SERUM LIPIDS, LIPOPROTEINS AND SEX-HORMONE BINDING GLOBULIN IN BREAST CANCER

A.Ray*, S.L.Dayalu Naik*, A.K.Bahadur, S.T.Pasha***, R.S.Rautela*** and B.K.Sharma***

*Institute of Cytology and Preventive Oncology (ICMR) and **Department of Radiotherapy, Maulana Azad Medical College, New Delhi – 110002; and *** Division of Biochemistry and Biotechnology, National Institute of Communicable Diseases, Delhi – 110054.

ABSTRACT

Serum levels of various lipid parameters, i.e., triglycerides, total cholesterol, alpha lipoprotein or high density lipoprotein (HDL)-cholesterol and beta lipoprotein or low density lipoprotein (LDL)-cholesterol were measured in a total of 138 histologically proven cases of breast cancer along with 146 control women. The mean levels of serum triglycerides, total cholesterol and LDL- cholesterol were found to be significantly higher in breast cancer cases as compared to controls. However, lower mean levels of serum HDL-cholesterol and sex-hormone binding globulin (SHBG) were observed in breast cancer patients than the control subjects. The results indicate a probable relation between serum lipids and the activity of sex-hormones. Moreover, the study reflects an overall disturbance of lipid metabolism in the pathological process of breast cancer.

KEY WORDS : Serum lipids, related proteins and female breast cancer.

INTRODUCTION

Carcinoma of the breast is the second commonest malignancy among Indian women (1). A number of clinico-epidemiological studies have been published in recent years showing several risk factors in relation with breast cancer. One of the major risk factors is diet. Several epidemiological evidences and animal studies have delineated that the increased intake of dietary fat is positively associated with breast cancer (2). Nevertheless, the relationship amongst dietary fat, serum lipids and the risk of breast cancer is still not clearly understood. A number of recently published studies have shown abnormalities in blood lipid levels in patients with breast cancer (2-5). Further, there is a strong interrelation between lipid metabolism and metabolism of sex-steroid hormones (including oestrogens) which play an important role in the pathological process of breast cancer (6).

A small proportion of oestrogen (up to 4%) is

present as unbound or free form in our blood. The remaining portions exist in bound state with serum proteins. In healthy women, oestradiol binds avidly to a transport globulin (approximately 37%) called sex-hormone binding globulin (SHBG), while rest is bound to albumin with less affinity. Further, a little percentage of oestrogen (0.1%) is present with corticosteroid-binding globulin (CBG or transcortin). Notwithstanding, phenomena like higher percentage of free oestrogen / higher percentage of oestrogen not bound to SHBG or low level of serum SHBG have been observed by several investigators (7-9) amongst patients with breast cancer. Logically, there should be an inverse relationship between percentage of free oestrogen and serum level of SHBG. Also, the SHBG is associated with blood lipid components in a perplexing manner (10,11). However, low serum levels of SHBG possibly signify their involvement with the pathogenesis of breast cancer which is closely related with oestrogen metabolism. Thus there is no unanimity regarding the relationship between serum lipids, serum lipoproteins, SHBG and breast cancer. We therefore decided to carry out a detailed study.

In the present study, different serum lipid fractions

Author for correspondence :

Dr. B. K. Sharma, Deputy Director, at above address,
Fax: 323 3406

and SHBG were determined among histopathologically proven cases of breast cancer patients and control women in order to evaluate their role in the pathological process of breast cancer.

MATERIALS AND METHODS

This study was conducted on serum samples of 138 breast carcinoma patients who were randomly selected from the Department of Radiotherapy, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi, from 1993-2000. The tumours which were histopathologically diagnosed as infiltrating duct carcinoma of the breast (belonged to clinical stages I and II only) were included in this study. In addition to above, 146 women with minor surgical ailments but without any history of breast disease who attended Surgery OPD of aforesaid hospital during the same period were also randomly selected as controls. Standard questionnaires relating to clinical and epidemiological parameters were used for all cases and controls.

