

Some Biochemical Parameters In Breast Cancer (Part I)

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ABSTRACT

The research was concerned with a study on the relationship between women breast cancer and some biochemical parameters. Blood samples have been drawn from (200) women breast cancer and (70) women apparently healthy as a control group; the age range was (35-70) years. The measured biochemical parameters included: the level of estrogen, progesterone hormones, total protein, albumin, globulin, cholesterol, triglycerides, lipoproteins (HDL, LDL) total lipids, some immunoglobulins (IgG, IgA and IgM) complement protein C₃, C₄ and peroxidase activity also had been measured in the study.

The results demonstrated significantly high values of estrogen, progesterone hormones, triglyceride, high density lipoprotein (HDL) cholesterol, total lipid, and significantly high values in immunoglobulins IgA, IgG, C₃ and C₄.

There were also high values of sera peroxidase activity in women breast cancer in comparison with control group, and significantly low values in low density lipoprotein (LDL) cholesterol, no significant difference in globulin, cholesterol and immunoglobulin IgM was observed.

*This work is taken from her Ph. D Thesis

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	(70)	(200)
:		(70 -35)
(HDL)	()	
IgM IgA IgG		(LDL)

C₄ C₃

(HDL) ()

. C₄ C₃ IgG IgA

(LDL)

. IgM

INTRODUCTION

Breast cancer is a major public-health problem in many countries. It is the most common malignancy and the most frequent cause of cancer death among females (Abdel-Fattah et al., 2000). It derives from the epithelial lining of the terminal mammary ducts or the glandular-like lobuli. The most frequently observed histological type is infiltrating ductal carcinoma (Cornelis, 1995).

The pathogenesis of breast cancer is not clear. There is now considerable evidence that hormonal factors contribute to its etiology and pathogenesis. It has been suggested that estrogens may act as promoters and, possibly, as initiators of the carcinogenic process. However, familial and genetic factors may also influence breast cancer development (Salmon, 1993). The aetiology of the majority of breast cancer is multifactorial, including both genetic and environmental factors, and it is possible that many of the factors responsible are still unrecognized. The existence of non-genetic determinants of breast cancer is also indicated by the large variation of breast cancer among different countries and over time within countries. These factors include: age, hormonal, family history and dietary factors (Dixon et al., 1999).

The present paper deals with determination the level of some : hormones, biochemical parameters, immunoglobulins and peroxidase in breast cancer patients compared to control individual.

MATERIALS AND METHODS

Patients: Patients were enrolled in the present study at the breast-examination unit in Al-Khansaa Hospital and Al-Hafith Hospital in Nineva Governorate. The total number of patients is (200), their age range ($30 \leq 70$) years. They are divided into three groups: 30-45, 46-55 and $56 \leq 70$ years. In addition to (70) cases as a control group.

Collection of Blood Samples: Venous blood samples (5 ml) were drawn from each patient then transferred immediately to a clean dry plain tube. After removing the needle, the blood was allowed to clot for at least (10-15) min. at room temperature and then centrifuged for (10) min. at (4000 xg). Serum was removed for the measurement of some biochemical parameters. (Bacchus et al., 1980).

Methods : Serum total protein (S.T.P.) was determined by Biuret method using kit manufactured by RANDOX (United Kingdom).

Serum albumin was determined by dye-binding method using kit manufactured by RANDOX (United Kingdom).

The concentration of serum globulin (Glb) was calculated for each person according to the following formula: $C \text{ globulin} = C \text{ total protein} - C \text{ albumin}$

Where C = concentration of serum globulin, total protein and albumin, expressed in g/dl (Tietz, 1987)

Serum total cholesterol was determined by an enzymatic method using kit manufactured by biocon (Germany).

Serum triglyceride were determined by an enzymatic method using a kit manufactured by BioMerieux (France).

Serum HDL-cholesterol was determined by an enzymatic method using a kit manufactured by bicon (Germany).

The concentration of LDL-cholesterol was calculated for each person according to the following formula without ultracentrifugation.

$$C \text{ LDL} = C \text{ serum} - C \text{ HDL} - TG/5$$

Where C = concentration of cholesterol in LDL, HDL, in serum, expressed in mg/dl.

TG = serum triglycerides concentration, expressed in mg/dl

(Tietz, 1987).

Serum total lipids (T.L.) were determined using colourimetric methods, which included heating a small amount of serum with concentrated sulfuric acid. The mixture was then treated with phosphovaniline reagent to give a red to violet coloured complex (Chabrol and Chardonnet, 1973).

