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Study of the Effect of Chemotherapy on Some Serum Antioxidants and Partly Purified Glutathione Peroxidase from Patients with Breast Cancer

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Abstract

Cancer is considered one of the most serious diseases that threaten the society at present. Accordingly, some antioxidants have been studied about breast cancer during chemotherapy and the effect of this treatment on antioxidants that protect cells from oxidation and damage associated with cancer. This study was conducted on (92) Participants; (52) of which for patients with breast cancer who are aged between (25-80) year. Additionally, (40) healthy people aged between (22-75) year were enrolled. The samples were obtained from the Center for Oncology in Azadi Teaching Hospital in Kirkuk. This research deals with the study of glutathione peroxidase (GPx) using gel filtration (Sephadex-50) and the dimension column (28×1.5) cm. The serum from a patient with breast cancer was partially purified and compared with that from healthy female control. The effect of chemotherapy on GPx, Catalase (CAT), Glutathione (GSH) and Uric acid (U. A) has studied as an antioxidant. The results showed, A substantial decrease in the catalase enzyme in the different groups of chemical doses (A, B, C and D) Group (A) consists of patients undergoing chemotherapy from (1-3) Chemotherapy doses. Group (B) consists from (4-6) Chemotherapy doses, Group (C) consists from (7-9) Chemotherapy doses, Group (D) consists from (10-12) Chemotherapy doses at a Statistical level ($p \leq 0.01$). On the other hand, the group (E) consists from (13-16) Chemotherapy doses has found no considerable difference, the drugs that make up the chemotherapy given to patients (Adriamycin, Cytoxan, methotrexate, Fluorouracil). the level of enzyme activity (GPx) at a Statistical level ($p \leq 0.01$). (C, D and E) groups is decrease, either groups represented by (A and B) with no significant difference was recorded, the level of enzyme activity (GSH) at a Statistical level ($p \leq 0.01$).

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(A) group is decrease, either groups represented by (B,C,D,E) groups with no significant difference was recorded,.Lastly, in the Uric acid, there are No significant differences in groups that differ in chemical doses.

Keywords: Breast cancer; Glutathione peroxidase; Glutathione; Catalase; Uric acid; Purification of glutathione peroxidase; chemotherapy.

1. Introduction

Breast cancer is one of the most serious diseases affecting the society. This research is due to the spread of this disease in our societies. This research investigates the effect of chemotherapy given to breast cancer patients and its effect on the body enzymes that act as antioxidants.

Cancer is a disease caused by abnormal divisions of the cells of the body in a disorderly and unstructured [1]. Each organ in the human body consists of a group of cells that suffer from limited and regular divisions and under full control to compensate for the shortage of damaged cells and maintain the body in a healthy manner [2]. Therefore, any disorder that occurs in the divisions of these cells leads to a defect in the size of the cell. Consequently, a breach occurs in the system of regular division of the cell [3], and hence starts the tumor as a result of the production of cells that do not need the member [1].

1.1 The Breast Cancer

It is a complex disease that requires mutual awareness between both doctors and patients [4] It's the most common disease in women with 1 out of every 9 women who are at risk of developing breast cancer [5]. Men may also suffer for every 200 infection in women there is one infection for men [6]. Breast cancer is a malignant disease in the form of a tumor that arises from the breast cells itself, and most cases of the breast cancer start from the small ducts [7].

1.2 Chemotherapy and Breast Cancer

It is a group of drugs used to get rid of cancer cells. They are given directly to the bloodstream and reach all parts of the body [8]. Therefore, it is the best treatment for the patient who suffers from the spread of the disease throughout the body [9]. Chemotherapy is given for breast cancer in the following cases:

- In the event of the spread of the disease throughout the body [10].
- Reduces the size of the tumor before the operation, which facilitates the tumor removal agent in the case of response of the tumor to the drug and its smaller size became. However, in the case of no size changes, this indicates the lack of response and the drug must be changed [11]
- After the mastectomy, chemotherapy doses are given to reduce tumor recurrence [12].

1.3 Breast Cancer and Oxidative Stress

An imbalance between effective free radicals and the antioxidant defense system[13]. It is known that free

radicals are ions, atoms or molecules containing one or more single electrons that give them high efficiency to interact with another free radical or molecule to reach stability [14]. These free radicals act on oxidation of cell membranes and important molecules within them (such as DNA, proteins and fats) causing significant damage to these molecules [15]. Causing significant damage to these molecules, evidence suggests the participation of free radicals in the transformation of cells of the body into cancer cells, which have a significant role in the process of development and formation of cancer cells[16].

