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Serum Lipid Profile of Breast Cancer Patients

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Abstract: The purpose of this study was to carry out a comparative study to investigate the effect of lipid profile, oestradiol and obesity on the risk of a woman developing breast cancer. This study was carried out at the Komfo Anokye Teaching Hospital (KATH), Peace and Love Hospital, Oduom, Kumasi and Redeemed Clinic, Nima, Accra between May 2002 and March 2003. In this study, 200 consented women comprising 100 breast cancer patients (43 pre- and 57 post-menopausal) and 100 controls (45 pre- and 55 post-menopausal) with similar age range (25 to 80 years) were assessed for lipid profile, oestradiol and BMI. There was a significant increase in Body Mass Index (BMI) ($p = 0.011$), Total Cholesterol (TC) ($p<0.001$), triglyceride ($p = 0.026$) and low density lipoprotein (LDL-cholesterol) ($p = 0.001$) of the breast cancer patients compared to the controls. With the exception of oestradiol (EST) that decreased, the lipid profile generally increased with age in both subjects and controls with the subjects having a much higher value than the corresponding control. There was also a significant positive correlation between BMI and TC ($r^2 = 0.022$; $p = 0.002$) and also between BMI and LDL-cholesterol ($r^2 = 0.031$; $p = 0.0003$). Apart from EST and LDL-cholesterol that were increased significantly only in the postmenopausal phase in comparison to the controls, BMI, TC and TG were increased in both pre-menopausal and post menopausal phases with HDL-cholesterol remaining unchanged. This study confirms the association between dyslipidaemia, BMI and increased breast cancer risk.

Key words: Lipid profile, oestradiol, obesity, breast cancer

INTRODUCTION

The breast is an external symbol of beauty and womanhood; however cancer of the breast is responsible for the death of millions of women worldwide every year. Malignancy of the breast is one of the commonest causes of death in women aged between 40-44 years (Wernberg *et al*., 2009). Cancer of the breast is so widespread that it has become a genuine problem for public health, with about one woman in ten developing it in her lifetime throughout the world. Its incidence increases with age, being uncommon below the age of 30; and its behaviour varies from a slowly progressive to a rapidly progressive disease despite all forms of treatment.

Breast cancer primarily affects women, however, it occasionally affects men. The female to male ratio of breast cancer prevalence is 100:1 (Wernberg *et al*., 2009). Breast cancer accounts for 0.2% of all cancer cases in men. The aetiology of the disease is unknown, although both low radiation and oncogenic viruses may play a role. A variety of interrelated genetic, hormonal, environmental, sociobiological and physiological factors exert an influence on the development of this disease (Li *et al*., 1983; Polednak, 1999). Despite the identification of high risk factors, only 35% of breast cancer may be explained by known or suspected risk factors, including modifiable behaviours involving diet, overweight, exercise and alcohol use (Polednak, 1999).

Breast cancer incidence, mortality and survival varies widely among women of different racial or ethnic background (Miller, 1993). There is a high mortality and poor survival among Africans both in the diaspora and on the mother continent. This has been attributed at least partially to low utilization of breast cancer screening measures to detect tumours at a more treatable stage (Gordon *et al*., 1992).

The incidence of breast cancer has been much lower in other parts of the world as compared to the United Kingdom and North America where it accounts for the largest number of deaths of approximately 34,000 per annum (Sainsbury, 1999). However, the incidence of this disease is rising in many countries such as Japan and developing nations such as Ghana. Available statistics estimates the incidence at 35 cases/100,000 women in Ghana in 1977 (Quartey-Papafio and Anim, 1980). Wiredu and Armah (2006) reported that breast cancer formed 11%

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of all cancers histologically diagnosed at the Pathology Department of Korle Bu Teaching Hospital (K'BTH) the period 1974-78. During a free physical examination of breast exercise carried out in Ghana, Baako (1999) detected 13 breast cancer cases out of 712 women aged 20-80 years (mean 39.9 years). Of the 13 breast malignancy 3 out of 412 cases were found in southern and 10 out of 300 in Northern Ghana.

