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SEX HORMONE BINDING GLOBULIN IN BREAST CANCER

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ABSTRACT

The present study was undertaken to determine the significance of sex hormone binding globulin, the major and specific binding protein for testosterone and estradiol, in breast cancer. Among breast cancer patients, lower serum levels of Sex hormone binding globulin and higher levels of testosterone were observed. Sex hormone binding globulin showed an inverse relationship with testosterone and total cholesterol, and a direct relation with HDL-cholesterol. By the western blot analyses, Sex hormone binding globulin was detected in all biological samples that we examined. In the breast tumor tissue sections, immuno-staining for Sex hormone binding globulin was confined in cell cytoplasm and 29% cases were positive, which showed no association with the investigated prognostic markers of breast cancer such as ER and HER-2/neu over-expression. In this study, decreased circulating levels of Sex hormone binding globulin in breast cancer patients possibly indicate higher bioavailable estrogens.

KEY WORDS

Breast cancer, Menopausal status, Sex hormone binding globulin, Hormones.

INTRODUCTION

Breast cancer is the most frequent cancer and the second leading cause of cancer deaths among women worldwide and an increasing incidence rate has been observed in Indian urban areas according to the reports of the National Cancer Registry Programme (2001). Several lines of evidence suggest that metabolism of sex steroid hormones, especially estrogen, is closely associated with the pathological process of this disease. In the blood, the major and specific binding protein for testosterone and estradiol is sex hormone binding globulin (SHBG), which is a 93 kDa homo-dimeric glycosylated protein. Until recently, it was generally accepted that the sole function of SHBG is to regulate the concentration of free steroid hormones in blood. The discovery of specific SHBG-receptor in cell membrane from a variety of tissues has suggested a broader role for this glycoprotein. SHBG binding was demonstrated in various tissues including MCF-7 breast cancer cells and normal breast (1). In a recent study, Catalano et al (2) observed that the interaction of SHBG with MCF-7 cell membrane causes inhibition of the anti-apoptotic effect of estradiol, which might account for SHBG's inhibitory effect on breast cancer cell growth.

The aim of the present study was to evaluate the significance of SHBG in breast cancer. (i) Circulating SHBG levels in patients with breast cancer and controls were measured in order to find out the role of SHBG and its relationship (if any) with serum testosterone, estradiol and different lipid fractions. (ii) Western blot analysis of serum from the above-mentioned subjects was performed to evaluate serum SHBG concentrations between patients and controls. (iii) Immunohistochemical analyses for SHBG, estrogen receptor (ER) and HER-2/neu (c-erbB-2 oncoprotein) were carried out on breast tumor tissue sections. (iv) Estrogen-dependent MCF-7 and estrogen-independent MDA-MB-231 human breast cancer cells were evaluated for the presence of SHBG. Mammary gland tissue from the mice was also examined for SHBG in order to understand its biological status in the normal breast tissue including adipose tissue.

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MATERIALS AND METHODS

In this case-control study, a total of 102 patients with breast cancer were selected from Lok Nayak Hospital, New Delhi. It may be worthy to mention that the case-control study is an important type of observational epidemiologic studies, where people with a disease of interest and a suitable control or reference group of people unaffected by that particular disease are compared for the occurrence of the possible cause (3). However, in the present study, only the histopathologically confirmed infiltrating duct carcinoma of stages I and II were included. The mean (±SD) and range of ages of the patients with breast cancer were 48.6 (±12.14) and 22-80 years. Among premenopausal patients, the mean ages and range were 38.5 (±5.99) and 22-47 years whereas the mean ages of the postmenopausal patients and their age range were 59.1 (±6.84) and 50-80 years (Table 1). All cases with family history of the breast cancer, chronic alcohol intake and smoking habit were excluded from the study. Patients had no history of any apparent hormonal disorder and they did not consume any hormonal preparations of any type at least for 3 months before the time of collection of blood samples. Further, blood samples were collected from patients before initiation of any systemic treatment and/or radiotherapy. The blood samples from premenopausal women were collected during the follicular phase of their menstrual cycle. Similarly, 100 women with minor surgical ailments (such as sebaceous cyst, keloid, paronychia, etc.) were selected as controls. Persons with obesity or cardiovascular disease and hypertension, habit of smoking/ chronic alcohol intake, benign breast lesion and family history of breast cancer were not included in the study. The basic information relating to epidemiological/socio-demographic aspects and clinical history were recorded by interview before collection of blood from both the groups. The questionnaires (and the study protocol) were approved by both the scientific advisory committee and the ethical committee of the Institute of Cytology and Preventive Oncology. After an overnight fast, 5 ml of peripheral venous blood samples were collected from all subjects. Serum was separated within 6 hours of collection of blood and serum samples were kept at -20°C until the laboratory investigation was carried out. For the western blot analysis, sera of 25 patients and controls were randomly selected from the order of enrolment (every 4^{th} subject). From the above-mentioned cases, the tissue sections (paraffin-embedded, 5 μm thick) were collected from 38 breast tumors, which were utilized for the immunohistochemical analyses. Paraffin tissue sections from 3 patients of lipoma were used as negative control. Furthermore, estrogen-dependent human breast cancer cell line MCF-7 and estrogen-independent cell line MDA-MB-231 were cultured in Eagle's Minimum Essential Medium (EMEM) and L-15 media, respectively. The cell lysates were used for the western blot analysis. Lysates of the mice mammary gland-associated tissue were collected for a separate study with the cell lines.

