

Detection of C Reactive Protein level in breast cancer

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Abstract:

Background: breast cancer is most prevalent malignancy in females in Sudan. The diagnosis and prognosis of breast cancer in Sudan need additional marker.

Objectives: The objectives of this study was to study association between breast cancer and CRP level, and also to compare CRP level between known diagnostic breast cancer women and healthy control women, in addition to asses uses of CRP test as diagnostic and prognostic marker in cancer.

Materials and Methods: A case-control study was conducted at National Cancer Institute – University of Gezira (NCI – UG) during 2013. The study involved 60 subjects (20 healthy women, who are in accordance with examined groups concerning age, 40 confirmed breast cancer cases).the level of CRP in serum determined by immunoturbidimetry method using biosystem analyzer.

Results: An average of CRP level in serum of the control group was 2.91 mg /L, , while the average of CRP level in serum of patient group was 17.68 mg /L, which is statistically much bigger in relation to control group ($P < 0.00$).

The CRP level was high in 31 cases (77.5 %), and normal in 9 cases (22.5 %) giving statistically significant correlation between values CRP level and breast cancer ($r = p$) was determined.

Conclusion: study results have shown that CRP level in serum can be a useful diagnostic and prognostic marker in inflammatory process in cancer.

Key words: C reactive protein level, breast cancer, Sudan

C-reactive protein (CRP) is one of an acute-phase reactant that is increased during bacterial infection, inflammatory disease, trauma, myocardial infarction, surgery, and cancer; liver is produced CRP in response to elevated cytokine levels after an inflammatory stimulus (1-4).

Breast cancer is one of types of cancer (malignant tumor originating from breast tissue and abnormally developed. It is a heterogeneous disease composed of a number of recognizable histological and intrinsic molecular subtypes (5-7).

Breast cancer is one of commonest female malignancy in the world, approximately 75% over age of 50 years (8-11), its incidence is rising at a rate of a proximately 2% per year in all populations(12).

Breast cancer it is the highest prevalent cancer among females in east Africa region (34.5%) (13), and It accounts for 34.5% of all female cancer in Sudan (14).

According to latest WHO data published in April 2001 breast cancer in Sudan reached 1,968 or 0.53% of total death (15).

A epidemiological study in the Sudan showed (reflect) that breast cancer is most prevalent malignancy in females, however it has been reported that it is rarely seen in women below 30 years age ($p = 0.006$) (16).

The essential problems concerning breast cancer relates to the lack of patients awareness about the disease. Limitations in implementing breast self-examination and mammography screening programs are the other important problems. Overall survival and mortality due to breast cancer are influenced strongly by the stage of the disease at diagnosis. About 54% of the women are diagnosed in stage II, while only 16% are diagnosed in stage I (17).

The essential Pathophysiology of breast cancer like other cancers occurs because defective gene, damage to the DNA,

genetic mutation and other factors which lead to uncontrollable tissue growth (18).

The diagnosis of breast cancer depend on clinical exam, imaging (ultrasound scanning – mammograms), tissue biopsy, genetic markers (as BRCA.1), CT scan, MRI and hormone receptor test (19).

Once breast cancer is diagnosed, it is divided as per the histological or cellular type, staged, and gave treatment. Breast cancer divided into many types as DCIS, LCIS, invasive ductable breast cancer, invasive lobular breast cancer, and IBC. Breast cancer staged and named by roman number from 0 to IV (19).

The treatment of breast cancer includes surgery, radiotherapy, chemotherapy, hormone therapy, and biological therapy (19, 20).

In previous studies, CRP levels have been found to be highly elevated in patients with cancer compared with healthy control subjects or subjects with benign conditions (21, 22).

Higher CRP expression in breast cancer is strongly predicting poor survival of patient (23).

In recent years, elevated levels of CRP have been reported as a risk factor for the development of breast cancer (24).

Cynthia, A. Thomson *et al* found CRP was moderately or severely elevated in 90.5% of the breast cancer patient (mean of 5.1-5.3mg=dL) in 2009 (8).

Kristine, H. A et al was found Elevated plasma levels of C-reactive protein (CRP) associated with breast cancer in 2011(25).

The breast cancer in Sudan increased every year and the methods of diagnosis and prognosis are more expensive and invasive. Therefore, the application of less invasive, quick and cheap technique is very consequential, particularly in a country like the Sudan, where many patients present from remote areas with poor health services. For that reason, the incidence and mortality of breast cancer are high, remarkably constant and

the frequency is increasing, particularly amongst younger women.