Diet may also be a factor in the variation of the incidence of breast cancer among women from different racial or ethnic communities (Armstrong and Doll, 1975; Polednak, 1999). There has been much debate regarding the correlation between the intake of total and saturated fat and the risk of breast cancer. Epidemiological studies have provided evidence on the postulated association between fat intake and breast cancer risk. Ecological studies have also shown that there is strong positive association between estimated per capita fat intake and breast cancer rates internationally and that there is a positive correlation between increase in fat consumption and increase in breast cancer rates over time in a number of countries (Prentice and Sheppard, 1990). Migrants from low-to-high-risk countries demonstrate substantial increase in breast cancer risk and corresponding increases in fat consumption, supporting the hypothesis (McMichael and Giles, 1988).

On the contrary, evidence from three prospective studies, each of which had at least 50 incident cases of breast cancer, did not support such an association (Hunter and Willett, 1993). Godwin *et al.* (1997) suggested that the positive association predicted by the international correlation study might have missed the link between breast cancer and dietary fat because of inadequate statistical power, lack of variation in diet within the cohort, impression of the dietary questionnaire, limited follow-up time or biased detection of breast cancer due to low use of mammography among women with high fat diets.

Dietary fat and obesity affects breast cancer by altering oestrogen level as a result of changes in the gut bacteria (Hill *et al.*, 1971; Rose *et al.*, 1987) or through an underlying hormonal enhancement of the disease (Senie *et al.*, 1992). The indicator of diet-related effect to date is the fairly consistent increase in breast cancer among women who are tall or obese (Kohlmeier and Mendez, 1997). Obesity is associated with decreased production of sex hormone-binding globulin, which results in significant increase in the biological active unbound form of oestradiol (Bernstein and Ross, 1993), which may promote further tumour growth in larger women.

Increased levels of circulating lipids and lipoproteins have also been associated with breast cancer risk, though published results have been inconsistent (Moysich *et al.*, 2000). The aim of this study, therefore, is to find out the effect of lipids and obesity on breast cancer risk.

MATERIALS AND METHODS

Subjects: This study was carried out at the Komfo Anokye Teaching Hospital (KATH), Kumasi-Ghana, Peace and Love Hospital, Oduom, Kumasi-Ghana and Redeemed Clinic, Nima, Accra-Ghana in the period between May 2002 and March 2003. The participation of the respondents was voluntary and informed consent was obtained from each of them.

The subjects were made up of 43 premenopausal and 57 postmenopausal breast cancer female patients, reporting to KATH, Peace and Love Hospital in Kumasi and Redeemed Clinic in Accra. Any subject on statin or any drugs that interfere with lipid metabolism were excluded from the study. The age group of the subjects ranged from 25 to 80 years. A control group consisted of 45 premenopausal and 55 postmenopausal normal females with similar age group as the breast cancer patients. These were apparently healthy volunteers who were not taking oral contraceptives or any form of hormonal medication.

Women were classified as postmenopausal if they had not had naturally occurring menstrual cycles during the preceding three years, or, if they had undergone a hysterectomy without complete oophorectomy before menopause and were 48 years of age or older. All subjects answered a questionnaire which contained details of age, age at menarche, age at first delivery, last day of menses and age at menopause.

Sample collection and preparation: Without the use of a tourniquet venous blood samples were collected into Vacutainer® plain tubes after an overnight fast (12-16 h) from the patients. The blood was allowed to clot, centrifuged at 500 g for 15 min within 30 min of sample collection and serum was collected and stored at -80°C until assayed.

Anthropometric variables: Subjects were weighed on a bathroom scale while barefooted. They were also made to remove all other extra outer clothes e.g., coats or turbans and asked to stand straight for the weight to be read on the bathroom scale. Measurement was done to the nearest 0.5 kg. The height was measured with a wall-mounted ruler. After removing footwear, the subject was made to stand up as straight as possible with feet together and with heels, buttocks and shoulders and back of the head touching the upright. The head was positioned such that the subject views horizontally. A direct height reading

was taken through the bar window after the metal piece was gently lowered to make contact with the head. When necessary, thick hairs were compressed so as to get contact with the top of the head. Measurement was done to the nearest 0.5 cm. BMI was calculated by dividing weight (kg) by height squared (m^2).

