European Journal of Research Development and Sustainability (EJRDS)
Available Online at: https://www.scholarzest.com
Vol. 5 No 05, May 2024
ISSN: 2660-5570

ISOLATION AND EVALUATION OF THE LEVEL OF LYSYL OXIDASE AND SOME BIOCHEMICAL PARAMETERS IN PEOPLE WITH BLADDER CANCER

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Article history:
Received: 30th March 2024
Accepted: 26th May 2024

Abstract:
The study aimed to measure some biochemical variables in the blood serum of patients with bladder cancers. The study was conducted on (90) healthy and bladder cancers patients, their ages ranged between (53–83) years, and the study indicated that there was a significant increase at the probability level ($p \leq 0.01$) for the activity of the lysyl oxidase like -2 (LOXL-2) enzyme in the blood serum of patients with bladder cancer of both genders. It also aimed to study the impact of oxidative stress on patients with bladder cancer and comparing it with the healthy ones, by measuring (14) variables of oxidation and antioxidants, also, measuring the enzyme activity. the results showed that there was a significant increase at the probability level ($p \leq 0.01$*) in the activity of (LOXL-2) enzyme, malondialdehyde, lipoxygenase and (tumor necrosis factor -a) in comparison with healthy people. Also, the results showed a significant decrease in the patients with bladder cancer in all antioxidants (vitamin C - vitamin D -albumin-SOD-glutathione-glutathione peroxidase–ceruloplasmin and zinc) compared to healthy people as a result of the increased oxidative stress. the effect of gender was also studied.

Keywords: the bladder cancer, the lysyl oxidase like -2, enzyme.

INTRODUCTION

Cancer It is a genetic disorder that occurs in the normal processes of cell division, in which several molecular changes are involved that stimulate normal cells to form cancer cells. Cancer is characterized by uncontrolled growth and spread of abnormal cells, as when control of 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 cancer worldwide (Antoni *et al.*, 2017). It ranks seventeenth in women's cancer cases and sixth in men's cancer cases (Omorphose *et al.*,2022). bladder cancer is the second most common urogenital malignancy, with about 90% of cases occurring in developed countries and having a urothelial origin (transitional cell carcinoma) (Omorphose *et al.*,2022). 25% of bladder cancers are muscle invasive, and 75% are non-muscle invasive (Siegel *et al.*, 2018). The majority of BCs develop as a result of external exposure to carcinogens through the skin, gastrointestinal tract, or respiratory system. Tobacco smoke, as well as carcinogens from the workplace and environment, are the most common risk factors for BC. 50% of BCs are caused by tobacco smoke, but the risk associated with it varies depending on factors like sex, smoking history, and type of tobacco used (black and blonde tobacco are cured by air and flue, respectively). Because black tobacco contains higher levels of nitrosamines, biphenyls, and arylamines, it is more carcinogenic. (Cumberbatch and noon., 2019). Tobacco smoking is the leading risk factor for bladder cancer, accounting for 50-65% of new cases annually. Smoking increases the risk by three to four times due to carcinogenic compounds in tobacco smoke. Occupational exposure to carcinogens, such as those found in industrial production, is also a significant preventable risk factor. (Saginala *et al.*,2020).

lysyl oxidase like -2 a copper-dependent amine oxidase, lysyl oxidase (LOX) is often referred to as protein-lysine 6-oxidase (EC 1.4.3.13). It facilitates the oxidative degradation of certain lysyl and hydroxylysyl residues found in elastin and collagen, which are the first phase of these extracellular molecules' covalent cross-linking ECM proteins in the matrix. (Vallet et al., 2019). is a disease biomarker; its blood concentration positively correlates with the advancement of cancer or fibrosis. A precise measurement of LOX concentration can help with diagnosis, tracking the course of a disease, or evaluating the efficacy of a treatment. (Yao et al., 2023). New roles for the LOX family include crosslinking of collagen and elastin, tumor growth (Saleem *et al.*,2020).

The aim of this study is: The aims of this research were a biochemical study of bladder cancer patients and its effect on gender

3. Materials and Methods:

3.1. The Subjects:

For this investigation, 90 blood samples from both the control group and BC patients were obtained.

3.2. Preparation of blood serum: Using a sterile needle (syringe) with a capacity of 5 milliliters, blood was drawn from the veins. After the drawn blood was allowed to clot, a sample was put in a tube that was tightly sealed to extract the serum. Consequently, the tube spent ten minutes in a water bath set at 37°C. After that, the tube was spun for 15 minutes at a speed of 3000 Xg while being centrifuged. After that, the serum was cautiously removed from the tube using a micropipette (Burtis *et al.*, 1999) and split into three sections within an Eppendorf Field. Until the exams were performed, these tubes were kept at -10°C.

