Al-Nahrain Journal of Science
ANJS, Vol.28 (4), December, 2025, pp. 55-66

CXCL12, CA15-3, and Liver Function Tests as Predictors of Liver Metastasis in Breast Cancer

Baqer A. Altimmime[1,*], Farah A. Rashid[2], Yaala Saady Raof[3], Omran Mansour[4]

[1]College of Pharmacy, Al-Nahrain University, Baghdad, Iraq.
[2]Department of Chemistry, College of Science, Al-Nahrain University, Jadiriya, Baghdad, Iraq.
[3]Al-Amal National Hospital for Cancer Patients, Baghdad, Iraq.
[4]Department of Environmental Science, Faculty of Sciences, Bani Waleed University, Bani Walid City, Libya.

Article's Information

Received: 17.05.2025
Accepted: 29.11.2025
Published: 15.12.2025

Keywords:
Tumors
Breast cancer
Liver metastasis
Chemokine

Abstract

Breast cancer liver metastasis (BCLM) is a multi-step process that characterized by the spread of breast cancer cells to the liver. The majority of breast cancer-related deaths are due to metastatic rather than primary breast tumors. CXCL12 is one of the chemokines, small proteins capable of migrating various types of immune cells to the site of inflammation. CXCL12 can affect several cell biological activities, such as migration and proliferation, when it binds to its receptors. The aim of this study is to investigate the predictive value of plasma CXCL12, CA15-3, and some liver-related parameter levels in breast cancer patients with liver metastasis. This study involves 94 women, 25 healthy controls and 69 patients with breast cancer, divided into three subgroups: 26 newly diagnosed women with primary breast cancer, 28 women with non-metastatic breast cancer undergoing chemotherapy, and 15 women with liver metastatic breast cancer. Plasma levels of CXCL12, CA15-3, albumin, ALP, ALT, and AST were measured using absorbance photometry and ELISA assays. The results of the study reveals that the level distributions of CXCL12 ($p>0.9999$) and CA15-3 ($p>0.9999$) showed no significant difference between breast cancer patients with and without liver metastasis. Albumin levels were significantly lower (=p0.0089) in women with liver metastatic breast cancer compared to those without metastasis. Levels of ALP ($p=0.0006$,(=ALT (p0.0015), and AST ($p<0.0001$) were significantly elevated in breast cancer liver metastatic patients compared to patients without metastasis. CXCL12 (AUC, 0.5840, $p=0.3788$) 3-and CA15 (AUC, 0.5787, $p=0.4099$) could not serve as reliable discriminatory parameters for liver metastasis. Albumin (AUC, 0.7267, $p=0.0176$) showed a moderate ability to distinguish between BC patients with and without BCLM. ALP (AUC, 0.8693, $p=0.0001$) and AST (AUC, 0.9453, $p<0.0001$) exhibited the highest diagnostic accuracies for liver metastasis. Together, none of the study parameters was identified as an independent risk factor for liver metastasis in breast cancer patients.

http://doi.org/10.22401/ANJS.28.4.05

***Corresponding author:** ba.altimmime1999@gmail.com

1. Introduction

In 2022 approximately 2.3 million women were diagnosed with breast cancer globally, with about 1.5 million women diagnosed every year. This suggest that breast cancer constitute about 25% of all cancer cases among women [1]. Breast cancer is primarily categorized by the presence of a tumor that develops in the breast tissue, which can originate from the lobes, ducts, or stroma [2]. Breast cancer is a disease with a high degree of diversity at the tissue level and clinical behavior. Several classification systems were obtained to provide additional information about each type and sub type of breast cancer. Breast cancers were divided histologically into invasive and non-invasive depending on the spreading of breast cancer cells

from the lobes or ducts to the surrounding tissues [3]. On the other hand, four types of breast tumors were obtained from the molecular classification. The tumors were categorized based on the presence and absence of the hormone receptors, resulting in luminal A, luminal B, HER2-positive, and triple-negative tumors [4]. Breast cancer is also associated with the second highest rate of death among women. Still, it is worth mentioning that metastatic breast cancer is responsible for 90% of cancer-related deaths rather than primary cancers [5]. The The non-metastatic breast cancer patients showed a 5-year survival rate of 99%, whereas those with metastatic breast cancer showed only 27% [6]. Statistical data indicated that 20-30% of the women with early-stage breast cancer develop metastasis later [7]. The molecular types of breast cancer exhibited different behaviors against metastasis, including the time to distant metastasis, the favored site for distant metastasis, and survival after the development of distant metastasis. Luminal A tumors of (ER+/HER2-) demonstrated a tendency to metastasize to the bone more than other sites. At the same time, the liver was the preferred organ to metastasize to in HER2-positive tumors of (ER-/HER2+) [8]. Breast cancer metastasis is a complex process involving several steps that must occur consecutively. Firstly, local invasion happens when breast cancer cells separate from the primary breast tumor. These detached cells migrate and enter the bloodstream through blood vessels. After that, extravasation takes place when the circulating tumor cells move out of the blood vessels. Finally, breast cancer cells travel to the new organ and start building their colonization [9]. Breast cancer cells should adapt to the new micro environment of the metastatic site. The micro environment includes hormones, cytokines, growth factors, and physical conditions such as PH and oxygen content [10,11]. Moreover, each micro environment comprises various cell types, such as leukocytes, fibroblasts, and adipocytes. The association between metastatic breast cancer cells and the new micro environment has been represented as seeds and soil [12]. Metastatic breast cancer cells need additional nutrients and oxygen to grow, so they promote angiogenesis, in which new vascular capillaries are formed [13]. The liver is one of the common sites to which breast cancer cells metastasize. About 40-50% of total metastatic breast cancer patients have liver metastasis [14]. Furthermore, the breast is the primary organ in 15% of the total women with liver metastasis [15]. Liver metastasis can affect the liver's functions and lead to liver failure. Untreated liver metastasis reduces the overall survival time to just a few months [16]. The treatment of breast cancer liver metastasis is a real challenge due to the absence of a standard therapy. Currently, the treatment involves radiotherapy, chemotherapy, and targeted therapy, depending on the hormone receptor status [17]. Chemotactic cytokines, or chemokines, a family of tiny proteins, play a key role in promoting breast cancer metastasis to distant organs, including the liver [18]. Chemokines are the communication language between immune cells. During inflammation, immune cells secrete chemokines in the bloodstream to attract additional immune cells to the injured site. Chemokines were classified into four groups (C, CC, CXC, and CX_3C) based on their structural features, especially the arrangement of their cysteine residues [19]. Chemokines can be inflammatory, produced in response to inflammation, or homeostatic, produced constantly under normal conditions [20]. Chemokines must bind to their receptors on the cell's surface to activate intracellular signaling. CXCL12 (C-X-C motif chemokine ligand 12) is a chemokine that belongs to the CXC group. CXCL12 was isolated for the first time from the stromal cells within the murine bone marrow, which led to its naming as stromal cell-derived factor 1 (SDF-1) [21]. Initially, CXCL12 was identified as a pre-B-cell growth-stimulating factor. The structure of CXCL12 involves 68 amino acids with a molecular weight of 8 kDa [22]. CXCL12 is a homeostatic chemokine with several biological functions, such as cell migration, immune cell production, stem cell trafficking, and blood vessel formation [23]. CXCR4 and CXCR7 are the receptors to which CXCL12 binds. Unlike other CXC chemokines, for which genes are located on chromosome 4q21, the CXCL12 gene is on chromosome 10q11 [24]. As of now, six CXCL12 isoforms (α, ß, γ, δ, ε, and θ) have been discovered as a result of alternative mRNA splicing. Many studies have highlighted the significance of CXCL12 in developing breast cancer liver metastasis through enhancing breast cancer cell migration, survival, and proliferation [25]. It was observed that breast tumor cells express CXCR4, while the liver was found to be rich in CXCL12 [26]. Blocking CXCR4 resulted in a decrease in breast cancer cell migration, as well as an improvement in response to therapy [27]. In addition, studies have shown that the CXCL12/CXCR4 signaling contributes to the extravasation of metastatic breast cancer cells in the liver [28]. Moreover, CXCL12 has shown an ability to inhibit T lymphocyte infiltration, and this helps to promote breast cancer metastasis [29].