Biochemical assays: Serum biochemistry was performed with an ATAC® 8000 Random Access Chemistry System (Elan Diagnostics, Smithfied, RI, USA). Parameters that were determined include Total Cholesterol (TC), triglycerides (TG), High Density Lipoprotein (HDL-cholesterol), Low Density Lipoprotein (LDL-cholesterol). The methods adopted by the automated instrument for the determination of the above parameters are according to the reagent manufacturer's instruction-JAS™ diagnostics, Inc. (JAS Diagnostics, Ine., Miami Florida, USA). Serum total cholesterol was estimated using Cholesterol Oxidase/Peroxidase method (Allain *et al.,* 1974; Trinder, 1969)

Sandwich enzyme immunoassay (SIA) for oestradiol: Serum oestradiol (E2) was determined by sandwich enzyme immunoassay (SIA) using NoviWell™ assay kits (HySkill Diagnostics, Bahlingen, Germany). Assays were carried out as described by the manufacturer. The assay is based on simultaneous binding of E2 to monoclonal antibodies; one is immobilized on the microplate, the other is soluble and conjugated with horseradish peroxidase (HRP). Briefly, 25 μL aliquots of standards and samples were dispensed into their respective wells in ready-to-use microtitre plates precoated with anti-hormone IgG antibodies. After the addition of 100 μL oestradiol-HRP conjugate reagent and 50 μL rabbit anti-oestradiol reagent to each well, the plates were incubated for 90 min at room temperature. The contents of the well were then aspirated and the wells washed twice with 200 μL of distilled water. The enzyme reaction was started by addition of 100 μL the chromogen (tetramethylbenzidine/hydrogen peroxide system) into each well. Plates were then incubated for 20 min. The reaction was stopped by addition of 100 μL of 1N HCl. Absorbance was measured at 450 nm in an ELx800™ Microplate Reader (Bio-Tek Instrument, Winooski, VT, USA).

RESULTS

Clinical characteristics: The breast cancer patients have significantly higher BMI as compared to the control group. The high BMI value falls within the overweight region. They also had a significantly higher total cholesterol, triglycerides and low density lipoprotein as compared to the control group (Table 1). Fifty five percent of the breast cancer patients had their serum total cholesterol greater or equal to the upper limit of the reference range (200 mg dL^{-1}) whilst 20% of the controls had theirs greater or equal to the upper limit of the reference range.

Table 1: Characteristics of whole study population

Parameters	Total	Control	Subjects
Age (years)	45.50±11.30	42.64±13.40	48.21±13.69
BMI (kg m^{-2})	25.60±4.80	24.90±4.80	26.40±4.70*
TC (mg dL^{-1})	188.30±49.30	174.50±40.50	202.00±53.60**
TG (mg dL^{-1})	107.80±59.30	99.60±47.20	115.90±68.60*
HDL (mg dL^{-1})	57.20±22.20	56.80±21.20	57.50±33.20
LDL (mg dL^{-1})	108.60±35.33	99.60±33.30	117.70±42.80**
EST (pg mL^{-1})	35.60±37.30	36.90±39.20	34.40±35.50

The data are presented as Mean±SD, BMI: Body Mass Index, TC: Total serum cholesterol, TG: Serum triglycerides, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, EST: Oestradiol, *p<0.05 and **p<0.001 when the subjects group was compared to the control group

Table 2: Comparisons of biochemical parameters between breast cancer subjects and control group divided into different ranges of age (years)

	Age groups (years)					
Parameters	25-30	31-40	41-50	51-60	61-70	71-80
Breast cancer patient						
TC (mg dL^{-1})	183.33	199.24	204.69	206.26	197.60	198.25
TG (mg dL^{-1})	95.30	101.60	117.52	139.08	98.95	136.35
HDL (mg dL^{-1})	56.00	57.84	57.49	53.63	64.80	56.50
LDL (mg dL^{-1})	108.43	117.15	116.06	126.40	113.02	114.25
Control subjects						
TC (mg dL^{-1})	165.69	173.62	175.47	175.05	205.67	105.00
TG (mg dL^{-1})	78.42	95.91	106.97	109.82	110.35	95.00
HDL (mg dL^{-1})	71.92	54.41	56.13	52.00	60.67	45.00
LDL (mg dL^{-1})	76.03	100.88	105.99	101.06	124.77	37.80

