

## Correlation of CA125, and Some Mineral in the Progression of Breast Cancer in Pre- and Postmenopausal Iraq Women

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### ABSTRACT

**Background:** Breast cancer presents a severe threat to women's health worldwide, as it is the most often diagnosed cancer and the primary cause of mortality for female patients.

The aim of this study was to study the relationship between Ca 125 and insulin with some chemical tests in order to know the relationship between them and therefore the speed and accuracy in diagnosing breast cancer in women early.

**Material and method:** In this research, there are 160 participants (age 21 - 61 years) divided into three groups. The first group included 50 (group A) that represented Premenopausal. The second group included 55 (group B) that represented postmenopausal the A and B group suffering breast cancer who visited Baghdad Medical City Hospitals-Bab Al-Moadham and the third group control group (55 participant). The blood (5ml) was drawn from every participant to separate the serum. The serum was used to measure (CA125, Insulin, C-RP, Mg, Iron and Ca). The results showed there is significantly an increase in the level of CA125 and C-RP in the serum of menopause women with breast cancer but the insulin non significantly different between study group. In addition, there is significantly slightly increase in the level of Mg, Iron and Ca in the serum of breast cancer women than control group.

**As conclusion:** The relationship between Ca 125 was positive and of clinical significance for disease progression. CR-P, which developed a lot because it was a strong indicator of inflammation. As for the prognostic elements (Mg, Iron and Ca), they were of great importance in their association with the disease, and therefore it may be an important indicator in the early diagnosis of the disease. The serum levels of CA125 and CR-P were demonstrated to be of great value in the management of patients with breast cancer, and could serve as predictive indicators and for monitoring the course of disease.

**Keywords-** Breast cancer, Mineral, Ca125, premenopausal, postmenopausal, CRP, Mg, Ca and Iron.

## I. INTRODUCTION

Breast cancer is one of the many common malignant tumors, which affect women. Numerous internal and external variables contribute to the development and occurrence of breast cancer. Its incidence is associated with social-psychological variables, environmental conditions, and poor lifestyle choices it has been demonstrated that 5% to 10% of breast cancers can be attribute to genetic mutations and family history, and 20% to 30% of breast cancers can be attributed to factors that may be modifiable (1). Breast cells are where breast cancer first develops. A collection of cancer cells known as a cancerous tumor is capable of spreading into and destroying nearby tissue. As well as spreading throughout the body, it can. Breast cells occasionally undergo changes that prevent them from growing or behaving normally. Non-cancerous breast conditions atypical hyperplasia and cysts may result from these changes (2). In the United States, 195 000 women are diagnosed with breast cancer annually: 25% are in their reproductive years and 70% of them in premature menopause following adjuvant chemotherapy (3). Menopause is defined by the World Health Organization (WHO) as the absence of menstruation for a year because of a drop in estrogen hormone levels due to ovarian function discontinuation.

The average age of menopause, which varies by society, is 51 in the world and 47 in Turkey. As a result, women should expect to spend a third of their lives dealing with menopause and its associated issues (4).

The period from normal ovulatory cycles before menopause to the end of menstruation is characterized by menstrual irregularities and is called the perimenopausal transition years. According to the definition of WHO, perimenopause is the period before menopause, which includes the period of one year following the last menstrual period from the onset of any clinical, biological and endocrinological signs of impending menopause. According to some authors, perimenopause is the period before and after the last menstruation (menopause), surrounding this event (5). Cross-sectional surveys suggest that symptoms of estrogen deficiency, which occur in up to 66% of women treated for breast cancer, are the most common side effects of adjuvant therapy. Furthermore, they may be more bothersome and persist for longer time as compared with the same symptoms experienced by healthy women (6). A 50-year-old woman has a 10% chance of developing breast cancer during her remaining life. In the first years of hormone therapy, uncontrolled, high-dose and long-term use of synthetic preparations and the increase in the incidence of breast cancer caused this treatment to be feared for many years. However, today's results reveal that the risk ratios are not high enough to prevent treatment, considering the balance of profit and loss (7).