Several studies were involved in the association between CXCL12 and breast cancer metastasis to the liver. However, these studies were limited to tissue-level analysis and did not extend to the cytoplasm. The current study aims to investigate whether the blood levels of CXC motif chemokine ligand 12 (CXCL12), carbohydrate antigen 15-3 (CA15-3), albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) can serve as potential indicators for liver metastasis in breast cancer patients.

2. Materials and Methods

A. Breast Cancer Patients

Ninety-four females were included in this pilot study, divided into two groups: twenty-five healthy women and sixty-nine breast cancer patients. Patient selection was carried out using simple random sampling. The breast cancer patient group involved twenty-six newly diagnosed women with primary breast cancer who had not yet started treatment, twenty-eight women with non-metastatic breast cancer undergoing chemotherapy (dose per 21 days), and fifteen women with liver metastatic breast cancer with chemotherapy (weekly dose). All the patients in this study were selected at Al-Amal National Hospital for Cancer Management, where they were diagnosed and treated. Breast cancer diagnosis was confirmed through pathological testing for all patients, while biopsy, MRI, or PET-CT were used to detect the presence of liver metastasis. Blood samples were collected from patients with liver metastatic breast cancer approximately four months following the diagnosis of liver metastasis. The exclusion criteria comprised patients with chronic diseases such as liver and kidney disorders, patients with more than one metastatic site, and patients with incomplete data. The study followed the approved protocol of Al-Nahrain University, and ministry of health ethical standards (31441; date, 2023/08/21).

B. Blood Samples

A total of 5 ml of blood was drawn from the vein of each study participant. Blood samples were collected using lithium-heparin tubes. After that, blood samples were centrifuged at 178.88 (R.C.F.) for 15 min to separate the plasma from the cellular components before storage. The plasma samples were transferred into microcentrifuge tubes and stored at -20 °C. Blood samples were collected from December, 2023 to May, 2024.

C. Investigated indices

A quantitative sandwich ELISA technique was employed to measure plasma CXCL12 and CA15-3 levels. ELISA kits for CXCL12 (Catalog No. EL0249Hu) and CA15-3 (Catalog No. QS0383Hu) were obtained from Sunlong Biotech, China. Albumin, ALP, ALT, and AST levels were measured using absorbance photometry assay via Cobas C111 instrument. These parameters were selected for their potential to provide valuable insights into liver metastasis in breast cancer.

D. Statistical Analysis

GraphPad Prism 9.5.0 software was used to perform all statistical processing. Data normality was tested using the Kolmogorov-Smirnov test. Categorical data were represented as frequencies and percentages, and the Chi-square and Fisher's exact tests were employed to assess the significance level. *p*-value was significant at $p<0.05$. For the continuous data that were not normally distributed, the Kruskal-Wallis and Dunn's tests were applied to compare the distributions between study groups. Group comparisons were made using the median value, along with the minimum and maximum values. Additionally, the diagnostic ability of the parameters was determined by the Receiver Operating Characteristic (ROC) curves. The cut-off value of each parameter was calculated using the Youden-J index. Potential associations between various parameters and the occurrence of liver metastasis were evaluated using simple and multiple logistic regressions.

3. Results

A. Patients' Characteristics

The study groups showed a matched age distribution ($p=0.2859$), indicating that age is not a determining factor in the groups' comparison. Luminal A was the most prevalent molecular type of breast tumor among both newly diagnosed and non-metastatic breast cancer women, whereas all tumors in the liver metastatic group were HER2-positive ($p<0.0001$ No surgery was .(performedin the newly ,diagnosed breast cancer groupwhereas all patients in the non-metastatic breast cancer group underwent surgery. Only 40% of the liver metastatic breast cancer group patients had surgery, making a significant difference between groups ($p<0.0001$.(No significant difference was observed in the comparisons of smoking or alcohol intake ($p>0.9999$), as there was no smoking or alcohol intake case among all the groups (Table 1).