The data are presented as Mean±SD, BMI: Body Mass Index, TC: Total serum cholesterol, TG: Serum triglycerides, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein

Comparisons between subjects with different age ranges: With increasing age ranges (Table 2), there were increasingly adverse lipid profiles as reflected by high TC, TG and LDL-cholesterol. These parameters increased with age up to 60 years in the breast cancer patients and up to the age of 70 years in the control group. However, for all parameters, the breast cancer patients have higher values than the control group at the corresponding age group. There is minimal change in the level of HDL-cholesterol as age increased for both breast cancer patients and the control as shown in Table 2.

Even though, oestradiol level decreases as the age progresses in both the breast cancer patients and the control group, breast cancer patients have higher level than the control at the corresponding age (Fig. 1).

BMI shows little variation with age in both the breast cancer patients and the control group. However, breast cancer patients have slightly higher BMI than their corresponding control at the various age groups (Fig. 2).

Table 3: Partial correlation between age, BMI, EST and biochemical parameters

Parameters	Age	BMI	EST
TC	0.31	0.02*	-0.02
TG	0.04*	0.01	-0.07
HDL	-0.01	-0.01	-0.02
LDL	0.04*	0.03**	0.03
EST	-0.11**	0.10	-

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed), BMI: Body Mass Index, TC: Total serum cholesterol, TG: Serum triglycerides, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, EST: Oestradiol

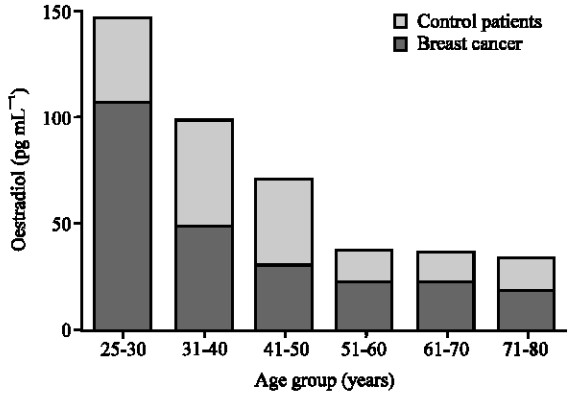

Fig. 1: Mean estradiol values for breast cancer patients and the control group

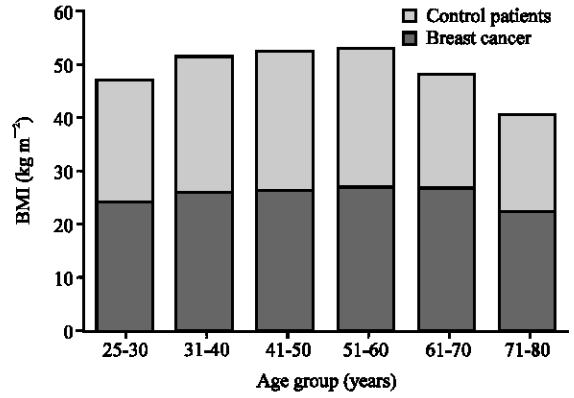

Fig. 2: Mean BMI values for breast cancer patients and the control group

Associations between age, BMI, EST and biochemical parameters: There was a significant positive correlation between age and TG; age and LDL-cholesterol and significant but negative correlation between age and oestradiol in this study. BMI also showed a significant but positive correlation with TC and LDL-cholesterol (Table 3).

Comparison of pre- and post-menopausal lipid values to the control: From Table 4, the breast cancer patients have significantly higher BMI, TC and LDL-cholesterol than the control group during both pre- and post-menopausal stage. The results demonstrated a 16% increase in total serum cholesterol levels of premenopausal patients compared to the control women. However, oestradiol and TG are only significantly raised during the postmenopausal stage and not the premenopausal stage.