3.3 Estimation of biochemical variables in serum:

3.3.1. Determination of the level of Lysyl oxidase like -2 (LOXL-2) from seurm of bladder cancer patient: The effectiveness of the enzyme Lysyl oxidase like -2 was determined based on the method followed by the researchers.

3.3.2. Determination of the level of Sirtuin 3 (SIRT3

SIRT3 concentration has been determined by the researchers (Gan and Patel, 2013).

3.3.3 Determination of the level of tumour necrosis factor-α (TNF-α)

TNF-α: concentration has been determined by the researchers by A sandwich ELISA assay was used to measure TNF-α levels in blood serum.

3.3.4. Determination of Lipoxygenase activity in serum

The effectiveness of the enzyme lipoxygenase (LOX) was determined based on the method followed by the researchers. (Shastry and Rao).

3.3.5. Determination of albumin in blood serum:

The albumin was determined using Bromocresol Green Method, according to the method used by (Tietz, 1982).as a readymade analysis kit.

3.3.6. Estimation of superoxide dismutase (SOD) activity in serum:

SOD concentration has been determined by the researchers (Brown *et al.,*1975).

3.3.7. Estimating the effectiveness of the enzyme glutathione peroxidase (GPX) concentration has been determined by the researchers (Sunderman and Nomoto, 1970)

3.3.8. Determination of serum Ceruloplasmin (Cp):

Cp concentration has been determined by the researchers (Sunderman and Nomoto, 1970)

3.3.9. Determination of reduced glutathione (GSH) level

Serum glutathione was determined using a modified method used by the researchers (Sedlak & Lindsay, 1968)

3.3.10. Determination of oxidant marker, Malondialdehyde (MDA)

The concentration of malondialdehyde in blood serum was estimated using the researcher's method (Buege and Aust, 1973)

3.3.11. Determination of the level of vitamin C concentration in the blood serum: Vitamin C concentration has been determined by the researchers (Omaye *et al.*,1970)

3.3.12. Determination of Vitamin D3

Vitamin D3 concentration has been determined by the researchers (Heijboer *et al.*,2012).

3.3.13. Determination of copper (Cu) level in blood serum

copper (Cu) concentration has been determined by the researcher (Noma, 1989).

3.3.14. Determination of zinc (Zn) level in blood serum:

zinc (Zn) concentration has been determined by the researchers (Eliasson, 1987).

4.Statistical analysis

The clinical examination results were analyzed using SPSS16 to determine the following:

1.Using a one-way analysis of variance, standard statistical methods can be used to calculate the mean and standard error of the mean.

2.The t-test was used to compare two variables and determine the significance of the difference (based on p-value).

3.The Duncan test and ANOVA test were used to compare multiple variables and identify significant differences between groups (P≤0.01) (Bewick *et al.,* 2004).

5. Results and discussion:

5.1. The results of the biochemical study

4.1.1. Level of lysyl oxidase like -2

The results confirmed the table (1-1) and showed a significant increase at the probability level (P< 0.01) in the concentration of lysyl oxidase like 2 in the patient group, in which the average concentration of the enzyme was (697.43pg/ml), while the average concentration of the enzyme in the group of healthy people was (166.78pg/ml). increase the concentration of lysyl oxidase like 2 in the patient group of bladder cancer These results are consistent with (Bomestein *et al.*,2014) In healthy adult tissues, Lysyl oxidase-like 2 (LOXL2) has been shown to be overexpressed in a variety of cancers, including bladder cancer, when compared to normal tissues. LOXL2 is a member of the lysyl oxidase (LOX) family that helps to remodel the extracellular matrix (ECM) by crosslinking collagen

and elastin. This activity promotes cell invasion, metastasis, and tumor microenvironment modulation (Wang *et al.*, 2022). increased expression of LOXL2 in cancerous tissues supports its potential role as a biomarker for bladder cancer prognosis and as a therapeutic target to inhibit tumor progression (Liburkin *et al.*,2022). Clinical studies have found elevated levels of LOX in serum and plasma in various fibrotic diseases and cancers. These results are consistent also with (Yao *et al.*,2023). Clinical studies have found elevated levels of LOX in serum and plasma in various fibrotic diseases and cancers. LOXL2 plays a crucial role in cancer progression by activating focal adhesion kinase (FAK)/Src, leading to tumor cell invasion and metastasis. This activation increases tumor cell proliferation and invasion, causing distant metastasis (Wu and Zhu,2015). LOXL-2 is linked to phosphatidylinositol 3-kinase (PI3K)/ Akt (PI3K/Akt) in cancer. A previous study (Pez *et al.*,2011) suggested that LOX regulates H2O2 production, a byproduct. The enzymatic reaction activates the PI3K/Akt signaling pathway, resulting in increased HIF-1α protein synthesis. This positive feedback loop promotes tumor proliferation. In a breast cancer model, LOX-mediated H2O2 production contributes to Src and FAK activation.