1.4 Glutathione Peroxidase-Enzyme

The primary Function of GPx is to counteract the oxidative attack [17]. It is classified into oxidation and reduction enzymes (E.C.1.11.1.9) [18]. Selenium element (Selenium-Se) plays a significant role in the concentration of peroxidase in the body because it is the main component of peroxidase [19]. There are many proteins in mammalian cells that can metabolize peroxide and lipid hydroxyl oxides. These include selenium-four proteins found in various cells and tissues in the body, and selenium can be obtained from the nutrient-rich in this element. The peroxidase is Subcutaneous protein units, each of which has one atom of selenium (Se) and is associated with cysteine. The total Molecular weight of the peroxidase is 44000 daltons [20].

1.5 Purification of glutathione peroxidase

The enzyme peroxidase is an essential and necessary enzyme that keeps the cell safe from damage and various diseases. The techniques used in the purification of this enzyme are gel filtration chromatography, and ion exchange Chromatography as well as membrane screening Dialysis[21]. In the purification process, chemicals are used to separate the enzymes based on the molecular weight and size of the constituent molecules[22]. The chemicals used in the separation process are Sephadex and (DEAE-cellulose) [23]. Then, deposition of the enzyme by the precipitates [24].

2. Materials and Methods

2.1 Sample of study

The study was conducted on (92) blood samples (52) for patients with breast cancer and (40) for healthy ones. The samples were obtained from the Center of Oncology / Azadi Teaching Hospital / Kirkuk. The patients ranged between (25-80) years To be subject to chemotherapy sessions, while for healthy people ranged between (22-75) years. Samples were collected from 1/2/2017 to 28/5/2017, The chemotherapy doses given to patients with breast cancer begin with (1-16) Chemotherapy doses, and the patients were divided into five groups according to the different chemical doses given to them,Group (A) consists of (1-3) Chemotherapy doses. Group (B) consists from (4-6) Chemotherapy doses, Group (C) consists from (7-9) Chemotherapy doses, Group (D) consists from (10-12) Chemotherapy doses at a Statistical level ($p \leq 0.01$). On the other hand, the group (E) consists from (13-16) Chemotherapy doses, The drugs that make up the chemotherapy given to patients (Adriamycin, Cytoxan, methotrexate, Fluorouracil).

2.2 Blood Samples Collection and Serum Preparation

Blood samples were collected by drawing blood from the vein using a disposable syringe with a thin lid (5 cm3). Blood was placed in plastic tubes that did not contain anticoagulants,The tubes were left at laboratory temperature until coagulated and then centrifuged for 10 minutes and at a speed of 4000 rpm. After the serum separation, it was withdrawn by a micro-pipette until the tests are carried out. Lastly, (-20C0) dry, clean and sterile tubes and kept in a freezing state.

2.3 Determination of the Activity of Catalase in Serum

The activity of the catalase enzyme is estimated Spectrometrically at a wavelength of (240 nm). The concentration of this enzyme depends on the consumption of hydrogen peroxide [25].

2.4 Determination of the Activity of Glutathione Peroxidase in Serum

The activity of the Gpx enzyme was estimated using the chromatic method on Green and Hill [26].

2.5 Determination of the Activity of Glutathione in Serum

The concentration of glutathione in blood plasma was measured by DTNB (Ellman's Reagent). Different disulfides are formed and the thiol anion when the compound was combined with the thiol group (SH) in the glutathione molecule in a basal medium (pH = 8). These have measured by the spectrophotometer at a wavelength of (420 nm) [27].

2.6 Determination of the Concentration of Uric Acid in Serum

Uric acid is oxidized by the urease enzyme to the Aerosol compound and hydrogen peroxide is released, which indicates the oxidation of (4-2 dichlorophenol) complex by the peroxidase enzyme and the presence of the colored solution)[28].

2.7 Purification of Glutathione Peroxidase

Glutathione Pyroxidase is purified by gel filtration chromatography technique, which is one of the techniques that depends on the molecular size and molecular weights, I use a (28 x 1.5 cm) thick column that contains the (DEAE Sephadex-50) gel which separates the compounds based on the size. The gel is placed at a height of 20 cm by pouring the gel on the walls of the column of the separation quietly and obliquely to prevent the formation of the bubbles, After placing an appropriate piece of glass wool at the end of the column, The blood serum was injected into the column of the separation in a circular manner and on the columns of the separation column followed by the procedure of the Rogan using the regulated solution with pH 7. The process was collected at a rate of (65.4 cm^3 / hour) ,(1.09 cm^3/ per minute) and manually. Calculation of the protein concentration by measuring the absorbance of the separated parts, and using the fulen method as in the above paragraph. In addition, the efficacy of Glutathione Peroxidase was monitored in each of the separated parts [29].

