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MATRIX METALLOPROTEINASE-2 LEVELS IN IRAQI BREAST CANCER WOMEN AND ITS ASSOCIATION WITH GENE SNPS AND OTHER TUMOR MARKERS

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ABSTRACT

Background: Matrix metalloproteinases (MMPs) are a family of extracellular matrix-degrading proteinases. Owing to their matrix-degrading abilities and high expression in advanced tumors, MMPs were originally implicated in cancer progression, invasion, and metastasis. **Objective:** The present work aims to investigate the role of the matrix metalloproteinase levels in pathogenesis of Iraqi breast cancer patients of breast cancer and to study its association with MMP-2 gene polymorphism and another tumor markers such as carcinoembryonic antigen (CEA) and cancer antigen 15.5 (CA15-3). **Materials and Methods:** Forty one women with breast cancer with age ranged between (16 – 82) years and 45 apparently control women with age ranged between (18 – 50) years were included in this case-control study performed during Oct., 2019 and July, 2020. All samples were obtained from Imam Al-Hassan Oncology Unit, Al-Hussein Teaching Hospital, Al-Hussein Medical City, Kerbala Health Directorate / Kerbala – Iraq. The relation between the levels of various tumor markers including MMP-2, CEA and CA15-3 and clinical pathological parameters were determined. The MMP-2 gene polymorphism was analyzed in order to interpret its roles in pathogenesis of breast cancer and correlation with tumor markers studied. **Results:** The amplicon size of the MMP-2 gene was 304 base pair, and the amplification results for amplification of the MMP-2 SNP gene rs243865 showed one wild type (CC), heterozygous (CT) and homozygous (TT) bands, after amplification reactions by allelic specific polymerase chain reaction. Non-significant association was found between serum levels of MMP-2, CEA and CA15-3 (P> 0.05). Elevated levels of serum CEA and CA15-3 of 41 postmenopausal patients were determined in (63.74%) and CAE (21.3%), respectively. Larger tumor size, advanced axillary lymph nodes and TNM stage showed higher incidence of elevated CEA and CA15-3 levels. The elevation of CA15-3 levels was significantly greater in patients with HER2-positive tumors 10 (24.39%), and the elevation of CA15-3 levels was significantly greater in ER 36 (87.8%) patients with breast and PR status 29 (70.7%). A lower sensitivity to CEA compared to CA15-3 in diagnosing breast cancer was determined. **Conclusions:** In the present study, the relation between levels of *MMP-2* gene polymorphism and serum marker of CA15-3, CEA and well clinic pathological features of breast carcinoma was shown, whereas the prognostic importance of CA15-3 and CEA was shown in the follow-up of patients with breast cancer.

KEYWORDS: Breast cancer, MMP-2, CEA, CA 15-3, allelic PCR.

INTRODUCTION

The aggressiveness of cancer derives from local tissue invasion and progression to remote areas. Invasive growth of neoplastic cells into the host tissues is a crucial phenomenon during cancer progression: it includes a number of complex interactions that arise at the tumor-host interface, including angiogenesis and substantial extracellular matrix (ECM) re-modeling.[1] This process involves many steps, including extracellular protease secretion and proteolysis, endothelial cell proliferation and migration to form capillary buds and lumen closure.[2,3] In the first phase, some proteases such as mineral matrix proteins (MMPs) are thought to play an important role in the vascular reaction.[4,5] The first step towards entering and penetrating cancer cells is the digestion of the basement membrane located under the endothelium. The main component of the basement membrane is type IV collagen, which forms the first basic layer that cancer cells reach when they become invasive. The mineral protein, that is, 72 kDa type IV collagen (MMP-2 or gelatin A), promotes its degradation.[6] The survival rates have increased in recent years, considering the growing prevalence of breast cancer, due to deep studies on the biological activity of breast cancer.[7] Typical pathological factors such as tumor size, tumor grade, status of the lymph node,

molecular markers including hormone receptor activity, and expression of human epidermal growth factor receptor 2 (HER2).[8] In screening, early detection of recurrence and treatment of certain malignancies, serum tumor markers play a significant role.[9,10]

The two most frequently used serum tumor markers in the therapeutic field for over 30 years are carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA15-3) in breast cancer. The prognostic importance of preoperative levels of CEA and CA15-3 in breast cancer has drawn a lot of interest in recent years. The study has shown that preoperative CEA data levels combined with levels of CA15-3 can provide valuable knowledge for breast cancer diagnosis and treatment.[11-13]

