A comparative study on serum levels of testosterone and SHBG in carcinomas of breast and uterine cervix

A Ray¹, S L D Naik¹, S Katiyar¹, A Kumar¹, N S Murthy¹, S Sharma¹, A K Bahadur†, S T Pasha †, S A Husain§ and B K Sharma *

¹Institute of Cytology and Preventive Oncology (ICMR), ²Department of Radiotherapy, Maulana Azad Medical College, New Delhi 110 002;
³Division of Biochemistry and Biotechnology, National Institute of Communicable Diseases, Delhi 110 054 and
§Department of Biosciences, Jamia Millia Islamia, New Delhi 110 025

Received 26 August 1999; revised 3 February 2000

Serum levels of testosterone and sex-hormone binding globulin (SHBG) were measured in 31 patients with breast carcinoma and 33 patients with carcinoma of the uterine cervix along with 30 healthy (control) women. Amongst the patients of breast cancer, significantly higher level of testosterone and a low level of SHBG were found in comparison with the other two groups. The study has revealed that testosterone and SHBG may have an indirect role in breast cancer, probably through their influences on the amount of bioavailable estrogen.

Carcinoma of the breast ranks as the second most common cancer in women in India. Several lines of research suggest that reproductive hormones influence the risk of breast cancer. The major sites of endogenous estrogen biosynthesis are the ovarian granulosa cells in premenopausal women and extraovarian adipose tissues in postmenopausal women. Androgens are converted into estrogen (aromatization) by the enzyme complex, aromatase in the above mentioned tissues. Increased peripheral conversion of adrenal androgens into estrogens in obese women is presumed to increase the risk. Therefore, obese postmenopausal women may be at 'higher risk' for breast cancer than postmenopausal women who are not obese. But there are many reports in which no correlation between endogenous hormones and the risk of breast cancer could be demonstrated. There are also several reports of women with breast cancer having higher levels of nonprotein-bound estrogens and lower level of SHBG in blood. Although more consistent results have been found in case-control studies on urinary or serum levels of testosterone, it is doubtful whether it is due to the stress associated with cancer. Hence, it may be more appropriate to compare the findings with another cancer as control. Many of the risk factors for cancer of the breast appear to be the inverse of those for cancer of the uterine cervix which is the commonest malignancy among Indian women and does not seem to be dependent on endogenous hormonal factors. The present comparative study was undertaken to determine the status of serum testosterone and SHBG in patients with carcinomas of the breast and uterine cervix which are of different etiopathogenesis.

Materials and Methods

A total of 31 cases of breast carcinoma and 33 cases of carcinoma of the uterine cervix were randomly selected from the Lok Nayak Hospital (Maulana Azad Medical College), New Delhi. The tumours were histopathologically diagnosed as infiltrating duct carcinoma of the breast and squamous cell carcinoma of the uterine cervix, and all cases belonged to stages I and II. In addition to above, 30 women with minor surgical ailments but without any history of breast disease who attended the same hospital during this period were randomly selected as controls. Standard questionnaires relating to clinical and epidemiological parameters were used for all cases and controls.

After overnight fast, peripheral venous blood samples were collected from controls and patients before initiation of any systemic treatment and/or radiotherapy. The blood samples from premenopausal women were collected during the first week of the follicular phase of their menstrual cycle, serum was separated within 6 hr and was stored at -20°C until the samples were analyzed.

*Author for correspondence
Phone: 323 1880, 323 1889. Fax: 323 3406.
Testosterone was measured by radioimmunoassay (RIA) method. The concentration of testosterone was estimated by using direct coated tube and necessary reagents were obtained from the Orion Diagnostica Spectra (Finland). In brief, serum samples and $^{125}$I-labeled testosterone were added to the tubes coated with a second antibody to which the primary polyclonal testosterone antibody had already been bound. After 1 hr incubation at 37°C, the tubes were decanted and washed. The radioactivity of the bound $^{125}$I-labelled testosterone was measured using $^{125}$I gamma counter (EC, Hyderabad, India) and testosterone was estimated from a standard curve constructed from which the SHBG concentration in unknown samples was interpolated.

