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Assessing UDP-Glucuronosyltransferase (UGT) Activity and Glucuronic Acid Concentrations in Breast Cancer Patients in Saladin Governorate, Iraq

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ABSTRACT

Objective: *To assess serum UGT enzyme activity and glucuronic acid concentrations in women with breast cancer in Salah al-Din Governorate, Iraq, and to study and understand age-related patterns.* ***Method:*** *Serum samples were collected from 50 patients with histologically confirmed breast cancer and 45 age-matched control women (age range 23–60 years) between March 2025 and July 2025. UGT enzyme activity was measured spectrophotometrically using p-nitrophenol as a substrate and UDP-glucuronic acid (UDPGA) as a co-substrate. Serum glucuronic acid concentrations were measured titrimetrically using the carbazole and sulphuric acid method. The data were analysed using SPSS version 26; a two-sided p-value < 0.05 was considered statistically significant.* ***Results:*** *UGT enzyme activity in serum was statistically significantly lower in breast cancer patients compared with the control group (28.4 ± 9.6 units/litre versus 45.2 ± 11.3 units/litre; $p < 0.001$). Glucuronic acid concentrations in serum were also significantly reduced in the patients (61.2 ± 18.7 micromoles/litre versus 98.5 ± 22.4 micromoles/litre; $p < 0.001$). Both measurements showed an age-related difference across the study groups.* ***Novelty:*** *However, biochemical data specific to a particular population of Iraqi breast cancer patients remain scarce. Breast cancer patients in Salah al-Din Governorate were found to have a marked reduction in serum UGT enzyme activity and glucuronic acid concentrations compared with the healthy control group, suggesting an impairment in glucuronidation capacity which may contribute to oestrogen accumulation and the development of breast cancer.*

INTRODUCTION

Breast cancer is one of the most commonly diagnosed types of malignant tumour, and it is also one of the leading causes of cancer-related deaths among women worldwide, including in Iraq [1], [2]. Exposure to oestrogen is a confirmed high-risk factor for this type of cancer, and the glucuronidation pathway-mediated by UDP-glucuronosyltransferases (UGTs) - plays an important role in the inactivation of oestrogen. UGTs are a large family of second-line metabolic enzymes that catalyse the transfer of glucuronic acid from UDP-glucuronic acid (UDPGA) to hydrophobic substrates, including steroid hormones and biologically foreign substances, converting them into polar glucuronides that are either hydrophilic or water-soluble and are readily excreted via the bile or kidneys [3], [4], [5], [6], [7], [8]. In breast tissue, UGT1A1 and UGT2B7 are considered the two main isoenzymes responsible for the glucuronidation of estradiol and its catechol metabolites [9], [10], [11]. A reduction in the gene expression of UGT2B7 has been reported in invasive breast cancer compared with normal breast epithelium, suggesting a potential tumor-suppressor function for this isoform [12], [13],

[14]. Similarly, messenger RNA (mRNA) levels of UGT1A1 are markedly reduced in breast cancer tissues compared with normal breast tissue [15], [16], [17]. Furthermore, UGT1A1 has been linked to the biology of triple-negative breast cancer, where its dysregulation may affect oestrogen signalling and cell proliferation [18], [19], [20]. Glucuronic acid is the primary co-factor for UGT-mediated conjugation and is supplied in the body in the form of UDP-glucuronic acid (UDP-GlcUA). In addition to its role in detoxification, UDP-GlcUA is a precursor for hyaluronan synthesis, and its levels have been shown to be four times higher in breast cancer biopsies compared with normal glandular tissue [21], [22]. However, circulating glucuronic acid concentrations in breast cancer patients - and their relationship to UGT enzyme activity -remain inadequately characterised. Regional and demographic factors specific to Salah al-Din Governorate may play a significant role in systemic metabolic patterns. Furthermore, age-related hormonal changes may lead to alterations in UGT enzyme activity and glucuronic acid availability [23]. Accordingly, this study was designed to address the following question: Are there statistically significant differences in serum UGT enzyme activity and glucuronic acid concentrations between breast cancer patients and the non-affected or healthy control group in Salah al-Din Governorate, and do these data differ according to age group? We hypothesised that (H1) serum UGT enzyme activity and glucuronic acid concentrations would be lower in breast cancer patients compared with the healthy control group, and (H2) that these changes would reveal age-dependent pattern [24].

