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ORIGINAL ARTICLE

THE SIGNIFICANCE OF SERUM MIDKINE IN THE DIAGNOSIS AND PROGNOSIS OF INVASIVE DUCTAL CARCINOMA OF THE BREAST

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Summary

Background and Objectives: Initial diagnosis of brest cancer (BC) is important for fate and prognosis of the diseases profile, we sought to identify the correlation between Midkine (MK) as a new biomarker with cancer antigen (CA)15-3, liver function test, renal function test, blood cells parameters in individuals with invasive ductal carcinoma.

Methods: The serum MK and CA15-3 of all subjects were measured by the ELISA technique, Liver enzymes were measured by colourimetric methods and neutrophils, and lymphocytes were measured by an Electrical Impedance Cell Counting method (automated machine).

Results: The results of the correlation among serum MK and other parameters in invasive ductal carcinoma of the breast showed a considerable positive correlation among MK and CA15-3 and measured white blood cells. Moreover, there were a weak correlation with Aspartate Aminotransferase (AST) and RBC, while there is no correlation between serum MK and other liver enzymes or blood parameters. **Conclusion:** The study results of the correlation between serum MK and other parameters in colorectal carcinoma patients show a significant positive correlation of MK with CA15-3 markers in invasive ductal carcinoma of the breast.

Key words: Invasive ductal carcinoma; Colorectal carcinoma; Liver enzymes; Lymphocytes; Neutrophils; Midkine

Introduction

Midkine (MK) is a heparin-binding growth element originally recognised as a gene significantly expressed during mouse embryogenesis (1). As a soluble excreted protein that is greatly raised in many disorders, including cancer, MK has the potential to be an important disease biomarker (2). The MK gene has been proven to be overexpressed in numerous cancer kinds (2), particularly as the tumour enters more severe stages(3). Noteworthy, blood tests have shown that MK expression is present in malignancies (4, 5), urinary(6), and tumour analysis (7).

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Even though some biomarkers have been thoroughly investigated, brest cancer (BC) is a genetically complex and highly widespread illness (8). Different BC subtypes exhibit varying clinical outcomes and may have various prognoses. Exploring new molecular targets for predictive biomarkers and novel therapeutic targets is urgently needed. First, compared to healthy people, BC patients' plasma and tissue MK levels were unusually raised (9, 10). they indicated that MK dysfunction occurs early in the course of disease progress.

Elevated plasma MK heights and traditional indicators (like CA15-3 and CEA) are also offered (9). Furthermore, in primary invasive mammary tumour without distant metastases, elevated MK heights were associated with menopausal status and nuclear grade (9). Although encouraging, more research is essential to determine the clinical importance of MK in breast tumour patients' plasma.

Materials and Methods

Subjects: The study was conducted on individuals who were clinically and laboratory diagnosed with colorectal cancer and who attended the Al Sadr Teaching Hospital and Middle Euphrates Hospital for Oncology and Hematology in Al Najaf Al Asharaf– Iraq; samples and all information were taken from the patients, and healthy subjects were selected for the study. Laboratory tests were carried out in the laboratories of the Clinical Biochemistry Branch / College of the Medicine / University of Al-Qadisiyah. Also, some laboratory tests were conducted in the Clinical Chemistry Unit / Laboratory Division of the Al Sadr Teaching Hospital and Middle Euphrates Hospital for Oncology and Hematology. One hundred and twenty-nine individuals participated in the study between September 2022 and May 2023 (for sample collection), divided into two groups: patients subject: sixty people are patients with colorectal carcinoma selected from the Al Sadr Teaching Hospital and Middle Euphrates Hospital for Oncology and Hematology, after confirming their clinical and laboratory diagnoses. Control subject: Sixty-nine healthy people who do not have any disease. They were established after asking people and conducting all required laboratory analyses.