After an overnight fast, peripheral venous blood samples were collected from controls and cases before initiation of any systemic treatment including radiotherapy. Sera were separated within 6 hours of collection and were stored at -20°C until the samples were analysed. Triglycerides, total cholesterol and HDL-cholesterol were estimated by enzymatic method. Triglycerides and total cholesterol were measured at 500nm by standard coupled enzymatic procedures (12,13). HDL-cholesterol was determined by the phosphotungstic acid and magnesium chloride precipitation method. After centrifugation, the supernatant contained HDL-fraction which was assayed for HDL-cholesterol using the cholesterol enzymatic method (14). LDL-cholesterol was calculated from the measured concentrations of total cholesterol, HDL-cholesterol and triglycerides, according to the formula of Friedewald et al (15).

The estimation of SHBG was done by immunoradiometric assay (IRMA) method (16). Appropriately diluted 100 μl of standards (six), controls and serum samples (1:100, i.e., dilution of 10 μl of

serum to 1ml with buffer) were pipetted and placed into duplicate tubes. A mixture (200 μl) of equal volumes of anti-SHBG antiserum and ^{125}I -labelled monoclonal antibody against SHBG was added to the tubes. The contents were mixed and incubated for one hour at room temperature. Then, 500 μl of secondary antibody (solid-phase), appropriately diluted with buffer, were added to each tube; followed by 2ml of 0.9% NaCl were added. After centrifugation, the supernatants were decanted and the radioactivity of the pellets was then measured in ^{125}I -gamma counter. The concentration of SHBG in serum samples was estimated from the standard curve which was constructed by calculating the bindings in standards.

Statistical analysis: Non parametric Mann-Whitney U-test was applied to test the difference between study and control groups for various parameters. Karl Person's correlation was calculated to find out the interrelationship and their significance was estimated by using the t-test.

RESULTS

The mean ages and standard deviation (S.D.) of the patients with breast cancer and controls were 47.40 (± 12.01) years and 41.06 (± 11.55) years respectively. Table 1 shows the mean and S.D. of the different serum lipid fractions studied. The mean triglycerides (190.70 $\pm$ 86.45 mg/dl), total cholesterol (200.60 $\pm$ 61.75 mg/dl) and LDL-cholesterol (122.50 $\pm$ 57.47 mg/dl) levels in patients with breast carcinoma were found to be significantly higher than the corresponding levels among control women ($p < 0.001$). Interestingly, patients with breast cancer showed a significantly lower ($p < 0.001$) mean level of HDL-cholesterol (39.82 $\pm$ 20.21 mg/dl) than controls (46.14 $\pm$ 15.20 mg/dl).

In this study, the patients with breast cancer revealed a lower mean level of serum SHBG (23.6 $\pm$ 10.92 nmol/l) in comparison with control women (44.1 $\pm$ 15.27 nmol/l). The difference between mean values of breast cancer patients and of control women was found to be statistically significant ($p < 0.001$). Further, serum SHBG showed a significant correlation with

serum HDL-cholesterol both in patients with breast cancer ($r=0.31$, $p<0.05$) as well as in control women

Table 1. Mean and S.D. of various serum lipid fractions in patients with breast cancer and control women.

Study Group	SHBG (n mol/l)	Lipid Fractions			
		Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)
Breast Cancer (n=138)	23.6 ± 10.92*	190.70 ± 86.45*	200.60 ± 61.75*	39.82 ± 20.21*	122.50 ± 57.47*
Control Women (n=146)	44.1 ± 15.27	110.50 ± 44.60	168.70 ± 40.53	46.14 ± 15.20	99.83 ± 38.07

* $p<0.001$

($r=0.25$, $p<0.05$). However, no statistically significant association was found between SHBG and other serum lipid fractions.