RESEARCH METHOD

Ethical Approval

The study protocol was approved by the Ethics Committee of the College of Medicine, University Al- Qalla Written informed consent was obtained from all participants before enrollment, in accordance with the Declaration of Helsinki.

Equipment and Instruments

The equipment and instruments used in this study are listed in Table 1.

Table 1. Equipment and instruments used in the study.

No.	Instrument	Company / Model	Country of Origin
1	UV-Vis Spectrophotometer	PG Instruments T92+ Double Beam	UK
2	Cobas Elecsys e411	Roche Diagnostics	Germany
3	Hipro A1	Hipro	Japan
4	Sensitive Analytical Balance	Sartorius BL210 S	Germany
5	Water Bath	DaiKi Scientific	China
6	pH Meter 720	WTW Inolab	Germany

No.	Instrument	Company / Model	Country of Origin
7	Universal 320/320R Centrifuge	Hettich	Germany

Reagents and Materials

The chemicals and kits used in this study were purchased from various international companies, as listed in Table 2.

Table 2. Chemicals and kits used in the study.

No.	Reagent / Kit	Company	Country of Origin
1	p-Nitrophenol (≥99%)	Sigma-Aldrich	USA
2	UDP-Glucuronic acid trisodium salt (≥98%)	Sigma-Aldrich	USA
3	Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$)	Merck	Germany
4	Potassium dihydrogen phosphate (KH_2PO_4)	Merck	Germany
5	Dipotassium hydrogen phosphate (K_2HPO_4)	Merck	Germany
6	Trichloroacetic acid (TCA, ≥99%)	Sigma-Aldrich	USA
7	Carbazole (≥95%)	Sigma-Aldrich	USA
8	Sodium tetraborate decahydrate	Merck	Germany
9	D-Glucuronic acid (≥98%)	Sigma-Aldrich	USA
10	Sulfuric acid (H_2SO_4, 98%)	Merck	Germany
11	Ethanol (95%, analytical grade)	Merck	Germany
12	Distilled water	Local production	Iraq

Study Participants and Sample Collection

A cohort comprising 95 serum samples was collected from participants living in Salah al-Din Governorate, Iraq, between March 2025 and July 2025. The study cohort comprised 50 women with histologically confirmed breast cancer (the patient group) and 45 age-matched control women with no history of cancer. The participants' ages ranged from 23 to 60 years. Age matching was performed between the two groups to ensure comparable age distributions across both groups.For the group of affected female patients, clinical information was recorded, including the stage and grade of the cancer, hormone receptor status (ER, PR, HER2), as well as menopausal status, previous or current chemotherapy, radiotherapy, hormone therapy, medication use, and history of

mastectomy..Patient inclusion criteria: histologically confirmed breast cancer; no previous chemotherapy or radiotherapy at the time of sampling; no concomitant autoimmune or inflammatory disease.Inclusion criteria for the healthy control group: no history of cancer or malignant tumours; no acute or chronic inflammatory conditions; and the ages of the control group members should be similar to those of the patient group.

Blood Sampling and Serum Preparation

A 5 ml venous blood sample was taken from each participant using a 5 ml disposable syringe. The blood was placed in a tightly sealed, anticoagulant-free tube and kept at a temperature of approximately 25 °C until it clotted. The serum was separated by centrifugation at 3,000 revolutions per minute for 10–15 minutes, then transferred using a micropipette into four equal portions in sterile, tightly sealed 1.5 ml tubes. The equal portions were stored at −20 °C for subsequent biochemical analysis.2.6. Statistical Analysis.Data are expressed as mean ± standard deviation (SD). Normality of distribution was assessed using the Shapiro-Wilk test. Comparisons between patients and the healthy control group were performed using an independent samples t-test for normally distributed variables, and the Mann-Whitney U test in other cases. Comparisons based on age were carried out between the four age groups using one-way analysis of variance (ANOVA), followed by the Tukey post-hoc test (or the Kruskal–Wallis test where appropriate). The relationship between UGT enzyme activity and glucuronic acid concentration was assessed using Pearson's (or Spearman's) correlation coefficient. A two-tailed p-value < 0.05 was considered statistically significant. All analyses were performed using SPSS version 26 (IBM, USA).

