Eur J Cancer Clin Oncol, Vol. 24, No. 8, pp. 1329–1335, 1988.
Printed in Great Britain

0277-5379/88$3.00 + 0.00

Serum Hormone Levels in Breast Cancer Patients and Controls in Egypt and Great Britain

The Anglo-Egyptian Health Agreement Collaborative Study

Abstract—*The levels of serum prolactin, progesterone, oestradiol and sex hormone binding globulin (SHBG) have been measured in premenopausal and postmenopausal normal women and patients with breast cancer living in Egypt or Britain. In addition, the percentage of non-protein-bound oestradiol (free oestradiol) was determined. The breast cancer patients had attended the Tanta Cancer Institute, Cairo Cancer Institute, Guy's Hospital, London or the Western Infirmary, Glasgow. The respective control women were unaffected volunteers living in Tanta, Cairo, Guernsey or Glasgow. The concentration of serum prolactin, oestradiol and progesterone was similar for cancers and controls within, or between centres. There was no difference between patients and their respective controls in SHBG levels for the four centres. In premenopausal women a comparison of combined patient and control groups showed that women from Tanta had significantly raised SHBG levels compared with similarly combined groups from the other three centres. Egyptian women (cancers plus controls) had a higher percentage free oestradiol* (1.61%) *than British women* (1.42%).

In all four centres there was a significant linear correlation between the percentage free oestradiol and SHBG levels for pre- and postmenopausal women. The regression line for British women was significantly lower than that of Egyptian women. Thus, for a given serum SHBG level, Egyptian women have a higher free oestradiol than British women. The results may be associated with the claim that Egyptian women present with a more aggressive form of breast cancer than British women.

INTRODUCTION

RECENT results indicate that increased blood levels of biologically available oestradiol, or 'free' oestradiol, are of importance in the aetiology of breast cancer in the United Kingdom [1, 2]. Furthermore, in studies on the relationship between endocrine status and geographical variation in the risk of developing breast cancer, Japanese and British women have been found to have profound differences in the amount of biologically available oestradiol [3].

Although there are no reliable data on the absolute incidence of breast cancer in Egypt, the results from the metropolitan area of Cairo show the disease to differ from that in the United Kingdom by being a predominantly premenopausal disease [4]. Thus the age-specific incidence for Egyptian women appears to reach a peak level between 40 and 60 years and declines rapidly thereafter. This contrasts with the continuous increase in incidence of breast cancer with increasing age in Britain. Additionally, it has been claimed that Egyptian patients, in common with women in the North African littoral, have a particularly aggressive form of breast cancer [5–8].

The purpose of this study is therefore to determine whether there are endocrine differences, especially those involved with biologically available oestrogens, which could be related to these differences in the patterns of incidence and behaviour of breast cancer in Egyptian and British women.

MATERIALS AND METHODS

Subjects

Of the patient groups only women with operable breast cancer were investigated. Egyptian subjects

Accepted 31 March 1988.
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were recruited from the Cairo Cancer Institute and the Tanta Cancer Institute. British patients were women attending either Guy's Hospital, London or the Western Infirmary, Glasgow.

Control women were volunteers who were ostensibly healthy, not taking oral contraceptives or any form of hormonal medication and matched for menopausal status. In the case of Guy's patients the controls were taken from the Island of Guernsey. This was thought to be justified since trends in reproductive history (age at menarche, parity, age at first full term birth and age at menopause) are similar for these two groups [9]. In the Egyptian centres there was a marked reluctance on the part of normal postmenopausal women to give blood samples which was attributed to the cultural attitudes of older Egyptian women.

