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The Effect of Breast Cancer on the Human Serum Albumin Structure

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Abstract

Background: Human serum albumin (HSA) can be defined as the major protein in plasma. Also, the ammonium sulfate precipitation is the procedure that is utilized for albumin from plasma, while delipidation has been done with the mixture of ethanol-ether.

Objective: To evaluate the effect of breast cancer on albumin structure.

Methods: Spectroscopy measurements and biochemical assays were used for determining the amounts regarding thiol, free amine, and carbonyl in purified HSA and total protein.

Results: The findings indicated that patients with breast cancer had significantly lower albumin concentrations, lower levels of free amine, and higher levels of carbonyl and thiol. Additionally, the patients' sera (total protein) had lower carbonyl and amine levels and higher thio levels. Also, our data demonstrated the delipidation process following

purification must not to be taken into account since it damaged the function as well as the structure regarding albumin.

Conclusion: The present work indicated that breast cancer affected the functional and structural integrity related to HAS in sera of patients with the disease as compared with normal women.

Keywords: A**lbumin, breast cancer, carbonyl, free amine, thiol.**

Introduction

Breast cancer

Breast cancer is the world's most frequently occurring cancer among women. Additionally, it is one of the leading cancer-related mortality causes [1]). In case of being diagnosed in a timely manner, breast cancer can be largely treated via surgery, radiation, additionally systemic therapy [2]. First childbirth after the age of thirty, nulliparity, alcohol use, early menarche, advanced age, late menopause, inactivity, postmenopausal obesity, and a breast cancer family history in both the mother and the father are risk factors for breast cancer. The risk of invasive breast cancer amongst women is further increased by atypical ductal carcinoma hyperplasia, ductal carcinoma in situ, and lobular carcinoma in situ. Early exposure to ionizing radiation is an additional risk factor [1].

Proteins

Proteins are big bio molecules, comprised of at least one long chain of amino acid residues. One type of globular protein is albumin. Every member of the albumin family of proteins is soluble in water.

Albumin

The most prevalent one of the proteins in plasma is human serum albumin (HSA), which typically ranges from 3.50 to 5g/100dl. With regard to children with age of no more than 3 years old, the normal range is broader (2.90–5.5g/dL) [2,3]. HSA is produced in the liver and it forms a large percentage of all plasma protein [4]. Its pharmacological and physiological characteristics were thoroughly examined over several decades [2].

Human serum albumin’s three-dimensional structure was specified crystallographically to a resolution of 2.8 Å (250 pm) [2]. Albumin’s isoelectric point is 4.90. Albumin is 65kDa-70kDa protein. Since albumin serum represents the primary protein in human

blood plasma, its primary role is to control the oncotic pressure of blood by binding water, cations (like Na+, Ca2+, and K+), hormones, fatty acids, bilirubin, thyroxine (T4), and medications (like barbiturates) [5].

In this study, HSA has been utilized as model protein, because it's the most abundant blood plasma protein constituent in human body and this study is comparative study between before after purified albumin by uses four parameters: -protein concentration, amine, thiol, and carbonyl group and study the structure of albumin between control and breast cancer patient.

Blood samples

Plastic syringe with gauge 21 stainless needles has been used in order to puncture the vein and draw five milliliters of venous blood. Blood has been separated in plain poly-ethylene tube, left to clot at room temperature, after that the tube has been centrifuged at (704xg) for a period of 10 mins, afterwards serum has been obtained by freezing at (-20^{o}C) to the time of analysis. Hemolyzed samples have been discarded. 50 blood samples, (25) from each of healthy and breast cancer subjects with a ranged of age between (20 and 63) have been utilized in the present study. The subjects of the study have visited the medical city, Kirkuk oncology hospitals throughout 1st of March 2026 to 31st of May 2026, the illness had been diagnosed by subspecialized doctors. Any cases that might have interfered with the study have been discarded, including "hypertension, heart disease, and diabetes mellitus".

Materials and method

The Lowry et al. Method has been used for the purpose of calculating serum samples' total protein concentration [6].