DISCUSSION

The data of the present study have shown an increase in the levels of serum triglycerides and total cholesterol among breast cancer patients when compared with control women. The results of our study confirm the findings of earlier studies as reported by Bani et al (17), Alexopoulos et al (18), Kumar et al (2), Ray et al (4) and Bahadur et al (5). However, only serum triglycerides levels in patients with breast cancer were found to be higher as reported by Knapp et al (19) and Kokoglu et al (20). Our findings further support the above observations on the status of serum triglycerides.

Boyd & McGuire (21) suggested a relationship between higher levels of serum HDL-cholesterol and risk of breast cancer. Contrary to this, Knapp et al (19) and Bahadur et al (5) observed a lower level of HDL-cholesterol among breast cancer cases. Further, Hoyer & Engholm (22) found an inverse association between serum HDL-cholesterol and risk of breast cancer. The present study also revealed a significantly lower level of HDL-cholesterol in patients with breast cancer as compared to control women. However, Ferraroni et al (23) did not notice any relation between HDL-cholesterol and breast cancer. Besides, our study revealed a significant association between serum levels of HDL-cholesterol and SHBG in both breast

cancer cases and controls. Several earlier studies (11,24) also observed a positive interrelationship between serum SHBG concentrations and HDL-cholesterol levels. Like HDL-cholesterol, serum SHBG among patients with breast cancer showed a statistically significant lower mean level in comparison with control women.

Hoyer & Engholm (22) observed no association between LDL-cholesterol and breast cancer risk, whereas, Bahadur et al (5) noticed a higher level of LDL-cholesterol in patients with breast cancer compared to normal healthy control women. However, the data of present study also indicated a significant increase in serum LDL-cholesterol level among breast cancer patients.

The study reflects an overall change in the levels of different blood lipid fractions in patients with breast carcinoma. These alterations may be the result of dietary factors or due to metabolic disturbances. Sex-steroids (including oestrogens) are related to lipid metabolism and are considered as an important aetiological agent in the pathological process of breast cancer. The altered level of lipids and lipoproteins may be considered as an indicator of disturbed metabolism of sex-steroid hormones and lipids in mammary carcinogenesis.

Acknowledgement

Authors thank Mrs. Sudershan Kaur for technical help.