According to the studies, although no risk increase was detected for five years of continuous use, it is suggested that there is an approximately 20-30% risk increase up to 15 years in use for more than eight years (8). The Million Women Study, undertaken in 2003, found that those who had recently undergone hormone therapy had a greater risk of breast cancer than women who had never gotten it. The risk was found to be 1.30 for those receiving estrogen, 2.0 for those receiving combined therapy, and 1.45 for those receiving Tibolone (9). The fact that progesterone increases mitotic activity in breast tissue suggests that progesterone will be risky.

Quantitative variations of serum tumor markers have been developed as non-invasive tools for the assessment of treatment efficiency in human malignancies. In breast cancer, cancer antigen 125 (CA125) is the most widely used serum tumor markers in clinical routine, although their usefulness remains controversial (10). The CA 125 test determines how much CA 125 protein (cancer antigen 125) is present in the blood. The CA 125 test can be used to track the progression of some cancers both during and after therapy. CA 125 may be used in some situations to search for early symptoms of ovarian cancer in patients who are at a high risk of getting it (11). CA125 is proposed as a serum biomarker for ovarian cancer, but elevated levels have been observed in up to 84% of metastatic breast patients (12).

Therefore the aim of this study to demonstrated that the level of some marker in breast cancer women patients can uses as biomarker to early diagnosed breast cancer in women.

## II. MATERIAL AND METHOD

### *Study population*

In this research, there are 160 participants (ranging in age from 21 to 61 years old) divided into three groups. The first group included 50 (group A) that represented Premenopausal. The second group included 55 (group B) that represented postmenopausal the A and B group suffering breast cancer who visited Baghdad Medical City Hospitals-Bab Al-Moadham. The third group included 55 (group C) who did not take any synthetic hormones and served as a control. All participants answered the questionnaire form that included their age, and any chronic illnesses.

### *Blood Sampling*

Following the acquisition of permission, five milliliters of blood drawn from each subject, control and patient groups, by venipuncture and put in a gel activator tube. The blood samples were allow coagulating for 15 minutes at 40°C and then were centrifuge at 3000 rpm for 10 minutes to separate the serum. The serum was put in Eppendorf tubes and refrigerated at -20°C until used. The serum was used to determine:

#### **1- The level of CA125 marker**

The Human Mucin 16 solid-phase sandwiches ELISA (enzyme-linked immunosorbent test) is use to determine how much of the target is bound between two antibodies. The wells of the supplied microplate have been pre-coat with a target-specific antibody. The wells are subsequently fill with samples, standards, or controls, which bind to the immobilized (capture) antibody. The second (detector) antibody is add to the sandwich, and then a substrate solution is add, which reacts with the enzyme-antibody-target complex to create a quantifiable signal. The intensity of this signal is relate to the target concentration in the original material.

#### **2- The level of Insulin hormone**

The Human Insulin Solid-Phase Sandwiches ELISA (enzyme-linked immunosorbent assay) is use to determine how much of the target is bound between two antibodies. The wells of the supplied microplate have been pre-coat with a target-specific antibody. The wells are subsequently fill with samples, standards, or controls, which bind to the immobilized (capture) antibody. The second (detector) antibody is add to the sandwich, and then a substrate solution is add, which reacts with the enzyme-antibody-target complex to create a quantifiable signal. The intensity of this signal is relate to the target concentration in the original material.

**3- The level of calcium, magnesium and Iron that determined by:**

A German-made Cobas 6000 analyzer (c501) is an entirely automatic analyzer by uses a proprietary Electro –chemi-luminescence (ECL) system and employs a competitive test principle by using a monoclonal antibody that is directed against these parameters.