B. Screening Tests

The median levels of CXCL12, CA15-3, albumin, ALP, ALT, and AST were not significantly different in healthy women (Table 1) compared to those of non-metastatic breast cancer (CXCL12 1296 vs 1403 pg/ml; p=0.5173) (CA15-3 15.81 vs 18.41 U/ml; p=0.2945) (albumin 3.713 vs 3.851 U/dl; p>0.9999) (ALP 63.80 vs 64.60 U/L; p>0.9999) (ALT 14.20 vs 15.60 U/L; p=0.3253) (AST 17.60 vs 18.20 U/L; p=0.8957). The newly diagnosed breast cancer group exhibited median levels of CXCL12 and CA15-3 of (1371 pg/ml and 14.54 U/ml), which were lower but not significantly so than the non-metastatic breast cancer group (p>0.9999 and p=0.0568), respectively. However in contrast, the median level of albumin was markedly decreased (p=0.0436) in newly diagnosed breast cancer women (3.584 g/dl) compared to those with non-metastatic breast cancer (3.851 g/dl). None of the median levels of ALP, ALT, or AST in the newly diagnosed breast cancer group demonstrated a significant difference from the non-metastatic breast cancer group (ALP 63.70 vs 64.60 U/L; p>0.9999) (ALT 14.80 vs 15.60 U/L; p>0.9999) (AST 18.00 vs 18.20 U/L; p>0.9999). No observed difference (p>0.9999) was shown in the median level of CXCL12 between the non-metastatic (1403 pg/ml) and liver metastatic (1404 pg/ml) breast cancer groups. Similar to CXCL12, the median level of CA15-3 was not significantly different (p>0.9999) in women with non-metastatic breast cancer (18.41 U/ml) compared to those with liver metastasis (17.32 U/ml). The liver metastatic breast cancer group exhibited a median level of albumin of (3.533 g/dl), significantly (p=0.0089) lower than the non-metastatic breast cancer group (3.851 g/dl). Median levels of ALP, ALT, and AST demonstrated a significant elevation in the liver metastatic breast cancer group compared to the non-metastatic group (ALP 112.8 vs 64.60 U/L; p=0.0006) (ALT 23.60 vs 15.60 U/L; p=0.0015) (AST 28.50 vs 18.20 U/L; p<0.0001). Figure 1 presents the levels of study parameters.

C. Diagnostic Accuracy

The area under the curve (AUC) of CXCL12 was 0.5840 (95% CI: 0.3876-0.7804; p=0.3788), with a sensitivity of 80.00% and a specificity of 44.00%. CA15-3 exhibited an AUC of 0.5787 (95% CI: 0.3975-0.7598; p=0.4099), and the sensitivity and specificity were 80.00% and 40.00%, respectively. The ROC curve analysis for albumin yielded an AUC of 0.7267 (95% CI: 0.5522-0.9011; p=0.0176), and the sensitivity and specificity were 66.67% and 80.00%, correspondingly. For ALP, the AUC was 0.8693 (95% CI: 0.7132-1.000; p=0.0001), achieving sensitivity and specificity values of 80.00% and 96.00%, respectively. The AUC of ALT was 0.8520 (95% CI: 0.7261-0.9779; p=0.0002), while AST showed an AUC of 0.9453 (95% CI: 0.8761-1.000; p<0.0001). The sensitivities of ALT and AST were 86.67% and 93.33%, and the specificities were 88.00% and 92.00%, respectively (Figure 2 and Table 2).Univariate Logistic Regression Analysis Simple logistic regression results revealed a non-significant relationship between CXCL12 and the risk of liver metastasis [OR=1.002, 95% CI: 0.9996-1.004; p=0.1398]. Similar to CXCL12, CA15-3 also did not show an observed association with liver metastasis risk [OR=0.9591, 95% CI: 0.8755-1.027; p=0.2891]. No significant correlation was observed between albumin and the risk of liver metastasis. [OR=0.1419, 95% CI: 0.01192-1.131; p=0.0864]. ALP, ALT, and AST were found to be significant predictors of liver metastasis risk [OR=1.089, 95% CI: 1.041-1.167; p=0.0031], [OR=1.196, 95% CI: 1.067-1.406; p=0.0105], and [OR=1.552, 95% CI: 1.237-2.288; p=0.0035], respectively (Table 3). Multivariate Logistic Regression Analysis Neither CXCL12 [OR=1.002, 95% CI: 0.9988-1.006; p=0.2069] nor CA15-3 [OR=1.018, 95% CI: 0.8767-1.148; p=0.7910] demonstrated a significant association with liver metastasis occurrence. Likewise, albumin was not significantly correlated with liver metastasis risk [OR=3.970, 95% CI: 0.08161-740.5; p=0.5067]. None of ALP [OR=1.067, 95% CI: 1.005-1.165; p=0.0668], ALT [OR=1.063, 95% CI: 0.8233-1.298; p=0.5526], or AST [OR=1.253, 95% CI: 0.9909-1.843; p=0.1024] were defined as independent risk factors for liver metastasis in breast cancer (Table 3).

4. Discussion

The expression of CXCR4 on the surface of breast cancer cells, the high abundance of CXCL12 in the liver micro environment, and the ability of CXCL12 to migrate cells to distant sites suggest that CXCL12 contributes to breast cancer metastasis to the liver. Several studies have investigated the relationship between CXCL12-CXCR4 signaling and liver metastasis. However, they have focused on the tissue level rather than the plasma level. Because biopsies can sometimes be difficult and costly, the current pilot study aims to provide preliminary data on the correlation between plasma levels of

CXCL12, CA15-3, albumin, ALP, ALT, and AST and the risk of liver metastasis in women with breast cancer. Based on the present results, plasma CXCL12 levels in women with non-metastatic breast cancer did not differ significantly from healthy women. This aligns with a previous study in which CXCL12 expression was not significantly different between normal and breast cancer tissues [30]. Conversely, another study reported that CXCL12 expression is significantly higher in breast cancer tissues compared to normal tissues [31]. In addition, the current results demonstrated no effect of chemotherapy on the plasma CXCL12 levels, which is in line with an earlier finding that showed chemotherapy courses are not related to CXCL12 tissue expression [32]. Moreover, there was no observed difference in plasma CXCL12 levels between the breast cancer women with and without liver metastasis. This result differs from an earlier finding in which CXCL12 expression was significantly lower in primary gastric cancer tissues compared to liver metastatic gastric cancer tissues [33]. The conflicting results of these studies may be due to the fact that CXCL12 expression is more prominent in tissues than in plasma. The current study indicated no significant difference in plasma CA15-3 levels between healthy women and those with non-metastatic breast cancer. In contrast, a previous study found that CA15-3 levels increase significantly in non-metastatic breast cancer women compared to healthy women [34]. No effect of chemotherapy on CA15-3 levels was noted, whereas an earlier study found a direct association between chemotherapy and elevated CA15-3 levels [35].