2.8 Statistical Analysis

The results were statistically analyzed using (ANOVA). The results showed by the mean values of (Mean ± SD). The (F-TEST) test was used to compare groups to analyze the results of patients with breast and healthy cancer, divided by the number of chemical doses assigned to each patient, Graphs were drawn using (Minitab ver) (2017)

3. Results and Discussion

3.1 The Results

Results showed a substantial decrease of the catalase enzyme in the different groups of chemical doses (A, B, C and D). However, in the (E) group, no significant difference was recorded at a Statistical level ($p \leq 0.01$) (There is no significant difference in E group only) so in the statistical analysis shows that there is a significant change meant for the rest of the groups and not E group

. These results are listed in the Table (1).

Table 1: The average standard deviation shows the level of antioxidant enzymes according to difference in chemotherapy doses

Parameters	**Patients / n=11 Group (A) (1-3)**	**Patients / n=15 Group (B) (4-6)**	**Patients / n=12 Group (C) (7-9)**	**Patients / n=4 Group (D) (10-12)**	**Patients / n=10 Group (E) (13-16)**	**P≤**
CAT (k/ml)	0.389 ±0.035	0.367±0.012	0.302±0.012	0.257±0.003	0.235±0.019	0.01
GPx (U/l)	0.3925±0.02316	0.38500±0.01883	0.33133±0.01876	0.276±0.012	0.198±0.010	0.01
U.A (mg/dl)	5.527±0.919	6.683±1.267	7.460±3.78	7.300±1.134	6.750±1.182	N.S
GSH (µmol/L)	4.568±0.239	3.852±0.280	3.660±0.219	2.717±0.047	2.7940±0.273	0.01

* Chemotherapy is given to patients with breast cancer only, so there is no healthy groups in this table

Additionally, for glutathione peroxidase, it showed significant decrease at a Statistical level ($p \leq 0.01$) in totals that vary in chemical doses and include these totals (C, D and E) either group represented by (A and B). Therefore, as shown in the Table (1), no significant difference was recorded. When moving from group A to group B did not notice any significant difference in the effectiveness of the enzyme as for the rest of the groups found a significant difference in the effectiveness of the enzyme.

Consequently, the results showed a significant decrease in the level of enzyme efficacy (GSH) at a Statistical level ($p \leq 0.01$). In a group that varies by chemical dose is the group (A) as groups (B, C, D and E) no significant difference was recorded. Meanwhile, the results of uric acid showed that there are no significant differences at a Statistical level ($p \leq 0.01$) in groups that differ in chemical doses.

As for the purification of glutathione peroxidase enzyme, the results were shown in the Figure (1), a single peak of the purified enzyme with a capacity of 0.367 units / l in patients with breast cancer. The same enzyme value was (0.266) units/litre for the healthy control group (gm/cm^3), which represents the highest amount of protein in the serology of patients with breast cancer.

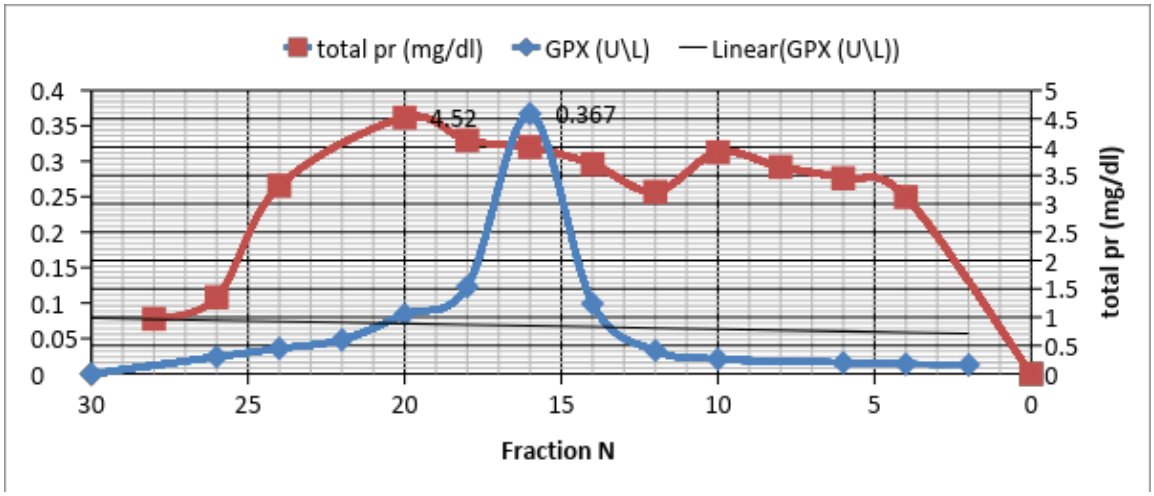

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Figure 1: glutathione peroxidase enzyme and concentration of protein from the separation column using Gel filtration chromatography using (DEAE Sephadex) for the sample (G2)

Table 2: Purification of glutathione peroxidase in the serum of a woman with breast cancer using gel filtration chromatography.