4.1.2. Level of Sirtuin 3 (SIRT3)

The results shown in Table (1-1) showed that the level of SIRT3 in the serum of people with bladder cancer (Bd) was significantly lower (P≤0.01) at 3.0056 ± 0.148 ng/ml than in the control group (15.1156± 0.364 ng/ml). Many factors, disrupted cellular signaling may be connected to the decrease in SIRT3 levels. In order to control various cellular signaling pathways that affect cellular metabolism, stress response, and lifespan, SIRT3 is essential. The Chronic inflammation causes a decrease in SIRT3 concentration. SIRT3 expression and activity can be disrupted by inflammation, which lowers serum levels of the protein (Shi *et al.*, 2017). In general, SIRT3 regulates mitochondrial metabolism by promoting oxidative phosphorylation and inhibiting glycolysis, many factors, such as mitochondrial dysfunction and a reduced ability to respond to oxidative stress, may be connected to the decrease in SIRT3 levels. Reduced SIRT3 levels are thought to be a factor in the unbalanced metabolism of cellular energy and increased susceptibility to oxidative stress. (Silaghi *et al.*, 2021). SIRT3 in the disease. SIRT3 contributes to cancer in two ways. SIRT3 functions as a tumor suppressor in the majority of cancers. SIRT3 can, on the one hand, prevent carcinogenesis and preserve the stability of the cancer genome. SIRT3, on the other hand, prevents the Warburg effect of cancer to stop tumor growth. Furthermore, SIRT3 has the ability to stop tumor growth and spread. Autophagy and apoptosis induced by SIRT3 were also important in this development. (Zhang *et al.*,2020). SIRT3 inhibits ROS-regulated tumorigenesis and metastasis either directly or indirectly, the elimination of ROS by SIRT3 (Zhang *et al.*,2020).

4-3-Tumor necrosis factor-α (TNF-α)

The results shown in the table (1-1) indicated that the level of tumor necrosis factor increased significantly at the probability level (P≤0.01) in the group of bladder cancer patients, giving an average of (363.36pg /ml), while the average concentration reached (8.63pg/ml) in the healthy group. The reason for increased (TNF-a) levels in peoples with bladder cancer is that (TNF-a) can act as an endogenous tumor growth factor by promoting cell survival through activation of anti-apoptotic pathways. Many in vitro and in vivo studies also indicate that (TNF-a) can aid in the development of cancer by causing DNA damage and inhibiting DNA repair by enhancing the production of genotoxic molecules such as (NO ،ROS) by the cancer cells themselves or bystander cells, such as the tumor. - Macrophages infiltrates (Dhar *et al.*, 2002). And the increased level of TNF-a in bladder cancer patients is due to that: Two TNF-α gene polymorphisms have been found to increase the risk of bladder cancer. Activated macrophages are the primary source of TNF-α. This might be because thymidine phosphorylase, an enzyme that has been demonstrated to be regulated by TNF-α}, is in the course of bladder cancer development. Angiogenesis and the growth of various tumor types are also encouraged by TNF-α (Ziyadullaev *et al.,* 2021).

4-4-Lipoxygenase (LOP)

Table (1-1) displays the results, which show that the group of bladder cancer patients had a significantly higher level of Lipoxygenase (LOP) at the probability level (P≤0.01), with an average of (144.40U/L); in contrast, the healthy group had an average concentration of (58.55 U/L). the reason for the increase in the LOP enzyme in bladder cancer patients is as a result of their exposure to external factors such as exposure to chemicals or smoking, which leads to the release of unsaturated fatty acids such as arachidonic acid (AA), which is the basic substance for the LOP enzyme, and then the oxidation process will begin, leading to the production of a number of Mediators of inflammation such as cytokines (Han *et al.,* 2021). Lipoxygenases, specifically 5-lipoxygenase (5-LOP), play an important role in the progression of bladder cancer. These enzymes are involved in the metabolism of arachidonic acid, which produces a variety of bioactive lipids that may influence tumorigenesis. (Wisastra & Dekker ,2014) Increased expression and activity of 5-lipoxygenase have been observed in bladder cancer, which is linked to increased production of leukotrienes, compounds that promote inflammation and can aid in cancer growth and metastasis. Studies have shown that higher levels of 5-LOP and its metabolites are associated with more aggressive cancer behavior (Moore & Pidgeon, 2017).