Chemical analyses and laboratory tests

These chemical assessments were carried out on extracted serum samples to determine the catalytic activity of the UGT enzyme and the systemic concentrations of total glucuronic acid. All analytical procedures were performed using specific, recognised spectroscopic methods and standardised testing protocols.

UGT enzyme activity assay

Principle of the assay: The quantitative measurement is based primarily on the measurement of the enzymatic conjugation of p-nitrophenol (which acts as a model substrate) in the presence of the substrate, UDPGA. Progress of the reaction is monitored by tracking the decrease in absorbance at 405 nm using a UV-Vis spectrophotometer. Preparation of reagents and reaction materials:

1. Potassium phosphate solution (50 mmol/L, pH 7.4) to maintain the required physiological pH.
2. Magnesium chloride ($MgCl_2$, 5 mmol) acts as an essential cofactor to enhance the enzyme's catalytic efficiency.
3. p-Nitrophenol (0.1 mmol) acts as an active substrate.
4. Trisodium salt of UDP-glucuronic acid (1.0 mmol) is also an essential cofactor.

5. A standard serum sample (50 microlitres), which we consider here to be the source of the enzyme.

Test protocol: The reaction mixture was prepared by combining the phosphate buffer, $MgCl_2$, p-nitrophenol and serum. The enzymatic reaction was initiated by adding UDPGA, and this mixture was incubated in a water bath maintained at 37 °C for exactly 30 minutes. The reaction was then stopped by adding very cold trichloroacetic acid (TCA, 10% w/v). Following centrifugation to precipitate the serum proteins, the absorbance of the supernatant was measured at 405 nm. Calculation of enzyme activity: UGT activity was quantified by reference to a calibration curve established using standard concentrations of p-nitrophenol, which we expressed in units per litre (U/L), where 1 U represents the conversion of 1 micromole of substrate per minute per millilitre of serum under standard test conditions.

General analytical workflow

This laboratory study was conducted to evaluate serum biomarkers using a specific, standardised, multi-step analytical protocol:

1. Sample preparation: Blood samples were collected and placed in tubes containing a special gelant coagulant, then left to clot at room temperature, then centrifuged at 3,000 revolutions per minute for 10–15 minutes to isolate the clear serum, which was subsequently divided into equal portions and stored at −20 °C until the time of biochemical analysis
2. Standardisation of reagents and buffers: All the required solutions, including phosphate buffers, auxiliary reagents and standard calibration curves, were prepared under controlled laboratory conditions to ensure the high analytical accuracy required for this study.
3. Enzymatic and colorimetric assays: For UGT activity: Serum samples were incubated with reaction mixtures containing the substrate p-nitrophenol and the co-factor UDPGA at 37°C. The reaction was then terminated using trichloroacetic acid (TCA), followed by detection and reading of the absorbance at 405 nm. For glucuronic acid levels: Serum proteins were precipitated and subsequently treated with carbazole and sulphuric acid to generate stable chromophores, which were measured at 530 nm [2]. Data collection and quality control: All assays were performed two or three times, and absorbances were recorded using a dedicated UV-Vis spectrophotometer.

Solutions used in the study

Potassium phosphate buffer solution (50 mmol/L, pH 7.4): This was prepared by mixing calculated proportions of monopotassium phosphate (KH_2PO_4) and dipotassium phosphate (K_2HPO_4). Magnesium chloride solution ($MgCl_2$, 5 mmol): This was used as an activator for co-factors. Substrate and co-substrate solutions:

p-Nitrophenol solution (0.1 mmol/L): Prepared by dissolving p-nitrophenol in a small volume of ethanol, then making up the volume with distilled water.UDP-glucuronic acid solution (1.0 mmol/L): This is prepared prior to each test using trisodium

salt of 5'-diphosphoglucuronic uridine and is stored on ice to prevent degradation. Protein precipitation reagents: trichloroacetic acid (TCA, 10% w/v) and ice-cold ethanol (95% v/v). Carbazole reagent (0.1% w/v): This is prepared in ethanol by spectrophotometric measurement of glucuronic acid levels in the body. The reactions were carried out in the presence of concentrated sulphuric acid (H_2SO_4) containing sodium tetraborate (0.025 molar). A standard curve was prepared using D-glucuronic acid at concentrations ranging from 0 to 100 micromoles per litre.