**Statistical analysis**

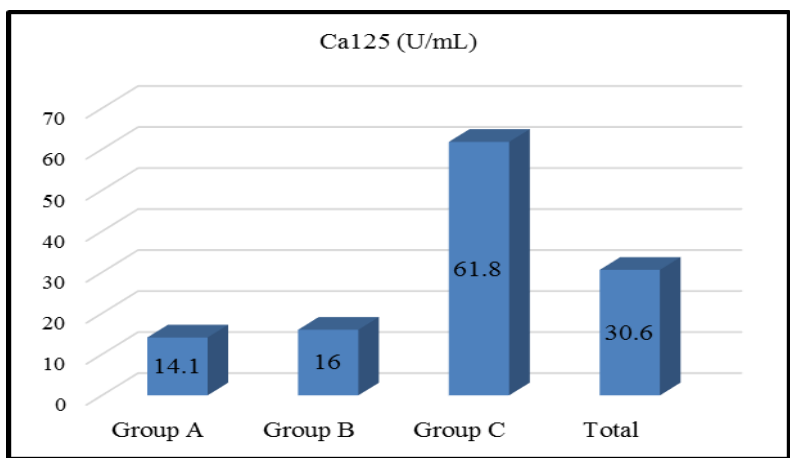
All experiments were conducted at least twice, unless otherwise stated. To express all quantitative data, the mean standard deviation (MeanSD) was employed. The data of healthy women was compared to diseased women's samples obtained from an oncology hospital. The chemistry data were analyzed with the SPSS 25 statistical program. As a statistical significance criterion, P values less than 0.05 were utilized. P-values and means were determined using the one-way ANOVA test.

**III. RESULT**

Table (1) show the comparing Group A to Group C, the mean Ca125 differs significantly when compared to Group A (14.1 ± 6.88) with Group B and C (16.0 ± 8.29, 61.8 ± 12.8 respectively) and total was (30.6 ± 24.3). The minimum value of group A, B, C and total was (3.53, 6.73, 44.90, 3.53 respectively) and the maximum value of group A, B, C and total was (25.80, 31.70, 79.20, 79.20 respectively).

**Table (1): The levels of Ca125**

CA125 (U/mL)	MEAN±SD	MIN.	MAX.	SIG.
Grop A (n = 50)	14.1 ± 6.88	3.53	25.80	0.001
Grop B (n = 55)	16.0 ± 8.29	6.73	31.70	0.001
Grop C (n = 55)	61.8 ± 12.8	44.90	79.20	0.001
Total (160)	30.6 ± 24.3	3.53	79.20	



**Figure 1: Mean Ca125 shows a significantly different for groups A, B and C**

According to Table 2: The mean insulin was a non-significantly between groups B and C (15.2 ± 6.52, 12.0 ± 4.51 ng/mL respectively) compared to group A (16.6 ± 4.20) and total was (14.6 ± 5.38). The minimum value of group A, B, C and total was (9.54, 5.84, 6.84, 5.84 respectively) and the maximum value of group A, B, C and total was (22.70, 26.00, 19.40, 26.00 respectively).

**Table (2): The levels of insulin (U/mL) in A, B and C group.**

INSULIN (ng/mL)	MEAN±SD	MIN.	MAX.	SIG.
Grop A (n = 50)	16.6 ± 4.20	9.54	22.70	0.138
Grop B (n = 55)	15.2 ± 6.52	5.84	26.00	0.138
Grop C (n = 55)	12.0 ± 4.51	6.84	19.40	0.138
Total (160)	14.6 ± 5.38	5.84	26.00	

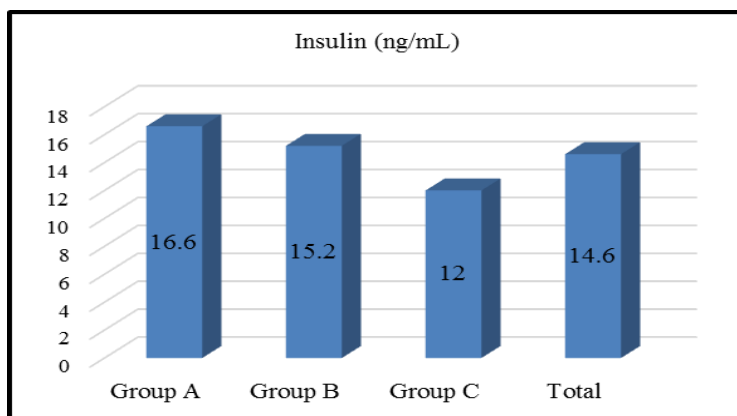


Figure 2: Mean insulin shows a non-significantly different for groups A, B and C

In table 3: show the mean C-RP differs significantly from Group A ( $1.97 \pm 1.14$ ) with Group B and C ( $2.80 \pm 1.61$ ,  $7.88 \pm 0.85$  mg/L respectively) and total was ( $4.21 \pm 2.91$ ). Clinically significant C-RP increase in breast cancer. The minimum value of group A, B, C and total was (0.74, 0.67, 6.50, 0.67 respectively) and the maximum value of group A, B, C and total was (3.73, 5.47, 8.95, 8.95 respectively).