4-5- Albumin

The results shown in Table (1-1) demonstrated a significant decrease (P ≤ 0.01) in the serum albumin level of patients with Bladder cancer disease (34.85±1.14 g/dl) in contrast to those in good health (60.68 ±0.75 g/dl). The patient's blood serum's decreased albumin levels were linked to increased degradation, insufficient synthesis, and poor antioxidant and mechanisms for scavenging free radicals. The blood's concentration of albumin decreased as a result of continuous exposure to oxidative stress. (Sun *et al.*, 2022). The reason for the decrease in albumin

concentration may be due to its antioxidant role and the suppression of free radicals that cause inflammation, as the state of albumin oxidation in the blood is considered a biological sign of systemic oxidative stress, as the redox state turns into a highly oxidative state as a result of the worsening of the disease condition (Tabata *et al.*,2021). The synthesis of cytokines like IL-6, which influence the production of albumin by liver cells, could be the cause of low blood albumin concentration. On the other hand, tumor necrosis factor might enhance microvascular permeability, which would enable more albumin to pass through the capillaries (Al-Ka'abi ,2011), additionally proved that albumin levels and inflammatory activity are inversely correlated (Bakkeheim *et al.*, 2011).

4-6-Superoxide dismutase (U/L)

The results shown in Table (1-1) indicate that there is a significant decrease in the activity level of the SOD enzyme in the blood serum of bladder cancer patients (2.63 (U/L)) at the probability level ($P \leq 0.01$) compared to the control group (4.43 U/L). The reason for the notable decline in the SOD enzyme's efficacy ($p < 0.01$) Low SOD levels can result in increased oxidative stress in the body. This elevated oxidative environment can harm cellular components such as DNA, proteins, and lipids, potentially leading to mutations and cancer progression. Oxidative stress has been linked to tumor growth and aggressiveness in bladder cancer. And may the majority of bladder cancer patients Were exposed to chemical substances or tobacco use, this leads to the accumulation of harmful chemical substances in the bladder. These harmful substances may lead to damage to the bladder lining, and by increasing the formation of free radicals, it leads to oxidative stress, Oxidative stress (OS) has previously been linked to the development and growth of tumors. Overproduction of reactive oxygen species (ROS) and nitrogen (RNS) was noted in this process, along with a concurrent decline in both enzymatic (SOD) and nonenzymatic antioxidant protection. Oxidative stress has the ability to break DNA, cause necrosis, which results in cell death, and prevent apoptosis (Choromańska *et al.*, 2021).

4-7- Glutathione peroxidase (U/L)

The results presented in Table (1-1) indicated a significant reduction ($P \leq 0.01$) in the level of Glutathione peroxidase in the serum of patients with Bladder cancer (81.64 U/L) when compared to healthy individuals (135.91 U/L). (GPx) is an essential antioxidant enzyme that protects cells from oxidative damage by lowering hydrogen peroxide and organic hydroperoxide levels. In the context of bladder cancer, low GPx levels have been linked to increased oxidative stress, which can contribute to cancer progression and poor patient outcomes (Karatas et al.,2003) Lower levels of GPx have been linked to more aggressive bladder cancer and poorer prognosis. According to studies, decreased GPx activity is associated with higher tumor grades and stages, an increased risk of recurrence, and a lower overall survival rate. Thus, GPx levels can be used as a prognostic biomarker in bladder cancer patients (Unt *et al.,* 2008).

4-8-Ceruloplasmin

The results presented in Table (1-1) indicated a significant reduction ($P \leq 0.05$) in the level of Ceruloplasmin in the serum of patients with Bladder cancer (0.4476 g/L) when compared to healthy individuals (1.0390g/L). The decrease in Cp levels may be related to oxidative stress, when there is an imbalance between the body's capacity to remove ROS and their production, oxidative stress results. them, causing harm to the cells. An antioxidant enzyme called Cp acts as a buffer against oxidative damage (Montes *et al.,* 2014). CP functions as an antioxidant that lowers the amount of free radicals produced in the body and stops oxidation from happening so low levels of ceruloplasmin can lead to increased oxidative stress, which can contribute to DNA damage, promoting tumor initiation and progression. In bladder cancer, oxidative stress is a known factor that can exacerbate cancer development and aggressiveness (Fang *et al.,*2002).

4.9. Level of Glutathione (GSH)

Table (1-1) displays the results, which show that the glutathione level in the serum of patients with bladder cancer disease was significantly lower ($P \leq 0.01$) at (11.34 µmol/l) than in the control group (45.37 µmol/l). The decline in GSH levels could be due to various factors. GSH is a non-enzymatic antioxidant that effectively neutralizes free radicals.

and their harmful consequences. In BC chronic inflammation and tissue damage from free radicals can deplete GSH levels. (Ribeiro, 2023). Furthermore, GSH can oxidize to form GSSG during the neutralization of free radicals. The reduction of active GSH levels may be gave more difficult by the accumulation of GSSG (Bjørklund *et al.*, 2021). The decrease in antioxidants, which promotes increased oxidative stress and tissue damage, can be attributed to the release of free radicals. Glutathione is instead oxidized to GSSG and/or GSSR, which are exported from cells, then absorbed and degraded by kidneys. (Teskey *et al.*,2018).