Estrogen hormone (Est. H) in serum or human plasma (heparin) was analyzed by mini VIDAS analyzer for the quantitative measurement of 17α -estradiol in serum or human plasma (heparin), using Enzyme Linked Fluorescent Assay (Butt and Blunt, 1988).

Progesterone hormone (Prog. H) in serum or in plasma (heparin or EDTA) was analyzed by mini VIDAS analyzer for the quantitative measurement in serum or in human plasma (heparin or EDTA), using ELFA technique (Diver, 1987).

Serum immunoglobulins were assayed using the method of Single Radial Immunodiffusion

(Zilva et al., 1988). The method was applied to the quantitative determination of human immunoglobulins, and other proteins in serum as IgG, IgM, IgA and complements C_3 , C_4 .

Determination of Peroxidase activity in Serum:

Peroxidase activity in serum was assayed colorimetrically at 470 nm which was applied for determining peroxidase activity from human placenta (Nelson and Kulkarni, 1990). The reaction mixture (working solution) contained:

1 ml 0.1 M of sodium phosphate buffer, pH = 7.2, 1 ml 13 mM of Guaiacol, and 1 ml 0.3 mM of H_2O_2 . The reaction was initiated by the addition of (50) μ L serum to the working solution, and all the assays were performed at 37 °C in a water bath. One unit of peroxidase

activity (U) represents the amount of enzyme catalyzing the oxidation of (1) μ mole of guaiacol in (1) min.

Statistical Analysis: In this study, paired t-test was used to compare subjects result for various parameters among different groups tested in the work. The difference is considered significant at $p \leq 0.05$ (Kirkwood, 1988).

RESULTS AND DISCUSSION

The results of the measured different biochemical parameters in the research were listed below for patients and control:

Table 1: Comparison between the first group of breast cancer and the control (at age 30-45 year) for all measured parameters

Parameters	Mean \pm S.D.		Z-value	P-value
	First group	Control group		
Est. H (Pg/ml)	136.20 \pm 48.03	42.20 \pm 20.95	4.26	< 0.0001
Prog. H. (ng/ml)	5.69 \pm 0.69	1.194 \pm 0.085	3.73	< 0.0001
S.T.P. (g/L)	60.56 \pm 8.61	69.23 \pm 6.17	4.91	< 0.0001
Alb. (g/L)	28.11 \pm 5.76	34.03 \pm 3.33	4.90	< 0.0001
Glb. (g/L)	32.45 \pm 7.07	35.56 \pm 6.36	1.87	> 0.05
Cholesterol (mmol/L)	4.75 \pm 0.97	4.81 \pm 0.66	0.5	> 0.05
Triglyceride (mmol/L)	2.27 \pm 0.54	1.29 \pm 0.39	6.46	< 0.0001
H.D.L. (mmol/L)	1.09 \pm 0.19	0.92 \pm 0.09	4.29	< 0.0001
L.D.L. (mmol/L)	3.20 \pm 0.67	3.63 \pm 0.49	3.32	< 0.001
T.L (g/L)	4.72 \pm 1.04	3.83 \pm 1.04	4.10	< 0.0001
IgA (g/L)	3.95 \pm 1.72	2.18 \pm 0.88	3.29	< 0.001
IgM (g/L)	1.73 \pm 1.14	1.30 \pm 0.28	0.789	> 0.05
IgG (g/L)	20.35 \pm 5.62	16.17 \pm 1.58	2.52	< 0.01
C ₃ (g/L)	1.70 \pm 0.32	1.29 \pm 0.11	3.40	< 0.001
C ₄ (g/L)	0.32 \pm 0.13	0.21 \pm 0.04	2.58	< 0.01
Peroxidase (U)	112.19 \pm 33.78	51.48 \pm 15.50	7.19	< 0.0001

$p > 0.05$: no significant difference, $p \leq 0.05$: significant difference, $p \leq 0.01$: highly significant difference, $p \leq 0.001$: very highly significant difference, U: one unit of peroxidase activity represents the amount of enzyme catalyzing the oxidation of 1 μmol of guaiacol in 1 min.