Steps	Total Volume (ml)	Protein conc. mg/ml	Total protein conc. mg/ml	Activity U/L	Total Activity x U/L	specific activity U/mg	Degree of purification	Yield %
Crude enzyme	1	3.84	3.84	0.421	0.421	0.109	1	100
Purified Enzyme	1.5	3.21	4.815	0.367	0.550	0.114	1.045	130.6

3.2 Discussion

For catalase enzyme, studies have shown that cancer of all types increases the stress and as survival rates are

still weak there is an urgent need to develop treatments and make them more effective[30], breast tumors work to increase oxidative stress and must be matched by enough antioxidants to protect[31]. The Previous studies showed` that an increase in the secretion of antioxidant catalase may control the spread of the tumor [32]. The level of catalase has been measured in combination with chemotherapy. The most paramount drugs included in the synthesis of chemotherapy are (Cisplatin, 5-Fluorouracil, paclitaxel , Daunorubicin & Hydroxyurea) [33]. Several studies have indicated that most chemotherapy works on the production of hydrogen peroxide and therefore the body needs to produce an increase of the catalase enzyme to get rid of the peroxide [34] . Thus, the above results show that the catalase enzyme starts to decrease as a result of increased peroxide [35], as well as studies have shown that cisplatin with catalase can eliminate cancer cells and prevent them from spreading [36]. As for the glutathione peroxidase enzyme, the results can be explained by the fact that chemotherapy works on the reduction of reduced glutathione and enzyme-based antioxidant glutathione peroxidase, which may be the result of high oxidation after chemotherapy. Free radicals increase and oxidative stress increases, causing significant and significant reduction in enzymatic antioxidants[37]. These enzymes protect the cells from harmful substances by stimulating their association with the reduced glutathione and prevent damage caused by the reactive oxygen species by reducing hydrogen peroxide, fat and phospholipid hydroperoxides, GPx works together with the associated enzymes as an antioxidant for reduced glutathione, acts as a high level of glutathione peroxidase in most studied human cancers but decreases after chemotherapy. This is confirmed by (Moutet) and others [38].

The non - significant reduction in glutathione is due to the increased oxidative stress that leads to(NADPH). Increased glutathione oxidation reduces its level and can overcome this oxidation through the production which acts as an adjunct to the elimination of oxidized glutathione. This study was agreed with (Jayasurya and others) [39]. (Zhang and his colleagues) [40] confirmed that the level of concentration of glutathione might be a predictive sign of the patient's response to treatment and its success. The lower enzyme activity indicated its increased consumption due to increased oxidative stress [41].

Several studies have confirmed that uric acid may rise due to oxidative stress or some inflammation but is not affected by chemotherapy [42-43]. Glutathione peroxidase was partially purified from the blood vessels of patients with breast cancer and healthy by using gelatin filtration technique. The Glutathione peroxidase was separated depending on the difference in their size. Large particles pass first through the column because they move between the granules of the gel. The small particles penetrate into the molecules, and the gel is delayed by degeneration [44]. As shown in the Figure (1), (DEAE Sephadex -50) is used as a molecular sugar, which acts as a molecular molecule through which different proteins can be separated by molecular weight and by the presence of a phosphate-regulated phosphate solution (PH= 7) [45].

4. Recommendation

Purification of glutathione peroxidase enzyme and isolation using other vital techniques such as ion exchange and other purifications for high purity.Studying the impact of drugs on the enzyme partially purified for patients with breast cancer.Inspecting the effect of chemotherapy and the damage done by the treatment of healthy cells in the body.Examining the influences of enzymatic antioxidants in the patients with breast cancer (Glutathione

S-transferases, Superoxide dismutase). Furthermore, studying the relationship between them and the enzyme glutathione peroxidase.and Conducting further studies on the enzyme purified molecule by using it by using it against different types of diseases.Determination of Trace elements that are included in the composition of antioxidants, such as selenium which is considered as the main component of the enzyme glutathione peroxidase.

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