REFERENCES

1. National Cancer Registry Programme (1988-89) Biennial Report, Indian Council of Medical Research, New Delhi.
2. Kumar, K., Sachdanandam, P. and Arivazhagan, R. (1991) Studies on the changes in plasma lipids and lipoproteins in patients with benign and malignant breast cancer. *Biochem. Int.* 23, 581-589.
3. Boyd, N.F., Connelly, P., Lynch, H., Knaus, M., Michal, S., Fili, M., Martin, L.J., Lockwood, G. and Tritchler, D. (1995) Plasma lipids, lipoproteins, and familial breast cancer. *Cancer Epidemiol. Biomarkers Prev.* 4, 117-122.
4. Ray, A., Sharma, B.K., Bahadur, A.K., Pasha, S.T., Bhadola, P. and Murthy, N.S. (1997) Serum lipid profile and its relationship with host immunity in carcinomas of the breast and uterine cervix. *Tumori* 83, 943-947.
5. Bahadur, A.K., Yadav, R., Ray, A., Pasha, S.T., Sharma, S. and Sharma, B.K. (1998) Alteration in serum lipids and host cell mediated immune response in patients of carcinoma breast before, during and after treatment. *J. Basic Appl. Biomed.* 6, 19-24.
6. Vatten, L.J. and Foss, O.P. (1990) Total serum cholesterol and triglycerides and risk of breast cancer: a prospective study of 24,329 Norwegian women. *Cancer Res.* 50, 2341-2346.
7. Kaaks, R. (1996) Nutrition, hormones, and breast cancer: is insulin the missing link? *Cancer Causes Control* 7, 605-625.
8. Zeleniuch-Jacquotte, A., Bruning, P.F., Bonfrer, J.M., Koenig, K.L., Shore, R.E., Kim, M.Y., Pasternack, B.S. and Toniolo, P. (1997) Relation of serum levels of testosterone and dehydroepiandrosterone sulfate to risk of breast cancer in postmenopausal women. *Amer. J. Epidemiol.* 145, 1030-1038.
9. Ray, A., Naik, S.L.D., Katiyar, S., Kumar, A., Murthy, N.S., Sharma, S., Bahadur, A.K., Pasha, S.T., Husain, S.A. and Sharma, B.K. (2000) A comparative study on the serum levels of testosterone and SHBG in carcinomas of the breast and uterine cervix. *Indian J. Biochem. Biophys.* 37, 210-215.
10. Takatani, O., Okumoto, T. and Kosano, H. (1991) Genesis of breast cancer in Japanese: a possible relationship between sex hormone binding globulin (SHBG) and serum lipid components. *Breast Cancer Res. Treat.* 18 (Suppl. 1), S27-29.
11. Pugeat, M., Moulin, P., Cousin, P., Fimbel, S., Nicolas, M.H., Crave, J.C. and Lejeune, H. (1995) Interrelations between sex hormone-binding globulin (SHBG), plasma lipoproteins and cardiovascular risk. *J. Steroid Biochem. Molec. Biol.* 53, 567-572.
12. Wahlefield, A.W. (1974) Triglycerides: determination after enzymatic hydrolysis. In, *Methods of enzymatic analysis*, Vol II. Ed. Bergmeyer, H. Academic Press, New York, p.1831.
13. Allan, C.C., Poon, L.S., Chan, C.S.G., Richmond, W. and Fu, P.C. (1974) Enzymatic determination of total serum cholesterol. *Clin. Chem.* 20, 470-475.
14. Lopes-Virella, M.F., Stone, P., Ellis, S. and Colwell, J.A. (1977) Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin. Chem.* 23, 882-884.
15. Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge. *Clin. Chem.* 18, 499-502.

16. Hammond, G.L., Langley, M.S. and Robinson, P.A. (1985) A liquid-phase immunoradiometric assay (IRMA) for human sex hormone binding globulin (SHBG). *J. Steroid Biochem.* 23, 451-460.
17. Bani, I.A., Williams, C.M., Boulter, P.S. and Dickerson, J.W.T. (1986) Plasma lipids and prolactin in patients with breast cancer. *Brit. J. Cancer* 54, 439-446.
18. Alexopoulos, C.G., Blatsios, B. and Avgerinos, A. (1987) Serum lipids and lipoprotein disorders in cancer patients. *Cancer* 60, 3065-3070.
19. Knapp, M.L., Al-Sheibani, S. and Riches, P.G. (1991) Alterations of serum lipids in breast cancer: effects of disease activity, treatment and hormonal factors. *Clin. Chem.* 37, 2093-2101.
20. Kokoglu, E., Karaarslan, I., Karaarslan, H.M. and Baloglu, H. (1994) Alterations of serum lipids and lipoproteins in breast cancer. *Cancer Lett.* 82, 175-178.
21. Boyd, N.F. and McGuire, V. (1990) Evidence of association between plasma high-density lipoprotein cholesterol and risk factors for breast cancer. *J. Natl. Cancer Inst.* 82, 460-468.
22. Hoyer, A.P. and Engholm, G. (1992) Serum lipids and breast cancer risk: a cohort study of 5,207 Danish women. *Cancer Causes Control* 3, 403-408.
23. Ferraroni, M., Gerber, M., Decarli, A., Richardson, S., Marubini, E., DePaulet, P.C., DePaulet, A.C. and Pujol, H. (1993) HDL-cholesterol and breast cancer: a joint study in northern Italy and southern France. *Int. J. Epidemiol.* 22, 772-780.
24. Haffner, S.M., Dunn, J.F. and Katz, M.S. (1992) Relationship of sex hormone-binding globulin to lipid, lipoprotein, glucose, and insulin concentrations in postmenopausal women. *Metabolism* 41, 278-284.