase, advanced glycation end products, carbonyls, malondialdehyde (MDA), peroxynitrite, and HO-1 (Dröge, 2002). The current results also agree with Fu et al. (2021), who stated that smoking showed a negative association with vitamin C concentration in the serum of bladder cancer patients.

The lack of homogeneity in the effect of smoking can be attributed to the regular use of non-steroidal anti-inflammatory drugs. As for the significant increase in the

concentration of cyclooxygenase-2 enzyme, it is known that the presence of the wild-type of chromosome p53 prevents the transcription of COX-2, and the loss or mutation of p53 leads to the regulation of COX-2 expression (Pruthi et al., 2004). The non-homogeneous effect of smoking works by abolishing the activation of the p53 pathway, leading to increased expression of COX-2 (Jiang et al., 2012).

3- Effect of gender

The gender difference in bladder cancer incidence is due to the hormonal differences between males and females, which suggest that estrogen has a protective role in females (Antoni et al., 2017) while androgen increases the risk of bladder cancer (Li et al., 2017). In addition to the differences in exposure to cancer-causing agents and cellular and physiological responses (Maderbacher, 2001)

The proportion of women was 9% of the total number of bladder cancer patients who were included in this study, meaning that the proportion of men exceeded ten times the proportion of women. This is higher than what was obtained by Jemal et al. (2009), who reported that bladder cancer is three times higher in men than in women, while Kirkali et al. (2005) reported that bladder cancer is more common in men by three to four times than in women. Our study found that all women with bladder cancer were smokers. In this direction, Castelao et al. (2001) reported that the risk of bladder cancer is higher in women than in men who smoke similar numbers of cigarettes.

Table 3-4 shows the results of the biochemical tests studied for the male and female groups of bladder cancer patients. It is noted that the gender factor had a significant effect at a probability level of ($P \leq 0.01$) in the concentration of cyclooxygenase-2 enzyme and malondialdehyde, and at a probability level of ($P \leq 0.05$) in the concentration of bilirubin and iron, as the male group recorded the highest averages for the concentrations of bilirubin, iron, and malondialdehyde, which were 0.203, 53.75, and 1.021 µmol/L, respectively. The female group recorded the highest mean for the concentration of cyclooxygenase-2 enzyme, which was 132.81 U/L. As for the other variables, the male group recorded the highest averages for the concentrations of total protein, zinc, and glutathione, while the female group recorded the highest averages for the concentrations of albumin, uric acid, vitamin C, tumor necrosis factor, and interleukin-6.

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Table 3-4: Levels of some biochemical variables in the serum of people with bladder cancer for males and females.

smoking categories (years) Biochemical variables	Mean men ± SD	Women's mean ± SD	Probability
Albumin (mg/dL)	3.74 ± 0.29	3.9 ± 0.42	0.49^{NS}
Bilirubin (µmol/L)	0.203 ± 0.03	0.150 ± 0.05	0.05*
Iron (µmol/L)	53.75 ± 0.35	51.22 ± 4.59	0.05*
Total protein (g/dL)	5.5 ± 0.28	5.4 ± 0.30	0.95^{NS}
Uric acid (µmol/L)	4.88 ± 0.53	4.9 ± 0.14	0.95^{NS}
Zinc (mg/L)	84.1 ± 0.28	82.74 ± 2.72	0.08^{NS}
Cyclooxygenase-2 (U/L)	122.04 ± 3.32	132.81 ± 3.93	0.002**
Malondialdehyde (µmol/L)	1.021 ± 0.23	0.729 ± 0.01	0.002**
Vitamin C (µmol/L)	0.105 ± 0.01	0.115 ± 0.01	0.24^{NS}
Glutathione (µmol/L)	8.581 ± 0.35	8.54 ± 0.29	0.87^{NS}
Tumor necrosis factor (pg/ml)	339.42 ± 6.69	345.89 ± 3.57	0.20^{NS}
Interleukin-6 (pg/ml)	182.29 ± 1.55	184.99 ± 3.01	0.24^{NS}

-** and * are significant at a probability level of 0.01 and 0.05, respectively.
-NS - No significant difference.

The results of this study are in agreement with the results of the study by Al-Ka'abi (2011), which showed no significant effect of gender on most of the biochemical variables studied. The results of his study showed that the male group recorded higher averages than the female group for the concentrations of total protein, haptoglobin, iron, and zinc, while the female group recorded the highest mean for the concentration of albumin. Toma (2022) also showed that the proportion of males in his study was 72.4%, and there was no significant difference in the effect of gender on the variables studied.

The reasons for the associations between elevated bilirubin levels and cancer are not fully understood, but it is thought that they may be due to a combination of factors, including bilirubin metabolism, production, and removal from the body. In an animal study, estrogen deficiency in female rabbits led to a significant decrease in prostaglandin E2 in the urinary bladder mucosa, and when treated with estrogen, prostaglandin E2 levels returned (Hass et al., 2009). It has also been reported that estrogen stimulates prostaglandin synthesis by activating COX-2. Therefore, it is possible that estrogen, through COX-2, can promote the development of bladder tumors (Jiang et al., 2012).

Conclusion:

The study showed that age, smoking, and gender have a significant effect on many biochemical variables in bladder cancer patients.

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