For estimation of SHBG and hormones, assay kits were obtained from the Orion Diagnostica (Finland). SHBG was measured by immunoradiometric assay (IRMA) method. The estimation of testosterone and estradiol-17β was done by radioimmunoassay (RIA) technique. Serum triglycerides and total cholesterol were measured according to the methods described by Wahlefield (4) and Allan et al (5), respectively. Serum high density lipoprotein (HDL-) cholesterol was determined by the enzymatic method (6). Further, serum low density lipoprotein (LDL-) cholesterol was calculated according to the formula of Friedewald et al (7). Serum samples were prepared for the polyacrylamide gel electrophoresis and subsequently, proteins were electrotransferred to PVDF membrane (8). Similarly, lysates of human breast cancer cell lines and mice mammary gland-associated tissue were analyzed by the western blot technique (9) using rabbit polyclonal antibody against SHBG (Santa Cruz, USA). Immunohistochemical staining was performed according to the method described by Ray et al (10).

Mann-Whitney test and Student's 't' test were applied to see the difference between patients and control groups for various parameters. Variables that were not normally distributed, tests

Table 1: Mean ± SD of Age and Hormonal Parameters

	Breast cancer (n=102)	Premenopausal patients (n=52)	Postmenopausal patients (n=50)	Controls (n=100)	Premenopausal women (n=65)	Postmenopausal women (n=35)
Age and range (years)	48.6±12.14 (22-80)	38.5±5.99 (22-47)	59.1±6.84 (50-80)	44.3±12.09 (21-70)	36.9±6.80 (21-46)	58.0±6.25 (48-70)
SHBG (nmol/l)	24.0±10.76*	24.6±8.36**	23.4±12.85***	41.0±15.78	40.8±15.84	41.3±15.90
Testosterone (nmol/l)	2.6±1.05*	2.8±1.12**	2.4±0.93***	1.8±0.64	1.9±0.63	1.8±0.67
Estradiol (pmol/l)	103.6±56.20	144.4±47.72**	61.2±23.34	91.0±51.70	109.1±53.38	57.4±25.06

Significant (p<0.05), comparison between cases and controls – among total subjects*, premenopausal** and postmenopausal groups***.

were applied after logarithmic transformation of the data. Further, Pearson's correlation coefficient was performed to see the association between two variables. The '*p*' value of <0.05 is considered as significant. Fisher's exact test of significance was employed to see the association of SHBG over-expression with ER and HER-2/neu expression in breast cancer tissues. Densitometry analysis for the western blot was performed using UN-Scan-IT gel digitizing software-Version 5.

RESULTS AND DISCUSSION

Table 1 shows the mean ±SD of serum levels of SHBG, testosterone and estradiol for both patients and control groups. Among various serum lipids, the mean triglycerides (184.6±83.00 mg/dl), total cholesterol (203.9±63.01 mg/dl) and LDL-cholesterol (126.5±61.07 mg/dl) levels in breast cancer patients were found to be significantly higher than the corresponding levels in controls (triglycerides: 113.2±52.10 mg/dl, total cholesterol: 169.9±47.49 mg/dl and LDL-cholesterol: 102.7±41.73 mg/dl). On the contrary, breast cancer patients showed a significantly lower mean level of HDL-cholesterol (40.6±21.28 mg/dl) compared to control women (44.7±15.82 mg/dl). Interestingly, SHBG in breast cancer cases revealed a statistically significant inverse relationship with testosterone (r = -0.25, $p<0.05$) and total cholesterol (r = -0.23, $p<0.05$); whereas, a positive correlation was noticed between serum HDL-cholesterol and SHBG (r = 0.42, $p<0.001$). However, SHBG did not show any significant relation with other studied parameters in control women.