4.10. Level of Malondialdehyde (MDA)

The findings presented in Table (1-1) demonstrated a statistically significant rise ($P \leq 0.01$) in the serum MDA level among Bladder cancer patients (2.83 µmol/l) in contrast to the control group (0.194 µmol/l). This growth may be associated with oxidative stress, which was defined as an imbalance between the body's capacity to neutralize ROS and their production. Lipid peroxidation in different tissues, such as the serum, can be caused by oxidative stress (Peña-Bautista *et al.,* 2019). The process by which reactive oxygen species (ROS) harm lipids—especially unsaturated fatty acids—and produce harmful byproducts is known as lipid peroxidation. These organisms could oxidize a variety of vital biomolecules, such as lipid membranes, because there aren't enough antioxidants to counter the increase in free radicals, which leaves fatty acids with insufficient protection towards oxidation. As a result, these fatty acids were oxidized, resulting in the formation of other secondary products and MDA as the main product of lipid peroxidation. (Gaschler and Stockwell, 2017).

4.11. Level of vitamin C

The findings displayed in Table (1-1) demonstrated a statistically significant reduction in the serum vitamin C level in patients with Bladder cancer group (0.075 mg/dl) in contrast to the control group (0.284 mg/dl) at (P ≤ 0.01). Oxidative stress is linked to this drop in vitamin C levels. The ability of vitamin C to neutralize harmful free radicals and reduce oxidative stress is largely dependent on its antioxidant properties. The decrease in the level of vitamin C in the group of bladder cancer patients compared to healthy people is due to it being a water-soluble nutrient and believed to be one of the most effective antioxidants in blood serum. Since oxidative stress is a major factor in causing cancer, vitamin C is one of the main physiological antioxidants by reducing or preventing excessive inflammation without reducing the defense capacity of the immune system (Vollbracht *et al.,*2018).

4.12. Level of vitamin D3

The results shown in table (1-1) indicate that there is a significant decrease in the level of vitamin D3 concentration at the level of probability (P≤0.01). in the blood serum of a patient (16.50 nmol/L) compared to the average concentration in the blood serum of the control group (25.11 nmol/L). This result is consistent with (Huang et al.,2020). Vitamin D plays multiple roles, as D3 deficiency can lead to an increase in (T-helper 2 cells) TH2 helper cells and a decrease in lymphocytes. Where Vitamin D works to prevent the production of proinflammatory cytokines, stimulates the synthesis of antimicrobial peptides, and reduces Tcells (Sikorska & Sozańska,2020).

4.13. Level of Zinc

The results of the table (1-1) showed that the concentration of zinc in the blood serum was significantly affected at the level of probability (p <0.01) due to bladder cancer, as the sick group recorded a significant decrease with an average of (99.22µg/dl), while the healthy group recorded with an average of (109.50µg/dl) This result is consistent with the researchers' study on bladder cancer and indicates the studies have shown that zinc levels are decreased in patients with bladder cancer compared to healthy controls (Zluza et al .,2023) . Unbalanced levels of zinc can lead to a variety of disorders because it is an essential element that affects normal cellular functions (Roohani *et al.,* 2017). As Zinc stabilizes the structures of ribosomes, DNA, and RNA, which has an anti-cancer effect. Many proteins and transcription factors that attach gene copies and identify particular DNA sequences depend on zinc for their proper operation. Because zinc guards against the damage caused by free radicals, it may be fatal. Because of the protective effect of zinc and the decrease in antioxidants, low zinc levels raise the risk of bladder cancer (Wu et al.,2004).

Table (1): biochemical variable levels in the blood serum of bladder cancer patients as compared to the control group.

Biochemical variable	Patient group No. (45)			Control group No (45)			P-value
	Mean	Std. Deviation	Std. Error	Mean	Std. Deviation	Std. Error	
LOXL2 (Pg /ml)	697.43	160.80	23.97	166.78	12.12	1.87	0.01**
Sirtuin 3 (ng /ml)	3.00	0.99	0.14	15.11	2.44	0.36	0.01**
Tumor necrosis factor - α (Pg /ml)	363.36	52.32	7.80	8.63	1.39	0.20	0.01**
Lipoxygenase (U/L)	144.40	77.98	11.62	58.55	5.835	0.86	0.01**
Albumin (g/dl)	34.85	7.69	1.147	60.68	5.06	0.75	0.01**
Superoxide dismutase (U/L)	2.63	0.71	0.107	4.43	1.10	0.16	0.01**
Glutathione peroxidase (U/L)	81.64	14.39	2.14	135.91	23.40	3.48	0.01**
Ceruloplasmin (g/dl)	0.44	0.08	0.013	1.03	0.49	0.07	0.01**
Glutathione (µmol/L)	11.34	2.15	0.32	45.37	8.27	1.23	0.01**
Malondialdehyde (µmol/L)	2.83	0.70	0.10	0.19	0.15	0.023	0.01**
Vitamin C (mg/dl)	0.07	0.028	0.004	0.284	0.148	0.022	0.01**
Vitamin D3 (nmol/L)	16.50	1.92	0.29	25.11	1.16	0.24	0.01**
Zinc (µg/dl)	99.22	8.62	1.30	109.5	5.74	0.85	0.01**
Copper (µg/dl)	174.33	19.90	2.96	231.96	25.91	3.86	0.01**