Amino groups' determination: -

The spectrophotometric measurement regarding amino groups has been carried out in the following method described before [7]: 100µL 1.0M phosphate buffer (pH 7.20), 100µL sample, and 40µL 0.10M p-benzoquinone in di-methyl sulfoxide have been combined and water has been added to 1500µL volume. The reagent blank has been utilized for the purpose of measuring absorbance at 480nm. A standard curve has been built with glysin in a range of concentration from 10–90mM (y =4.5533x+0.0239 r=0.9867, p>0.01

Assay for free sulfhydryl group

Ellman [8] technique, as modified by others [9], was used to analyze sulfhydryl groups. In short, cuvettes have been filled with 50µl of 10mmole\L DTNB in methanol after 1ml of a buffer that contains 10 mmole\L EDTA, 0.1 mol\L tris, 50µl serum, and PH 8.2 was added. With the exception of the absence of DTNB in methanol after a 15min period of incubation at the temperature of the room, blanks have been performed for every sample generated as previously detailed in this paper. Sample absorbance has been measured at 412 nm. Sample as well as reagent blanks have been also subtracted. TNB molar extinction coefficient of 14,100M^{-1} CM^{-1} has been utilized in order to calculate the sulfhydryl group concentration. Results have been expressed as (µmol\L) [10].

Determination of carbonyl levels

With minor adjustments, Levine et al. [11] approach was used to quantify protein carbonyls. In short, 0.5ml of serum (1mg/ml) was treated with 0.5ml of TCA (20% solution) for 10 minutes at room temperature and then centrifuged for 30 minutes at 3,000rpm. The pellet has treated with 0.50ml of 10mM DNPH in 2M HCL or with 0.5ml HCL only for the blank sample have been incubated for 30min at room temperature in dark, and treated after that with 20% TCA and centrifuged at 3000rpm for 10min. The pellet has been washed 3 times in ethanol\ethyl acetate (v\v); and 1.5ml of 1NaOH has been added to the pellet followed by incubation at 37^{o}C for 15min. the concentration of Carbonyl had been determined with the use of molar absorption coefficient of Ɛ370 =22,000M^{-1} cm^{-1} and using UV-spectrophotometer and expressed as nano-mole of carbonyls per mg of the protein.

Purification of HSA and globulin from serum by precipitation

Human globulin has been purified from serum by using the sodium sulfate–sulfite precipitation method [12]. The sodium sulfate–sulfite reagent was prepared by dissolving 208 g of sodium sulfate and 70 g of sodium sulfite in approximately 900 mL of distilled water with continuous stirring. Subsequently, 2.0mL of concentrated H2SO4 was added, and the solution was transferred to a volumetric flask and diluted to the required final volume with distilled water. The pH of the prepared reagent was adjusted to 7.0. For globulin precipitation, 2 mL of the sodium sulfate–sulfite reagent was mixed with 1 mL of serum, followed by adding 2mL of ether. The mix has been gently inverted 20 times in order to ensure adequate mixing. Following the

centrifugation at 3000rpm for 10 minutes, the precipitated globulin was separated, and the supernatant was carefully collected.

Human albumin has been purified from serum by using the ammonium sulfate (AS) precipitation method [13]. A saturated ammonium sulfate solution (767g/L) was prepared, and its pH has been adjusted to 7.4 using ammonium hydroxide (NH_4OH). In the subsequent step, the saturated ammonium sulfate solution was gradually added to the collected supernatant under sterile conditions while maintaining the mixture in an ice bath until a final ammonium sulfate concentration of 70% was achieved. The resulting HSA precipitate has been recovered by centrifugation at 5000g for 10min, after which the supernatant has been discarded. The HSA pellet was then re-suspended in 0.1M sodium phosphate buffer (pH 7.40). for the purpose of eliminating residual ammonium sulfate, the HSA solution was washed three times and subsequently concentrated by ultrafiltration using an Amicon 10 kDa filter (Millipore).