Table (3): The levels of C-RP (mg/L) in A, B and C group

C-RP (mg/L)	MEAN±SD	MIN.	MAX.	SIG.
Grop A (n = 50)	$1.97 \pm 1.14$	0.74	3.73	0.007
Grop B (n = 55)	$2.80 \pm 1.61$	0.67	5.47	0.007
Grop C (n = 55)	$7.88 \pm 0.85$	6.50	8.95	0.007
Total (160)	$4.21 \pm 2.91$	0.67	8.95	

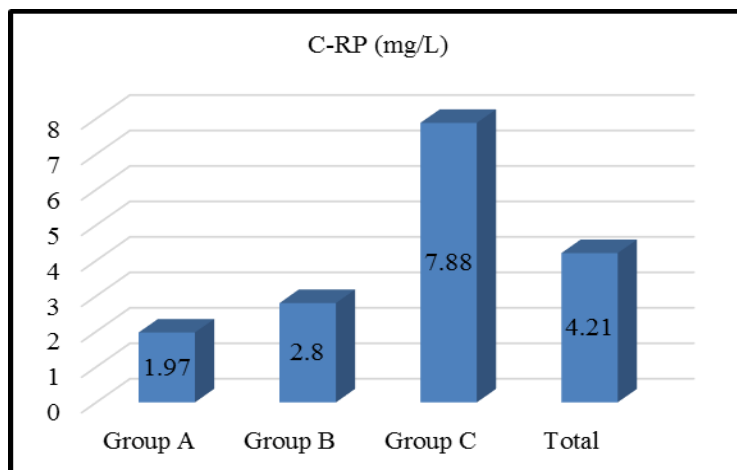


Figure 3: Mean C-RP (mg/L) shows a significantly different for groups A, B and C

In table 4: In same study, the mean calcium (mg/dl) differs significantly between groups B and C ( $8.68 \pm 0.89$  and  $7.88 \pm 0.85$  respectively) compared to group A ( $9.18 \pm 1.09$ ) and total was ( $8.58 \pm 1.07$ ). The minimum value of group A, B, C and total was (7.95, 7.58, 6.50 and 6.50 respectively) and the maximum value of group A, B, C and total was (10.89, 10.30, 8.95 and 10.89 respectively).

Table (4): The levels of Ca (mg/dl) in A, B and C group

Ca (mg/dl)	MEAN±SD	MIN.	MAX.	SIG.
Grop A (n = 50)	$9.18 \pm 1.09$	7.95	10.89	0.017
Grop B (n = 55)	$8.68 \pm 0.89$	7.58	10.30	0.017
Grop C (n = 55)	$7.88 \pm 0.85$	6.50	8.95	0.017
Total (160)	$8.58 \pm 1.07$	6.50	10.89	

In table 5: The mean iron (mg/dl) differs non-significantly between groups B and C ( $41.8 \pm 4.20$  and  $41.6 \pm 4.78$  respectively) compared to group A ( $39.2 \pm 4.17$ ) and total was ( $40.9 \pm 4.40$ ). The minimum value of group A, B, C and total was (31.70, 37.40, 32.60 and 31.70 respectively) and the maximum value of group A, B, C and total was (44.90, 48.30, 47.20 and 48.30 respectively)