By western blot analysis, we detected SHBG in serum samples (Fig 1 A); however, this is not a suitable method for the quantitative measurement of a circulating protein according to the results of our study. Also, we noticed that both estrogen-dependent MCF-7 cells (Fig 1B I) and estrogen-independent MDA-MB-231 cells (Fig 1 B II) revealed the presence of SHBG by western blot analysis. The observed SHBG might be produced by these cells (11-13) or could be derived from the serum component of culture media; it has been mentioned earlier that SHBG can bind to the cell membrane. Interestingly, mice mammary gland-associated tissue showed high concentrations of SHBG (Fig 1 B III-VI). In the present study, out of 38 breast cancer cases, which were stained immunohistochemically, 11 (29%) showed positive immuno-staining for SHBG (Fig 2). The immunoreactivity for SHBG was found in the cytoplasm as reported previously by Meyer et. al. (14). The positive staining in cytoplasm may be due to receptor-mediated endocytosis (internalization into cytoplasm) of SHBG-steroid-complex and/or endogenous production of SHBG by the cells. Perhaps, over-expression of SHBG, its expression pattern and frequency may depend on the tumor microenvironment, i.e., local phenomena, particularly changes in the cell membrane properties (15,16). Probably, for this reason, the serum SHBG levels in immunohistochemically positive patients in the present study (24.3±7.32 nmol/l) did not differ significantly in comparison with the corresponding serum levels of patients negative for SHBG immuno-staining (23.8±8.71 nmol/l). In this study, positive staining for SHBG did not show any association with the over-expression of either ER or HER-2/neu/c-erbB-2 oncoprotein (Table 2). It is worthy to mention that ER expression in breast cancer tissue is considered as a marker for good prognosis. On the contrary, HER-2/neu over-expression is a poor prognostic factor. However, similar to the findings of the current study, Meyer et. al. also observed that SHBG-staining was unrelated to ER (14).

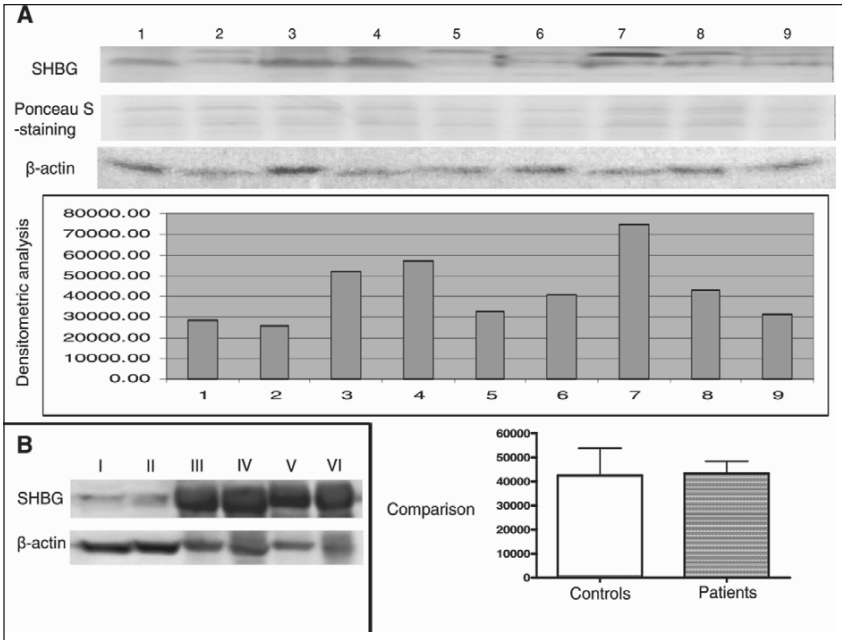

Fig 1. A — Representative western blot analysis of serum samples. Lanes 1 and 2: premenopausal controls, 3-5: premenopausal patients, 6 and 7: postmenopausal controls and, 8 and 9: postmenopausal patients [lanes 1, 3 and 4: wild-type, 2 and 9: homozygous, and 5-8: heterozygous].
B — Western blot analysis of cell/tissue lysates. Lane I: estrogen-dependent MCF-7 breast cancer cells, II: estrogen-independent MDA-MB-231 breast cancer cells, III and IV: mammary gland-associated tissue from obese mice and, V and VI: mammary gland-associated tissue from lean mice.