Therefore we made this study to assist in diagnosis and prognosis with simple, quick, cheap, and non invasive test.

Patients and Methods:

Patients:

The study involved 60 subjects who were divided into two groups. In the first group (40 subjects) were female patients suffering from breast cancer with average age of 46 years, (range from 26 To 71 years), hospitalized in the National Cancer Institute – University of Gezira (NCI – UG) in Gezira state in the Sudan.

The first group has 3 stages which were stage II (5 subjects) about 12.5%, with average age of 33 Years, and they were from 35 to 45 Years old. Stage III (16 subjects) about 40%, with average age of 48.8 Years, and they were from 37 to 71 Years old. Stage IV (19 subjects) about 47.5%, with average age of 45.4 Years and they were from 26 to 70 Years old.

All subjects (patients) were diagnosed with breast cancer by specialist's oncologist.

The second group was control group which consisted of 20 subjects (women) who are in accordance with examined groups concerning age. With average age of 45.4 Years, and they were from 33 to 70 Years old with no family members suffering from breast cancer.

Personal data and disease information was recorded using a questionnaire. It included age, stage of disease, occupation, residence, and past medical history. Three mL of venous blood were taken in plain container.

Measurement of CRP:

Procedure of CRP level in serum was measured by preparing working reagent (1 ml of reagent B + 4 ml of reagent A), then zero instrument with D.W, pipette one ml of working reagent

into three tubes labeled blank – standard – sample, add 0.007 ml from reagent S to standard tube, add 0.007 ml from sample into sample tube, mix gently, measure the absorbance of sample and standard against blank at 540 nm immediately (A1) and then after 2 minute (A2), and finally calculate result sample.

$$A \text{ sample} = A2 - A1$$

CRP level = A sample / A standard * concentration of sample.
Referent interval of CRP level for this method was up to 6mg/L.

Statistical Analysis:

Study results were entered in SPSS version 20 and were expressed in form of arithmetic mean \pm standard deviation, the data were analyzed using chi – square test, compare mean one sample T test, correlation, and cross tabulation.

Results:

An average CRP level in serum of the control group was 2.91 mg /L, the range of obtained values was from 0.95 To 5.08 mg/L, and The average of CRP level in serum of patient group was 17.68 mg /L, the range of obtained values was from 2.84 To 69.55 mg/L, what is statistically much bigger in relation to control group ($P < 0.00$).

The mean of CRP level in patients was 17.67 mg/L and 2.91 mg/L in healthy controls (table 1).

Table 1. Comparison of CRP test between patients and healthy controls.

Sample	No of cases	Mean
Patients	40	17.67
Healthy Controls	20	2.91

P value = 0.006 (P value less than 0.05)

The number of patient group with normal CRP level in serum was 9(about 22.5 %), and the average of CRP level was 4.12 mg

/L, the range of obtained values was from 3.0 To 5.20 mg/L, and The number of patient group with high CRP level in serum was 31 (about 77.5 %) and the average of CRP level was 21.61 mg /L, the range of obtained values was from 6.01 To 69.55 mg/L, which is statistically much bigger in relation to patient with normal CRP group ($P < 0.00$).

The CRP level was high in 31 cases giving percentage of 77.5 %, and normal in 9 cases giving percentage of 22.5 % (table 2).

Table 2. Association of the CRP test and breast cancer.

CRP	No of case	Percentage %
Normal	9	22.5 %
High	31	77.5 %
Total	40	100 %

The average of CRP level in serum of stage II group was 16.28 mg /L, the range of obtained values was from 3.94 To 47.63 mg/L, The average of CRP level in serum of stage III group was 15.12 mg /L, the range of obtained values was from 2.84 To 47.15 mg/L, and The average of CRP level in serum of stage IV group was 20.20 mg /L, the range of obtained values was from 3.0 To 69.55 mg/L, which is statistically, is much bigger in relation to stage III and IV group ($P < 0.00$).

The CRP level was high in 4 cases of stage II and normal in 1 case ($p = 0.32$), high in 12 cases of stage III and normal in 4 cases ($p = 0.016$), and high in 15 cases of stage IV and normal in 4 cases ($p = 0.001$) (table 3).