Cancer antigen15-3 is also a protein, which is a substance from natural breast tissue. If the breast contains cancer, the amount of CA 15-3 can increase as the number of cancer cells increases. It is a monoclonal antibody made from human breast cells (molecular weight: 300-450 kDa).[14] As far as CA15-3 is concerned, it is a mucino genic antigen product of the MUC1 gene which is not confirmed, however recent evidence indicates that it plays a role in cell adhesion, leading to decreased interactions between cell-cell and extracellular matrix, immunity, and metastasis.[15]

Carcinoebyrionic antigen (CEA) is one of the first markers of the carcinogenic glycoprotein to be identified and used, which is expressed in normal mucosal cells and is overexpressed by adenomas, especially the colon, rectum, breast, pancreas, and lung. CEA and long-chain gamma IGG show that CEA is part of the "superfamily" immunoglobulin gene. The European group on tumor markers has recommended that CEA and CA15-3 levels can be used for assessing prognosis, the early detection of disease progression, and treatment monitoring in breast cancer.[16] As a result, the American Society of Clinical Oncology (ASCO) guidelines do not currently recommend the use of serum CA 15–3 and CEA for or screening, diagnosis, staging, or routine surveillance of breast cancer patients after primary therapy.[17,18] There is no evidence for efficacy of screening with this marker in breast cancer: CA15-3, in fact, is elevated in only 3% of patients with localized cancer while it is elevated in up to 70% of patients with metastatic disease.

For this reason, a review of the clinic-pathological data of breast cancer patients was performed in the current study to investigate the associations between preoperative serum CEA, CA15-3 levels and clinicopathological parameters with the MMP-2 levels and its gene polymorphism before chemotherapy in different stages and the prognostic importance of these biomarkers with the development of breast cancer and its association with pre- and postmenopausal patients.

MATERIALS AND METHODS

This study include forty-one women with breast cancer underwent surgery with age ranged between (16-82) years and another 45 individuals as apparently control with age ranged between (18-50) years during Oct., 2019 to July, 2020 at Imam Hussan Oncology Center, Al-Hussein Teaching Hospital, Al-Hussein Medical City / Kerbala Health Directorate / Kerbala- Iraq. Before treatment for stage I–III invasive breast cancer informed consent for intravenous blood was obtained from all patients. Inclusion criteria were: female; invasive breast cancer; underwent mastectomy or breast-conserving surgery; CEA and CA15-3 levels were determined before surgery; tumor completely removed by surgery with pathologic evaluation; before chemotherapy, adjuvant radiotherapy; complete results of estrogen receptor, progesterone receptor, HER2, and histologic grade. Exclusion criteria were: Patients with hyperprolactinemia, thyroid diseases, diabetes mellitus, and ischemic heart diseases. Samples of whole blood were collected from patient and healthy control group in the EDTA tubes and gel tube.

All patients had an anatomically confirmed diagnosis of primary breast cancer and did not receive any treatment chemotherapy or radiotherapy, and for all patients, the histological diagnosis and stage of cancer were determined by evaluation. and were serum samples collected from patient, blood collected in gel tube without anticoagulant was centrifuged at 1600 × g for 10 min at 4°C one hour after collection and transferred into tubes and kept at −20 °C . Serum CEA and CA15-3 levels were determined using an automatic electrochemistry luminescence immunoassay analyzer the cut-off values of CEA. MMP-2 was also analyzed at known concentrations with the level of estrogen and progesterone receptors.

This study was carried out in accordance with the ethical guidelines and was of the approved by the Medical Ethics Committee at College of Medicine/University of Kerbala and from Immam Al-Hassan Oncology Unit, Al-Hussein Teaching Hospital, Al-Hussein Medical City, Kerbala – Iraq.

RESULTS

Table.1: Levels of tumor markers in sera of breast cancer and control.

