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Sex Hormone Binding Globulin (SHBG) in Breast Cancer: a Correlation with Obesity but not with Estrogen Receptor Status*

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Abstract—*Plasma sex hormone binding globulin (SHBG) levels were determined in a group of 82 patients suffering from breast carcinoma with a known estrogen receptor status (ER). Overweight (>20% of ideal weight) premenopausal patients had a significantly lower SHBG plasma level than their non-obese counterparts* (43 pmol/ml *vs* 72.7 pmol/ml, $P < 0.001$). *No difference in plasma levels of SHBG was found between obese and non-obese postmenopausal patients. No correlation was found between SHBG levels and ER status either in non-obese premenopausal patients or in postmenopausal patients in general. Breast carcinoma patients had significantly higher SHBG plasma levels than a group of normal controls* (57.9 pmol/ml *vs* 40.6 pmol/ml, $P < 0.01$), *but the stage of the disease did not influence the SHBG level within the breast carcinoma patients. Results of this study do not support a correlation between SHBG levels and ER status. SHBG plasma levels are significantly influenced by a patient's weight, particularly in those who are premenopausal.*

INTRODUCTION

THE IMPORTANCE of a tumor's estrogen receptor (ER) status in planning therapy for breast carcinoma patients is being increasingly recognized. The presence of these receptors in breast tumors represents a reliable indicator of hormone dependency [1]. Since ER determination requires tumor tissue samples that are not always available, an alternate but equally useful indicator would be of great value.

This study was undertaken in a group of breast carcinoma patients to assess whether there is any correlation between a tumor's ER status and plasma levels of sex hormone binding globulin, which helps to maintain adequate circulating levels of testosterone and estrogen. Reports from the literature with regard to this correlation are conflicting. Harris *et al.* [2] have suggested that SHBG is of little value as a prognostic indicator in breast cancer. Mason *et al.* found no difference in plasma SHBG concentration in a group of 44 breast carcinoma patients, all postmenopausal, who were either ER-positive or -negative [3]. To the contrary, a direct correlation between SHBG plasma levels and tumor ER status has been reported by Murayama *et al.*, first in postmenopausal patients only [4] and recently in premenopausal patients as well [5]. These authors measured SHBG titers in 35 patients with primary breast carcinoma and found that in both pre- and postmenopausal patients who were ER-positive, SHBG plasma levels were significantly higher than in their counterparts who were ER-negative. In addition, when analyzing the response to a variety of hormonal manipulations, ablative or additive, these authors found that patients who responded had a significantly higher SHBG that non-responding patients, regardless of their menopausal status [5]. Finally, as further evidence of SHBG's prognostic significance in breast carcinoma, Murayama *et al.* have suggested that patients with high plasma levels may have a longer disease-free interval than patients with a

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low SHBG [6], which is also true for ER-positive tumors [7].

In this paper we have measured SHBG plasma levels by the filter assay method, with a correction for non-specific binding [8], in 82 patients with breast carcinoma at different stages of the disease and of known ER status. We have analyzed the data to establish the value of SHBG plasma levels as a possible indicator of hormone dependency in breast cancer.

MATERIALS AND METHODS

Patients

SHBG was determined in 82 women suffering from breast carcinoma, with known estrogen receptor (ER) status, who have been followed in the Department of Radiation & Clinical Oncology of the Hadassah University Hospital. The group's median age at the time of SHBG determination was 53 yr (range, 33–71 yr). Thirty-six patients were premenopausal, 11 estrogen-receptor-positive (ER+) and 25 estrogen-receptor-negative (ER–). Forty-six patients were postmenopausal, 18 with an ER+ tumor and 28 were ER–. Fifteen of the premenopausal patients (39%) were more than 20% overweight [9], as were 26 (57%) of the postmenopausal women. Control SHBG values are from a randomly selected healthy female population.

Disease staging was done according to the TNM classification of the WHO [10]. Three patients had stage I disease and were followed in the Outpatient Clinic without further treatment after mastectomy; 33 patients had a mastectomy for stage II disease and were receiving adjuvant chemotherapy with cyclophosphamide, methotrexate and 5-fluorouracil (CMF) [11] at the time of SHBG determination. Fifteen patients had locally advanced, stage III disease; all were given radiation therapy to the tumor area following a biopsy for histologic documentation of malignancy and for estrogen receptor determination. They were undergoing chemotherapy with CMF at the time SHBG was measured. Finally, 31 patients had stage IV disease, with widespread, measurable metastases, who were under treatment with systemic chemotherapy with either CMF or an adriamycin-based combination at the time SHBG was obtained. None of the patients were on chronic steroid therapy at the time of SHBG determination. Student's t test was used to test for statistical significance.

