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Assessment of bone profile and Liver Enzymes among Triple negative versus Hormone Receptor Positive Sudanese Breast Cancer Patients

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Abstract:

Background: Some serum biochemical parameters may be used as prognostic markers of breast cancer; these are studied broadly, but not studied yet in Sudan.

Objective: In this study we aimed to assess calcium, phosphate, Alkaline phosphatase (ALP), Gamma glutamyle trans peptidase (GGT), and Lactate dehydrogenase (LDH) among hormone breast cancer subtypes.

Methods: In a comparative cross-sectional 178 BC patients, ages ranged from 28 to 95 were randomly selected. Bone minerals and liver enzymes were measured using Mindray-BS-480 Clinical Chemistry Analyzer.

Results: In total, 30.3% were triple negative, were 69.7 % hormone receptor positive, and52.8 % out of total was late stage. T. test analyses show significant difference in GGT between triple negative and hormone receptor positive also between late stage and early stage (P = 0.017 and 0.016, and 0.018), respectively.

Conclusion: Hormone receptor positive and late stage BC patients have increased GGT activity.

Key words: Liver enzymes, Bone, Triple negative, Hormone, Receptor

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Introduction

Breast cancer (BC) is the most prevalent malignancy and a significant factor in both morbidity and mortality among women. More than 90% of mortalities are due to metastasis(1). Since Sudan lacks a population-based national cancer registry, hospital case series are typically used to estimate cancer rates in Sudan (2). According to data from the Khartoum State Malignancy Registry for the years 2009 to 2010, BC was the most prevalent cancer among Sudanese women. According to data from the National Cancer Institute—University of Gezira (NCI-UG), 34% of female cancer patients in 2017 had BC (2). In spite of 20% to 30% of people experience its recurrence in distant locations, researchers observed that, the death rate was decreased during the past ten years, which attributed to the early detection and intervention (3).

BC molecular subtypes include: (luminal A, luminal B, Human epidermal growth factor

receptor 2 [HER2]-enriched and basal-like) based on the status of estrogen receptor (ER), progesterone receptor (PR), and HER2 (3, 4). Besides their different ability to metastasize to distant organs, these subtypes possess different pathways to the preferred metastatic sites, as well as differing survival responses after relapse (5). Several factors can increase the likelihood of developing a metastatic breast cancer MBC, including age, race, ethnicity, endogenous hormones, menopause, smoking, first-degree relatives, number of metastatic sites, length of breastfeeding, mutations, and the grade and size of the initial tumor (6).

Bone is the most frequent location for both metastasis and the first distant recurrence (3,7,8). The three most typical signs of bone metastases are severe osteopenia, pathologic fractures, and nerve compression. However patients continue to present with no clinical symptoms and miss detection at the diagnosis, therefore the bone metastasis associated with worse outcomes and lower quality of life (9, 10).

Furthermore, the rate-limiting factor for patient survival is hepatic metastases, which involve in about half of all cases (11).The early detection of liver metastases is essential for prompt treatment to improves outcomes. Fine-needle aspiration cytology (FNAC) of hepatic lesions has become a common diagnostic tool (11). Serological examination is used to monitor metastatic disease during treatment with low sensitivity and specificity. Liver function test that includes estimation of enzymes activity, showed poor results in 92% most cases, the glutamyl transferase (GGT) and alkaline phosphatase (ALP) are exhibit higher levels (11). The alteration in the levels of serum bone minerals is also noticed in BC patients. The hypercalcemia in BC has been attributed in part to osteolytic bone metastases (12). This study hypothesis that, bone minerals (Calcium & Phosphate) and liver enzymes {alanine aminotransferase ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH)} are useful for prognosis and screening of bone and liver metastasis in breast cancer patients.

Study populations

Eighty-ninediagnosed adult female patients with breast cancer who were presented to Radiation and Isotope centre at Khartoum (RICK) a period of consecutive 24 months from December 2018 till December 2020 were recruited into this cross-sectional study after informed consent. The clinco-pathological data (stage, number of treatment doses and hormone receptor status) and demographic data (age, BMI and marital status) were recorded from the pathology reports of the patients. Patients diagnosed with bone disease, asthma, autoimmune disease and other types of cancer were excluded, all participants were candidates for chemotherapy with or without adjuvant. The study populations were classified in to groups according to hormone receptor status (Triple negative, hormone receptor positive group) and stage (Early, Late group). These groups were age and BMI matched.