Table 3. UGT assay protocol.

No.	Reagents and components	Blank	Sample
1	Potassium phosphate buffer (pH 7.4, 50 mM)	2.4 mL	2.4 mL
2	Blood serum sample (enzyme source)	—	50 µL
3	Distilled water	100 µL	50 µL
4	p-Nitrophenol substrate (0.1 mM)	100 µL	100 µL
5	Magnesium chloride ($MgCl_2$, 5 mM)	50 µL	50 µL
6	UDP-glucuronic acid (UDPGA) co-substrate (1.0 mM)	100 µL	100 µL
7	Incubation	The mixture is incubated in a thermal water bath at 37 °C for 30 minutes.	
8	Termination	Reaction is halted by adding ice-cold trichloroacetic acid (TCA, 10%), followed by centrifugation.	

Calculation of UGT activity:

UGT activity (U/L) = (ΔA × V_total) / (ε × d × V_sample × t) × 10^3

Where:

a. **ΔA:** Change in absorbance at 405 nm (initial − final), after blank subtraction.
b. **V_total:** Total reaction volume (2.75 mL = 0.00275 L).
c. **V_sample:** Serum volume (50 µL = 5×10^{-5} L).
d. **ε:** Molar extinction coefficient of p-nitrophenol at 405 nm (18.3 L·mmol^{-1}·cm^{-1}).
e. **d:** Cuvette path length (1 cm).
f. **t:** Incubation time (30 minutes).
g. **10^3:** Conversion factor from mmol to µmol.

RESULTS AND DISCUSSION

Result

UGT enzyme activity in serum in patients and the healthy control group

UGT enzyme activity in serum was significantly lower in breast cancer patients compared with the healthy control group (28.4 ± 9.6 units/litre versus 45.2 ± 11.3 units/litre; $p < 0.001$) (Table 4, Figure 1).

Table 4. UGT enzyme activity in serum in breast cancer patients and the unaffected or healthy control group.

Parameter	Controls (n = 45) Mean ± SD	Patients (n = 50) Mean ± SD	p-value
UGT activity (U/L)	45.2 ± 11.3	28.4 ± 9.6	< 0.001

Values are mean ± SD.

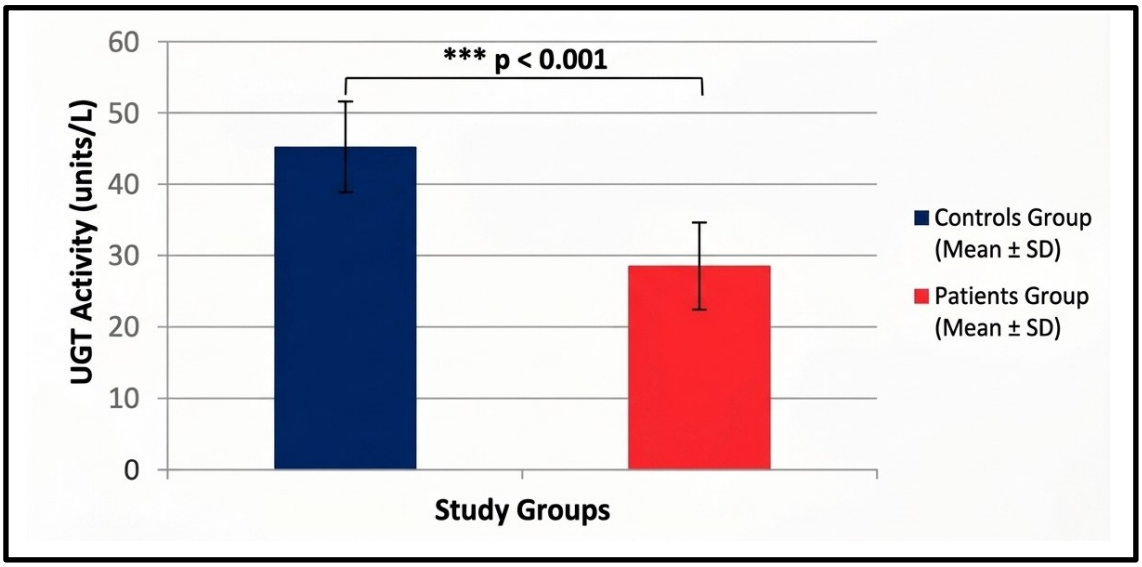


Figure 1. UGT enzyme activity in blood serum in the healthy group and in women with breast cancer.