6. Study of some factors affecting the biochemical variables measured in the blood serum of people with bladder cancer and healthy people

6.1. Effect of Gender

The gender disparity in the incidence of bladder cancer is due to hormonal differences between males and females, which indicate that estrogen has a protective role in females (Antoni et al.,2017), while androgens increase the risk of

bladder cancer (Li et al.,2017). As well as differences in exposure to carcinogens and cellular and physiological responses (Madersbacher,2001). Table (1-4) shows the results of the biochemical tests studied for the categories of men and women patients with bladder cancer. It is noted that the gender factor had a significant effect at the probability level (P≤0.01) on the concentration of the LOXL-2 enzyme and in most of the biochemical variables studied (sirt3, TNF-a, LOP, SOD, CP, GSH, Vitamin C, Vitamin D3, Zn and Cu). and at the probability level (P≤0.05) on the concentration of glutathione peroxidase, malonaldehyde and There was no significant difference of gender on the concentration of albumin, while the group of women recorded the highest average albumin concentration, lipoxygenase, tumor necrosis factor–a and glutathione concentration wichit reached to (34.92, 223.17, 415.86 and 12.09) respectively. If the group of men with bladder cancer recorded the highest averages in most of the biochemical variables studied. Tables (1-2) and (1-3) show a significant increase (P ≤0.01) in lysyl oxidase like-2 levels in male and female patient groups compared to the control group. lysyl oxidase like-2 levels were also higher in male cancer patients than in female patients. Several biological, genetic, and hormonal factors could explain the higher levels of lysyl oxidase 2 (LOX2) in men than in women with bladder cancer: Sex Hormones, differences in sex hormone levels between men and women may influence LOX2 activity. Testosterone in men and estrogen in women may regulate different genes involved in ECM remodeling, including LOX2. Androgens, such as testosterone, may increase LOX2 expression, whereas estrogens may inhibit it, (Wen et al.,2015) Lifestyle and Environmental Factors: Men and women may be exposed to different risk factors for bladder cancer, such as smoking and occupational hazards, which could influence the cancer's molecular characteristics, including LOX2 expression. Tables (4-2) and (4-3) show a significant decrease (P ≤0.01) in SIRT3 levels in male and female patient groups compared to the control group. SIRT3 levels were also lower in female cancer patients than in male patients. Additionally, the findings underline the impact of gender on BC, Hormonal Influence, Estrogens and androgens, which vary significantly between men and women, can impact the expression of various genes, including those involved in cancer metabolism and mitochondrial function, where SIRT3 plays a critical role. Hormonal differences can lead to differential regulation of SIRT3 expression. The estrogen receptor-binding motif in the Sirt3 gene promoter has the potential to influence SIRT3 expression. PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha) is a transcriptional coactivator that regulates cellular energy metabolism. Its role in mitochondrial biogenesis, oxidative phosphorylation, and overall cellular energy homeostasis makes it extremely relevant in the context of cancer, including bladder cancer. Here are some key points about PGC1α and its possible differences in expression or function between male and female bladder cancer patients: Estrogen has minimal effect on Sirt3 promoter activity without PGC-1α, a coactivator. regulates mitochondrial activity and metabolism. (Torrens-Mas *et al.,*2020) PGC-1α stimulates SIRT3 mRNA expression and may work with estrogen to promote SIRT3 transcription. Males have higher levels of PGC-1α, which could explain their higher SIRT3 protein levels. (Zawada et al., 2015). Regarding the concentration of TNF-a levels there was a significant increase (P ≤0.01) in male and female patient compared to control group and in females is more increase compared to male patients because of TNF-a can enhance the formation of new blood vessels (angiogenesis) that supply nutrients and oxygen to tumors, facilitating their growth and potential spread (metastasis). (Wang & Lin, 2008). hormonal differences between females and males can influence the expression and activity of TNF. Estrogen, for example, has been shown to interact with inflammatory pathways, possibly affecting TNF levels and activity in bladder cancer. TNF alpha, a tumor promoter, may be suppressed by estrogen, resulting in a stronger anti-tumor immune microenvironment in female gonad bladder tumors. (Doshi et al., 2023). The results also indicated that levels of oxidation and antioxidants (albumin, superoxide dismutase, Glutathione peroxidase, vitamin C, Ceruloplasmin and GSH)) significantly decreased (P ≤0.01) in the patient group (males and females) compared to the control group (males and females). GSH promotes intracellular antioxidant defense by scavenging ROS and regenerating other antioxidant molecules (Shukla *et al.,*2020). The decrease can be attributed to increased depletion in combating and removing free radicals caused by oxidative processes. As we age, our bodies generate more free radicals, which can cause cellular damage. Free radicals can overpower the Reduced activity reduces their ability to counteract oxidative stress. Reduced activity of antioxidant enzymes, including SOD, glutathione peroxidase, and Cp, contribute to this imbalance. These enzymes are essential for neutralizing free radicals and maintaining a healthy antioxidant balance. When their Oxidative damage affects the redox properties of cells. Reduced activity leads to decreased ability to combat oxidative stress. (Chang and Chen ,2020). In terms of antioxidants within the patient group, the findings indicate that albumin, exhibited similar patterns between male and female patients. Similarly, no significant differences were observed in albumin levels. However, in the case of MDA and lipoxygenase the results is significant increase (P ≤0.01) in male and females patients compared to the control group and in male is more increase. This discrepancy may be attributed to smoking habits among male patients, which can contribute to reduced antioxidant levels and the generation of numerous free radicals (Rafikov et al., 2019). increased levels of lipoxygenase, an enzyme involved in the metabolism of polyunsaturated fatty acids to bioactive lipid mediators, have been associated with various cancers, including bladder cancer. This enzyme, particularly the isoforms 5-lipoxygenase (5-LOX) and 12-lipoxygenase (12-LOX), has been implicated in cancer development and progression due to its role in promoting inflammation, cell proliferation, and angiogenesis. The study found that both male and female patients had significantly increase (P≤0.01) levels of MDA compared to the control group. This increase can be attributed to the oxidative stress caused by Patients' bodies experience increased oxidation processes. MDA is a lipid peroxidation indicator that interacts with DNA, indicating DNA damage. Female patients had higher concentrations of MDA compared to healthy females (Gaschler and