Table 4: Comparison of pre- and post-menopausal lipid values to the control

Parameters	PRE.MC	POS.MC	PRE.MS	POS.MS
BMI (kg m^{-2})	24.90±4.400	24.80±5.400	26.50±4.500	26.30±4.900†
TC (mg dL^{-1})	173.52±34.00	179.91±41.26	202.00±64.70**	202.21±44.02††
TG (mg dL^{-1})	92.01±37.01	102.92±31.44	102.65±51.14*	125.91±78.21†
HDL (mg dL^{-1})	59.33±21.41	54.40±18.81	55.61±19.62	58.92±25.72
LDL (mg dL^{-1})	95.20±32.20	104.80±33.70	116.62±49.73	118.50±37.31†
EST (pg mL^{-1})	50.00±44.44	14.50±4.110	50.90±42.90	20.90±12.01††

The data are presented as Mean±SD, BMI: Body Mass Index, TC: Total serum cholesterol, TG: Serum triglycerides, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, EST: Oestradiol, PRE.MC: PRE-menopausal control, PRE.MS: Pre-menopausal subjects, POS.MC: Post-menopausal control, POS.MS: post-menopausal subjects, *p<0.05 and **p<0.001 when pre-menopausal compared to control, †p<0.05 and ††p<0.001 when postmenopausal compared to control

DISCUSSION

In this study 200 women comprising 100 breast cancer patients and 100 controls were assessed to find out the relationship between Body Mass Index (BMI), lipids and oestradiol and breast cancer risk.

The mean age at diagnosis of breast cancer patients selected at random was 48.0 years (Table 1), the youngest patient was 25 years and the oldest 80 years. This is in agreement with the finding of Gapstur *et al.* (1996), who reported 46.29 years per woman in Ghana as compared to 60 years per woman in whites and 56 years in African-American black women. Majority of the women with breast cancer were found to be within the age group 30-50 (70%). This situation is rather unfortunate since this is the age when women are found to be most active as mothers, wives, *et cetera*. Out of this number 65% were not aware that they had breast cancer.

The higher BMI in the breast cancer patients as compared to the control and the significantly raised BMI level in the breast cancer patients during the pre- and post-menopausal period indicates a strong association between increased BMI and breast cancer risk. This observation is in agreement with the findings in previous studies (Kohlmeier and Mendez, 1997). Although, very weak or no association has also been reported (Tornberg *et al.*, 1988), it has also been hypothesized that the adult weight gain or increased BMI is a strong predictor of postmenopausal breast cancer risk (Ballard-Barbash, 1994).

The significantly increased level of TC in the breast cancer patients compared to the controls and its significant positive correlation with BMI in these patients,

indicates that, there is an association between TC, BMI and breast cancer risk. This study has also demonstrated a 16% increase in total serum cholesterol levels of the premenopausal patients compared to the control group which is in agreement with a 15% increase in total serum cholesterol levels for premenopausal patients reported by other studies Abu-Bedair *et al.* (2003) and Bani *et al.* (1986). Some other researchers could not however establish any association between total serum cholesterol levels of premenopausal women and breast cancer risk (Gaard *et al*., 1994).

This study also demonstrated a significant difference between total serum cholesterol levels of postmenopausal cases and the controls. Several other case-control and prospective studies have also reported that elevated total serum cholesterol is associated with increased breast cancer risk (Qi *et al*., 1994). This is in contrast with the non-significant change in total serum cholesterol of postmenopausal case reported by Gaard *et al.* (1994) and Kokoglu *et al.* (1994). Hence, the association between total serum cholesterol levels and breast cancer risk still seems to be controversial and published results are inconsistent. In spite of these, a major link has been established between cell growth and cholesterol biosynthesis. If cholesterol synthesis is inhibited and no exogenous cholesterol is available, cell growth will be blocked (Buchwald, 1992; Soma *et al*., 1992). Buchwald (1992) proposed that cholesterol inhibition, either by decreasing cholesterol availability (lowering of plasma cholesterol) or by decreasing intracellular cholesterol synthesis could inhibit tumour cell growth and possibly prevent carcinogenesis.