Delipidation of HSA: -

Complete delipidation of plasmatic purified albumin was carried out using the ethanol–ether extraction method [14]. Initially, approximately 6mL of the HSA sample (30g/L) has been pre-washed with 5% aqueous methanol by using Amicon ultra-filtration units (Amicon 10 kDa, Millipore) to eliminate salts and other low-molecular-weight contaminants. The subsequent delipidation procedure has been performed using 250 µL aliquots of the albumin solution. Every aliquot has been combined with 500µL of ice-cold ethanol ether (3:1, v/v), incubated at −25°C for 2 hrs, and centrifuged after that at 6000 rpm for 30sec at 4°C. Following centrifugation, the supernatant containing the extracted lipids has been discarded. The resulting protein pellet has been resuspended in 500µL of ice-cold ethanol ether (3:2, v/v) and incubated again at −25°C for 1 hour. The final protein pellet has then been re-suspended in phosphate-buffered saline (PBS). After completion of either the procedure of albumin purification or delipidation, the protein and albumin concentrations have been quantified with the use of the bromocresol green (BCG) assay.

Results

Measurement of protein, amino, thiol, and carbonyl concentration in serum

Table 1 shown the protein concentration, amino group, thiol group and carbonyl group in 25 patients in breast cancer and 25 normal between (26-62)y expressed as mean ± SD in g\dl, mmole\ l, µmole\l and nmole\ l respectively in serum.

Table 1. Protein, amino, thiol and carbonyl concentration in control and breast cancer patients in serum

Groups (number)	Amino (mean ± SD) mmole\l	Carbonyl (mean ± SD) nmole\l	Protein (mean ± SD) g\dl	Thiol (mean ± SD) µmole\l
Patients (25)	0.3534 ± 0.03463	0.01778 ± 0.001049	8.673 ± 0.3345	0.01237 ± 0.0007452
Control (25)	0.2357 ± 0.03822	0.0196 ± 0.001169	8.157 ± 0.3151	0.0146 ± 0.001109
P value	0.8046	0.4926	0.6394	0.1112
P value summery	Ns	Ns	Ns	Ns

Ns = non-significant

Measurement of protein, amino, thiol and carbonyl concentration in purified HSA.

Table .2 shown the protein concentration, amino group, thiol group and carbonyl group in 3 patients and 3 normal in breast cancer between (26-62)y expressed as mean ± SD in g\dl, mmole\l, µmole\l and nmole\l respectively in albumin that purified by AS

Table .2 Protein, amin, thiol and carbonyl concentration in control and breast cancer patient in purified HAS.

Group	Patients	Control
Plasma volume(ml)	1	1
Conc.HSA,g\dl By lwory	2.777±0.326	3.623±0.708
Conc.HSA,g\dl By BCG	2.8±0.342	3.46±0.625
Amino group Mmol\l	0.044±0.076	0.119±0.086
Thiol group µmol\l	0.049±0.0012	0.015±0.00052
Carbonyl group nmol\l	0.690±0.258	0.011±0.005

Discussion: -

Measurement of protein, amino, thiol, and carbonyl concentration in serum

Results that have been listed in table.1 showed that most patients who have breast cancer as well as healthy individuals had amino, total protein, thiol, and carbonyl groups. The findings in table.1 showed non-significant increase ($p \geq 0.050$) in total protein concentration regarding breast cancer patients when put to comparison with the healthy subjects. The findings were in line with findings of a research that was performed by Bae *et al*. [15] that showed a TP concentration increase in patients who have brain cancer.

A study that has been carried out by Gao *et al* [16] indicated that there has been an increase in TP concentration in patients experiencing lung cancer. Serum circulates through tissues and collects proteins which have been released from their original locations as a result of various physiological events, such as tissue remodeling, cell death, and trauma. This causes a total serum protein increase, which is why the TP concentration has increased. It is still difficult to distinguish between the proteins that are actually present in the serum and those that are there after being released as a result of physiological processes. The findings of this investigation, however, were at odds with those of a research by Al-Muhtasseb [17] and Al Ardee [18], who examined serum and/or tissue proteins in patients who have breast cancer and gynecological malignancies and discovered decreases in the concentrations of TP.

