ISSN : 0974 - 7427 Volume 6 Issue 3

Trade Science Inc.

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 6(3), 2012 [94-99]

Lipid ratios in premenopausal females with breast carcinoma in North Indian population

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Received: 1st March, 2012 ; Accepted: 1st April, 2012

ABSTRACT

Background: Breast cancer is present for many years before it can be diagnosed. This implies that breast cancer cells, during their subclinical period, are likely to have been exposed for a considerable time to lipids. So, we planned this study to evaluate the role of various lipids individually and their integrated ratios in premenopausal females. **Methods:** A case control study was performed in 75 premenopausal females with breast cancer at the time of diagnosis (Group A). Lipid profile was assessed and lipid ratios were calculated. Their results were compared with a group of 75 age- & sex-matched healthy controls (Group B). **Results:** All the lipid ratios in patients were significantly high using 't' test and ANOVA. Area under receiver operating characteristic curve was > 0.7 for all the lipid ratios. Regression analysis showed that all the values had high significance (0.000) except for TG/HDL ratio which suggests that change in lipid ratio has significant impact on occurrence of breast cancer. **Conclusions:** Screening of females at young age for dyslipidemia and interventions to normalize the lipid profile may prove helpful in interrupting the pathophysiological cascade of events induced by lipoproteins and lipids and this could enable major life and cost savings.

KEYWORDS

Cholesterol;
High density lipoprotein-cholesterol (HDL-C);
Low density lipoprotein-cholesterol (LDL-C);
Riglyceride;
Apolipoprotein A;
Apolipoprotein B;
Breast cancer.

INTRODUCTION

Cancer is a group of diseases characterized by uncontrolled cell division leading to growth of abnormal tissue[1]. Worldwide, breast cancer comprises 10.4% of all cancer incidences among women, making it the most common type of non-skin cancer in women and the fifth most common cause of cancer death[2]. In 2004, breast cancer caused 519,000 deaths worldwide (7% of cancer deaths; almost 1% of all deaths)[3].

The ratio of female to male breast cancer approximates 100:1[4]. Breast cancer is generally more aggressive in the premenopausal females with higher production of angiogenic growth factors, a higher proliferation rate, and a higher degree of lymph node involvement and lymphovascular invasion[5].

Various etiological factors implicated are – Caucasian race, family history of first relation of breast carci-

noma, prior breast disease, mutation of BRCA1 and BRCA2 *gene*, post menopausal females on hormone replacement therapy (HRT), radiation exposure, increased fat intake, moderate to heavy alcohol intake, obesity and less breast feeding[6].

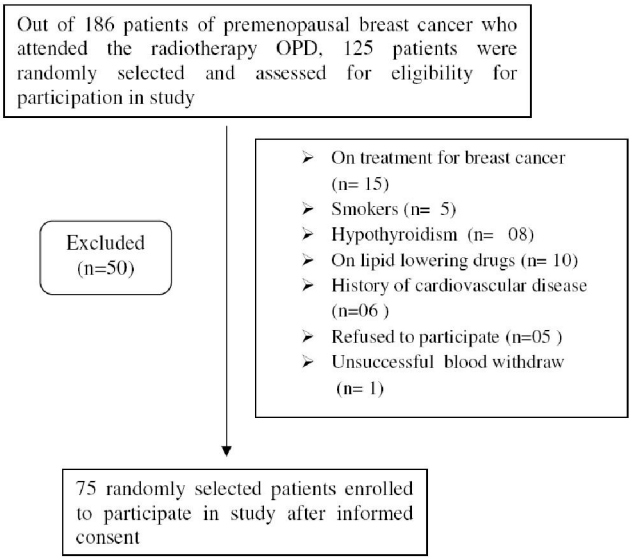
Recently, increased intake of saturated fatty acids has been linked to the promotion of breast cancer in females[7]. Sofi and colleagues showed that obesity, lack of physical activity and abnormal lipid profile are associated with increased risk of breast cancer and moderate intensity of physical activity decreases the chances of breast cancer[8]. Obesity affects the lipid profile and decreases levels of HDL-cholesterol[9]. Recent research suggests that low HDL-cholesterol, a traditional cardiovascular disease risk factor, may be associated with increased incidence of breast cancer[10].

The relationship between serum cholesterol levels and the risk of cancer in humans is an area of considerable research and debate, especially in the current era of intensive lipid modifying therapy and more aggressive cholesterol goals to reduce the risk of cardiovascular disease. To date, the literature on cholesterol and cancer has focused predominantly on total serum cholesterol, demonstrating an inverse relationship between serum cholesterol levels and incident cancer[11]. More recently, it has been reported that serum levels of low-density lipoprotein cholesterol (LDL-C) are significantly and inversely related to cancer in large randomized controlled trials (RCTs) of hydroxymethylglutaryl coenzyme A reductase inhibitors (statins), such that lower levels of LDL-C are associated with higher rates of incident cancer[12].