Table 1. Patient Characteristics and Screening Tests for the Study Groups.

Parameters	Control Group n=25	Newly Diagnosed BC Group n=26	Non-Metastatic BC Group n=28	Liver Metastatic BC Group n=15	p-value
Age (years) median (min-max)	48.00 (35.00-70.00)	45.00 (24.00-70.00)	50.00 (36.00-62.00)	51.00 (45.00-59.00)	NS
Tumor Types, n (%) Luminal A Luminal B HER-2 TNBC	–	21 (81) 0 (0) 4 (15) 1 (4)	17 (60) 1 (4) 6 (2) 1 (4)	0 (0) 0 (0) 15 (100) 0 (0)	<0.0001
Surgery Yes, n (%) No, n (%)	–	0 (0) 26 (100)	28 (100) 0 (0)	6 (40) 9 (60)	<0.0001
Smoking Yes, n (%) No, n (%)	0 (0) 25 (100)	0 (0) 26 (100)	0 (0) 28 (100)	0 (0) 15 (100)	NS
Alcohol Intake Yes, n (%) No, n (%)	0 (0) 25 (100)	0 (0) 26 (100)	0 (0) 28 (100)	0 (0) 15 (100)	NS
CXCL12 (pg/ml) Median (min-max)	1296 (1138-1638)	1371 (1179-1688)	1403 (1217-2471)	1404 (1096-2813)	NS[a] NS[b] NS[c]
CA15-3 (U/ml) Median (min-max)	15.81 (11.60-27.04)	14.54 (9.320-23.31)	18.41 (10.23-57.65)	17.32 (10.76-39.91)	NS[a] NS[b] NS[c]
Albumin (g/dL) Median (min-max)	3.713 (3.136-4.234)	3.584 (3.505-4.507)	3.851 (3.022-4.357)	3.533 (3.168-4.632)	NS[a] 0.0436[b] 0.0089[c]
ALP (U/L) Median (min-max)	63.80 (47.60-114.7)	63.70 (47.40-154.8)	64.60 (51.80-89.70)	112.8 (51.01-160.3)	NS[a] NS[b] 0.0006[c]

ALT (U/L) Median (min-max)	14.20 (11.10-30.30)	14.80 (9.200-35.90)	15.60 (12.20-38.40)	23.60 (14.90-47.20)	NS[a] NS[b] 0.0015[c]
AST (U/L) Median (min-max)	17.60 (13.50-22.40)	18.00 (12.00-30.70)	18.20 (15.10-30.40)	28.50 (19.40-43.70)	NS[a] NS[b] <0.0001[c]

p-value is statistically significant at p≤ 0.05.
NS stands for Not Significant.
[a]p indicates a comparison between the control group and the non-metastatic BC group.
[b]p indicates a comparison between the newly diagnosed BC group and the non-metastatic BC group.
[c]p indicates a comparison between the liver metastatic BC group and the non-metastatic BC group.
Normal Ranges: CA15-3 <30 U/ml, albumin 3.4-4.5 g/dl, ALP 35-120 U/L, ALT 0-45 U/L, AST 0-35 U/L.

Table 2. Assessment of CXCL12, CA15-3, albumin, ALP, ALT, and AST as indicators for liver metastasis.

Parameter	AUC	95% CI	Sensitivity %	Specificity %	Cut-off value	p-value
CXCL12 pg/ml	0.5840	0.3876-0.7804	80.00	44.00	> 1340	NS
CA15-3 U/ml	0.5787	0.3975-0.7598	80.00	40.00	< 22.04	NS
Albumin g/dl	0.7267	0.5522-0.9011	66.67	80.00	< 3.565	0.0176
ALP U/L	0.8693	0.7132-1.000	80.00	96.00	> 88.10	0.0001
ALT U/L	0.8520	0.7261-0.9779	86.67	88.00	> 19.35	0.0002
AST U/L	0.9453	0.8761-1.000	93.33	92.00	> 23.75	<0.0001

p-value is statistically significant at p≤ 0.05.
NS stands for Not Significant.
AUC: Area Under the Curve, CI: Confidence Interval

Additionally, no observed difference was reported between women with and without liver metastasis. This agrees with a former study in which women with non-metastatic breast cancer exhibited plasma CA15-3 levels that did not notably differ from those with liver metastasis [36]. Another finding investigated CA15-3 levels in breast cancer patients with and without liver metastasis and demonstrated no relationship between the presence of liver metastasis and CA15-3 levels [37]. On the other hand, an earlier study showed a significant elevation in CA15-3 levels in women with breast cancer who had liver metastasis compared to those who did not develop metastasis [38]. Although some studies have considered CA15-3 an indicator of liver metastasis in breast cancer, oncologists still prefer using additional tests, such as MRI and PET-CT, to detect the presence of liver metastasis and use CA15-3 levels for monitoring therapy response.

Table 3. Univariate and Multivariate Logistic Regression with Parameters to Predict Liver Metastasis.