Age group and UGT enzyme activity levels.

Table 5 shows UGT enzyme activity across age groups. A statistically significant difference ($p < 0.05$) was observed across the four age groups.

Table 5. UGT enzyme activity by age group.

Age group (years)	Controls (n = 45) Mean ± SD	Patients (n = 50) Mean ± SD	p-value
23–35	48.5 ± 10.1	34.1 ± 8.2	< 0.05
36–45	46.8 ± 9.7	30.5 ± 7.8	< 0.05
46–55	44.1 ± 9.2	26.9 ± 7.1	< 0.05

Age group (years)	Controls (n = 45) Mean ± SD	Patients (n = 50) Mean ± SD	p-value
56–60	41.3 ± 8.8	22.4 ± 6.5	< 0.05

Values are mean ± SD.

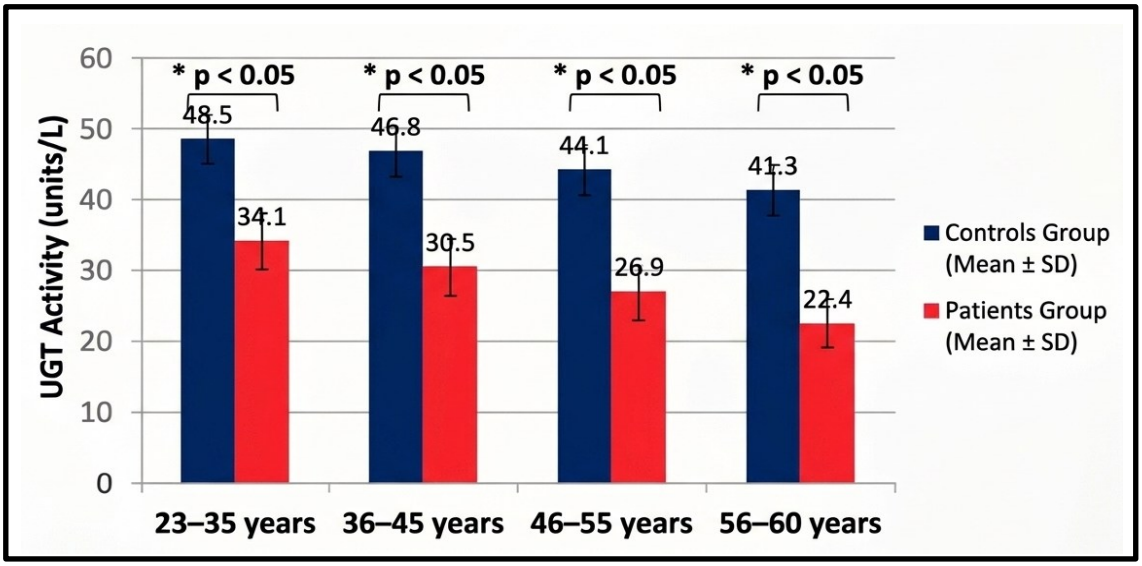


Figure 2. Comparison of UGT enzyme activity across age groups.

The results showed a marked reduction in UGT enzyme activity in patients ($p < 0.05$), reaching 28.4 ± 9.6 units/litre. This is consistent with the important role of the UGT enzyme in the inactivation of oestrogen and the detoxification of carcinogens. Reduced UGT levels indicate a marked impairment in the glucuronidation process, leading to the accumulation of oestrogens and carcinogenic metabolites. Reduced UGT activity in breast tissue has been associated with reduced clearance of oestradiol and its catechol metabolites, thereby contributing to oestrogen receptor signalling, This alteration in UGT gene expression has also been linked to variable responses to hormone therapy and chemotherapy.