Does changes in lipid profile increases the chances of breast cancer is still a matter of debate and conflicting results have been reported on the association between lipids and risk of breast cancer in women. Therefore, the major aim of this study is to determine the role of alterations in lipid profile in breast cancer and to calculate lipid ratios to assess which one is related more with the chances of breast cancer.

MATERIALS AND METHODS

This case control study was performed in the 75 female patients of breast cancer with stage I or stage II at the time of diagnosis (Group A) attending the Radiotherapy department of Pt. B.D. Sharma PGIMS, Rohtak. Lipid profile was assessed after 8-10 hr fasting. Their results were compared with a group of 75 age matched healthy controls (Group B). Patients taking drugs known to affect lipid metabolism, with history of hypertension, diabetes, hypothyroidism, liver or renal failure, smoking, alcohol consumption, personal or family history of hyperlipidemia were excluded. An informed consent was taken from all patients and healthy volunteers. Patients with history of cardiovascular disease



Flow chart of participants

Methodology

Venous blood was collected aseptically from antecubital vein. Serum was separated by centrifugation (2000 rpm for 15 minutes).

Lipid profile

Serum triglycerides, total cholesterol, high density lipoprotein-cholesterol (HDL-C) were estimated using Randox kit on random access auto analyzer (Konelab 30i) and very low density lipoprotein-cholesterol (VLDL-C), low density lipoprotein –cholesterol (LDL-C) were calculated.

A. Estimation of total cholesterol

Determination of total serum cholesterol was done enzymatically. It was based on the development of quinoneimine whose absorption was read at 510nm, which is directly proportional to the concentration of cholesterol (mg/dL)[4].

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Intra-assay precision 3.73% at 66.023 mg/dL
3.84% at 297.297 mg/dL
Inter-assay precision 1.33% at 64.47 mg/dL
1.39%at 290.35 mg/dL

B. Estimation of HDL - C

Magnesium chloride and sodium phosphotungstate was used to precipitate low density and very low density lipoproteins, which were precipitated and removed by centrifugation at 3000rpm. The HDL-C left in the supernatant was estimated by the method as described above for total cholesterol[5].

Intra-assay precision 1.80%at 30.42 mg/dL
3.11%at 77.22 mg/dL
Inter-assay precision 3.81%at 31.54 mg/dL
2.73%at 77.606 mg/dL

C. Estimation of triglyceride

Triglyceride was estimated enzymatically. It was based on the development of quinoneimine whose absorption is read at 520nm, which was directly proportional to the concentration of triglyceride[6].

Intra-assay precision 3.29%at 27.26 mg/dL
1.77%at 496.46 mg/dL
Inter-assay precision 3.51%at 56.8 mg/dL
1.33%at 268.14 mg/dL

D. VLDL-C

VLDL-C will be calculated by Friedwald formula[7]

E. LDL-C

LDL-C will be calculated by standard formula as follows[8]:

F. Estimation of apolipoprotein[9]

Apolipoproteins A-I and B were analyzed using immunoturbidimetric immunoassay (Randox-make) run in random access auto analyzer (Konelab 30i).

Apo A-I Intra-assay precision 2.67 - 4.10 mg/dL
Inter-assay precision 3.18 - 3.22 mg/dL
Apo A-I Intra-assay precision 3.86 - 4.13 mg/dL
Inter-assay precision 1.79 - 2.73 mg/dL

STATISTICAL ANALYSES

Values are expressed as mean ± SD. Statistical analyses were performed using SPSS version 17.0 (SPSS, Inc., Chicago, Illinois). We used unpaired 't' test to compare individual lipid ratio's between patients and controls. Doing multiple t-tests can result in an increased chance of committing type I error. For this reason, we used ANOVA to compare means of all the lipid ratios. The F-ratio was calculated to predict how different the means are relative to the variability within each sample. Regression analysis was done and unstandarized coefficient (B) was calculated to predict the dependent variable from the independent variable. Standardized coefficient (Beta) was obtained by standardizing all the variables in the regression, including the dependent and all the independent variables. Area under Receiver operating characteristic curve was calculated; which is an effective method to evaluate the performance of each individual diagnostic test.

RESULTS

The p value was calculated using unpaired 't' test with equal variance. The mean age of patients was 42.93 ± 0.46 years while the mean age of controls was 41.97 ± 0.49 years. Total cholesterol, LDL-C, VLDL-C, HDL, TG, Apo A1, Apo B levels were significantly high in the patients when compared to controls (TABLE 1).