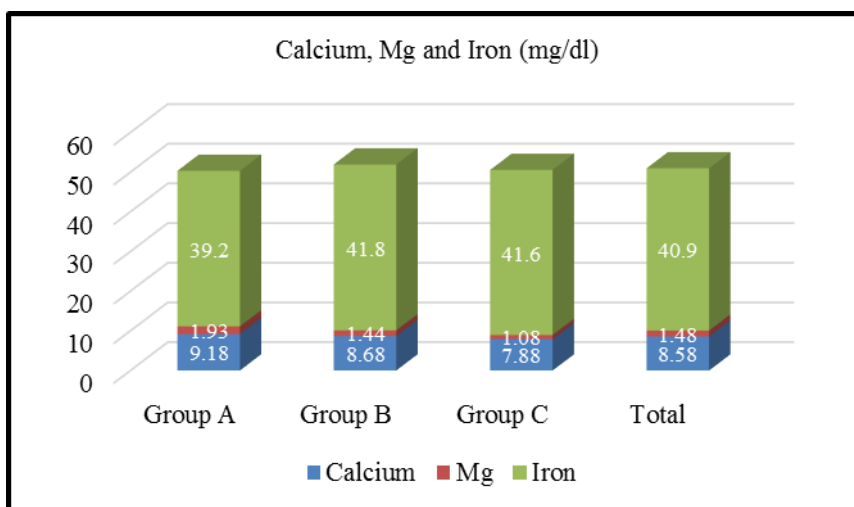
**Table (5): The levels of IRON (mg/dl) in A, B and C group**

IRON (mg/dl)	MEAN±SD	MIN.	MAX.	SIG.
Grop A (n = 50)	$39.2 \pm 4.17$	31.70	44.90	0.360
Grop B (n = 55)	$41.8 \pm 4.20$	37.40	48.30	0.360
Grop C (n = 55)	$41.6 \pm 4.78$	32.60	47.20	0.360
Total (160)	$40.9 \pm 4.40$	31.70	48.30	

In table 6: show a compared to Group A, the mean Mg (mg/dl) exhibits a substantial difference, Group A ( $1.93 \pm 0.36$ ) with Group B and C ( $1.44 \pm 0.53$  and  $1.08 \pm 0.43$  respectively) and total was ( $1.48 \pm 0.56$ ). The minimum value of group A, B, C and total was (1.64, 0.75, 0.74 and 0.74 respectively) and the maximum value of group A, B, C and total was (2.84, 2.25, 1.96 and 2.84 respectively)

**Table (6): The levels of Mg (mg/dl) in A, B and C group**

Mg (mg/dl)	MEAN±SD	MIN.	MAX.	SIG.
Grop A (n = 50)	$1.93 \pm 0.36$	1.64	2.84	0.001
Grop B (n = 55)	$1.44 \pm 0.53$	0.75	2.25	0.001
Grop C (n = 55)	$1.08 \pm 0.43$	0.74	1.96	0.001
Total (160)	$1.48 \pm 0.56$	0.74	2.84	



**Figure 4: Mean Ca, Mg and Iron for groups A, B and C**

#### IV. DISCUSSION

One cancer that is becoming more common in women is breast cancer, whose morbidity and death rates are rising year. Patients with distant metastases of breast cancer have a higher death rate, and even those in stage I have a higher chance of recurrence. Effective diagnosis of distant metastasis of breast cancer is of great significance for guiding clinical treatment, improving prognosis, and reducing mortality (13).

In our study, patients with breast cancer in menopause period show significantly high level of CA125 than premenopausal patient and control group this finding was agreed with Previous study (12)(13). Another study indicated that CA 125 production has been demonstrate in the normal breast it has been reported most often as a marker of pleural involvement with metastatic breast cancer (14). Previous study suggested that the serum levels of this tumor marker (CA125) might be more efficient for monitoring advanced tumors than early (12). With the improvement of screening techniques for tumor detection, the detection rate of breast cancer has been steadily increasing in the last two decades, and early breast cancer accounted for a large proportion. Since elevated levels of these markers are related to late tumor stage,

poor outcome may be predict, and more comprehensive therapy for the patients may be developed. (15). our findings showed there is no significantly different in insulin level in both pre- and post-menopausal women with breast cancer. This finding agreed with previous study that conducted in 50 years old women and demonstrated no significantly different in insulin level in breast cancer women (16). Although in a relevant study, positive association was found between insulin level and breast cancer in both pre- and post-menopausal women Insulin has been shown to stimulate cell proliferation in normal breast tissue and in human breast cancer cells (17). These results justify further investigation of insulin as a biomarker of long-term prognosis in early breast cancer in prospective trials.