Ethics Approval: The study protocol was approved by the Scientific Research Committee and Institutional Review Board (AL-Neelain University) IRB serial No: NU-IRB-17-10-10-106 and National Health Research Ethics Committee – Sudan. All participants were informed by the aim of the study.

Methods

Under aseptic condition 2.5 ml blood was withdrawn on lithium heparin container, plasma was obtained after centrifugation at 3000 rpm and kept at -20°.

Measurement of serum parameters: Parameters were measured using Mindray-BS-480 Clinical Chemistry Analyzer-Shenzhen Mindray Bio-Medical Electronics Co., Ltd.

Statistical analysis: Data analysed using Statistical Package for Social Sciences (SPSS Inc, ver. 23, IL-Chicago- USA). The descriptive results were presented as percentages mean ± SD. Independent t.test was employed to compare between two groups. *P value* of ≤0.05 was considered significant.

Results

One hundred and seventy-eight breast cancer female's patients**;** mean age 48 (range; 28 - 95) years, were included in this study. The descriptive results showed that the percentage of triple negative is30.3% in Sudanese BC while the hormone receptors positive is 69.7%; and the early stage was 47.2 % while 52.8 % was late stage(**Table 1**).The serum level in tables 2, 3 and 4 were analyzed using independent sample *t*-test. GGT was significant different between hormone receptor groups and Stage groups (*P value* 0.017, 0.016, and 0.018) (table 2, 3 and 4). While there was no significant difference in Ca, Pi, ALP, and LDH (*P value* 0.518, 0.347, 0.077, and 0.300) between hormone receptor groups (**Table 2**); also Ca, Pi, ALP, and LDH showed no significant difference between stage groups (*P value* 0.518, 0.347, 0.077, 0.300) and (0.459, 0.500, 0.129, 0.810) triple negative and hormone receptor positive respectively (**Table 3**& 4).

Table (5) showed significant positive correlation between cancer stage, Ca level and GGT activity while the other biochemical's showed no correlation. Also there is no correlation between age and all investigated parameters.

Table (1): Characteristics of Breast Cancer Patients

Percentage (%)	Frequency	Class
30.3%	54	Triple Negative
69.7%	124	Hormone. R Positive
47.2 %	84	**Early stage**
52.8 %	94	**Late stage**

Table (2): Differnces in bone profile and liver Enzymes between hormone receptor positive and triple negative BC patients

PARAMETER	Number	Mean ± SD	*P value*
CaTriple negative	54	8.5 ± 1.11	0.518
Hormone.R positive	124	8.6 ± 1.7	
PiTriple negative	54	3.9 ± 0.67	0.347
Hormone.Rpositive	124	3.8 ± 0.73	
ALP Triple negative	54	41 ± 42	0.077
Hormone.R positive	124	55 ± 46	
GGTTriple negative	54	25 ± 13	0.017
Hormone.Rpositive	124	46 ± 30	
LDH Triple negative	54	230 ± 114	0.300
Hormone.R positive	124	201 ± 181	

Ca = calcium, Pi = phosphate, ALP = alkaline phosphatase, GGT = gama glutamayl transpeptidase, LDH = lactate dehydrogenase.

Table (3): Comparison of Bone profile and liver Enzymes between early and late stage Triple negative BC patients

PARAMETER	Number	Mean ± SD	P value
Ca Early	24	8.3 ± 1.4	0.459
Late	30	8.6 ± 0.66	
Pi Early	24	3.8 ± 0.83	0.500
Late	30	3.9 ± 0.50	
ALP Early	24	33 ± 20	0.129
Late	30	51 ± 12	
GGT Early	24	21 ± 16	0.016
Late	30	30 ± 9.6	
LDH Early	24	226 ± 97	0.810
Late	30	233 ± 126	

Ca = calcium, Pi = phosphate, ALP = alkaline phosphatase, GGT = gama glutamayl transpeptidase, LDH = lactate dehydrogenase.