Table 3. Association between the CRP and stages of breast cancer patients.

Stages	Stage II	Stage III	Stage IV	Total
Normal	1	4	4	9
High	4	12	15	31
P value	*0.032	*0.016	*0.001	

* Sig. P value > 0.05 .

The high age distribution among the patient group was between 41 – 50 years (24 subjects) about 60 %, and low age distribution was between 20 -30 years (one subject) about 2.5 %. The age distribution among the breast cancer patients was 1 case in group 1(20 – 30 years) giving percentage of 2.5 %, 7 cases in group 2 (31 – 40 years) giving percentage of 17.5 %, 24 cases in group 3 (41 – 50 years) giving percentage of 60 %, 4 cases in group 4 (51-60 years) giving percentage of 10 %, and 4 cases in group 5 (<60 years) giving percentage of 10 % (table 4).

Table 4: Age distribution among the breast cancer patients.

Age Range	20-30	31 – 40	41 – 50	51 – 60	<60
Age group	Group 1	Group 2	Group 3	Group 4	Group 5
No of patients	1	7	24	4	4
Percentage %	2.5 %	17.5 %	60 %	10 %	10 %

The high stage distribution among the patient group was stage IV (19 subjects) about 47.5 %, and low stage distribution among the patient group was stage II (5 subjects) about 12.5 %.

The stage distribution among the breast cancer patient was 5 cases of stage II giving percentage of 12.5 %, 16 cases of stage III giving percentage of 40 %, and 19 cases of stage IV giving percentage of 47.5 % (table 5).

Table 5. Stage distribution among the breast cancer patients.

Stage	Stage II	Stage III	Stage IV
No of patients	5	16	19
Percentage %	12.5 %	40 %	47.5%

The average of CRP level in serum of 20 – 30 years old distribution group was 16.09 mg /L, for 31 – 40 years old distribution group was 15.64 mg /L. the range of obtained values was from 3.80 To 50.15 mg/L, for 41 – 50 years old distribution group was 19.0 mg /L. the range of obtained values was from 3.79 To 69.55 mg/L, and for 51 – 60 years old distribution group was 20.82 mg /L. the range of obtained values was from 6.15 To 47.15 mg/L, and for < 60 years old

distribution group was 11.35 mg /L. the range of obtained values was from 3.0 To 17.98 mg/L.

Discussion:

Breast cancer remains the most common cancer in women in the world (8-10).

This study was performed to assess and evaluate association between the CRP and the breast cancer, and also to compare the CRP between 40 breast cancer patient and 20 healthy controls analyzing by immunoturbidimetric method.

The CRP level was increased about 3 fold in 77% of the breast cancer patients of total breast cancer patients; furthermore we found CRP was highly increased (8 fold in concentration) in breast cancer patients compared with healthy controls this finding was in agreement with Cynthia et al (2009) (8) who found the CRP was moderately or severely elevated in 90.5% of the breast cancer women, and Praveen et al (2011), Kristine, H. A et al, and Pierce et al. who found the serum levels of CRP increased significantly in the breast cancer patients (24, 25).

The mean age of breast cancer patients in this study was found to be 46 years old (ranged from 26 to 71), this finding might be due to a lack of the awareness and knowledge concerning breast cancer and delay of the detection of breast cancer, this findings are the same as that seen in Arab where the mean age of breast cancer was 48 years (26), and are the same as that seen in Ghana where the mean age at diagnosis was 48 years (27), Similar findings were also seen in Tanzania where the mean age at diagnosis was 47.8 years (28), and are the same as that seen in Nigeria and Senegal where the mean age at diagnosis was 44.8 years (29), and Similar findings were also seen in Gazeira State in the Sudan where the mean age of patients of breast cancer was 48.7 years in (30).

Most patients in this study (90%) were found in late stages (28, 31), this finding might be due to a lack of the

awareness and knowledge concerning breast cancer, delay of the detection of breast cancer and Limitations in implementing breast self-examination and mammography screening programs (10, 17, 30).

The CRP level was found significantly ascending increased in all stages of breast cancer patients because the inflammation (severity) increased with stages (from acute to chronic); This finding was in agreement with Praveen et al (2011) who reported the median levels of CRP increased with develop stage of the disease (23).

From the above discussion we Conclude CRP has critical value in diagnosis and prognosis of breast cancer as an inflammatory biomarker (32).

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Competing interests: none declared.

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