Blood Sample Collection: Initially, six milliliters (ml) of blood is needed – two ml into an ethylenediaminetetraacetic acid (EDTA) tube for haematological investigations and four ml into another type of gel tube used specifically for biochemical analysis. Following extraction, both types of samples are immediately transported separately but together to the laboratory section. Here they undergo thorough processing steps tailored to individual tests before being analyzed further. While serum extracted by centrifugation (Centrifuge, Bioneer, Korea) from gel tubes will be subjected to biochemical analyses (renal function test, liver function test, CA15-3, and MK) and those in EDTA tubes would go through haematology examinations. These processes include techniques such as spectrophotometry or enzymatic assays depending upon the nature of the test. However, while the serum undergoes immediate frozen after collection for future analysis, the whole blood undergo immediate processing due to its fragility and potential degradation if left untreated too quickly. During processing, whole blood sample remains stored at sub-zero temperatures, this storage method helps maintain the integrity of the cells within, preventing any damage during prolonged periods without proper handling.

Inclusion Criteria: Patients with and without metastatic invasive ductal carcinoma of the breast without mastectomy.

Exclusion Criteria: Patients were excluded if they had cancer (colorectal, ovarian, pancreatic, lung, kidney, and prostatic cancers), Inflammatory bowel disease (Crohn’s disease and ulcerative colitis), neurological disorders (Alzheimer’s disease, Parkinson’s disease and multiple sclerosis), cardiovascular diseases (coronary artery disease, acute myocardial infarction and heart failure) and Renal diseases (diabetic nephropathy, glomerulonephritis and renal fibrosis). These diseases might interfer with the laboratory test for detection of serum MK in the BC.

Biochemical analysis: The biochemical investigations was done by fully automated machine (biochemistry analyzer Cobas, Roche, Germany), which based on the principle of Electrochemiluminescence (ECL) technology provides superior analytical performance. The concentration of sample determined through interpolation to the standards concentration which was fully automated by Cobas.

Haematological analysis: The haematological investigations was done by fully automated machine (haematology analyzer CBC, Swelab Lumi, Swedish), which based on the principle of Electrical Impedance Cell Counting methods.

Statistical analysis: Data were collected and gathered using Microsoft Office Excel (2013) Spreadsheet. For statistical analysis the statistical package for social sciences (SPSS) version 25 were used. In order to effectively communicate quantitative findings, The initial step involves converting all categorical variables into numerical form using specific algorithms. For instance, gender might become '0' representing male, '1' signifying female, while missing responses would be labeled as '9'. These digitized values then allow for easy manipulation and comparison between different groups. In addition, they facilitate the application of various tests including chi-square analyses which examine associations between two categorical variables, enabling researchers to identify significant relationships.

Numerical variables present another challenge altogether due to their potential deviation from a Gaussian distribution curve, commonly referred to as a bell curve (1, 2). To verify if any particular variable adheres to this pattern, statisticians employ the Kolmogorov-Smirnov Test. Should the results indicate non-normality, alternative measures come into play (5). Specifically, instead of relying solely on mean and standard deviation statistics typically associated with normally distributed data, median and inter-quartile range calculations offer valuable insights regarding central tendency and spread respectively (3). The Chi-square test assessed the association between any two category variables when less than 20% of cells had an expected count of less than 5 (7). Samples from various sources If the numeric variables were normally distributed, the t-test was used to assess the difference in mean between any two groups; if they were not, the Mann-Whitney U test was applied instead. Receiver operator characteristic (ROC) curve analysis with its related area under the curve (AUC), accuracy level, sensitivity, specificity, and degree of significance (P) was utilized to determine the cutoff value that predicts a positive finding. P-values equal to or less than 0.05 were used to indicate significance. P-values equal to or less than 0.01 were considered high significance (11, 12).

Results

As indicated in Table 1, There was no considerable variance in mean age among patients and healthy subjects, 54.33 ± 12.12 years versus 53.81 ± 12.61 years, respectively ($p = 0.451$).

Table 1. Correlation of mean age between individuals with invasive ductal carcinoma and control subject.