Measurement of SHBG

SHBG was measured by a modification of the method of Mickelson and Petra [8]. Serum was diluted 1:100 with 0.01 M Tris–HCl buffer, pH 7.4. Two sets of duplicate samples of the diluted serum were incubated for 15 min at room temperature with an equal volume of labeled [^{3}H]-dihydrotestosterone (DHT), prepared in the same buffer. The first set was incubated with about 10 nM DHT, a concentration sufficient to saturate the SHBG binding sites. The second set was incubated with an excess of DHT, 3.5 mM, to determine the non-specific binding of DHT. The tubes were then transferred to an ice bath for an additional 15 min incubation. One hundred-microliter aliquots of the mixture were then pipetted on top of DE-81 filter discs and washed 5 times with 2 ml of buffer by suction. The bound DHT was determined from the counts remaining on the disc after the correction for the non-specific binding. All results are expressed as pmol DHT bound/ml of serum. A serum pool was used as a control, and was run in every assay, the average for 10 separate determinations of this serum being 87.7 ± 3.5 pmol/ml (mean ± S.D.) and the interassay variance is about 4%.

Estrogen receptors

Estrogen receptors were determined by the dextran-coated charcoal competitive protein binding assay [12]. Tumors containing less than 10 fmol of estrogen receptor protein/mg of cytosol protein were considered ER– and those with higher values ER+.

RESULTS

Being overweight was an important variable influencing the results of this study, as shown in Table 1. Overall, and regardless of estrogen receptor status, obese premenopausal patients had a significantly lower SHBG serum concentration than their non-obese counterparts ($P < 0.001$). Furthermore, Fig. 1 shows that in the premenopausal subgroup of patients there is an inverse linear correlation ($r = -0.59$) between SHBG levels and the percentage of the ideal weight for each patient. This relationship was not found in the postmenopausal subgroup. While no significant difference in SHBG was observed between pre- and postmenopausal patients who were overweight, non-obese premenopausal patients had a significantly higher SHBG serum level than non-obese postmenopausal women ($P > 0.05$). Within the postmenopausal patients, no difference in SHBG levels was found according to the degree overweight.

Table 2 compares SHBG serum levels by estrogen receptor status in both pre- and postmenopausal patients both overweight and not. While normal weight premenopausal patients had a higher SHBG than obese patients (Table 1), estrogen receptor status *per se* did not result in any significant difference in SHBG

Table 1. Levels of SHBG in pre- and postmenopausal breast cancer patients according to their nutritional status

Nutritional status	Premenopausal No. of patients	Premenopausal SHBG pmol/ml (mean ± S.E.M.)	Postmenopausal No. of patients	Postmenopausal SHBG pmol/ml (mean ± S.E.M.)	*P*
Overweight	14	43.0 ± 4.7	26	54.3 ± 4.2	NS
Normal	22	72.7 ± 5.0	20	56.5 ± 5.6	<0.05
P, overweight vs normal		<0.001		NS	

NS = not significant.

Table 2. SHBG levels according to menopausal, nutritional and estrogen receptor status

Menopausal status	Nutritional status	Estrogen receptor status	No. of patients	SHBG pmol/ml (mean ± S.E.M.)	*P*
Premenopausal	overweight	ER+	3	39.0 ± 9.8	
		ER-	11	44.5 ± 5.5*	NS
	normal	ER+	8	83.5 ± 9.7	
		ER-	14	67.0 ± 5.2*	NS
Postmenopausal	overweight	ER+	10	59.0 ± 6.7	
		ER-	16	51.3 ± 5.3	NS
	normal	ER+	8	49.7 ± 6.2	
		ER-	12	59.4 ± 7.8	NS

NS = not significant.
*$P < 0.001$, ER- groups in premenopausal patients.

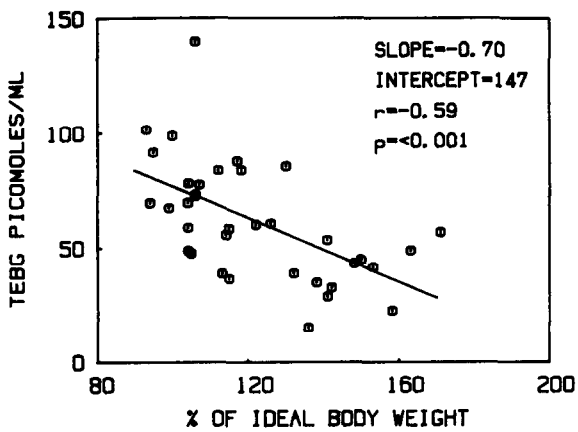

Fig. 1. The relationship between SHBG levels and body weight status in 39 premenopausal patients suffering from breast carcinoma. The ordinate scale is expressed as a percentage of actual weight over ideal weight, as determined from accepted weight tables [9].

concentration, neither within the non-obese subgroups of ER+ and ER- patients nor within the obese patients. ER- non-obese premenopausal patients had a significantly higher SHBG than ER- obese premenopausal patients ($P < 0.001$). A comparison between obese and non-obese ER- premenopausal patients was not valid because of the small number in the obese subgroup of patients. With regard to postmenopausal patients, no significant difference in SHBG serum levels was observed between those whose tumors were ER+ or ER-, regardless of their nutritional status. No difference was observed between ER+ obese and non-obese patients nor between those who were ER-.