Tumor Marker	Mean ± SD		P value
	Patients N = 41	Control N = 45	
CA 15–3, U/ml	25.45 ± 20.41	14.1 ±7.05	≤ 0.01
CEA, ng/ml	3.58 ± 2.92	2.15 ± 1.56	≤ 0.01

The data shown in (table.1) indicate the mean ± SD level of serum tumor markers in breast cancer as compared with control women, in a patient the serum marker CA15-3 is (25.45 ± 20.41 U/ml) and CEA in the patient was (3.58 ± 2.92 ng/ml) were elevated and significantly correlated with the apparent control (14.1 ± 7.05 U/ ml) (2.15 ± 1.56 ng/ml) group respectively ($P \leq 0.01$). Our analysis also focused on 41 female breast cancer patients with a mean age (47.36 years ± 14.29 years) and a mean age of 45 control women (32.6 ± 7.02 years). On women with breast cancer, the concentrations of CEA and CA 15-3 were compared between different tissues (invasive ductal carcinoma 95%) with (invasive lobular carcinoma 5%) in (Figure 1). The cancer stage was (stage II versus 65.85%) compared with the rest of the stages, and the presence of estrogen receptors (87.8%) or progesterone receptors (70.73%) was observed, did not reveal any statistically significant difference with respect to any status of estrogen or progesterone in Ultimately, histo-type or disease stage. It is important to note levels of data in patients whose tumor progression was not significantly different from patients with tumor progression and to assess disease progression risk associated with serum markers.

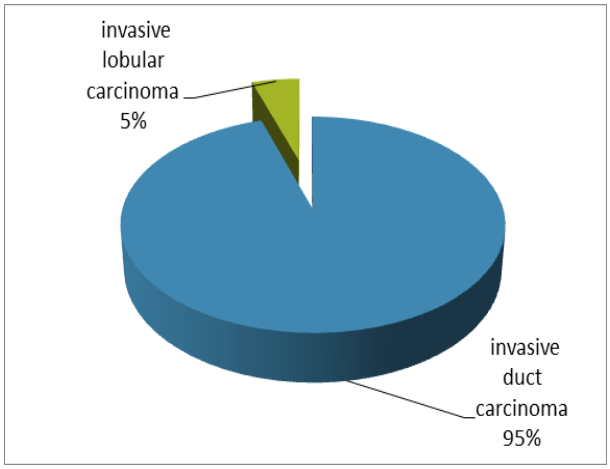

Fig.1: Chart represents the histopathological types of tumor specimens.

The data observed regarding the degree and stage of breast cancer indicate that stage (ll) or the second stage was higher than the other stages and the degree of this stage was higher than the other grades due to the first manifestations of signs and pain in **(Fig. 2)**. The number of patients in second stage were 27/41 (65.85%)), followed by the third stage patients which represents 10/41 (24.39%) and then finally the first stage which represents 4/41 (9.75%), while the fourth stage which represents 0/41 (0%) as shown in **(Fig 3).**

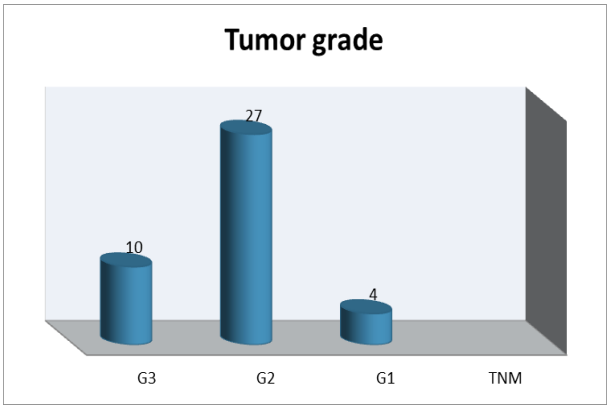

Fig.2: Classification of grade depended on number of breast cancer women.

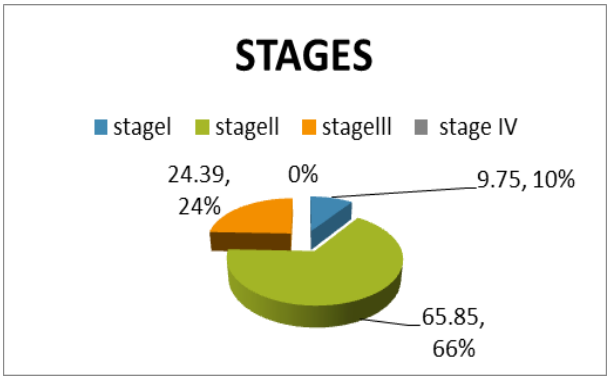

Fig.3: The different tumor stages of the tumor specimens.