SHBG was estimated by immunoradiometric assay kit (IRMA) obtained from the Orion Diagnostica (Finland). Serum samples, known reference standards and controls were diluted appropriately (10 μl to 1 ml with assay buffer) and 100 μl aliquots were added in duplicate to test tube. A mixture of $^{125}$I-labelled monoclonal SHBG antibody and anti-SHBG antiserum was added, mixed and incubated at room temperature for 1 hr. A second antibody covalently bound to solid particles was next added and after 15 min, 0.9% NaCl (2ml) was added, centrifuged and the supernatants were decanted. De paraffinization and rehydration in xylene and ethanol, endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol, followed by incubation in normal rabbit serum. Optimally diluted (1:30) primary rabbit monoclonal antibody against aromatase was applied to each section. After incubation with secondary bridging antibody, peroxidase-antiperoxidase complexes were used. The reaction was visualized by substrate diaminobenzidine (DAB, 0.1% solution in PBS with 0.05% H$_2$O$_2$). Intensity of immunoreactivity was scored semi-quantitatively by classifying the sections as no detectable expression (-) or low (+), moderate (+++) or high staining intensity (+++).

Statistical analysis

Comparison of mean±SD amongst the three groups was carried out by Kruskal-Wallis one way analysis of variance. Further, Mann-Whitney's test of significance was employed to test for significance between the groups. Simple correlation coefficients were estimated between different parameters to understand the relationship quantitatively.
Results

The mean ±SD age of the patients with breast cancer, cervical cancer and normal controls were 49.3 ± 10.5, 48.8 ± 11.2 and 48.1 ± 11.7 years respectively. The differences in mean age amongst the groups were found to be statistically non-significant (p > 0.05). Out of 31 breast cancer patients, 16 (51.6%) were postmenopausal whereas 19 (57.6%) patients with cervical cancer and 15 (50%) control women had attained the menopause. The differences were found to be statistically non-significant.

Table 1 shows the mean, standard deviation (SD), and range of values of serum testosterone and SHBG levels for different groups. It was observed that mean testosterone levels of breast cancer patients (3.12 ± 1.20 nmole/l) were observed to be higher as compared to patients of carcinoma cervix (1.74 ± 0.96 nmole/l) or control subjects (2.16 ± 0.63 nmole/l) (Kruskal-Wallis H = 31.055, p < 0.01). Same trend was noticed with median values also (breast cancer-3.35, cervical cancer-1.40 and controls-1.98 nmole/l). Further, the mean and median serum testosterone levels of normal control women were observed as higher in comparison with cervical cancer patients. The difference in mean values was statistically significant (p < 0.01).

Patients with breast carcinoma showed a lower concentration of SHBG (24.2 ± 8.47 nmole/l) than patients with cervical carcinoma (27.0 ± 10.2 nmole/l) and normal control women (46.2 ± 19.15 nmole/l). The difference in mean values between breast cancer vs control women was found to be significant (p < 0.01). The mean values of cervical cancer patients and control women also showed statistically significant difference (p < 0.01). Further, SHBG concentration of breast cancer patients did not alter significantly in relation to SHBG concentration of cervical cancer patients (p > 0.05).

It has also been observed in our study (Table 1) that high density lipoprotein (HDL) cholesterol level in blood of control women was higher (51.2 ± 12.6 mg/dl) as compared to HDL-cholesterol levels of breast cancer patients (39.1 ± 10.2 mg/dl) and patients with carcinoma of the uterine cervix (35.1 ± 10.1 mg/dl). HDL is rich in protein content; therefore, this lipoprotein may be considered as an indicator of the nutritional status of a person. The differences in mean values of HDL-cholesterol between normal control women and breast cancer patients as well as control women and cervical cancer patients were found to be statistically significant (p < 0.01). But like SHBG level of serum, HDL level of breast cancer patients did not show significant change in comparison with the patients of cervical cancer.