Table 2: Immunochemically over-expression of SHBG and its association with ER and HER-2/neu status in breast cancer

	SHBG	
	Positive (n=11)	Negative (n=27)
ER		
Positive (n=15)	6	9
Negative (n=23)	5	18
HER-2/neu		
Positive (n=14)	3	11
Negative (n=24)	8	16
Menopausal status		
Premenopausal (n=22)	7	15
Postmenopausal (n=16)	4	12

Epidemiological data have indicated that an increased cumulative exposure to estrogen increases risk for breast cancer. Different prospective as well as case-control studies have suggested an association between higher levels of circulating estradiol and breast cancer (17-19). In the present study, statistically significant higher levels of serum estradiol were found only in premenopausal breast cancer patients compared to premenopausal controls. Furthermore, the results of our study revealed significantly higher levels of serum testosterone both in premenopausal and postmenopausal patients with breast cancer compared to controls. Most probably, the contribution of androgens to breast cancer risk is largely through their role as a substrate for estrogen production (18). In the present study, significantly lower levels of serum SHBG were observed among patients with breast cancer. Manjer et. al. (20) concluded that low levels of SHBG may indicate high levels of bioavailable steroid hormones. Perhaps, the results of our study also have denoted a similar condition.

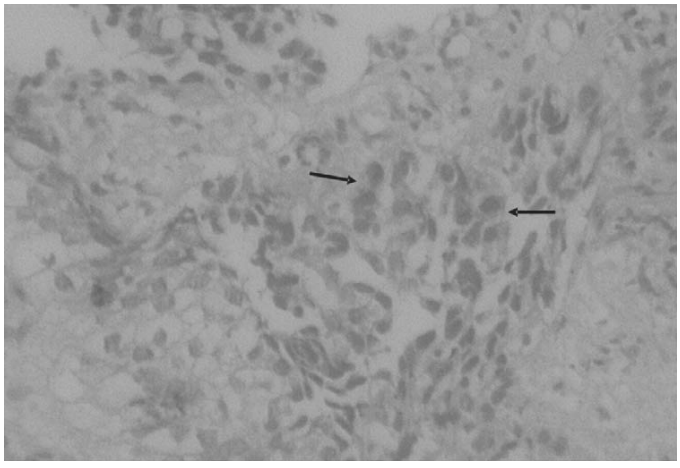

Fig 2. Shows over-expression of SHBG in breast cancer tissue section (magnification x400).

Sex hormones play an important role in etiopathogenesis of breast cancer. SHBG modulates the functions of testosterone and estradiol by altering their bioavailability to target tissues. In recent time, it has been thought that SHBG also functions as a regulator of the steroid hormone signaling system in the cells, by binding to its specific membrane receptor. Interestingly, different studies have provided evidence that hormonal environment can be altered with changes in lifestyle factors. Western lifestyle, characterized by reduced physical activity and a diet rich in fat, refined carbohydrates, and animal protein is associated with high prevalence of overweight, metabolic syndrome, insulin resistance, and high plasma levels of several growth factors and sex hormones, most of these factors are associated with breast cancer risk (21). Recent investigations have revealed that lower levels of SHBG were associated with all components of the metabolic syndrome and insulin resistance (22,23). On the other hand, studies on migrant populations have demonstrated that the metabolisms of steroid hormones as well as the incidence of breast cancer are influenced by environmental factors (24,25). The effect of diet on SHBG has been the subject of investigation in many interventional and cross-sectional studies over the recent years (26,27). However, our study indicates that low levels of SHBG in breast cancer are associated with the disease process probably through their influences on bioavailable steroid hormones. The observed relationship of SHBG with blood testosterone and lipids in the present study signifies that hormonal levels could be altered in a favorable manner by modifying lifestyle factors. However, only a precise biochemical understanding about sex steroid hormones could lead to an effective preventive strategy against breast cancer.

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