Table (4): Comparison of Bone profile and liver Enzymes between early and late stage Hormone receptor positive BC patients

PARAMETER	Number	Mean ± SD	P value
Ca Early	56	8.8 ± 0.7	0.541
Late	60	8.5 ± 2.2	
Pi Early	56	3.7 ± 0.73	0.803
Late	60	3.8 ± 0.74	
ALP Early	56	57 ± 24	0.570
Late	60	52 ± 37	
GGT Early	56	31 ± 20	0.018
Late	60	57 ± 25	
LDH Early	56	168 ± 82	0.047
Late	60	234 ± 117	

Ca = calcium, Pi = phosphate, ALP = alkaline phosphatase, GGT = gama glutamayl transpeptidase, LDH = lactate dehydrogenase.

Table (5): Correlation between Bone profile and liver Enzymes and cancer stage among BC patients

		Stage	Age
Ca	Pearson's correlation (r)	0.311**	0.075
	Sig (*P.value*)	0.000	0.166
	N	178	178
Pi	Pearson's correlation (r)	0.050	-0.065
	Sig (*P.value*)	0.270	0.199
	N	178	178
ALP	Pearson's correlation (r)	0.069	0.005
	Sig (*P.value*)	0.199	0.475
	N		178
GGT	Pearson's correlation (r)	0.263**	-0.055
	Sig (*P.value*)	0.000	0.239
	N	178	178
LDH	Pearson's correlation (r)	0.033	-0.114
	Sig (*P.value*)	0.342	0.060
	N		178

Ca = calcium, Pi = phosphate, ALP = alkaline phosphatase, GGT = gama glutamayl transpeptidase, LDH = lactate dehydrogenase. *P.value* less than 0.05 is considerd significant

Discussion:

In clinical practice, ALT, AST, GGT, ALP, and LDH are five serum biochemical markers that have been examined. All concern liver function (13,14), however, the latter three have also being looked into potential tumor invasion markers (15).

The present study detected that GGT was significantly higher in hormone receptor positive when compared to triple negative BC.This might be owing to Long-term estrogen inhibition can cause fatty liver disease (non-alcoholic fatty liver disease; NAFLD) (16); and Serum GGT activity is a sensitive marker of NAFLD (17)

Also this study observed significant increase in GGT in late stage compared to early stage. This is explicable by the function of GGT in the re-synthesis of GSH, which forms the cornerstone of cellular resistance to a number of electrophilic chemicals and is assumed to be implicated in the cellular defense mechanism (15). Chemotherapeutic resistance and a worse linked to increasing GGT may be explained by increased GSH, which metabolizes toxic compounds, including chemotherapy drugs. (18, 19). Additionally, it has been noted that GGT-mediated metabolism of GSH may result in a low but persistent amount of oxidants, which activate defense mechanisms. In fact, numerous studies showed that cells that overexpress GGT are less sensitive to a number of cytotoxic medicines (18).

Also this studty showed that LDH in Late stage hormone receptor positive patients was significant higher than in early stage hormone reeptor positiveBC patients. This can be explained by how LDH regulates the conversion of glucose to lactic acid, which explains why higher levels of LDH in cancer cells were linked to worse prognosis and higher rates of cell

proliferation (20, 21). Similar to earlier research, which showed that elevated serum LDH levels were frequently observed in cancer patients and were associated with a poor prognosis and therapy resistance (20).

In this study the correlation results showed significant positive correlation between BC stage, calcium level and GGT activity; the reason for high GGT activity was explained before. While the reason of hypercalciumcemia with stage is related to the pathological bone resoption caused by cytokines that stimulate osteoclast activity; one of these cytokines is parathyroid hormone related protein (PTHrP) (22). In other hand the remaining parameters (Pi, ALP, LDH) showed no correlation with stage.

One of the limitation of this study it was a cross sectional hospital‐based study. Second, we did not measure the bone density, ALP and LDH isoenzymes which might help in the explanation of the results.

Conclusion

The data of present study suggests that, Hormone positive and late stage BC patients have increased GGT activity. Therefore, GGT activity might use as metastatic and prognostic markerfor BC**.**

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