	Univariate Analysis		Multivariate Analysis	
Parameter	OR (95%CI)	P-value	OR (95%CI)	*p*-value
CXCL12 pg/ml	1.002 (0.999-1.004)	NS	1.002 (0.998-1.006)	NS
CA15-3 U/ml	0.959 (0.875-1.027)	NS	1.018 (0.876-1.148)	NS
Albumin g/dl	0.141 (0.011-1.131)	NS	3.970 (0.081-740.5)	NS
ALP U/L	1.089 (1.041-1.167)	0.0031	1.067 (1.005-1.165)	NS
ALT U/L	1.196 (1.067-1.406)	0.0105	1.063 (0.823-1.298)	NS
AST U/L	1.552 (1.237-2.288)	0.0035	1.253 (0.990-1.843)	NS

p-value is statistically significant at *p*≤ 0.05.
NS stands for Not Significant.
OR: Odd Ratio, CI: Confidence Interval
Univariate analysis: simple logistic regression, multivariate analysis: multiple logistic regression

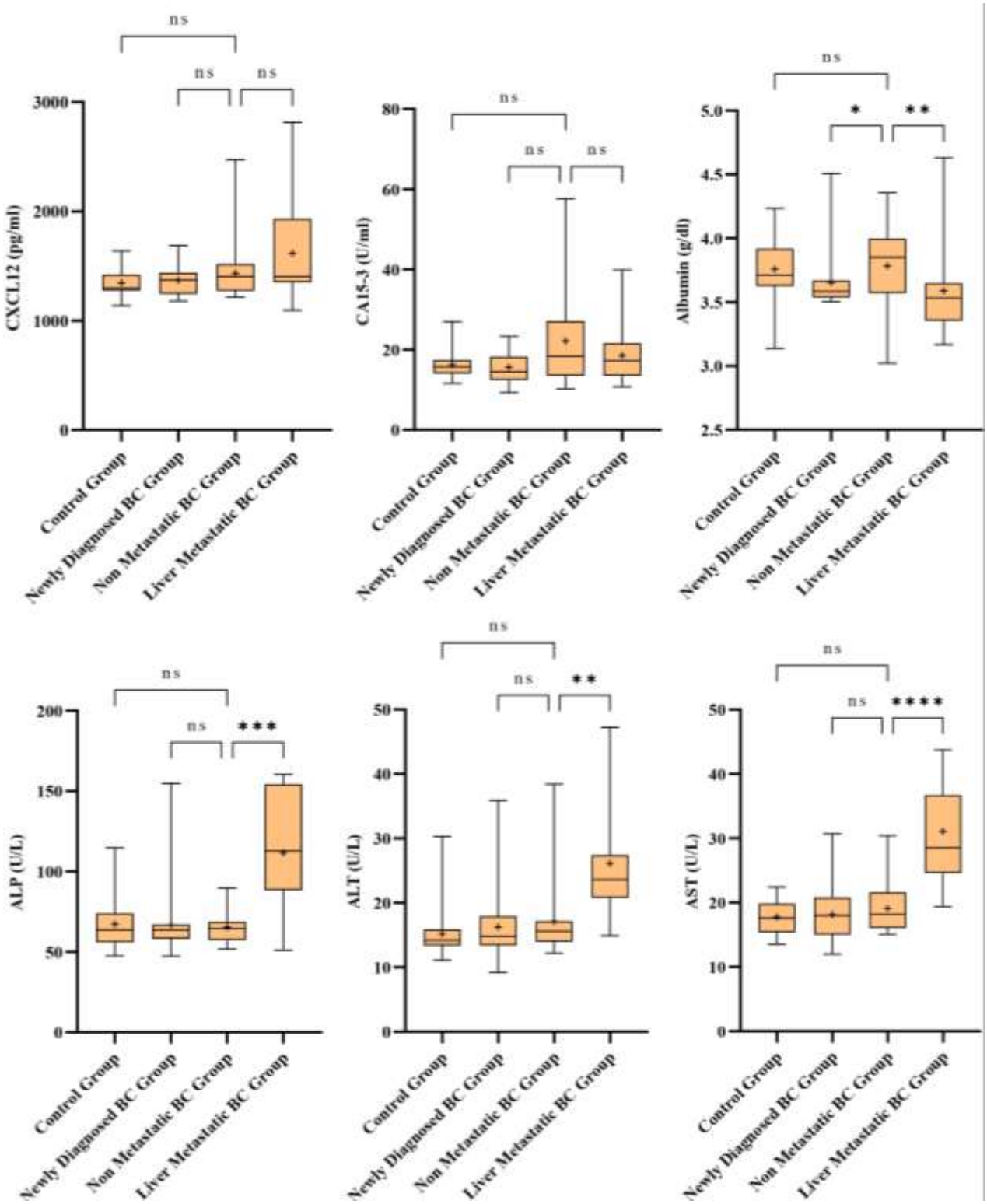

Figure 1. The Distribution of CXCL12, CA15-3, Albumin, ALP, ALT, and AST Levels Among Study Groups.