Characteristic	Invasive breast carcinoma ($n = 60$)	Control group $n = 69$	p
Age (years)			
Mean ± SD	54.33 ± 12.12	53.81 ± 12.61	0.451 I
Range	32 - 73	21 - 65	NS

n: number of cases; **SD**: standard deviation; **I**: independent samples t-test; **NS**: not significant

The present study included 19 cases with moderately differentiated tumours (grade II), accounting for 31.7 % and 41 cases of poorly differentiated tumours (Grade III), accounting for 68.3 % (Figure 1A). In addition, this study included 26 status stage II diseases (43.3 %), 24 status stage III conditions (40.0 %) and ten status stage IV diseases (16.7 %) (Figure 1B).

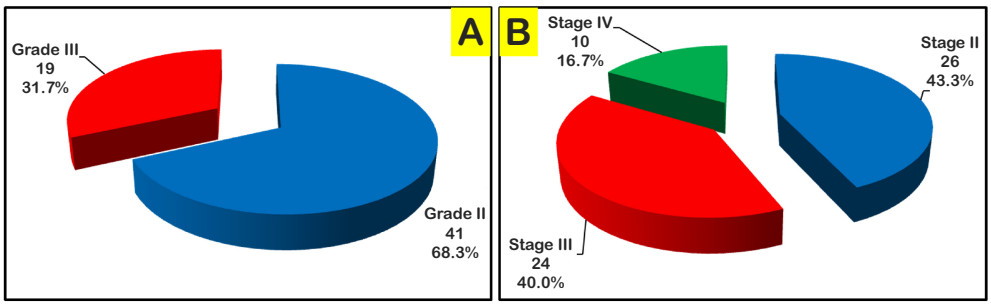

Figure 1. The frequency distribution of women with invasive ductal carcinoma (A) according to a grade of disease, (B) according to a grade of disease.

As indicated in Table 2, There was no significant variance in mean WBC count between the patients' subject and control subject, 5.78 ± 2.28 $X10^9$/L versus 6.18 ± 1.11 $X10^9$/L, respectively ($p = 0.202$). However, the mean RBC count was significantly lower in the patients' subjects compared to the healthy subject, 4.05 ± 0.59 $X10^{12}$/L versus 4.26 ± 0.58 $X10^{12}$/L, respectively ($p = 0.047$). In addition, the mean haemoglobin level was significantly lower in the patients' subject compared to a control subject, 10.66 ± 1.25 g/dl against 11.16 ± 0.77 g/dl, respectively ($p < 0.001$). There was no significant variance in mean platelet count between the patients' and healthy subjects, 288.12 ± 159.75 $X10^9$/L versus 291.00 ± 67.99 $X10^9$/L, respectively ($p = 0.892$).

Moreover, the mean neutrophil count was considerably higher in the patients' subject than the control subject, 8.02 ± 4.17 $X10^9$/L versus 3.92 ± 2.07 $X10^9$/L, respectively ($p < 0.001$). However, the mean lymphocyte count was significantly lower in the patients' subject compared to a control subject, 1.85 ± 1.03 $X10^9$/L vs. 2.81 ± 1.24 $X10^9$/L, respectively ($p < 0.001$). Therefore, the mean neutrophil/lymphocyte ratio count was considerably higher in the patients' subjects compared to the control group, 12.08 ± 7.15 versus 2.03 ± 0.91L, respectively ($p < 0.001$).

Table 2. Correlation of some haematological parameters between individuals with invasive ductal carcinoma and control subject.

Characteristic	Invasive breast carcinoma *n* = 60	Control group *n* = 69	*p*
WBC $X10^9$ (cells/L)			
Mean ± SD	5.78 ± 2.28	6.18 ± 1.11	0.202 I NS
Range	1.9 - 12.8	4.6 - 9	
RBC $X10^{12}$(cells/L)			
Mean ± SD	4.05 ± 0.59	4.26 ± 0.58	0.047 I *
Range	2.7 - 5.6	3.2 - 5.4	
Haemoglobin (g/dl)			
Mean ± SD	10.66 ± 1.25	11.16 ± 0.77	< 0.001 I ***
Range	9 - 14.2	9 - 12.5	
Platelet count $X10^9$(PLT/L)			
Mean ± SD	288.12 ± 159.75	291.00 ± 67.99	0.892 I NS
Range	64 - 920	178 - 399	
Neutrophils $X10^9$(cells/L)			
Mean ± SD	8.02 ± 4.17	3.92 ± 2.07	<0.001 I ***
Range	2.9 - 14.3	2.6 - 6.5	
Lymphocyte $X10^9$(cells/L)			
Mean ± SD	1.85 ± 1.03	2.81 ± 1.24	<0.001 I ***
Range	0.2 - 4.3	0.9 - 4.7	
NLR			
Mean ± SD	12.08 ± 7.15	2.03 ± 0.91	<0.001 I ***
Range	0.58 - 37	0.54 - 5.7	

