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A Biochemical Study of The Levels of Some Liver Functions Tests for Breast Cancer Patients Undergoing Chemotherapy in A Najaf Al-Ashraf City

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ABSTRACT: Cancer is one of the main causes of death in the world, is the second leading cause of death in the world, where in 2018 it is estimated that one out of every 6 deaths, which means about 9.6 million deaths. Data in the National Cancer Registry of Iraq shows that there were extra than 31,500 cases of cancer and cancer-related tumors in Iraq as of 2017-2018. It gives to an estimated 11% of all deaths in Iraq. Cancer is the result of multi-stage transformations that generally start from a pre-cancerous lesion to a malignant tumor that transforms normal cells into cancer cells. In women, the breast creates and supplies milk to feed babies and newborns. The size of each breast is determined by the quantity of fatty tissue in the breast. The breast is an organ located above the upper ribs and chest muscles. There are present on both sides, and they both have ducts, glands, and mainly adipose tissue. Factors that cause breast cancer are family history, smoking, alcohol drinking, obesity, tall stature, late menopause, early menstruation, lifestyle, therapy of inactivity and hormone replacement. Chemotherapy, also known as chemo, is a drug treatment in which powerful chemicals are used to kill rapidly growing cells in the body, Chemotherapy is a method of treating cancer cells grow and multiply much more quickly than other cells in the body, which drugs are used to deactivate or kill cancer cells. It also destroys some healthy cells because it does not distinguish between healthy cells and cancerous cells, but healthy cells can repair themselves after a period of time, or new compensatory cells can be created. There is a set of tests to evaluate liver function and estimate the percentage of damage to it depending on the percentage of the increase in these parameters, including the enzyme alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), acid phosphatase (AP), total bilirubin, creatinine kinase (CK), total protein, globulins, and albumin. Subjects: The samples were selected depending on the doctors' diagnosis, all patients were married, and their ages ranged from 25 to 65 years. The results of the analyzes were also compared with twenty-five healthy women as a control group who were selected under strict conditions and with specifications close to those of patients, where the cases were no chronic diseases, no diabetes, no smoking, no alcohol consumption, and they had not undergone surgery for a year. The sample was taken at similar ages to the patients. Most of the liver function tests were measured for the groups under study using colorimetric reaction methods are including the enzyme alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, creatinine kinase (CK), total protein, globulins, and albumin. Result: The results of the current study were that there was a significant increase when compare between patients with one chemotherapy dose and patients with multi chemotherapy dose for all the ALT,AST, ALP, CK and AP tests, while was significant

decrease for the Total Protein, Bilirubin, Albumin and Globulins tests, on the other hand, where the comparison results was a significant increase between patients with one chemotherapy dose and control groups except for globulins tests was no significant ($p=0.076$). While the results of the comparison between patients with multiple chemotherapy doses and healthy group was a significant increase in CK, Bilirubin and Globulins, on other side was a significant decrease in ALT and Albumin tests, then no significant ($p=0.662$) of AST and ALP, ($p=0.025$) of AP and ($p=0.028$) of total protein.

INTRODUCTION:

Cancer is one of the main causes of death in the world, is the second leading cause of death in the world, where in 2018 it is estimated that one out of every 6 deaths, which means about 9.6 million deaths⁽¹⁾ accounting for nearly 10 million deaths in 2020⁽²⁾. Data in the National Cancer Registry of Iraq shows that there were extra than 31,500 cases of cancer and cancer-related tumors in Iraq as of 2017-2018. It gives to an estimated 11% of all deaths in Iraq⁽³⁾. Among men, the most common cancers in lung, stomach, prostate, liver and colorectal cancer, while among women were colorectal, breast, thyroid, cervical, and lung cancer are the utmost common. There are more than 100 kinds of cancers that are categorized depending on the injured organ or tissue in the human body. Cancer is the uncontrolled development and growth of cells in the body. Cancer, a disease affected by many factors, depends on changes in the genome as a result of environmental influences of the patient. Cancer is the result of multi-stage transformations that generally start from a pre-cancerous lesion to a malignant tumor that transforms normal cells into cancer cells. These alterations are the result of the interaction between hereditary factors and external factors and include three types:

physical exposure, such as ionizing radiation and UV; chemicals hazards that cause carcinogenic such as tobacco components, asbestos, alcohol, and arsenic; and biological hazards, such as infections by viruses, bacteria, or parasites⁽⁴⁾. Characteristics of cancer are uncontrolled proliferation, resulting in detention of cell division, confrontation to apoptosis, insistent angiogenesis, failure to react to growth signals and capability to permeate other tissues. At present, the risk of cancers can be treated or reduced by both surgery, chemotherapy and radiotherapy, so there are complementary methods, including Nano therapy, immunotherapy, hormonal therapy, and others. The goal of these treatments is to prevent the proliferation of tumors or unhurried the level of cell growth, however they are not able to give complete safety⁽⁵⁾.