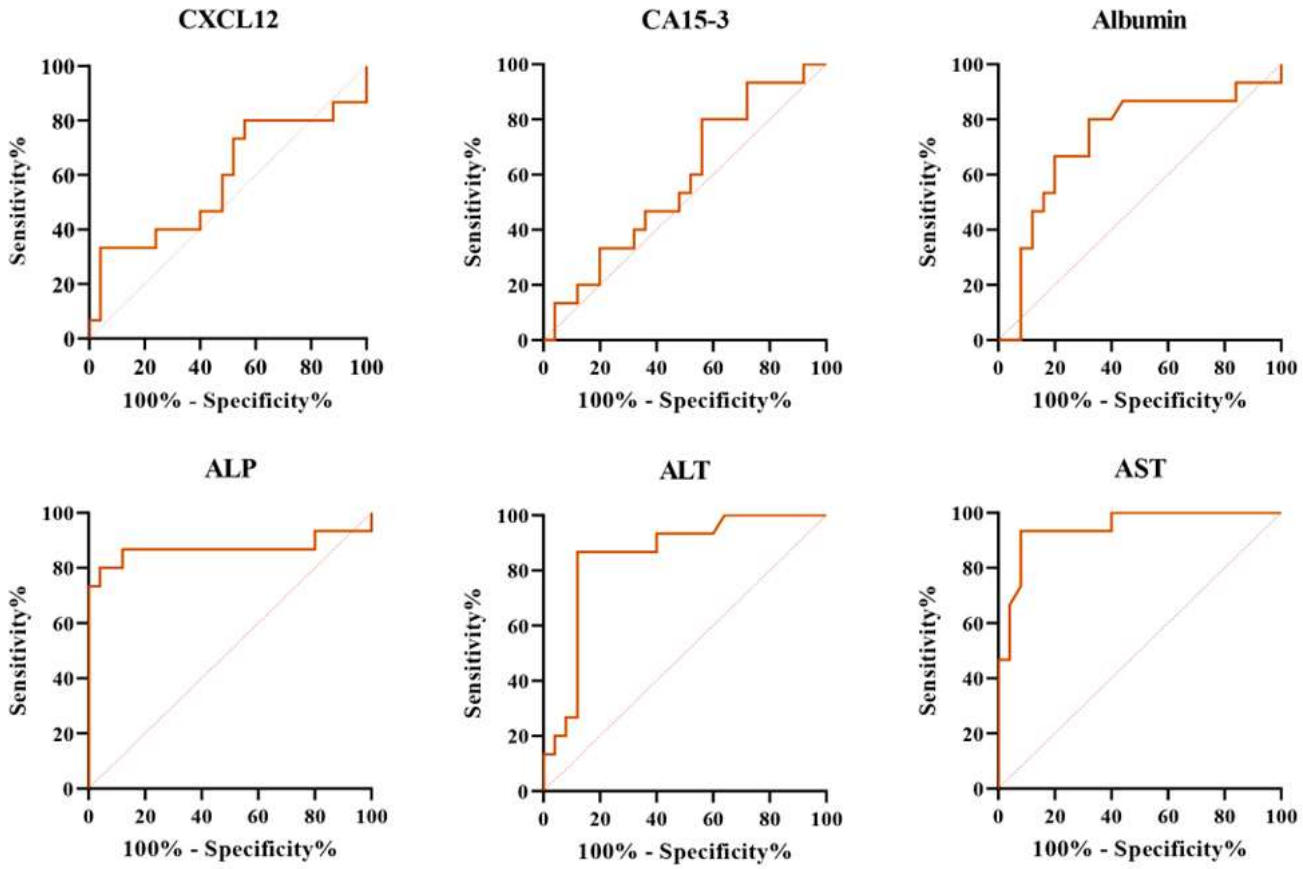

Figure 2. The ROC curve of CXCL12, CA15-3, Albumin, ALP, ALT, and AST as indicators for liver metastasis.

Healthy women did not exhibit an observed difference in albumin levels compared to women with non-metastatic breast cancer. A previous finding has indicated no association between albumin levels and breast cancer risk [39]. The current results found a significant decrease in albumin levels in women with breast cancer undergoing chemotherapy compared to those who had not yet started chemotherapy. Albumin is associated with nutritional status, liver function, and inflammation. Therefore, albumin levels can be altered by chemotherapy, as one of its side effects is inflammation. It can also affect liver function, subsequently impacting protein synthesis. The albumin levels in women with breast cancer and liver metastasis were significantly lower than in women with primary breast cancer. This indicates that liver metastasis has affected liver function, including albumin production. According to the present results, there was no significant difference in ALP, ALT, or AST levels between healthy women and those with primary breast cancer. This agrees with an earlier study that found no correlation between ALP, ALT, and AST levels and breast cancer [40]. Moreover, another study demonstrated that testing ALP, ALT, and AST levels is not useful in newly diagnosed breast cancer patients and does not lead to meaningful consequences [41]. None of the ALP, ALT, or AST levels differed notably in women with primary breast cancer undergoing chemotherapy from newly diagnosed women who had not started any treatment. This finding is consistent with a previous study in which ALP, ALT, and AST were not reliable parameters for monitoring chemotherapy [42]. ALP, ALT, and AST levels were significantly higher in women with breast cancer and liver metastasis than in women with non-metastatic breast cancer. This was in agreement with a prior study that found an observed increase in ALP, ALT, and AST levels in women with liver metastatic breast cancer compared to those without metastasis [43]. Another finding aligned with the current results, showing that women with breast cancer and liver metastasis

exhibited significantly higher levels of ALP, ALT, and AST compared to women with primary breast cancer [44]. In a different study, only AST levels were significantly higher in women with breast cancer who developed liver metastasis than in women with non-metastatic breast cancer [45]. Both CXCL12 and CA15-3 showed poor diagnostic accuracy for liver metastasis; however, the sensitivities were very good, while the specificities were low. When the diagnostic accuracy was investigated, sensitivity referred to the ability of the parameter to correctly identify women with breast cancer who have liver metastasis. Specificity indicated the ability to correctly identify women with breast cancer who do not have liver metastasis. Albumin exhibited a moderate predictive value in detecting liver metastasis, with good specificity and moderate sensitivity. Based on the results, ALP, ALT, and AST were identified as reliable indicators of liver metastasis. These parameters showed high values in detecting liver metastasis and distinguishing between women with and without it. When liver metastasis alters liver function, the levels of liver enzymes such as ALP, ALT, and AST will be affected, depending on the degree of liver damage. AST appeared to be more sensitive to liver cell damage than ALP, and ALT. The correlation between CXCL12, CA15-3, albumin, ALP, ALT, and AST with liver metastasis risk was assessed using logistic regression analysis. ALP, ALT, and AST were significantly associated with liver metastasis risk, with a direct association: as the levels of ALP, ALT, and AST increase, the risk of liver metastasis also increases. None of these parameters was identified as an independent risk factor for liver metastasis in breast cancer patients. The current study has some limitations. One of the main limitations is the small sample size, which persists even though it was designed as a pilot study. Another limitation would be the absence of tumor stage among the patients. Furthermore, the significant difference in tumor molecular types between breast cancer patients with and without liver metastasis may lead to an imprecise comparison.

5. Conclusions

With the background knowledge that CXCL12 contributes to metastasis to the liver in breast cancer patients, this pilot study investigated the relationship between CXCL12 and other biochemical parameter levels in breast cancer patients' plasma and liver metastasis risk. In breast cancer patients with liver metastasis, CXCL12 levels were not significantly different from those with non-metastatic breast cancer. On the other hand, it was found that ALP, ALT, and AST were significantly more sensitive to the presence of liver metastasis. Further studies considering additional variables or different methodologies may obtain more insight into the potential role of CXCL12 in liver metastasis detection.

Acknowledgment: Authors would like to thank all the patients who generously volunteered their blood samples for this study. Their participation was invaluable. We also extend our thanks to the authors for their contributions to the study.

Funding: This study did not receive any funding, grants, or financial support.

Availability of data and materials: Availability of the dataset for review can be requested.

Authors' contributions: BAA and YSR designed the study and conducted the experimental part. BAA collected the blood samples. FAR performed the statistical analysis and wrote the manuscript. All authors contributed to the final approval of the manuscript.