n: number of cases; **WBC:** white blood cells; **RBC**: red blood corpuscles; **SD**: standard deviation; **I**: independent samples *t*-test; **NS**: not significant; *****: significant at p ≤ 0.05; *******: significant at p ≤ 0.001

As indicated in Table 3, There was no significant variance in mean blood urea between individuals with breast carcinoma and the healthy subject, 25.23 ± 8.27 mg/dl versus 27.00 ± 6.80 mg/dl, respectively ($p = 0.186$). Moreover, there was no significant variance in mean serum creatinine between individuals with breast carcinoma and the healthy subject, 0.55 ± 0.37 mg/dl versus 0.59 ± 0.09 mg/dl, respectively ($p = 0.329$).

Table 3. Correlation of blood urea and serum creatinine between individuals with invasive ductal carcinoma and control subject.

Characteristic	Invasive breast carcinoma *n* = 60	Control group *n* = 69	*p*
Blood urea (mg/dl)			
Mean ± SD	25.23 ± 8.27	27.00 ± 6.80	0.186 I NS
Range	11 - 62	16 - 42	
Serum creatinine (mg/dl)			
Mean ± SD	0.55 ± 0.37	0.59 ± 0.09	0.329 I NS
Range	0.2 - 2.7	0.5 - 0.8	

n: number of cases; **SD**: standard deviation; **I**: independent samples *t*-test; **NS**: not significant

Liver parameters results: As indicated in Table 4, the Mean AST level was considerably higher in individuals with invasive ductal carcinoma compared to a control subject, 24.03 ± 17.31 IU/L versus 22.02 ± 12.21 IU/L, respectively ($p = 0.025$). However, there was no significant variance in mean ALT between individuals with breast carcinoma and the control subject, 25.23 ± 8.27 IU/L versus 27.00 ± 6.80 IU/L, respectively ($p = 0.442$).

In addition, there was no significant variance in mean ALP between individuals with breast carcinoma and the control subject, 102.62 ± 53.47 IU/L versus 95.08 ± 27.26 IU/L, respectively ($p = 0.306$). Moreover, there was no significant variance in mean TSB between individuals with breast carcinoma and the healthy subject, 0.50 ± 0.31 mg/dl versus 0.43 ± 0.38 mg/dl, respectively ($p = 0.496$).

Table 4. Correlation of liver parameters between individuals with invasive ductal carcinoma and control subject.

Characteristic	Invasive breast carcinoma *n* = 60	Control group *n* = 69	*p*
AST (IU/L)			
Mean ± SD	26.38 ± 15.13	21.73 ± 7.40	0.025 I *
Range	9.6 - 81	10.3 - 43	
ALT (IU/L)			
Mean ± SD	24.03 ± 17.31	22.02 ± 12.21	0.442 I NS
Range	8 - 78	4.7 - 61.4	
ALP (IU/L)			
Mean ± SD	102.62 ± 53.47	95.08 ± 27.26	0.306 I NS
Range	45 - 312	42.2 - 169.3	
TSB (mg/dl)			
Mean ± SD	0.50 ± 0.31	0.43 ± 0.38	0.496 I NS
Range	0.1 - 3.9	0.1 - 2.6	

n: number of cases; **SD**: standard deviation; **AST**: aspartate aminotransferase; **ALT**: alanine aminotransferase; **ALP**: alkaline phosphatase; **TSB**: total serum bilirubin; **I**: independent samples t-test; **NS**: not significant; *: significant at $p \leq 0.05$

As indicated in Table 5, Mean serum CA15-3 was considerably higher in individuals with invasive breast carcinoma compared to a control subject, 87.17 ± 63.56 U/ml versus 1.77 ± 1.07 U/ml, respectively ($p < 0.001$).