Stockwell, 2017). Regarding the concentration of lipoxygenase levels there was a significant increase (P ≤0.01) in male and female patient compared to control group and in females is more increase compared to male patients. Regarding Zn and Cu and vitamin D3 the results indicated a significant decrease (P≤0.01) in the level of Cu, Zn and Vitamin D3 in the patient group (males and females) compared to the control group (males and females) and in female patients are more decreased than male patients, Cancer patients often suffer from malnutrition due to various factors such as reduced food intake, altered metabolism, and the side effects of treatments. This can lead to deficiencies in essential nutrients, including zinc and copper. Overall, the decrease in zinc and copper in bladder cancer patients can be attributed to a combination of metabolic alterations, increased oxidative stress, impaired immune function, nutritional deficiencies, and chronic inflammation associated with the disease.

Table (2): Biochemical variable levels in the blood serum of men with bladder cancer disease were compared to those in healthy men.

Biochemical variable	Patient group No. (23) Males			Control group No (23) Males			p-value
	Mean	Std. Deviation	Std. Error	Mean	Std. Deviation	Std. Error	
LOXL2 (pg /ml)	852.79	8.369	1.745	156.06	4.062	0.847	< 0.01**
Sirtuin 3 (ng /ml)	3.94	0.257	0.053	17.39	0.684	0.142	< 0.01**
Tumor necrosis factor - α (Pg /ml)	313.14	4.582	0.955	7.46	0.650	0.135	< 0.01**
Lipoxygenase (U/L)	69.06	3.723	0.776	53.13	1.898	0.395	< 0.01**
Albumin (g/dl)	34.79	8.920	1.860	58.87	4.738	0.988	< 0.01**
Superoxide dismutase (U/L)	3.21	0.383	0.079	5.40	0.568	0.118	< 0.01**
Glutathione peroxidase (U/L)	95.01	4.115	0.858	156.34	4.070	0.848	< 0.01**
Ceruloplasmin (g/dl)	0.52	0.033	0.007	0.85	0.402	0.083	< 0.01**
Glutathione (µmol/L)	10.62	1.650	0.344	48.17	5.565	1.160	< 0.01**
Malondialdehyde (µmol/L)	2.92	0.660	0.137	0.19	0.119	0.025	< 0.01**
Vitamin C (mg/dl)	0.09	0.021	0.004	0.26	0.150	0.031	< 0.01**
Vitamin D3 (nmol/L)	18.35	0.466	0.099	26.50	0.781	0.163	< 0.01**
Zinc (µg/dl)	107.29	1.081	0.225	115.06	1.060	0.221	< 0.01**
Copper (µg/dl)	193.53	1.56511	0.323	257.00	1.229	0.252	< 0.01**

Table (3): Biochemical variable levels in the blood serum of female bladder cancer patients compared to females in good health.