Table 2: Comparison between the second group of breast cancer and the control (at age 46-55 year) for all measured parameters

Parameters	Mean \pm S.D.		Z-value	P-value
	Second group	Control group		
Est. H (Pg/ml)	138.86 \pm 43.85	82.11 \pm 41.52	3.26	< 0.001
Prog. H. (ng/ml)	3.65 \pm 0.53	1.20 \pm 0.62	2.40	< 0.01
S.T.P. (g/L)	61.06 \pm 6.15	70.76 \pm 4.62	5.63	< 0.0001
Alb. (g/L)	26.40 \pm 4.09	34.84 \pm 3.07	6.11	< 0.0001
Glb. (g/L)	34.89 \pm 6.52	35.92 \pm 5.07	0.57	> 0.05
Cholesterol (mmol/L)	4.73 \pm 0.85	4.38 \pm 0.51	3.03	< 0.001
Triglyceride (mmol/L)	2.53 \pm 0.48	1.26 \pm 0.37	6.62	< 0.0001
H.D.L. (mmol/L)	1.12 \pm 0.16	0.87 \pm 0.13	5.27	< 0.0001
L.D.L. (mmol/L)	3.10 \pm 0.59	3.25 \pm 0.30	1.95	< 0.05
T.L (g/L)	4.63 \pm 1.12	4.12 \pm 0.78	2.419	< 0.01
IgA (g/L)	3.76 \pm 1.93	2.18 \pm 0.92	2.17	< 0.05
IgM (g/L)	1.93 \pm 0.81	1.73 \pm 0.36	1.73	> 0.05
IgG (g/L)	17.74 \pm 4.93	15.16 \pm 3.47	1.98	< 0.05
C ₃ (g/L)	1.61 \pm 0.34	1.40 \pm 0.21	2.25	< 0.05
C ₄ (g/L)	0.43 \pm 0.10	0.28 \pm 0.04	3.94	< 0.0001
Peroxidase (U)	108.94 \pm 29.89	46.15 \pm 13.70	6.50	< 0.0001

$p > 0.05$: no significant difference, $p \leq 0.05$: significant difference, $p \leq 0.01$: highly significant difference, $p \leq 0.001$: very highly significant difference, U: one unit of peroxidase activity represents the amount of enzyme catalyzing the oxidation of 1 μmol of guaiacol in 1 min.

Table 3: Comparison between the third group of breast cancer and the control (at age $56 \leq 70$ year) for all measured parameters

Parameters	Mean \pm S.D.		Z-value	P-value
	Third group	Control group		
Est. H (Pg/ml)	57.36 \pm 25.79	20.77 \pm 14.16	2.41	< 0.01
Prog. H. (ng/ml)	0.85 \pm 0.12	0.21 \pm 0.10	2.57	< 0.01
S.T.P. (g/L)	61.43 \pm 6.59	69.14 \pm 4.64	5.10	< 0.0001
Alb. (g/L)	28.66 \pm 4.24	35.68 \pm 3.75	5.12	< 0.0001
Glb. (g/L)	32.94 \pm 6.76	33.48 \pm 4.75	0.63	> 0.05
Cholesterol (mmol/L)	4.59 \pm 1.12	4.22 \pm 0.57	2.46	< 0.01
Triglyceride (mmol/L)	2.39 \pm 0.61	1.24 \pm 0.34	6.23	< 0.0001
H.D.L. (mmol/L)	1.03 \pm 0.16	0.84 \pm 0.12	5.37	< 0.0001
L.D.L. (mmol/L)	3.08 \pm 0.83	3.13 \pm 0.38	1.976	< 0.01
T.L (g/L)	4.92 \pm 1.25	3.88 \pm 0.61	2.56	< 0.01
IgA (g/L)	4.43 \pm 2.23	2.03 \pm 0.94	2.72	< 0.01
IgM (g/L)	1.20 \pm 0.79	1.09 \pm 0.46	0.76	> 0.05
IgG (g/L)	20.28 \pm 5.69	14.82 \pm 0.23	2.88	< 0.001
C ₃ (g/L)	3.50 \pm 4.36	1.41 \pm 0.23	2.82	< 0.05
C ₄ (g/L)	0.55 \pm 0.05	0.26 \pm 0.02	2.90	< 0.01
Peroxidase (U)	104.00 \pm 24.38	58.24 \pm 24.32	5.53	< 0.0001

$p > 0.05$: no significant difference, $p \leq 0.05$: significant difference, $p \leq 0.01$: highly significant difference, $p \leq 0.001$: very highly significant difference, U: one unit of peroxidase activity represents the amount of enzyme catalyzing the oxidation of 1 μmol of guaiacol in 1 min.