Table 5. Correlation of serum CA15-3 between individuals with invasive ductal carcinoma and control subject.

Characteristic	Invasive breast carcinoma *n* = 60	Control group *n* = 69	*p*
CA15-3 (U/ml)			
Mean ± SD	87.17 ± 63.56	1.77 ± 1.07	<0.001 I ***
Range	35.8 - 297.1	0.18 - 4.2	

n: number of cases; **SD**: standard deviation; **I**: independent samples t-test; ***: significant at p ≤ 0.001

After analyzing the data collected, they discovered significant differences in serum MK concentrations between the two groups. Specifically, mean serum MK levels were considerably higher among Invasive Ductal Carcinoma patients than the control group, 4.02 ± 1.60 ng/ml versus 1.83 ± 0.92 ng/ml, respectively ($p < 0.001$). To further evaluate the predictive value of MK, Receiver Operating Characteristic (ROC) curves were plotted based on the obtained results. ROC analysis allows for determining the most effective cutoff point that maximizes both sensitivity and specificity values, leading to accurate prediction of BC presence. Based on this methodology, it was found that a MK threshold of > 3.02ng/ml could successfully discriminate between Invasive Ductal Carcinoma patients and non-cancerous subjects. Furthermore, the AUC value of 0.868 indicated strong association strength, while *p*-value less than 0.001 signified statistically significant difference. These figures suggest that MK may serve as a robust indicator for identifying BC at an earlier stage where treatment options are more viable and successful. However, despite these promising findings, caution should be exercised before implementing MK testing into clinical practice. Additional studies involving larger sample sizes and diverse populations are necessary to validate these initial observations and ensure reliable application across all demographics. In conclusion, MK shows considerable potential as a diagnostic biomarker for BC. Its ability to differentiate between Invasive Ductal Carcinoma patients and normal individuals provides hope for improved early detection strategies. However, extensive research and validation are required to establish its reliability and feasibility within routine medical settings (Table 6, Table 7, Figure 2).

Table 6. Correlation of serum MK between individuals with invasive ductal carcinoma and control subject.

Characteristic	Invasive breast carcinoma *n* = 60	Control group *n* = 69	*p*
Serum MK (ng/ml)			
Mean ± SD	4.02 ± 1.60	1.83 ± 0.92	<0.001 I ***
Range	0.56 - 6.54	0.34 - 4.01	

n: number of cases; **SD**: standard deviation; **I**: independent samples t-test; ***: significant at p ≤ 0.001

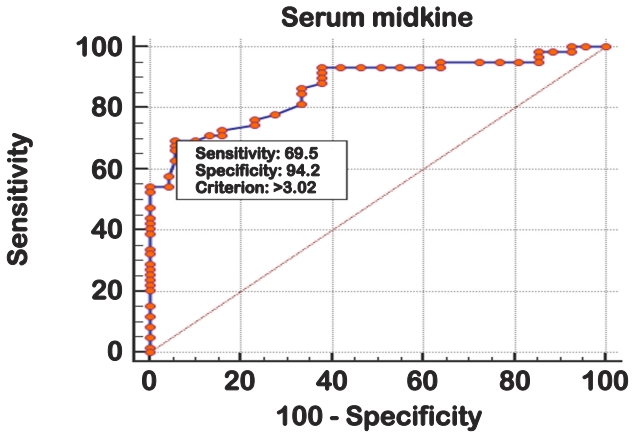

Figure 2. Receiver operational characteristic (ROC) curve analysis to find the best cutoff value of serum MK that can foresee a positive diagnosis of invasive ductal carcinoma of the breast in terms of sensitivity and specificity.

Table 7. Characteristics of ROC analysis concerning serum MK.