Table 3 compares results of SHBG serum levels and tumor estrogen receptor status in our group of breast carcinoma patients with control groups of healthy pre- and postmenopausal women. All subgroups of breast carcinoma patients, pre- and postmenopausal, with and without estrogen receptors, had significantly higher SHBG levels than their healthy counterparts. This finding prompted us to investigate the influence of disease activity upon SHBG serum concentration. Pre- and postmenopausal patients were divided into two subgroups: (a) with (stage IV) or (b) without metastatic disease (stages I and II); stage III patients were excluded. Results are shown in Table 4. SHBG levels did not differ significantly in patients with or without active disease.

DISCUSSION

This study was undertaken to assess whether plasma levels of SHBG correlate with the tumor's content of estrogen receptors in a group of breast carcinoma patients. If such a correlation could be

Table 3. SHBG levels in breast carcinoma and normal controls

Menopausal status	Estrogen receptor status	No. of patients	SHBG pmol/ml (mean ± S.E.M.)
Premenopausal	ER+	11	71.3 ± 9.6
	ER−	25	56.7 ± 4.3
	combined	36	61.3 ± 4.2
	normal	33	46.8 ± 3.0*
Postmenopausal	ER+	18	55.2 ± 4.7
	ER−	28	54.6 ± 4.5
	combined	46	54.8 ± 3.3
	normal	70	34.5 ± 1.4*

*$P < 0.01$, normal vs breast carcinoma patients.

Table 4. SHBG levels in pre- and postmenopausal patients with or without metastasis

Tumor stage	Estrogen receptor status	Premenopausal No.	Premenopausal SHBG pmol/ml	Postmenopausal No.	Postmenopausal SHBG pmol/ml
I and II	ER+	8	72.4 ± 13.2	10	50.5 ± 7.4
	ER−	11	61.5 ± 5.1	7	54.9 ± 8.5
	combined	19	66.1 ± 6.2	17	52.3 ± 5.4
IV	ER+	1	84.1	3	53.2 ± 11.7
	ER−	11	49.4 ± 7.7	16	50.9 ± 6.2
	combined	12	52.3 ± 7.7	19	51.3 ± 5.4

The results are expressed as the mean ± S.E.M. for each group.

demonstrated, SHBG could help to predict a tumor's hormone dependency, even in the absence of tumor tissue for estrogen receptor determination. Unfortunately, our results do not support the claim that SHBG plasma levels correlate with tumor estrogen receptor status. This is in agreement with some previously published studies [2, 3] but is opposed to Murayama *et al.*'s data [4–6].

Premenopausal patients who were not overweight had significantly higher SHBG levels than their postmenopausal counterparts, and also higher levels than obese premenopausal patients. Therefore a comparison of SHBG plasma levels without taking into account the effect of obesity is of no value. Recent reports emphasize the fact that SHBG levels are decreased when overweight [13, 14]. Moreover, Kopelman *et al.* demonstrated an increase in SHBG in obese individuals after substantial weight reduction [15].

Similarly, Mason *et al.* found no significant correlation between body weight and tumor estrogen receptor concentration in a group of breast carcinoma patients, strengthening our assumption that SHBG plasma levels and ER status are independent variables [16].

Siiteri *et al.* found significantly lower SHBG levels in obese pre- and postmenopausal women than in their non-obese counterparts, and concluded that both the increased availability of free estrogen in women with low SHBG levels and the increased estrogen synthesis in the peripheral tissues of obese women may explain a higher incidence of breast cancer in overweight women [17]. Furthermore, the author's preliminary data in breast cancer patients show a similar correlation between obesity, decreased SHBG levels and increased free estradiol [17].

The reason for higher SHBG levels observed in our group of patients suffering from breast carcinoma when compared to normal controls regardless of menopausal and nutritional status and the presence or absence of estrogen receptors remains unclear. It should be noted, however, that contrary to our findings, Gaidano *et al.* reported a significantly lower SHBG level in premenopausal breast carcinoma patients than in normal controls or in patients with fibrocystic breast disease matched by age [18].

When we analyzed the possible relation of SHBG levels with stage of disease, no difference was found between those patients with advanced metastatic disease and those with localized disease. Therefore, even though breast carcinoma patients have higher SHBG serum levels than controls, SHBG is not a useful indicator of tumor activity in breast cancer.

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