Biochemical variable	Patient group No.22)(Females			Control group No (22) Females			p-value
	Mean	Std. Deviation	Std. Error	Mean	Std. Deviation	Std. Error	
LOXL2	535.00	5.686	1.212	178.52	4.553	0.993	< 0.01**

Sirtuin 3 (ng /ml)	2.02	0.206	0.440	12.73	0.648	0.138	< 0.01**
Tumor necrosis factor - α (pg /ml)	415.86	8.086	1.724	9.85	0.764	0.162	< 0.01**
Lipoxygenase (U/L)	223.17	3.608	0.769	64.22	1.291	0.275	< 0.01**
Albumin (g/dl)	34.92	6.377	1.359	62.57	4.788	1.020	< 0.01**
Superoxide dismutase (U/L)	2.01	0.387	0.082	3.42	0.329	0.070	< 0.01**
Glutathione peroxidase (U/L)	67.66	4.012	0.855	114.57	13.999	2.984	< 0.01**
Ceruloplasmin g/dl)	0.36	0.029	0.006	1.22	0.522	0.111	< 0.01**
Glutathione (µmol/L)	12.09	2.392	0.510	42.45	9.671	2.062	< 0.01**
Malondialdehyde (µmol/L)	2.74	0.753	0.160	0.19	0.188	0.040	< 0.01**
Vitamin C (mg/dl)	0.06	0.027	0.005	0.30	0.146	0.031	< 0.01**
Vitamin D3 (nmol/L)	14.66	0.475	0.101	23.66	0.708	0.151	< 0.01**
Zinc (µg/dl)	90.38	1.313	0.286	103.87	0.965	0.151	< 0.01**
Copper (µg/dl)	154.26	1.317	0.280	205.79	0.817	0.174	< 0.01**

Table (4): Biochemical variable levels in the blood serum of females diagnosed with bladder cancer compared to males with the same disease.

Biochemical variable	Patient group (No.23) males			Patient group No (22) females			p-value
	Mean	Std. Deviation	Std. Error	Mean	Std. Deviation	Std. Error	
LOXL2	852.79	8.36907	1.74507	535.00	5.686	1.212	<0.01**
Sirtuin 3 (ng /ml)	3.94	0.25730	0.05365	2.02	0.206	0.044	<0.01**
Tumor necrosis factor - α (pg /ml)	313.14	4.58256	0.95553	415.86	8.086	1.724	<0.01**
Lipoxygenase (U/L)	69.06	3.72388	0.77648	223.17	3.608	0.76	<0.01**
Albumin (g/dl)	34.79	8.92041	1.86003	34.92	6.377	1.359	0.957NS
Superoxide dismutase (U/L)	3.21	0.38326	0.07991	2.01	0.387	0.082	<0.01**
Glutathione peroxidase (U/L)	95.01	4.11564	0.85817	67.66	4.012	0.855	0.02*

Ceruloplasmin (g/dl)	0.52	0.03383	0.00705	0.36	0.029	0.006	<0.01**
Glutathione (µmol/L)	10.62	1.65035	0.34412	12.09	2.392	0.510	<0.01**
Malondialdehyde (µmol/L)	2.92	0.66000	0.13762	2.74	0.753	0.160	0.039*
Vitamin C (mg/dl)	0.09	0.02133	0.00445	0.06	0.027	0.005	<0.01**
Vitamin D3 (nmol/L)	18.35	0.46602	0.09936	14.66	0.475	0.101	<0.01**
Zinc (µg/dl)	107.29	1.08179	0.22557	90.38	1.313	0.286	<0.01**
Copper (µg/dl)	193.50	1.565	0.326	154.26	1.317	0.280	<0.01**

CONCLUSION: There is a significant increase in each of the (enzyme LOXL-2, Lipoxygenase, malondialdehyde, and Tumor necrosis factor-a) in the serum of bladder cancer patients compared to the control group, while antioxidants (vitamin 3D, C, glutathione, albumin, SOD, glutathione peroxidase, ceruloplasmin, zinc and copper) showed a decrease in their levels in bladder cancer patients compared to healthy subjects. The results showed that the gender factor showed a clear and significant effect on the levels of each of the variables that were studied, the study found significant differences in demographic, clinical, biomarker, and lifestyle characteristics between bladder cancer patients and a control group. These findings focus on the importance of early detection, targeted screening, and lifestyle changes in lowering risk and improving prognosis for bladder cancer. Future research should concentrate on the creation of non-invasive diagnostic tools and personalized treatment plans based on the identified risk factors and biomarkers.

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