Serum estradiol-17β levels of the breast cancer patients were found to be higher (101.2 ± 60.2 pmole/l) than cervical cancer patients (79.3 ± 40.6 pmole/l) and normal controls (91.5 ± 55.9 pmole/l), although the differences did not reach statistical significance. The mean ± SD estradiol levels of premenopausal (n = 15) and postmenopausal (n = 16) patients with breast cancer were 151.1 ± 45.5 and 54.4 ± 22.3 pmole/l respectively. Whereas, amongst cervical cancer patients, the premenopausal (n = 14)

<table>
<thead>
<tr>
<th>Table 1 — Levels of testosterone, SHBG, estradiol, HDL cholesterol, triglycerides and total cholesterol in serum in breast cancer and cervical cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Values represent mean ± SD. n indicates number of cases analysed]</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Testosterone (n mole/l)</td>
</tr>
<tr>
<td>SHBG (n mole/l)</td>
</tr>
<tr>
<td>Estradiol (p mole/l)</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
</tr>
<tr>
<td>(n=31)</td>
</tr>
<tr>
<td>3.1±1.2 (1.2-5.4)**</td>
</tr>
<tr>
<td>24.1±8.4 (10.0-44)*</td>
</tr>
<tr>
<td>101.2±60.2 (26.0-218)</td>
</tr>
<tr>
<td>39.1±10.2 (21-72)*</td>
</tr>
<tr>
<td>182.8±66.1 (111-395.3)*</td>
</tr>
<tr>
<td>195.7±41.2 (117-298.1)*</td>
</tr>
</tbody>
</table>

* p<0.01 (compared to normal control women)  
# p<0.05 (compared to cervical cancer patients)
level was 87.5±39.9 pmole/l; it was 72.4±41.0 pmole/l for postmenopausal group. The mean ± SD estradiol level of premenopausal control women (n=15) was 118.5±64.4 pmole/l and the level in postmenopausal controls was 64.5±27.6 pmole/l.

Significant aromatase activity was detected in 19/31 (61.3%) breast cancer cases. The postmenopausal group showed slightly higher proportion of aromatase activity (10/16) than premenopausal cases. On the other hand, aromatase immunoreactivity was observed in all breast cancer cases. Immunostaining was observed in the cytoplasm of stromal or interstitial cells. Twelve cases (38.7%) showed significant immunoreactivity (Fig. 1), while 10 (32.3%) and 9 (29%) cases revealed moderate and low immunoreactivity respectively.

Serum testosterone and SHBG did not reveal any statistically significant relationship in cervical cancer patients (r=-0.1351) as well as in control women (r=-0.0970). Interestingly, a significant inverse relationship was observed between testosterone and SHBG (r=-0.39, p<0.05) among patients with carcinoma of the breast. Further, no statistically significant association was found between hormonal parameters and different lipid fractions.

Discussion

Risk of breast cancer is more in women under longer exposure of endogenous ovarian hormones, so early menarche or late menopause increases the risk. Majority of epidemiological studies indicate a tendency of elevated levels of testosterone or androgens in breast cancer patients. The increased rate of conversion of androgens into estrogens in peripheral adipose tissues (aromatization) may be responsible for higher risk for breast cancer. Dorgan et al. described that risk of breast cancer was positively and significantly associated with serum androgen levels. Similarly, many investigators have reported that risk of breast cancer was related to increased level of circulating testosterone. However, some others did not observe such association. Interestingly, Jemstrom et al. observed a significantly decreasing level of testosterone in relatives of breast cancer patients. However, we have noticed an increased level of blood testosterone in patients with breast carcinoma as compared to normal control women and patients with cervical cancer. Contrary to the opinion that higher level of testosterone is related to stress associated with cancer, our study did not detect any such effect of stress as evident by significantly higher level of testosterone only in breast cancer patients compared to patients with cervical cancer which is not dependent on endogenous sex hormones. Androgens might be taken up from blood, and thus there could be a storage of these steroid hormones inside the breast tissue and/or perhaps some alterations in their local metabolism; androgens could play a different role in breast carcinogenesis in relation to the circulating levels of estrogen and to the expression of estrogen receptors. Bryan et al. suggested that androgens might stimulate breast epithelium directly through binding to androgen receptors. Alternatively, it is also hypothesized that androgens stimulate the synthesis of epidermal growth factor (EGF) and possibly of other growth factors inside breast epithelium and thus play a role in the autocrine and paracrine growth regulation of human breast cancer. Testosterone is the principal circulating androgen in normal women. Both the ovaries and the adrenals normally secrete testosterone. Secreto et al. suggested that high levels of testosterone and other androgens might indicate the presence of underlying subclinical ovarian conditions. Chronic stimulation of luteinizing hormone (LH) leads to increased ovarian androgen secretion. Some investigators observed that breast cancer patients with elevated urinary testosterone levels at the time of diagnosis experienced a dramatic decrease in the excretion of this hormone after surgical removal of
the ovaries, while no change was observed in patients with normal levels at the time of diagnosis prior to oophorectomy. Histological examination of the excised ovaries revealed hyperplasia of interstitial cells in all hyperandrogenic patients. Thus the source of androgens may be the interstitial tissue of the ovary. Alternatively, the adrenal may contribute to the observed hyperandrogenic state. Disturbances in extra-adrenal regulators, like corticotropin or ACTH, may lead to excessive adrenal sex steroid production.