The immunohistochemical study of breast specimen indicated that 36/41(87.8%) were positive for estrogen receptor whereas 5/41(12.19%) cases were negative to progesterone receptors, while 29/41 (70.73%) were positive for the progesterone receptor. Dual positivity for both estrogen and progesterone receptors were encountered in 35/41 (85.36%) cases, while dual negativity was observed in 4/41(9.75%) cases as show in **(Fig.4).**

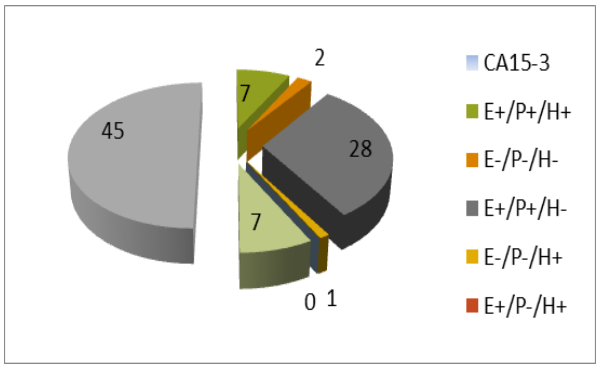

Fig. 4: The percentage distribution of subtypes of breast cancer according to ER, PR and HER2 molecular IHC staining reactions.

The mean ± SD of serum CA15-3 level was (26.72 ± 22.18) U/ml and (27.18 ± 18.91) U/ml for premenopausal and postmenopausal patients,

respectively. These findings revealed that significantly elevation of premenopausal and postmenopausal patients (***P***≤0.01) relative to stable control patient. The mean ± SD of the serum CEA level for premenopausal and postmenopausal in breast cancer was (3.04 ± 1.67) ng/ml and (3.74 ± 2.38) ng /ml respectively. These findings revealed that premenopausal and postmenopausal patients with breast cancer have importantly significant higher levels (***P***≤ 0.01) as compared with the apparently control group.

In table-2 a highly significantly different statistical relationship was found between the MMP-2 proteases, CA 15-3, MMP-2 and CEA in the CC allele (***P***≤ 0.05), and there was a non-significant correlation in the CT and TT alleles. The levels of CA15-3 and CEA were significantly higher, and the correlation coefficients enabled us to note that the higher values of the data were in association with high protease concentration.

Table 2: Levels of Tumor Markers Studies as compared with MMP2 Genotypes.

MMP2 (-1306 C>T) (rs243865) Genotype	**CA 15–3 Breast Cancer Mean ± SD**	**CA 15–3 Control Mean ± SD**	**P value**
CC	39.4 ± 51.7	15.2 ± 7.6	≤ 0.05
CT	41.7 ± 77.2	12.9 ± 5.8	0.2
TT	45.6 ± 59.7	10.02 ± 6.9	0.4
MMP2 (-1306 C>T) (rs243865) Genotype	**CEA Breast Cancer Pre- PCOS Mean ± SD**	**CEA Control Mean ± SD**	**P value**
CC	12.02 ± 24.96	2.03 ± 1.2	≤ 0.05
CT	12.6 ± 22.9	2.6 ± 1.6	0.1
TT	15.2 ± 32.4	1.5 ± 0.9	0.5

A relationship between tumor markers determined (Mean ± SD) and alleles of MMP2 gene in patient and control were observed with allele (CC,CT,TT) regarding CA15-3. The significant results (***P*** ≤ 0.05) were observed between patient (39.4 ± 51.7 U/ml) and control (15.2 ± 7.6 U/ml) in CC alleles of MMP2 gene.

A non–significant result was obtained between patient of breast cancer (41.7 ± 77.2 U/ml) and control (12.9 ± 5.8 U/ml) in according to alleles of MMP2 gene in CT allele (*P* = 0.2). The correlation between CA15-3 and alleles of MMP2 gene in breast cancer patient was (45.6 ± 59.7 U/ml) as compared with control (10.02 ± 6.9 U/ml) in according to TT allele which were revealed a non–significant result (***P***=0.4), and the (mean ± SD) of CEA and alleles of MMP2gene in patient and control allele (CC, CT and TT). The highly significant result (***P*** ≤ 0.05) that shown between breast cancer patient (12.02 ± 24.96 ng / ml) and control (2.03 ± 1.2 ng/ml) in CC alleles of MMP-2 gene. The non–significant result that shown between patient breast cancer (12.6 ± 22.9 ng/ml) and control (2.6 ± 1.6 ng/ml) alleles of MMP-2 gene in CT allele ***P*** ≤0.1. The correlation between CEA and alleles of MMP-2 gene in patient (15.2 ± 32.4 ng /ml) and control (1.5 ± 0.9 ng/ml) TT allele were revealed non-significant result (***P*** = 0.5).