In women, the breast creates and supplies milk to feed babies and newborns. The size of each breast is determined by the quantity of fatty tissue in the breast⁽⁶⁾. The breast is an organ located above the upper ribs and chest muscles. There are present on both sides, and they both have ducts, glands, and mainly adipose tissue^(6,7). The breast consists of lobules, which create milk, and there are about one million in each breast. The ducts are tubes that transport milk from the lobules to the nipple. adipose tissue surrounding the lobules and ducts to provide support, blood vessels and lymph vessels that carry lymph nodes in the armpit and above the clavicle and chest as well as there are nerves and muscles⁽⁸⁾.

Factors that cause breast cancer are family history, smoking, alcohol drinking, obesity, tall stature, late menopause, early menstruation, lifestyle, therapy of inactivity and hormone replacement⁽⁹⁾. Breast cancer is considered one of the strongest causes of death in women in the world. The difficulty in treating and managing this disease was caused by a group of problems, including resistance of multidrug, late diagnosis, and complications associated with the stage of breast tumor⁽¹⁰⁾.

Chemotherapy, also known as chemo, is a drug treatment in which powerful chemicals are used to kill rapidly growing cells in the body. Chemotherapy is a method of treating cancer cells grow and multiply much more quickly than other cells in the body, which drugs are used to deactivate or kill cancer cells. It also destroys some healthy cells because it does not distinguish between healthy cells and cancerous cells, but healthy cells can repair themselves after a period of time, or new compensatory cells can be created. Chemotherapy can be taken in a number of ways, Either through intravenous injections, injections, or pills, and this treatment is the only one for some cancers, and it can be given with surgical intervention, immunotherapy, hormonal, and radiation, often involves more than one drug^(11,12). Among the most common side effects that occur to the patient after chemotherapy are nausea or vomiting, hair loss, constipation or diarrhea, feeling tired and weak, fever, skin problems, mouth ulcers⁽¹³⁾. Chemotherapy is often given in the form of doses during different periods called cycles, where the patient is given a dose and left for a period of time to rest until he gets rid of the side effects and recovers, and so on⁽¹³⁾.

The liver is responsible for many functions, the most important of which is the synthesis of proteins, digestive enzymes, and detoxification. It also has a fundamental role in metabolic processes and regulation of red blood cells. The liver is located below the diaphragm in the upper right quadrant of the body under rib cage and it is the second in terms of size in relation to other body parts ⁽¹⁴⁾.

There is a set of tests to evaluate liver function and estimate the percentage of damage to it depending on the percentage of the increase in these parameters, including the enzyme alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), acid phosphatase (AP), total bilirubin, creatinine kinase (CK), total protein, globulins, and albumin⁽¹⁵⁾. **Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)** are synthesized in the liver in the cytosol. The concentration levels of these enzymes in serum is low, typically less than 30 IU/L. However, AST and ALT are release of at higher levels in process that results in loss of necrosis or integrity of hepatocyte membrane ^(16,17). **Alkaline phosphatase (ALP)** is an enzyme found in the cells that formed the bones and liver in greater concentrations than in the other parts of the body. High levels of ALP in the blood are often caused by bone disorders or liver disease. In the liver, ALP is found on cells that fuse to form the bile ducts, which are small tubes that carry bile from the liver to the intestines, where it helps in the digestion of fats in the diet. ALP is formed in bones by special cells called osteocytes that participate in bone formation. Each different tissue type has distinct forms of ALP called isoenzymes⁽¹⁸⁾. **Creatine kinase (CK)**, It is an intracellular enzyme that is found in greater concentrations in the heart, skeletal muscles, and brain. While the lowest concentrations in other visceral tissues⁽¹⁹⁾. **Acid phosphatase (AP)** is an active enzyme in acidic medium that catalyzes the hydrolysis of phosphate esters in. This reaction is critical for numerous metabolic processes. Research has shown in humans the AP plays a essential role in several physiological processes, including immune defenses, bone resorption, regulation of epithelial growth, pathogen clearance, and iron transport⁽²⁰⁾. **Total protein** concentration is determined for all types of proteins in the serum. Proteins are essential for the body to function. The two main types of proteins found in the blood are albumin and globulin. Measuring the total protein level as well as the albumin-to-globulin ratio can help diagnose and detect several types of diseases, including nutritional deficiencies and liver and kidney disease⁽²¹⁾. **Bilirubin** is a yellow substance that is produced naturally during the process of breaking down red blood cells. Bilirubin is found in bile, which is secreted by the liver to help digest food. A healthy liver gets rid of most of the bilirubin outside the body, but if the liver is damaged, the bilirubin is secreted from the liver into the bloodstream, causing what is known as jaundice, a condition that leads to yellowing of the whites of the eyes and skin. It is worth mentioning that the results of the bilirubin blood test in addition to The symptoms of jaundice enable the doctor to know that there is a problem with the liver⁽²²⁾.