Characteristic	Result
Cutoff value	> 3.02 ng/ml
AUC	0.868
95 % CI	0.797 to 0.921
p	< 0.001***
Sensitivity %	69.5
Specificity %	94.5
Accuracy %	86.8 %
AUC: area under curve; **CI**: confidence interval; ***: significant at p ≤ 0.001	

As indicated in Table 8, the grade of disease was positively correlated to WBC count, neutrophil count and neutrophil to lymphocyte ratio. The stage of disease was positively related to CA-15-3, NLR, AST, ALT, ALP and TSB.

Table 8. Correlations of grade and stage of the disease to other characteristics.

Characteristic	Grade		Stage	
	r	*p*	*r*	*p*
Age	-0.165	0.208	0.200	0.126
MK	0.030	0.820	0.045	0.732
CA15-3	-0.064	0.626	0.922	<0.001***
WBC	0.281	0.029*	-0.013	0.923
RBC	-0.240	0.064	0.124	0.344
HB	-0.110	0.404	0.086	0.514
PLT	-0.030	0.820	-0.014	0.913
Neutrophil	0.355	0.037*	0.261	0.118
Lymphocyte	0.223	0.251	0.246	0.165
NLR	0.685	0.025*	0.272	0.026*
Blood Urea	0.055	0.677	0.161	0.218
Serum Creatinine	0.123	0.351	0.146	0.265
AST	0.025	0.850	0.281	0.029*
ALT	-0.022	0.869	0.358	0.005**
ALP	0.199	0.128	0.438	<0.001***
TSB	-0.041	0.754	0.275	0.033*
*: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $\mathbf{p} \leq 0.001$				

Discussion

The mean age of individuals with invasive mammary carcinoma in the present study is comparable to that obtained by (13), who collected registered data for BC cases from the Iraqi Cancer Registry/Ministry of Health, enrolling 23,792 patients. They found that the mean old was 52 years. In addition, the mean age in this study was approximately similar to that obtained by Stiff *et al*. (2018)(14), who carried out a retrospective descriptive study in which medical notes and histopathological reports of individuals with confirmed diagnoses of breast tumours between January 2011 and December 2015 were reviewed in Basra and they found a mean age of 50 years.

Elevated midkine levels have been found in breast cancer tissues compared to normal breast tissues, indicating its potential as a diagnostic marker. Furthermore, higher midkine expression has been associated with advanced tumor stage, lymph node metastasis, and poor overall survival, suggesting its significance in predicting prognosis. As for treatment, targeting midkine signaling pathways could be a promising therapeutic approach. Preclinical studies using midkine inhibitors have shown promising results in reducing tumor growth and metastasis in breast cancer models. Additionally, midkine-targeted immunotherapy has shown potential in enhancing the immune response against cancer cells. However, further research is needed to fully understand the role of midkine in IDC and its potential as a diagnostic tool, prognostic marker, and therapeutic target.

Worldwide, about 80% of women with BC are individuals aged more than 50 years. Overall, tumour in elder age is not just restricted to mammary tumours; the accumulating large quantity of cellular alterations and exposure to possible carcinogens cause carcinogenesis to elevate over time (15). It appears that Iraqi women often present when a tumour is beyond stage I, which makes disease control a difficult mission and the survival shorter than that obtained in developed countries. The proportions of disease grades and locations in the current study are comparable to those in other Iraqi studies conducted by Yousif *et al*. (2019) and Al-Asadi and Al-Mayah (2020) (16, 17).

In line with the present study results, Akinbami *et al*. (2013) (18) described that leukocyte counts were higher in individuals with mammary tumours than in healthy subject. However, according to (19), they conducted a large case-control study and associated leukocyte counts between individuals with mammary tumours (n=4,402) and propensity score-matched controls (n=4,402) selected from the Korean National Health and Nutrition Examination Survey; they found that WBC count mean in patient's group was considerably lower than that of a control subject in clear controversy to current study finding.