The thiol content regarding breast cancer patients was found to be non-significantly higher ($p \geq 0.050$) than that of healthy participants, as listed in table-1. Those findings could suggest that antioxidant protection plays a role in the disease. Numerous systemic illnesses come from the body's oxidative stress, which causes imbalance between antioxidants as well as reactive oxygen radicals [19].

The decrease in the concentration of thiol groups can be explained by the possibility that oxidants will react with thiol to create disulfide bonds (also known as covalent bonds or disulfide bridges) [20]. This research's thiol concentration results have been consistent with those of Karaoğlanoğlu et al. [21], Chahla C. [22], and Titkte et al. [23]. They have demonstrated that thiol oxidation reduces in patients with primary osteoarthritis, type 1 diabetes, rheumatoid arthritis, and prostate biopsies.

The findings in table (1) showed that the amount of carbonyl concentration protein in patients with breast cancer was non-significantly higher ($p \geq 0.05$) than in healthy individuals. Oxidative stress, an imbalance towards pro-oxidant side regarding pro-oxidant/antioxidant homeostasis occurring in a number of human inflammations and diseases, could be the cause of such declines. These findings contradicted those of a study by Dalle *et al*. [24], who have discovered that patients with chronic kidney illness had higher carbonyl concentrations.

In the case when examining the in vitro effects regarding Fenton reaction on HSA, Khosravifarsani et al. [25] came to the same conclusion as the aforementioned researchers when calculating carbonyl concentrations. The findings in table (1) showed that there was non-significant increase ($p \geq 0.050$) in the concentration of free amino groups in patients with breast cancer in comparison with healthy individuals. The findings were in contrast to Dyachenko et al.'s studies [26], which indicated a reduction in concentration of free amine groups in DM disease in comparison with healthy subjects. This increase in concentration of free amine groups could be caused by cancer cells in basal medium.

Measurement of protein, amine, thiol, and carbonyl concentration in HSA

Due to the lack of a significant observation result in the measurement of chemical agents in the serum, the albumin was separated from the serum because it is the optimal part to determine the concentration of amine, thiol and carbonyl because the albumin is the most concentrated protein in the blood and containing multiple amino acids. Some diseases are caused by a defect in their chemical structure. this thesis selected albumin and purified from human serum by precipitate by ammonium sulphate and delipidation by ethanol – ether extraction method and measured the albumin concentrated by BCG. Table (2) revealed the result of patients with breast cancer and control subject that the albumin concentration is a significant increase in control subject compared as patients and the amine is cleared a significant increase in control compared as patients with breast cancer This shows that the glycation actually occur in breast cancer patients because of the lack of free amino groups because of the free amino group bonded with sugar carbonyl and its formed Amadori product.Carbonyl group is an important group in albumin its result revealed in the table (2) that explains carbonyl group concentration is significant increase in patient with breast cancer compared as control subject and this result agreement with Khosravifarsani *et al* studies [25]. Patients' inflammation and

oxidative stress could be the cause of the increase in carbonyl concentration [(24]. Additionally, the thiol group result showed in table (2) that the patient's thiol concentration was significantly higher than that of control subject. This outcome is consistent with research by Khosravifarsani et al. [25], who assessed the impact regarding in vitro Fenton reaction on HSA and verified the antioxidant activity on HSA.

ETHICAL APPROVAL

The research protocol was approved by the Ethical Research Committee of the Kirkuk Health Authority. We declare that the Helsinki Declaration had been followed in the study.

INFORMED CONSENT

Participants were aware of the purpose of the study and provided informed consent prior to the participations.

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Data Availability: The data available on request

All authors reviewed the results and approved the final version of the manuscript.

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The authors utilized DeepSeek-V3 (DeepSeek AI Inc.) for assistance in structuring the statistical analysis pipeline, generating code, and interpreting the output. All analytical decisions were made by, and the final responsibility for the analysis lies with, the human authors. The authors defined the research questions, designed the analytical strategy, prompted the AI, and critically reviewed, validated, and interpreted all output. The final manuscript was written and approved by the human authors.

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