Subjects & Methods : During the period from the beginning of October 2022 to June 2023, fifty blood samples were collected from women patients with breast cancer at the Center for Cancer Treatment in Al-Najaf Al-Ashraf, where five milliliters of intravenous blood samples were drawn from each patient, and they were classified according to the beginning of diagnosis and after the first, second, and third doses. The samples were selected depending on the doctors' diagnosis, all patients were married, and their ages ranged from 25 to 65 years. The results of the analyzes were also compared with twenty-five healthy women as a control group who were selected under strict conditions and with specifications close to those of patients, where the cases were no chronic diseases, no diabetes, no smoking, no alcohol consumption, and they had not undergone surgery for a year. The sample was taken at similar ages to the patients. A centrifuge was used at a speed of 3000 xg for a period of 15 minutes after the samples were left to coagulate at laboratory temperature. Most of the liver function tests were measured for the groups under study using colorimetric reaction methods are including the enzyme alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, creatinine kinase (CK), total protein, globulins, and albumin

RESULTS & DISCUSSION:

Demographic data were collected. Full information on the criteria for selecting individuals in the two groups are explained in subjects and methods section of the current study. Fact information for the individuals in each of the two groups are shown in **Table 1**

Test	The study groups		<i>p-value</i>
	Patients (50) Mean $\pm$ S.D. Min.- Mix. Range	Controls (25) Mean $\pm$ S.D. Min.- Mix. Range	
Age (year)	47.00 $\pm$ 12.778 25-73 48	47.96 $\pm$ 12.071 25-68 43	0.756
Weight (Kg)	75.56 $\pm$ 15.296 47-115 68	62.56 $\pm$ 4.691 54-70 16	0.000
Length (cm)	156.52 $\pm$ 5.694 142-168 26	174.04 $\pm$ 9.676 155-188 33	0.000

Table 1: Baseline Demographic Characteristics of The Participants in The Groups

The beginning of the current study included categorization the information contained in the questionnaire form designated for samples. Table 1 shows that there is no statistical significance when comparing the age ($p=0.756$), while significant increase ($p=0.000$) when comparing length, and weight of the two study groups. The figure (1) show the different between two groups

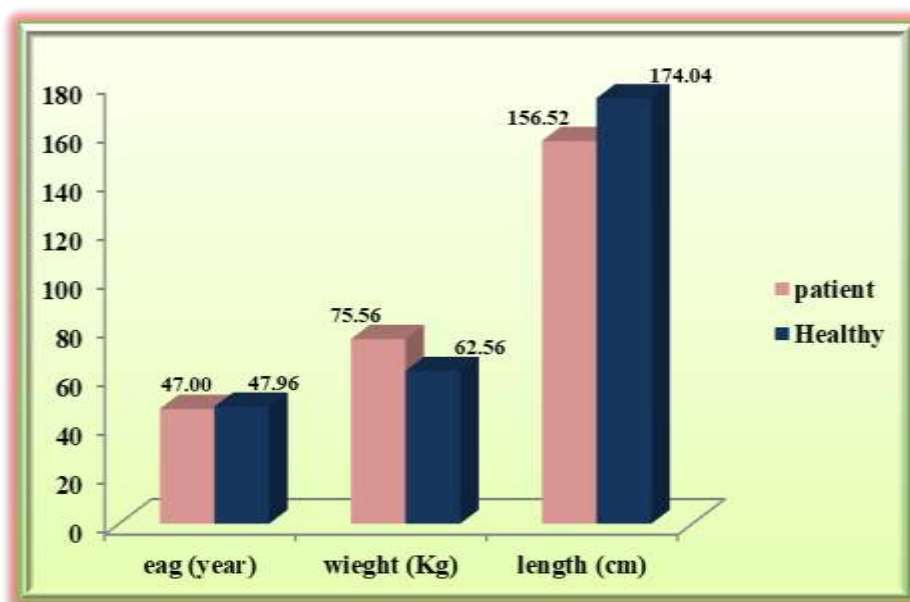


Figure (1) : show the different between two groups

This indication means that the people most affected are those with a high body mass index (they are obese).Where, some of the biological mechanisms that explain the relationship between cancer and obesity. Mass enlarged of white adipose tissue (WAT) appears as association between inflammatory dysregulation and tumor biology and metabolic in obesity. White

adipose tissue involves of endothelial cells, adipocytes, mature adipocytes, fibroblasts, pericytes, and immune cells. Obesity also stimulates hyperlipidemia, a concern of adipose tissue of disordered that can support the excretion of inflammatory cytokines⁽²³⁾.