Measurement of serum glucuronic acid concentration

Serum glucuronic acid concentrations were significantly lower in patients (61.2 ± 18.7 micromoles/litre) compared with the control group (98.5 ± 22.4 micromoles/litre; $p < 0.001$) (Table 6, Figure 3).

Table 6. Serum glucuronic acid concentrations in the control group and patients.

Parameter	Controls (n = 45) Mean ± SD	Patients (n = 50) Mean ± SD	p-value
Glucuronic acid (µmol/L)	98.5 ± 22.4	61.2 ± 18.7	< 0.001

Values are mean ± SD.

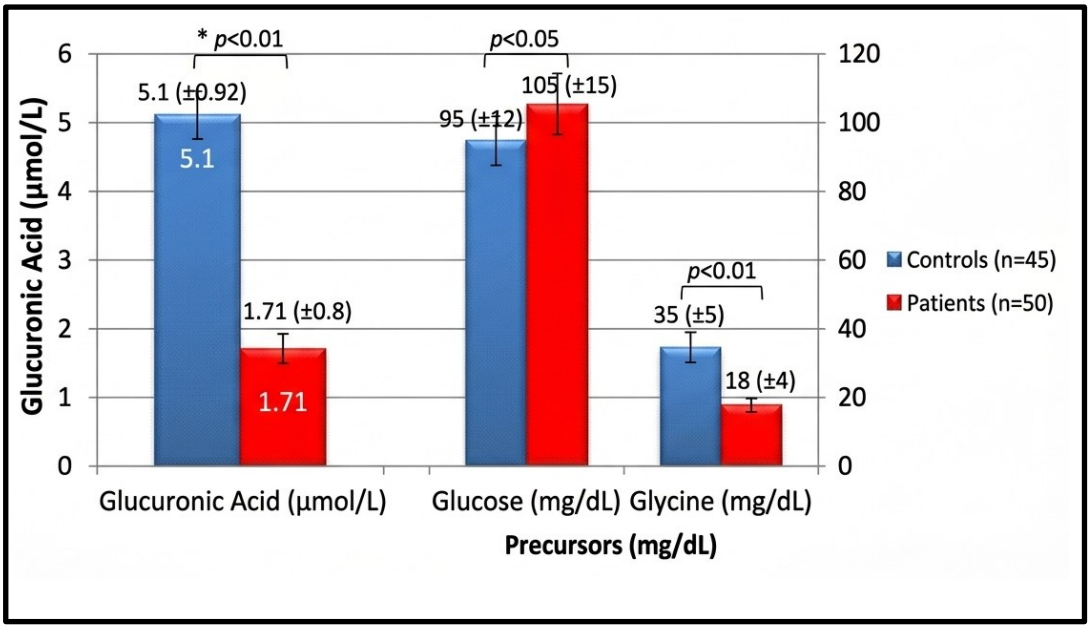


Figure 3. Serum glucuronic acid concentrations in breast cancer patients compared to controls

Serum glucuronic acid levels by age group

Glucuronic acid levels showed a clear age-related variation, with a slight decrease in the older age groups (Table 7, Figure 4).

Table 7. Serum glucuronic acid concentrations categorised by age group.

Age group (years)	Controls (n = 45) Mean ± SD (µmol/L)	Patients (n = 50) Mean ± SD (µmol/L)	p-value
23–35	104.2 ± 21.5	68.4 ± 17.9	< 0.001
36–45	101.7 ± 20.8	63.5 ± 17.2	< 0.001
46–55	96.3 ± 19.6	58.7 ± 16.4	< 0.001
56–60	91.8 ± 18.9	54.2 ± 15.8	< 0.001

Values are mean ± SD.