Ethics approval and consent to participate: The Ethics Committee of Al-Nahrain University, College of Science, approved this study. All participants provided informed consent to participate in the study.

Conflict of Interest: The authors declare that they have no conflicts of interest.

References

[1] Shi, W.; Jiang, J.; Yao, P.P.; Zhu, H.P.; "Risk factors and preventions of breast cancer". Int. J. Biol. Sci., 13(11): 1387, 2017.

[2] Palacios-Arreola, M.I.; Nava-Castro, K.E.; Castro, J.I.; García-Zepeda, E.; Carrero, J.C.; Morales-Montor, J.; "The role of chemokines in breast cancer pathology and its possible use as therapeutic targets". J. Immunol. Res., 2014(1): 849720, 2014.

[3] Nolan, E.; Lindeman, G.J.; Visvader, J.E.; "Deciphering breast cancer: from biology to the clinic". Cell, 186(8): 1708–1728, 2023.

[4] Duan, X.F.; Dong, N.N.; Zhang, T.; Li, Q.; "The prognostic analysis of clinical breast cancer subtypes among patients with liver

metastases from breast cancer". Int. J. Clin. Oncol., 18(1): 26–32, 2013.

[5] Wang, L.; Zhang, S.; Wang, X.; "The metabolic mechanisms of breast cancer metastasis". Front. Oncol., 10: 602416, 2021.

[6] Park, H.A.; Brown, S.R.; Kim, Y.; "Cellular mechanisms of circulating tumor cells during breast cancer metastasis". Int. J. Mol. Sci., 21(14): 5040, 2020.

[7] Chen, W.; Hoffmann, A.D.; Liu, H.; Liu, X.; "Organotropism: new insights into molecular mechanisms of breast cancer metastasis". npj Precision. Onc., 2(1): 4, 2018.

[8] Savci-Heijink, C.D.; Halfwerk, H.; Hooijer, G.K.; Horlings, H.M.; Wesseling, J.; van de Vijver, M.J.; "Retrospective analysis of metastatic behaviour of breast cancer subtypes". Breast Cancer Res. Treat., 150(3): 547–557, 2015.

[9] Méndez-García, L.A.; Nava-Castro, K.E.; Ochoa-Mercado, T.D.; Palacios-Arreola, M.I.; Ruiz-Manzano, R.A.; Segovia-Mendoza, M.; Solleiro-Villavicencio, H.; Cázarez-Martínez, C.; Morales-Montor, J.; "Breast cancer metastasis: are cytokines important players during its development and progression?". J. Interferon Cytokine Res., 39(1): 39–55, 2019.

[10] Soysal, S.D.; Tzankov, A.; Muenst, S.E.; "Role of the tumor microenvironment in breast cancer". Pathobiology, 82(3–4): 142–152, 2015.

[11] Altimmime, B.A.; Rashid, F.A.; "CXCL12 as a Metastasis Inducer Chemokine of Breast Cancer". Al-Nahrain J. Sci., 27(5): 49–57, 2024.

[12] Terceiro, L.E.; Edechi, C.A.; Ikeogu, N.M.; Nickel, B.E.; Hombach-Klonisch, S.; Sharif, T.; Leygue, E.; Myal, Y.; "The breast tumor microenvironment: A key player in metastatic spread". Cancers, 13(19): 4798, 2021.

[13] Madu, C.O.; Wang, S.; Madu, C.O.; Lu, Y.; "Angiogenesis in breast cancer progression, diagnosis, and treatment". J. Cancer, 11(15): 4474, 2020.

[14] Langley, R.R.; Fidler, I.J.; "The seed and soil hypothesis revisited: The role of tumor-stroma interactions in metastasis to different organs". Int. J. Cancer, 128(11): 2527–2535, 2011.

[15] Tsilimigras, D.I.; Brodt, P.; Clavien, P.A.; Muschel, R.J.; D'Angelica, M.I.; Endo, I.; Parks, R.W.; Doyle, M.; de Santibanes, E.; Pawlik, T.M.; "Liver metastases". Nat. Rev. Dis. Primers, 7(1): 27, 2021.

[16] Bale, R.; Putzer, D.; Schullian, P.; "Local treatment of breast cancer liver metastasis". Cancers, 11(9): 1341, 2019.

[17] Liu, C.; Mohan, S.C.; Wei, J.; Seki, E.; Liu, M.; Basho, R.; Giuliano, A.E.; Zhao, Y.; Cui, X.; "Breast cancer liver metastasis: Pathogenesis and clinical implications". Front. Oncol., 12: 1043771, 2022.

[18] King, J.; Mir, H.; Singh, S.; "Association of cytokines and chemokines in pathogenesis of breast cancer". Prog. Mol. Biol. Transl. Sci., 151: 113–136, 2017.

[19] Miller, M.C.; Mayo, K.H.; "Chemokines from a structural perspective". Int. J. Mol. Sci., 18(10): 2088, 2017.

[20] Janssens, R.; Struyf, S.; Proost, P.; "Pathological roles of the homeostatic chemokine CXCL12". Cytokine Growth Factor Rev., 44: 51–68, 2018.

[21] Nagasawa, T.; "CXCL12/SDF-1 and CXCR4". Front. Immunol., 6: 301, 2015.

[22] Mortezaee, K.; "CXCL12/CXCR4 axis in the microenvironment of solid tumors: A critical mediator of metastasis". Life Sci., 249: 117534, 2020.

[23] Mezzapelle, R.; Leo, M.; Caprioglio, F.; Colley, L.S.; Lamarca, A.; Sabatino, L.; Colantuoni, V.; Crippa, M.P.; Bianchi, M.E.; "CXCR4/CXCL12 activities in the tumor microenvironment and implications for tumor immunotherapy". Cancers, 14(9): 2314, 2022.

[24] Righetti, A.; Giulietti, M.; Šabanović, B.; Occhipinti, G.; Principato, G.; Piva, F.; "CXCL12 and its isoforms: different roles in pancreatic cancer?". J. Oncol., 2019(1): 9681698, 2019.

[25] Altimmime, B.A.; Rashid, F.A.; "Evaluation of CXCL12, carbohydrate antigen CA15.3, calcium and parathyroid hormone as potential biomarkers for bone metastasis in patients with breast cancer". World Acad. Sci. J., 7(2): 21, 2024.