Evidence is mounting that prolonged low-grade inflammation may contribute to the aetiology of several malignancies (20-22). leukocyte count, an inflammatory parameter, has emerged as a helpful indicator of infection and a predictor of other diseases (19). Even within the normal range, a high leukocyte count has been linked to atherosclerotic cardiovascular disorders, tumour incidence, and mortality (23, 24). In the context of well-known impact modifiers for mammary tumour growth, the function of leukocyte counts as a representation of inflammation has not been investigated (19). In line with the present study, it has been shown that mean RBC count and mean haemoglobin are lower in individuals with invasive ductal carcinoma compared to the control subject in several previous reports (18, 25).

Anaemia is frequently seen in cancer patients, with proportions ranging from 22.7% (26) to 63% (27) and up to 89% after chemotherapy (28). Elevated incidence of anaemia has been associated with reduced quality of life and drug response in individuals with breast tumours (29, 30) and increases the burden of the tumour (31).

The propagation of anaemia in cancer patients is related to many factors. The most frequent causes are biological causes (32), chemotherapy (33), demographic reasons (34), and forms of cancer (34). The occurrence of anaemia in cancer patients was significantly predisposed by socio-demographic characteristics, such as increasing age (34, 35); race, like Hispanics (34); and sex, like women. Additionally, the number of chemotherapy regimens (36, 37), the type of chemotherapy (34, 36), and the delay or reduction of chemotherapy dose (38) all contributed to an increase in the incidence of cancer-related anaemia.

Moreover, anaemia in cancer patients is strongly correlated with both the disease itself (36, 39) and the kind of cancer (40). Furthermore, cancer treatment (surgery, hormone therapy, radiation, and targeted therapy), the impact of cancer (through direct invasion of the bone marrow), and the impact of cytokines secreted by cancer cells all contributed to an increase in the occurrence of anaemia in cancer patients (41).

Regarding platelet count, and in agreement with the current study finding (40), found no significant variance in mean platelet count between individuals with BC and healthy subjects (43); however, reported significantly less mean platelet count in women with breast carcinoma compared to a control group and this is inconsistent with current study observation. Indeed, most previous authors linked the platelet count to disease behaviour, such as grade of disease, stage of disease and survival rates (44-48).

Regarding neutrophil count, lymphocyte count and NLR in this study are consistent with previous reports (49-51). The reason behind modulation in the level of inflammatory cells have been attributed to the fact that cancer produced tissue hypoxia and thereby increase inflammatory cytokine release which stimulate increased NLR (49), however, genetic differences, heterogeneity of BC, and ethnical origin have been also reported as being related to modulation of NLR (50, 51). No consensus has reached about explanation of modulated NLR in cancer patient.

There is much evidence to suggest that cancer led to liver impairment (55). Reports show this liver dysfunction is linked to worse overall survival and higher cancer-related mortality (56). Significantly higher ALT levels in the cancer subject compared to a control subject. Previous reports evaluated the liver function test in BC patients in association with liver metastasis or chemotherapy effect (56), and these two patterns were not among the goals of the present study. However, The liver dysfuction has been linked to anticancer used rather than cancer itself (56), however, when cancer-induced rat model used, liver dysfunction has been shown linked to cancer itself (16, 55).

The effectiveness of assessing CA15-3 levels for BC individuals remains debated. European Group on Tumor Markers has suggested the CEA and CA15-3 levels be utilised for evaluating prognosis, the initial detection of disease progression, and cure monitoring in mammary tumours. The American Society of Clinical Oncology and the National Comprehensive Cancer Network index do not currently recommend the usage of serum CA15-3 and CEA for mammary tumour screening and directing therapy (57). These disagreements may be partly due to the conflicting decisions of research (58, 59). The low positive degree of serum tumour parameters is also a possible cause. But several investigations found that continuous CA15-3 rise usually occurred in patients with disease advance (60).