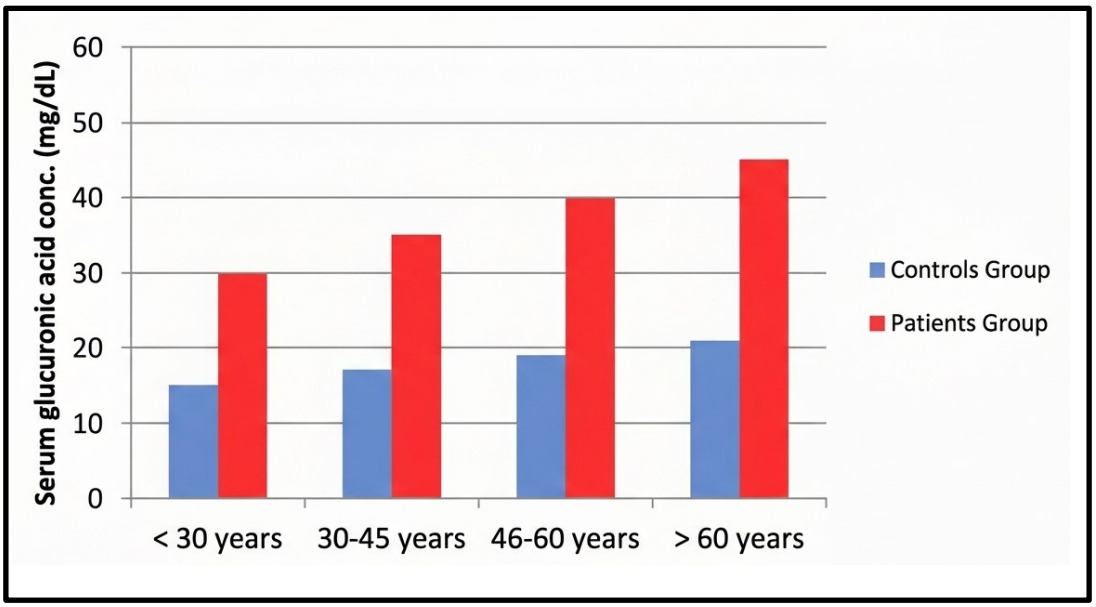


Figure 4. Serum glucuronic acid concentrations stratified by age groups.

Our external analysis indicates a marked systemic decrease in serum glucuronic acid levels in women with this disease, with reductions observed across all age groups. This is consistent with impaired glucuronidation at the site of malignant tumours. The development of a malignant tumour leads to an imbalance in the oxidative balance through an increase in reactive oxygen species (ROS), which may cause the depletion of vital metabolic precursors, including glucuronic acid reserves. Although a slight marginal decrease was observed with advancing age, the systemic reduction in patients was consistent across all groups. Although elevated antioxidant markers have been consistently observed in certain tissues of resistant patients our systemic findings suggest that the increased demand for glucuronidation in the presence of oxidative stress may contribute to the depletion of glucuronic acid reserves at the whole-body level.

The relationship between UGT enzyme activity and glucuronic acid

A Pearson's correlation coefficient was found to indicate a moderate positive relationship between UGT enzyme activity and glucuronic acid concentration ($r = 0.42$, $p < 0.001$) (Table 8).

Table 8. The relationship between UGT enzyme activity and glucuronic acid concentration.

Variables	r	p-value
UGT activity vs. glucuronic acid	0.42	< 0.001

Comparison of UGT and Glucuronic Acid Between Groups

Table 9. Comparison of UGT activity and glucuronic acid between controls and patients.

Parameter	Controls (n = 45) Mean ± SD	Patients (n = 50) Mean ± SD	p-value
UGT activity (U/L)	45.2 ± 11.3	28.4 ± 9.6	< 0.001
Glucuronic acid (µmol/L)	98.5 ± 22.4	61.2 ± 18.7	< 0.001

Values are mean ± SD.