We observed higher level of blood estradiol among breast cancer patients than cervical cancer and normal controls, although the differences were not statistically significant. Premenopausal patients with breast carcinoma showed an increased level of blood estradiol as compared to premenopausal patients with cervical carcinoma and premenopausal normal controls. Surprisingly, this level among postmenopausal women of breast cancer was lower than even postmenopausal control women and postmenopausal cervical cancer patients. However, on subgrouping the study-subjects taking into consideration the menstrual status, the number of cases as well as controls become inadequate to draw any conclusion. Also, Helzlouer et al. and Recchione et al. did not observe higher blood estrogen levels amongst postmenopausal women with breast cancer. Perhaps, intratumoral aromatase enzyme is more important in the pathological process of postmenopausal breast cancer in comparison with premenopausal cases. Despite the low levels of circulating estrogens, postmenopausal breast tissue can accumulate estrogens due to the intense enzymatic activities of the tissue itself. Locally produced estrogens may therefore play a significant role in the development of breast cancer.

The major binding proteins for steroid hormones are corticosteroid-binding globulin (CBG) which binds both cortisol and progesterone and SHBG which binds testosterone and estradiol. In the blood, androgens and estrogens exist in either a free (unbound) state or bound to serum proteins. It is a common finding that breast cancer is positively associated with percentage of free estradiol or percentage of estradiol not bound to SHBG. Kaaks noticed that breast cancer risk is related with decreased level of plasma SHBG. Schapira et al. reported that premenopausal breast cancer patients showed significantly lower levels of SHBG as compared to age-matched and weight-matched controls. Also, the present study revealed significantly lower level of SHBG in breast cancer patients than normal control women. Further, the levels of SHBG in patients with carcinomas of breast and uterine cervix showed similar changes as compared to normal controls. Interestingly, SHBG concentrations of normal control women were found to have higher level in comparison with patients of cervical cancer which is not dependent on sex hormones.

Like low serum SHBG protein concentration in cervical cancer, HDL which is rich in protein content also showed a low level among patients with carcinoma of the uterine cervix. It is well known that cervical cancer is more common in women of lower socio-economic status where under-nutrition is a common phenomenon. The overall decrease of SHBG concentration in cervical cancer patients perhaps related with nutritional deficiency. The relationship of SHBG to lipid and lipoprotein is complex in nature. Several studies report that SHBG is positively associated with HDL-cholesterol. Haffner observed a negative relationship between SHBG and triglycerides. Further, HDL concentrations may be reduced due to androgens and also an inverse relationship may exist between SHBG and androgens or testosterone in our physiological system. In the present study, we noticed elevated levels of testosterone and triglycerides and low levels of SHBG and HDL-cholesterol among breast cancer patients; yet, excepting a negative association between testosterone and SHBG, no other statistically significant correlation could be found. In the case of cervical cancer patients and normal controls there was no correlation between hormone levels and cancer. In a study on breast cancer patients, Lonning et al. also observed that SHBG correlated negatively with plasma androstenedione which is the immediate precursor of testosterone. Our findings indicate that the high level of serum testosterone and low level of SHBG among breast cancer patients possibly signify their involvement with the pathological process of mammary carcinogenesis which is closely related with estrogen metabolism.

References
3 Love R R (1990) J Natl Cancer Inst 82, 18-21
5 Kaaks R (1996) Cancer Causes Control 7, 605-625
33 Bezwoda W R, Mansoor N, Dansey R & Esser J D (1987) Oncology 44, 30-33