In line with the current study observation (61), reported that serum MK in women with breast carcinoma was considerably higher than that of a control subject. In addition, the present study results agree with (62), who performed a study on 40 females with invasive breast neoplasm and 20 healthy control groups and found similar results of considerably higher serum MK level in patients compared to the healthy group. Moreover, we found significantly higher serum MK levels in individuals with invasive breast tumours compared to a control group; therefore, which is in agreement with previous study (61). We also found a cutoff value for serum MK of > 1.4 ng/ml to differentiate malignant breast lesions from benign ones with a sensitivity level of 95 %, a specificity level of 97 % and an accuracy level of 94 %, thus support our opinion in that serum MK can be used as a diagnostic aid tool to confirm a diagnosis of breast carcinoma (62).

In the present study, the grade of disease was positively correlated to WBC count, neutrophil count and neutrophil to lymphocyte ratio. In contrast, the stage of illness was positively related to CA-15-3, NLR, AST, ALT, ALP and TSB. The positive correlation of grade of disease with leukocyte count may be due to its positive correlation with neutrophils, as neutrophils are the largest fraction of leukocyte count. The positive correlation of the stage of disease with liver enzymes can be attributed to liver metastasis, destroying liver cells and liberating their enzymes into circulation.

The positive correlation of grade and disease stage with increasing NLR follows the findings of several previous researchers (64). High condensation of blood neutrophils is seen in individuals with progressive tumour and are related to worse persistence (65, 66). Similarly, there is an abundant index for a negative predictive significance of NLR on mammary tumours. Multiple investigations have presented that higher NLR was related to worse persistence (49, 67-71), and the latest meta-analysis establish that higher NLR was related to both bad disease-free persistence and overall persistence (69). Numerous previous investigations have found that higher NLR was also related to more progressive or advanced mammary tumours (66, 71-73). Besides the logical explanation mentioned earlier regarding MMK's association with cancer, there is another fascinating aspect to consider. Cancer cells tend to grow at an abnormal rate, leading to a lack of oxygen supply, also known as hypoxia. Surprisingly, this hypoxia can induce changes in the release of various trophic factors by cancer cells. These factors play a crucial role in regulating cell growth and differentiation, making them a subject of great interest in cancer research (74-76).

One major limitation is the heterogeneity of cancer itself, this heterogeneity makes it challenging to develop universal treatments that effectively target all types of cancer. Small sample size and unicentre dependent collection of data make it difficult to shape a clear conclusion about the measurement of MK as a part of diagnostic tool for BC.

When investigating a complex and multifactorial disease like invasive breast ductal cancer, it is essential to ensure an adequate sample size to capture the heterogeneity of the population under study. A larger sample size is generally preferred as it increases the precision of estimates and enhances the statistical power to detect meaningful associations or differences. With a larger sample size, the study is more likely to have sufficient statistical power to detect even small effects, reducing the risk of type II errors (false negatives). Additionally, a larger sample size allows for subgroup analyses, which can help identify variations in treatment response or disease outcomes among different patient populations. Conversely, a smaller sample size may compromise the statistical power of the study, making it more challenging to draw meaningful conclusions. In the context of an invasive breast ductal cancer study, a smaller sample size may limit the ability to identify significant differences between the experimental group and the control group, leading to inconclusive or biased results. Therefore, researchers must carefully consider the sample size when designing a study to ensure that it is adequately powered to address the research question and provide reliable evidence for clinical decision-making.

Conclusion

There are significant changes in the mean haemoglobin, and red blood cells count in patients with invasive ductal breast carcinoma compared to the healthy control persons. There are no considerable variations in serum level of urea in addition to creatinine in patients subject in contrast to the healthy subject. In comparison, liver function tests showed a significant elevation in the mean AST level in patients with invasive ductal carcinoma. At the same time, there are no significant differences in serum levels of ALT between the patients and the control groups. Moreover, there are no significant TSB changes between patients and the control group. Compared to the control group, there is a high serum CA15-3 and MK concentration in patients with invasive ductal carcinoma, which might be a good predictor of invasive ductal breast carcinoma.

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Conflict of interest

The authors declare no conflict of interest concerned in the present study.

Adherence to Ethical Standards

The study was approved by the College of Medicine/ University of Al-Qadissiya with approval number (UoQ/CoM 30/4408 on 28.11.2022).

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