TABLE 1 : Comparison of lipid profile in premenopausal breast cancer patients with healthy controls(values are expressed in mg/dL as mean± SE)

Parameters	Breast Cancer (n= 75)	Healthy controls (n= 75)	p Value
Total cholesterol	204.04 ± 3.148	175.77 ± 1.598	<0.001*
LDL-C	154.97 ± 4. 7810	99.94 ± 2.389	<0.001*
VLDL-C	28.55 ± 0.563	24.94 ± 0.286	<0.05**
TG	135.05 ± 17.53	124.75 ± 1.43	<0.05**
HDL-C	32.64 ± 0.572	44.71 ± 0.497	<0.001*
Apo A1	135.46 ± 0.786	153.83 ± 1.027	<0.001*
Apo B	96.39 ± 1.919	86.72 ± 0.844	<0.001*

*** Highly significant; ** Significant**

TABLE 2 : Comparison of lipid ratios in premenopausal breast cancer patients with healthy controls

Ratio	Breast cancer patients	Healthy controls	p Value
TC/HDL	6.1 ± 0.96	3.91 ± 0.48	< 0.000*
LDL/HDL	4.74 ± 0.76	2.27 ± 0.45	< 0.000*
TG/HDL	4.14 ± 0.85	2.95 ± 1.39	< 0.000*
Apo B/Apo A1	0.67 ± 0.08	0.56 ± 0.05	< 0.000*

*** Very highly significant**

In our study, we found patients had significantly high lipid ratio as compared to controls (TABLE 2).

ANOVA test was highly significant which predicts that means differ more than would be expected by chance alone. Using the enter method a significant model emerged with adjusted R square=0.856 which suggests that 85.6% of variance in acne vulgaris can be predicted from the different lipid ratios. We obtained a large value of F (F=254.6, p<0.000), which is highly significant and suggests that there is greater likelihood that

TABLE 3 : ANOVA

Model	Adjusted R Square	F	Sig.
Enter	0.856	254.645	0.000*

*** Very highly significant**

the differences between the means are due to something other than chance alone (TABLE 3).

Regression analysis was done and unstandarized coefficient (B) was calculated to predict the dependent variable from the independent variable. The coefficient (LDL/HDL) is 0.169. So, for every unit increase in breast cancer lesion, a 0.169 unit increase in LDL/HDL, 0.895 unit increase in Apo B/Apo A1 is predicted, holding all other variables constant (TABLE 4).

Regression analysis showed that all the values had high significance (0.000) except for TG/HDL ratio which

TABLE 4 : Regression analysis

Lipid ratio	Unstandarized coefficient	Standarized coefficient	Significance
	B	Beta	
LDL/HDL	0.169	0.534	0.000*
TC/HDL	0.075	0.269	0.001**
TG/HDL	0.018	0.027	0.483***
ApoB/ApoA1	0.895	0.182	0.000*

*** Highly significant; ** Significant; *** Not significant**

suggests that change in lipid ratio has significant impact on occurrence of breast cancer (TABLE 4).

Area under receiver operating characteristic curve was calculated (AUC). AUC was >0.7 for all the lipid ratios and maximum for Apo B/Apo A1 which suggests that each individual lipid ratio can be used to evaluate risk of dyslipidemia in patients with severe acne vulgaris and best to use is Apo B/Apo A1.

DISCUSSION

Breast carcinoma is the most common malignancy and most common cause of cancer deaths among woman worldwide. It occurs more commonly in the developed countries, accounting for 3-5% of deaths while it is 1-3% in the developing countries. Carcinoma of breast is extremely rare in less than 20 years of age but thereafter the incidence steadily rises. Early menarche and late menopause, nulliparous females and elderly primigravida predispose to breast carcinoma[1].

The average age of patients in our study was 43 years which is almost 10 years lower than the West. This was in consistence with the study conducted by Sandhu and colleagues in north Indian population. The difference might be the result of the age structure of the Indian population, which is a bottom-heavy (predominantly young) pyramid[17].

Only 2 patients reported to have a positive family history of breast cancer. Although this could indicate the low incidence of hereditary breast cancer in this population, it could also be the result of the retrospective nature of the study and the incompleteness of history taking and record keeping.

Females with sedentary life style are more prone to breast cancer. In our study we found that 92.5% of females gave the history of sedentary life style. Lack of exercise results in decreased insulin sensitivity and increased insulin concentrations. The overabundance of insulin decreases binding protein for insulin-like growth factor-I (IGF-I) thus increasing the bioavailability of IGF-I. IGF-I and insulin together have been shown to stimulate motility in human breast cancer cell lines, an effect that could enhance migration and invasion. IGF-I also promotes cell proliferation and angiogenesis in the breast tissue[18].