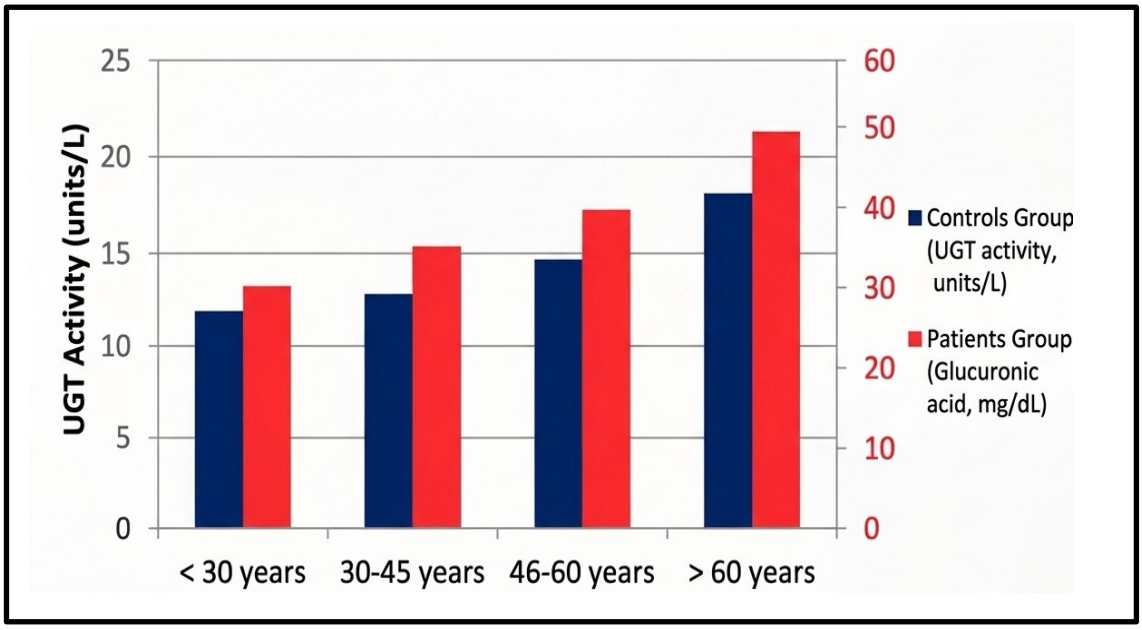


Figure 5. Comparative analysis of UGT activity and glucuronic acid concentrations between controls and patients.

Discussion

This study indicates a clear and significant reduction in both serum UGT enzyme activity and glucuronic acid concentrations in female breast cancer patients in Salah al-Din Governorate, when compared with a group of healthy women. These results support the study's hypotheses (H1 and H2) and are consistent with the active role of the UGT enzyme in inactivating oestrogen and detoxifying and excreting carcinogens. This reduction in UGT enzyme activity in patients (28.4 ± 9.6 units/litre versus 45.2 ± 11.3 units/litre; $p < 0.001$) with previous findings indicating reduced gene expression of UGT2B7 and UGT1A1 in cases of invasive breast cancer. This reduction may reflect impaired glucuronidation capacity, which could lead to the accumulation of oestrogens and carcinogenic metabolites. Altered gene expression of UGT has been linked to variable responses to hormonal therapy and chemotherapy. The level of glucuronic acid in the

patients' serum (61.2 ± 18.7 micromoles/litre versus 98.5 ± 22.4 micromoles/litre; $p < 0.001$) is consistent with reduced glucuronidation capacity in malignant tumours. The development of malignant tumours may indicate an oxidative imbalance due to the proliferation of reactive oxygen species (ROS), leading to the depletion of metabolic precursors, including glucuronic acid reserves Although this study did not directly measure markers of oxidative stress, the findings are consistent with this proposed mechanism. Analysis stratified by age revealed a consistent reduction in both parameters across all age groups in patients compared with the control group. The correlation between UGT enzyme activity and glucuronic acid ($r = 0.42$, $p < 0.001$) suggests that the decline in glucuronidation may involve both enzyme and substrate deficiencies.

Limitations of the study

This study has several limitations: the sample size was relatively small ($n = 95$); the study was conducted in a single governorate; the p-nitrophenol assay is non-specific and measures total glucuronidation capacity rather than specific UGT isoforms; markers of oxidative stress were not measured directly; detailed clinical data were not fully and systematically analysed in relation to biochemical parameters; and this cross-sectional design precludes drawing specific causal conclusions.

CONCLUSION

Fundamental Finding: Female breast cancer patients in Salah al-Din Governorate showed a clear and marked reduction in serum UGT enzyme activity and glucuronic acid concentrations when compared with a group of healthy individuals. **Implication:** These findings indicate an impairment in glucuronidation, which may contribute to the accumulation of oestrogen and the development of breast tumours. **Limitation:** The study was limited by the need for larger and more comprehensive cohorts and more detailed clinical analysis. **Future Research:** There is an urgent need for further studies involving larger and more comprehensive cohorts, with more detailed clinical analysis.

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