Serum triglyceride in postmenopausal cancer patients were higher than the control the percentage increase of triglyceride levels (22%) in this study is consistent with an earlier report of 22% presented by Bani *et al.* (1986), but much lower than the percentage increase of triglyceride levels (31%) observed by Abu-Bedair *et al.* (2003). On the other hand, there was no significant change in serum triglyceride levels between the premenopausal patients and controls, a finding which is in agreement with the results of a study conducted by Gaard *et al.* (1994). However, Goodwin *et al.* (1997) have reported elevated serum triglyceride levels in premenopausal breast cancer patients. In a case-control study, Moyisch *et al.* (2000) reported that women with high serum triglyceride levels have an increased breast cancer risk and it was suggested that this risk may be modified by apo-E4 genotype.

No significant difference was observed in HDL-cholesterol levels between the breast cancer patients and controls in this study. This is in agreement with the observation of Moorman *et al.* (1998). There was however, an increased level of LDL-cholesterol between the cases and controls. The increase in LDL-cholesterol levels of premenopausal patients was (22%) and that of postmenopausal patients was (12%) compared with the controls. The elevated serum LDL-cholesterol, which is more susceptible to oxidation, may result in high lipid peroxidation in breast cancer patients. This may cause oxidative stress leading to cellular and molecular damage thereby resulting in cell proliferation and malignant conversions.

Several studies have investigated the role of diet especially dietary fat, in the aetiology of breast carcinoma, but its significance has remained controversial (Kolonel *et al*., 1983; Wu *et al*., 2000). Although, the relationship between diet and serum lipid levels is complex, diets containing a large amount of saturated fats may lead to higher lipid levels, particularly cholesterol (Bani *et al*., 1986). Kolonel *et al.* (1983) suggested that elevated lipid levels precede the development of obesity and breast cancer and thus, may have an aetiological or predictive significance.

Obesity is not only associated with decreased production of sex hormone binding globulin (Bernstein and Ross, 1993), which results in a significant increase in biologically active unbound form of oestradiol, but also results in the increased production of oestrone, which is produced by aromatization of androstenedione in peripheral adipose tissue. It therefore leads to an overall increase in the active levels of circulating oestrone and oestradiol which may promote the growth and metastatic potential of breast tumour in larger women.

In this study, no significant change was observed in oestradiol levels between the premenopausal cases and the controls. This finding is inconsistent with a study conducted by England *et al.* (1974), who found a 15% average elevation of total plasma oestrogen in a small study consisting of 40-49 year old patients. During the postmenopausal phase however, this study demonstrated a significant increase in the level of oestradiol compared to the controls. There was a 50% increase in oestradiol which is much higher than the 30% reported by England *et al.* (1974). Data on total oestrogens from two studies (Hankinson *et al*., 1998; Kabuto *et al*., 2000) also suggested increased levels of oestrogen in the cases. It has been hypothesized that the risk of breast cancer is essentially determined by the intensity and duration of exposure of breast epithelium to menopausal oestrogen (De-Waard *et al*., 1977).

Oestrogen, like all other steroid hormones is able to cross cell membranes and bind in a specific manner to their receptors to form specific hormone-receptor

complexes. These complexes bind to specific DNA sites in oestrogen dependent tissues called Hormone Responsive Elements and cause increased transcription of various genes. The end result is increased cell growth, proliferation and protein synthesis and enzyme synthesis (Kumar *et al*., 1987).

CONCLUSION

The findings of this study confirm the detrimental effect of increased BMI or obesity on breast cancer risk. Thus higher BMI is associated with breast cancer risk. Obesity leads to overall increase in the active levels of circulating oestrone and oestradiol, which may promote the growth and metastatic potential of breast tumours in larger women. The results also indicate an increased risk of breast cancer with increasing serum oestradiol levels especially during the postmenopausal stage. The mean age of onset of breast cancer from this study (48.0 years) is earlier than in other populations.

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