Serum Estrogen Hormone in Breast Cancer:

The results in Tables (1, 2, 3) showed that there were a significant increase ($P < 0.0001$, $P < 0.001$, $P < 0.01$) in serum estrogen in the first, second and third groups of breast cancer women compared to the control group. These results were in good agreement with those obtained by other investigators (Schairer et al., 1998). It was reported that the increase in estrogen hormone is a good marker for increasing the risk factor of breast cancer (Herman et al., 2002).

This defect may demonstrate that estrogens play central roles in the development of breast carcinoma. The presence or absence of estrogen receptors in the cytoplasm of tumor cells is of prime importance in managing patients with breast cancer. Patients whose primary tumors are receptor-positive have a more favorable course than those whose tumors are receptor-negative (Al-Mudhaffar and Al-Samarai, 2001).

On the other hand, many studies suggested that breast cancer cells under estrogen control containing estrogen-receptors, which binds with estrogen hormone to form a complex. Then this complex is bound to promoter regions of specific genes to activate transcription of new mRNA and continue this process (Lindsay et al., 2002).

Serum Progesterone Hormone in Breast Cancer:

The results in Table (1, 2, 3) showed that there was a significant increase ($p < 0.0001$, $p < 0.01$, $p < 0.01$) in serum progesterone hormone in all groups of breast cancer women compared to the control group. Similar results have been reported by other investigators (Danova et al., 1998). These results demonstrated that the increase in ovarian secretion of progesterone hormone might lead to breast cancer in many women. The fact that progesterone does not down-regulate progesterone-receptors in the breast might contribute to its adverse effects (Stewart et al., 2001).

Total Serum Protein in Breast Cancer:

The statistical analysis of data listed in Tables (1, 2, 3) show that there was a significant difference ($p < 0.0001$) in total serum protein between breast cancer women and the control groups. These results were found to be compatible with the result obtained by other investigators (Al-Mudhaffar and Al-Samarai, 2001), who found that total serum protein was lower than the normal range in breast cancer women. Furthermore sometimes total serum protein decreased in abnormal level when there was weight loss in breast cancer patients (Monnin et al., 1993).

Serum Albumin in Breast Cancer:

The results listed in Tables (1, 2, 3) showed that there was significant decrease ($p < 0.0001$) in serum albumin in the first, second and third groups of breast cancer women

compared to the control. Similar results were arrived at by other investigators (Goodwin et al., 1998).

The reduction of serum albumin in breast cancer women could be attributed partly to nutritional deficiency and weight loss in association with psychological distress and lower quality of life (Ovesen et al., 1993). In addition, these results might be attributed to the abnormal renal function of those patients (Goodwin et al., 1998).

Serum Globulin in Breast Cancer:

The results of globulin in the first, second and third groups of breast cancer women are shown in Tables (1, 2, 3). The statistical analysis of data showed that there was no significant difference ($p > 0.05$) between these groups. The results of all groups were in the normal range, which are in agreement with others (Al-Mudhaffar and Al-Samarai 2001). This might be due to the fact that the groups with a lower concentration of albumin possess a higher concentration of globulin since globulin was as follows: $C_{glob} = C_{total\ protein} - C_{albumin}$

Serum Cholesterol in Breast Cancer:

The results in Table (1) showed that there was no significant difference ($p > 0.05$) in serum cholesterol in the first group of breast cancer. This result indicates that the cholesterol level is unaffected and remains its normal range. Similar results were obtained by other investigators (Riley et al., 1999). Who found that the cholesterol level was unaffected in breast cancer.

On the other hand, the results in Table (2, 3) showed that there was a significant increase ($p < 0.01$) in serum cholesterol in the second and third groups of breast cancer women as compared to the control. This increase might be due to the analogue between serum cholesterol and the menopausal status (Stanford et al., 1995). Moreover, several studies have shown a positive correlation between cholesterol and plasma or urinary estrogen levels (Gauley, 1999).

Serum Triglyceride in Breast Cancer:

As shown in Tables (1, 2, 3), there was a significant increase ($p < 0.0001$) in serum triglyceride in the first, second and third groups of breast cancer women as compared to the control group. This increase might be due to the intake of tamoxifen (King et al., 2002). It is known that tamoxifen is prescribed for patients with breast cancer as a chemotherapy treatment. However, it was reported that such a drug has a side effect and alters the level of cholesterol and triglyceride (King et al., 2002).