DISCUSSION

In the current study, involving 41 patients, the predictive significance of preoperative and prior evaluation of the chemotherapy serum tumor marker CEA and CA 15-3, the results showed that preoperative serum CEA data and CA15-3 levels were independent variables influencing prognosis. The efficacy of calculating CEA and CA15-3 levels in breast cancer patients remains controversial. CEA and CA15-3 levels have been recommended by the European Oncology Marker Group for prognosis, early diagnosis of disease progression, and patient monitoring of breast cancer.[15] The American Society of Clinical Oncology (ASCO) and recommendations The National Systemic Cancer Network (NCCN) do not formally recommend the use of serum CA 15-3 and CEA for breast cancer screening and control.[17] On the other hand, the reason may be partly due to the conflicting conclusions obtained from various works.[11,12,27-29] The frequency of breast cancer triumphs has risen gradually over the last two decades, but the survival rates of breast cancer have improved in recent years due to the early diagnosis and increased use of more successful systemic treatment, and early breast cancer accounts for a significant proportion. Previous studies have shown that CEA and CA15-3 levels are correlated with markers of tumor load, including tumor size and lymph node status[28,30], and locally advanced breast cancer patients have slightly higher CEA and CA 15-3 levels.[31,32]

The present studies also found that higher levels of CEA and CA 15-3 were observed and are more than that found in normal. As predicted, the incidence of irregular serum CA 15-3 and CEA decreased with the growth of early breast cancer patients which does not indicate that therapeutic benefit is therefore low. The **MMP-2** gene was the most distinct of the more than 15 types of MMPs and has been reported to play a major role in the degradation of macromolecule structures of connective tissue such as collagen, proteoglycans, laminin and

fibronectin.[18] Stromal ECM development includes stromal ECM modification and fibrinogenic reaction often occur in the adjacent stroma due to malignancy[19], and tumor stroma formation is used as a non-specific effort by the host to the tumor wall and is considered to have a deleterious effect on growth from the tumor. Malignant cells detach from the primary tumor during the course of metastasis, infiltrate the stromal tissue, enter the bloodstream, stop at the peripheral vascular bed, infiltrate, and invade the interstitial tissue and parenchyma of the target organ, forming a diffuse colony. Cancer cells must avoid immune monitoring of the host, thus successfully initiating only a small fraction of the circulating cancer cells and enabling endothelial cell migration and formation of new blood vessels through stealing.[20,21] The observed data obtained from various studies, reflected tumor tissue expression, and measured serum levels of MMPs and CA15-3, CEA to assess their role in tumor growth.[22]

Determination of blood levels of angiogenic cytokines can assist in determining which of them may be used as diagnostic and prognostic markers and to assess the efficacy of MMP inhibitors.[23,24] The association between serum expression of gelatinase and serum CEA and CA 15-3 levels was explored in this research. Our data indicate that a non-correlation between the levels of MMP-2 in the blood, indicating that this gelatinase does not play a significant role in tumor growth. There was a non-correlation between serum CA15-3 and CEA levels and disease stage or survival, although the small number of patients could explain these results. Our observations do not indicate a significant association between CA15-3 and CEA levels after surgery and disease outcome. Several authors have recorded evidence that CA 15-3 is one of the most specific neoplastic markers in separating malignant neoplasms from benign diseases, particularly breast cancer. We observed that levels of CA 15-3 metastasized were higher in patients with advanced or metastatic breast cancer. MMPs are not produced directly by tumor or stromal cells, but other sites may be responsible for the increased levels of MMP-2 data that correlate with the presence of tumor tissue. The absence of a link between the clinical features of MMP-2, CEA, CA15-3, and CA15-3 indicates that these proteases and cytokines depend on tumor activity and not on disease stage or tumor histological form. Conclusion, this report indicates high data levels of CA 15-3 and MMP-2 in patients with breast cancer serum, as described in our previous study.[25] MMP-2 is predictive of more aggressive tumor colonies, associated with poor prognosis and survival.[26]

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