The levels of most liver function tests were measured for all samples that included the breast cancer patient group after the chemotherapy doses and compared with the control group. The results were as shown in Table 2

Test	The Study Groups			<i>p-value</i>	
	Patients with one dose (50) Mean±S.D. Min.- Mix. Range	Patients with multi dose (50) Mean±S.D. Min.- Mix. Range	Controls (25) Mean±S.D. Min.- Mix. Range		
Alanine Aminotransferase (ALT)	26.88±1.58	30.89±2.41	28.48±3.04	1vs2	0.000
	24.0- 29.2	27.5- 39.0	23.0-34.0	1vs3	0.005
	5.2	11.5	11.0	2vs3	0.000
Aspartate Aminotransferase (AST)	27.73±1.36	29.33±8.53	29.68±3.47	1vs2	0.000
	24.7-29.7	0.9-35.2	24.0-38.0	1vs3	0.002
	5.0	34.3	14.0	2vs3	0.662
Alkaline Phosphatase (ALP)	8.52±0.91	9.73±1.39	9.59±1.94	1vs2	0.000
	6.5-10.1	7.9-13.5	7.4-13.0	1vs3	0.002
	3.6	5.6	5.6	2vs3	0.662
Creatinine Kinase (CK)	42.48±14.49	105.82±8.10	128.56±38.00	1vs2	0.000
	27.0-89.0	86.0-121.0	14.0-182.0	1vs3	0.000
	62.0	35.0	168.0	2vs3	0.000
Acid Phosphatase (AP)	0.32±0.06	0.45±0.11	0.50±0.12	1vs2	0.000
	0.24-0.50	0.23-0.63	0.36-0.70	1vs3	0.000
	0.26	0.40	0.40	2vs3	0.025
Total Protein	7.83±0.20	6.91±0.33	6.77±0.25	1vs2	0.000
	7.4-8.1	6.3 -7.5	6.2-7.2	1vs3	0.000
	0.7	1.2	1.0	2vs3	0.028
Bilirubin	0.91±0.16	0.73±0.18	0.8±0.2	1vs2	0.000
	0.7-1.3	0.0-0.9	0.6-1.3	1vs3	0.000
	0.6	0.9	0.7	2vs3	0.000
Albumin	5.51±0.48	4.34±0.34	4.00±0.27	1vs2	0.000
	4.9-6.3	4.0-5.0	3.5-4.5	1vs3	0.000

	1.4	1.0	1.0	2vs3	0.000
	2.63±0.29	2.38±0.27	2.75±0.23	1vs2	0.000
Globulins	2.1-3.1	2.0-2.9	2.2-3.1	1vs3	0.076
	1.0	0.9	0.9	2vs3	0.000

Table 2: Levels (Mean ± S.D.) of ALT, AST, ALP,CK, AP,TP, Bilir, Alb and Glob. in The Sera of (1)patients with one chemotherapy dose,(2) a group after more than one chemotherapy doses and (3) a healthy group

The results of the current study were that there was a significant increase when compare between patients with one chemotherapy dose and patients with multi chemotherapy dose for all the ALT,AST, ALP, CK and AP tests⁽²⁴⁾ while was significant decrease for the Total Protein, Bilirubin, Albumin and Globulins tests, on the other hand, were the compare results was a significant increase between patients with one chemotherapy dose and control groups except for globulins tests was no significant ($p=0.076$). While the results of the comparison between patients with multiple chemotherapy doses and healthy group was a significant increase in CK, Bilirubin and Globulins, on other side was a significant decrease in ALT and Albumin tests, then no significant ($p=0.662$) of AST and ALP, ($p=0.025$) of AP and ($p=0.028$) of total protein. Pre-hepatic hyperbilirubinemia may be a result of hemolytic anemia as a result of chemotherapy, or it may be due to the effect of cancer cells in the breast⁽²⁵⁾. This result was consistent with the previous study as an abnormal liver function test in patients with cancer in the stage of chemotherapy^(26,27).

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