Serum Lipoproteins in Breast Cancer:

The results in Tables (1, 2, 3) showed that there was a significant increase ($p < 0.0001$) in serum high-density lipoprotein (HDL) cholesterol in the first, second and third groups of breast cancer women compared to the control groups. A similar finding has been reported by other investigators (Shlipak, 2000), where serum (HDL) is highly than the normal range in breast cancer women. The increase was attributed to the effect of high level

of estrogen, progesterone hormones and the drug which was taken by the patient (Shyamala et al., 1992).

On the other hand, the statistical analysis of data showed that there was a significant decrease ($p < 0.01$) in serum low-density lipoprotein (LDL) cholesterol in the first, second and third groups of breast cancer women compared with the control groups. These results were found to be compatible with the results obtained by other investigators (Shlipak, 2000), where serum (LDL) was lower than the normal range in breast cancer women. This effect might be due to the level of estrogen, progesterone hormone and drug treatment where HDL increased and LDL decreased in breast cancer women (Khan et al., 1998).

In the present study, the increase in HDL might be due to the influence of tamoxife, which is taken as a treatment to decrease the risk of breast cancer and increase the (HDL) substantially (Ridker et al., 1995).

Serum Total Lipid in Breast Cancer:

The results in Tables (1, 2, 3) showed that there was a significant increase ($p < 0.0001$) in serum total lipid in the first group of breast cancer women, and a significant increase ($p < 0.01$) in serum total lipid in the second and third groups of breast cancer women as compared to the control groups. These results agree with those obtained by other investigators (Plaza and Fernandez, 2000). The increase might be due to the high animal fat intake and weight gain by these patients, which is considered as one of the factors which increase the abnormal level of lipid (Schwarz et al., 1996).

Serum Immunoglobuline IgG, IgM and IgA in Breast Cancer:

The results in Tables (1, 2, 3) showed that there were significant differences ($p < 0.001$, $p < 0.05$, $p < 0.01$) in the first, second and third groups of breast cancer women respectively and the control groups in serum immunoglobulin IgA, IgG. On the other hand, the results also showed that there was no significant difference ($p > 0.05$) in serum immunoglobulin IgM. Though some investigators report that the advancing metastatic breast cancer is associated with high serum immunoglobulin levels of IgG and IgA, other suggests a defense reaction against increasing tumor load or the secretion of immunoglobulin by the tumor (Cochran, 2002).

Serum Complement Proteins C₃, C₄ in Breast Cancer:

The results in Tables (1, 2, 3) also showed that there was a significant increase in serum levels of C₃ and C₄ complement protein in the first, second and third groups of breast cancer women compared to the control groups. A similar finding had been reported by other investigators (Al-Mudhaffar and Al-Samarai, 2001; Cochran, 2002).

In this study the increase in C₃ and C₄ levels might be due to the over function of immunoglobulin IgG or might be an indicator of increase in the normal function of monocyte cells against tumor mass (McKenzie et al., 1987).

Serum Peroxidase Activity in Breast Cancer:

The results in Tables (1, 2, 3) showed that there was a significant increase ($p < 0.0001$) in serum peroxidase activity in the first, second and third groups of breast cancer women as compared to the control group. These results seem to agree with those obtained by other investigators (Shomon, 2004).

The increase in serum peroxidase activity in breast cancer women might be due to tumor, and as a result of this tumor the peroxide products increased intercellularly (Mittra et al., 1998). On the other hand, several studies observed that the peroxidase activity was high in erythrocytes breast carcinoma. It is unclear, and might be predicted that the increase in peroxidase activity to prolong ovarian function results in elevated circulating level of estrogen and of impaired responses to estrogen-mediated oxidative stress (Jun and Alan, 2003).

The overall results of this study showed that there was no significant difference ($p > 0.05$) between the first and second group of breast cancer women in all measured parameters. A similarly there was no significant difference ($p > 0.05$) between the first group and second group of breast cancer women in all measured parameters except in serum estrogen and progesterone hormones. This result might be due to the menopausal state and hormonal changes (Markopoulos et al., 1998).

On other hand, there was no significant difference ($p > 0.05$) between the second group and third groups of breast cancer women in all measured parameters except, in serum estrogen, progesterone hormones, serum albumin and globulin. This result might be due to the menopausal state and in few cases hypertension (Garber, 2000).

The second part of the present study focused on isolation and partial purification of peroxidase from breast cancer tissues. This enzyme had already been isolated and its optimum conditions had been investigated in our laboratory from different plant sources (Ahmad and Hamody, 2001; Ahmad and Mahammad, 2001).

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