C-reactive protein (CRP) is an acute phase reactant influenced by inflammation and tissue damage. It has been demonstrated that elevated CRP levels are associated with poor outcome of cancer including metastatic breast cancer. However, evidence regarding an impact of CRP levels on outcome in early breast cancer (EBC) are missing (18). In current study show that there is significantly different in CRP protein in pre-and postmenopausal women with breast cancer when compared with control group. This meta-analysis assessed the association between CRP levels and breast cancer risk. Overall, the result supported a significant positive association between the elevated levels of CRP and an increased risk of breast cancer. The findings of this meta-analysis indicated that elevated CRP levels was associated with increased risk of breast cancer, especially among the Asian population (19).

Elevated levels of calcium in serum are frequently observe in advanced breast cancer. The hypercalcemia observed in metastatic breast cancer is commonly the result of osteolysis caused by bony metastases. However, elevated serum calcium levels may occur in the absence of bony metastases and are cause by the production by the breast tumors of humoral factors (humoral hypercalcemia of malignancy (20). In current study, there is different in the level of Ca in pre- and postmenopausal women with breast cancer when compared to control group. In previous study conducted in 555 women aged 40–79 with newly diagnosed, untreated breast cancer, they demonstrated a significant positive association between tumor volume and serum calcium levels (20). Hypercalcemia is a frequent complication of breast cancer, particularly in women with bony metastases (21). Another study reported that higher pre-diagnostic serum calcium levels were associated with a significantly increased risk of subsequent breast cancer among overweight and postmenopausal women in Sweden (22).

Iron is an essential cofactor needed for normal functions of various enzymes and its depletion lead to increase DNA damage, genomic instability, deteriorate innate, adaptive immunity, and promote tumor development. It is also linked to tumorigenesis of breast cancer cells through enhancing mammary tumor growth and metastasis. In Saudi study conducted in pre- and postmenopausal women, suffering breast cancer demonstrated that there is significantly different in Iron level in these women. In addition, support the idea of utilizing iron serum level as an early risk measure for assessing possibility of breast cancer especially in young women (23). Previous study reported that iron deficiency and high estrogen levels in premenopausal women could lead to breast cancer due to stimulated production of vascular endothelial growth factor (VEGF)(24).

Path analysis revealed that dietary magnesium affected breast cancer risk both directly and indirectly by influencing the CRP level. The results indicate that a direct negative association and an indirect association through influencing the CRP level were observe between dietary magnesium intake and breast cancer risk (25). In our study exhibits a substantial difference in the level of Mg in pre- and postmenopausal patient when compare to control group. In previous study, the results showed an inverse association between dietary magnesium intake and overall breast cancer risk. Consistent with our result, a case–control study found that the serum magnesium level was significantly lower among breast cancer patients than among control subjects. Magnesium deficiency has been found to be involve in both the risk and prognosis of cancers, including breast cancer (26).

This pilot retrospective study investigated the association between C125 level, insulin, CRP level and some mineral and the risk of developing breast cancer among pre and postmenopausal patients. Findings from this study recommend that clinicians and pathologists should pay closer attention to their patients as they could have higher risk of developing breast cancer.

## V. CONCLUSION

When breast cells change and develop into malignant cells that proliferate and create tumors, it is known as breast cancer. Breast cancer typically affects women at age 50 and older, but it is also effected younger women. The relationship between Ca 125 was positive and of clinical significance for disease progression. To clearly define its involvement in the treatment of breast cancer more information about CA 125 is need. Insulin was not important in the development of breast cancer, according to the study we conducted, in contrast to CR-P, which developed a lot because it was a strong indicator of inflammation. As for the prognostic elements (Mg, Iron and Ca), they were of great importance in their association with the disease, and therefore it may be an important indicator in the early diagnosis of the disease. Our findings suggest that high levels of iron and calcium in benign breast tissue may be linked to a slight increase in the risk of later breast cancer.

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