There are conflicting results regarding association of lipid profile and breast cancer in different geographical areas. The present study was planned to identify whether there is such an association between alterations in lipid profile in carcinoma breast in a north Indian population.

In our study plasma cholesterol levels were significantly increased in the patients when compared with controls. Total cholesterol levels may affect development of breast cancer because cholesterol derived from

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the plasma is the immediate precursor of androgens in adrenal gland. So increase in cholesterol levels would lead to increased production of androgens[19]. Circulating androgens are exposed to the mammary tissue. Normal mammary tissue and peripheral adipose tissue contains aromatase cytochrome P450 enzyme that catalyzes the conversion of androgens into estrogens thus increasing the estrogen level. Serum estrogen enters in the cell by free diffusion & its intracellular concentration is enhanced in those organs and tissues that express estrogen receptors (ERs). Breast tissue expresses estrogen receptors so increase in serum estrogen leads to increase in estrogen levels in breast tissue[20]. Estrogen increases the chances of carcinoma by influencing the expression and transcription of growth factors, by activation of protooncogenes and oncogenes (e.g. c-fos, c-myc) and nuclear proteins. Estrogens and some of their metabolites may also be directly genotoxic[21,22].

Low density lipoprotein-cholesterol (LDL-C) levels in breast cancer patients were significantly increased compared to controls. LDL-C is a calculated parameter, increased cholesterol level and decreased HDL-C leads to increase in LDL-C. This finding is in contrast to the study done by Alsheikh and coworkers[23]. They reported significant inverse relation between LDL-C and breast cancer. This difference might have occurred due to selection criteria. In our study we have only selected premenopausal females with stage I or stage II at the time of diagnosis whereas Alsheikh and coworkers had included patients with different stages of breast cancer and also included post menopausal females.

In our study, we found patients had significantly decreased high density lipoprotein-cholesterol (HDL-C) levels compared to controls. This was in corroboration with the study done by Anna and colleagues[24]. Apolipoprotein AI levels in breast cancer patients were significantly low as compared to healthy controls.

The primary mechanism by which HDL-C exerts its atheroprotective effects is via reverse cholesterol transport, but HDL-C has also been shown to have other beneficial effects via its anti-inflammatory and antioxidant properties[19,25,26]. Cancer is a proinflammatory state, in which inflammatory cells actively participate in the neoplastic process, allowing tumor cell proliferation, survival, and migration[27-29]. Therefore, it is plausible that HDL-C, by mechanisms that are not yet known, may influence some of the proinflammatory mediators involved in carcinogenesis. Further work will need to be done to elucidate these potential mechanisms.

HDL-cholesterol levels have also been found to be inversely associated with levels of insulin-like growth factor-I (IGFI) in some studies[30-32]. Breast carcinoma patients with sedentary lifestyle and low HDL-C cholesterol levels have high IGF-I levels as explained earlier, which promotes carcinogenesis in these patients[18].

Serum triglyceride levels were found to be high in patients compared to controls. Serum triglyceride levels are known to be directly related to intake of fat rich food[19]. Diet has long been hypothesized to be one of the primary reasons of breast cancer with strong correlation between average fat intake and breast cancer[33].

It is difficult to explain the reason for increase in TC/HDL levels but this increase may affect the development of breast cancer lesions because increase in cholesterol level would lead to increase synthesis of androgens and normal mammary tissue contains aromatase cytochrome P450 enzyme that catalyzes the conversion of androgens into estrogens thus increasing the estrogen level, which increases the risk of breast cancer[18].

Cholesterol ester transfer protein (CETP) could play a plausible role in increased LDL/HDL levels. It transfers the esterified cholesterol from HDL (HDL_2) to VLDL and LDL and replaces it with triacylglycerol. LDL, so altered, is a potential substrate for hepatic lipase. The enzyme plays a major role in lipoprotein metabolism as a lipolytic enzyme and hydrolyzes triglycerides and phospholipids in chylomicron remnants, IDL, and HDL and generates smaller, denser LDL particles. This subfraction binds less well to the LDL receptor in comparison with its larger counterparts, which has the consequence of prolonging its lifetime in the circulation and thus increasing its levels[11].

Apo B is the main apolipoprotein of LDL and Apo A1 is the main apolipoprotein of HDL. Increase in LDL/HDL might be the reason for increase in Apo B/Apo A1[34].

The present study shows dyslipidemia may increase the chances of breast cancer in females without any family history. Screening of females at young age for

dyslipidemia and interventions to normalize the lipid profile may prove helpful in interrupting the pathophysiological cascade of events induced by lipoproteins and lipids and this could enable major life and cost savings. The sample size taken in the study was small and further long-term prospective research is needed to reveal the predictive value of the dyslipidemia in increasing the risk of breast cancer.

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