[26] Ma, R.; Feng, Y.; Lin, S.; Chen, J.; Lin, H.; Liang, X.; Zheng, H.; Cai, X.; "Mechanisms involved in breast cancer liver metastasis". J. Transl. Med., 13(1): 64, 2015.

[27] Chen, I.X.; Chauhan, V.P.; Posada, J.; Ng, M.R.; Wu, M.W.; Adstamongkonkul, P.; Huang, P.; Lindeman, N.; Langer, R.; Jain, R.K.; "Blocking CXCR4 alleviates desmoplasia, increases T-lymphocyte infiltration, and improves immunotherapy in metastatic breast cancer". Proc. Natl. Acad. Sci. U.S.A., 116(10): 4558–4566, 2019.

[28] Zielińska, K.A.; Katanaev, V.L. "The signaling duo CXCL12 and CXCR4: chemokine fuel for breast cancer tumorigenesis". Cancers, 12 (10): 3071, 2020.

[29] Liu, X.; Chen, Y.; Tang, J.; Zhang, H.; Zhang, X. "CXCL12 inhibited T lymphocyte infiltration and promoted breast cancer metastasis".

[30] Wu, W.; Qian, L.; Chen, X.; Ding, B. "Prognostic significance of CXCL12, CXCR4, and CXCR7 in patients with breast cancer". Int. J. Clin. Exp. Pathol., 8 (10): 13217, 2015.

[31] Zhao, S.; Chang, S.L.; Linderman, J.J.; Feng, F.Y.; Luker, G.D. "A comprehensive analysis of CXCL12 isoforms in breast cancer". Transl. Oncol., 7 (3): 429–438, 2014.

[32] Pfeffer, U.; Mirisola, V.; Zuccarino, A.; Bachmeier, B.; Sormani, M.P.; Falter, J.; Nerlich, A. "CXCL12/SDF1 expression by breast cancers is an independent prognostic marker of progression". Eur. J. Cancer Suppl., 7 (2): 141, 2009.

[33] Xin, Q.; Zhang, N.; Yu, H.B.; Zhang, Q.; Cui, Y.F.; Zhang, C.S.; Ma, Z.; Yang, Y.; Liu, W. "CXCR7/CXCL12 axis is involved in lymph node and liver metastasis of gastric carcinoma". World J. Gastroenterol., 23 (17): 3053, 2017.

[34] Ali, H.Q.; Mahdi, N.K.; Al-Jowher, M.H. "The value of CA15-3 in diagnosis, prognosis and treatment response in women with breast cancer". J. Pak. Med. Assoc., 63 (9): 1138–41, 2013.

[35] Ryu, J.M.; Kang, D.; Cho, J.; Lee, J.E.; Kim, S.W.; Nam, S.J.; Lee, S.K.; Kim, Y.J.; Im, Y.H.; Ahn, J.S.; Park, Y.H. "Prognostic impact of elevation of cancer antigen 15-3 (CA15-3) in patients with early breast cancer with normal serum CA15-3 level". J. Breast Cancer, 26 (2): 126, 2023.

[36] Gaughran, G.; Aggarwal, N.; Shadbolt, B.; Stuart-Harris, R. "The utility of the tumor markers CA15.3, CEA, CA-125 and CA19.9 in metastatic breast cancer". Breast Cancer Manage., 9 (4): BMT50, 2020.

[37] Fatima, H.; Maqbool, A.; Siddiqui, M.; Sohail, H.; Saleem, O.; Ahmed, A. "Significance of Tumor Marker CA15-3 in Metastatic Breast Cancer". J. Can Tumor Int., 4 (3): 1-12, 2016.

[38] Haider, G.; Shaikh, Z.; Memon, P.; Shahid, A.; Rahul, R.; Kumar, P.; Beg, S.; Parkash, J. "Significance of CA15-3 in carcinoma of the breast with visceral metastases". J. Ayub Med. Coll. Abbottabad, 35 (4 Suppl 1): 710–714, 2023.

[39] Yang, Z.; Zheng, Y.; Wu, Z.; Wen, Y.; Wang, G.; Chen, S.; Tan, F.; Li, J.; Wu, S.; Dai, M.; Li, N. "Association Between Pre-diagnostic Serum Albumin and Cancer Risk: Results from a Prospective Population-Based Study." Cancer Med., 10(12): 4054–4065, 2021.

[40] Neelam, A.; Ali, A.; Alam, R.; Mujtaba, G.; Malik, I.R. "Study of diagnostic and prognostic parameters in breast cancer patients without metastasis". Adv. Life Sci., 7 (3): 177–180, 2020.

[41] Louie, R.J.; Tonneson, J.E.; Gowarty, M.; Goodney, P.P.; Barth Jr, R.J.; Rosenkranz, K.M. "Complete blood counts, liver function tests, and chest x-rays as routine screening in early-stage breast cancer: value added or just cost?". Breast Cancer Res. Treat., 154 (1): 99–103, 2015.

[42] Ali, A.H.; Abdusalam, F.M.; Abdullah, F.M.; Abdulrahim, E.A. "Study of some liver function tests in patients with breast carcinoma during chemotherapy treatment at oncology center in Sebha city". J. Pure Appl. Sci., 19 (5): 68–73, 2020.

[43] Cao, R.; Wang, L.P.; "Serological diagnosis of liver metastasis in patients with breast cancer". Cancer Biol. Med., 9 (1): 57–62, 2012.

[44] Kimchy, A.V.; Singh, H.; Parikh, E.; Rosenberg, J.; Sanghavi, K.; Lewis, J.H. "The predictive value of liver tests for the presence of liver metastases". Hepat. Oncol., 10 (3): HEP48, 2023.

[45] Leser, C.; Dorffner, G.; Marhold, M.; Rutter, A.; Doeger, M.; Singer, C.; Koenig-Castillo, D.M.; Deutschmann, C.; Holzer, I.; König-Castillo, D.; Gschwantler-Kaulich, D. "Liver function indicators in patients with breast cancer before and after detection of hepatic metastases—a retrospective study". PLoS One, 18 (3): e0278454, 2023.