All volunteers answered a questionnaire and gave a sample of blood. In patients this blood was taken 1–2 days before surgery. All blood specimens were taken in the morning. Serum prepared from the blood was stored at −20°C in 2–3 ml aliquots. The blood samples were collected from Egypt during the period 1983–1985. The bloods were shipped to the U.K. in solid CO_2. These samples were brought as personal luggage of the authors who were travelling to the U.K. This meant that the transit time was always less than 10 h which ensured that samples remained frozen. On arrival samples were stored again at −20°C. The London and Glasgow blood samples were assayed in batches with each batch having random samples from the four centres.

Table 1 shows the mean ages, heights and weights for cases and controls. In premenopausal women these statistics were similar between cases and controls within the four centres. Furthermore, the average age and height between centres was similar. However, Egyptian women were significantly heavier than British women (70.4 kg and 63.9 kg; $P < 0.001$). In postmenopausal women the average age, height and weight of British cases and controls were similar between and within centres. Allowing for the small numbers of controls, the same appears to be the case for Egyptian volunteers. However, comparing Egyptian and British women the former were on average younger (55.5 years compared with 62.4 years) and heavier (71.4 kg compared with 64.9 kg; $P < 0.001$).

ASSAYS

Prolactin

Serum prolactin was assayed using a second-antibody radioimmunoassay method available from Amersham International plc, Bucks. (Cat. No. IM 1060). For the range of titres estimated in this study the within- and between-assay reproducibility had a coefficient of variation of less than 10%. The specificity of the assay was satisfactory, its cross-reaction with growth hormone and gonadotrophins being 0.2% or less.

Progesterone and oestradiol

Serum progesterone and oestradiol were assayed using a solid-phase radioimmunoassay purchased

Table 1

	Guy's		Glasgow		Cairo		Tanta	
	N	Ca	N	Ca	N	Ca	N	Ca
Premenopausal								
Age	41.7 ± 5.1	40.3 ± 5.8	42.1 ± 5.8	45.9 ± 4.7	35.6 ± 7.4	40.9 ± 4.6	38.9 ± 3.7	39.3 ± 6.1
Height (cm)	163 ± 5.8	164 ± 6.9	160 ± 6.3	162 ± 8.2	159 ± 7.8	163 ± 7.5	159 ± 8.4	161 ± 6.6
Weight (kg)	66.4 ± 9.9	62.2 ± 9.1	64.9 ± 15.1	61.4 ± 12.3	67.2 ± 15.6	74.9 ± 15.0	71.5 ± 13.2	67.8 ± 18.1
Number	21	21	19	14	27	23	25	25
Postmenopausal								
Age	62.4 ± 7.2	64.7 ± 8.5	60.8 ± 9.4	61.7 ± 7.3	55.3 ± 4.5	54.2 ± 4.7	53.4 ± 6.2	58.0 ± 6.7
Height (cm)	157 ± 6.5	158 ± 3.8	162 ± 7.3	161 ± 9.7	162 ± 5.5	158 ± 4.4	162 ± 6.8	160 ± 5.7
Weight (kg)	63.8 ± 12.0	66.5 ± 11.0	66.1 ± 13.6	66.1 ± 12.7	75.3 ± 15.8	72.1 ± 17.8	72.2 ± 9.7	69.5 ± 12.7
Number	20	20	20	20	3	19	5	15

All values are expressed as mean ± 1 S.D.

from Diagnostic Products Corporation, Wallingford (progesterone, Cat. No. TKPG2; oestradiol, Cat. No. TKE21). The specificity of the assays were satisfactory. In the case of the progesterone assay the cross-reactions with cortisol, 5α-pregnan-3,20-dione and 5β-pregnan-3,20-dione were 1.3% or less. For the oestradiol assay the cross-reactions with oestrone and oestriol were 1.1% and 0.32%, respectively. The between- and within-assay variation for the progesterone assays was less than 10%. In the case of oestradiol this was 8% and 15% for pre- and postmenopausal women, respectively.

Sex hormone binding globulin

Sex hormone binding globulin (SHBG) was measured using an immunoradiometric assay (Farmos Diagnostica). The inter- and intra-assay variation was less than 10%. In terms of specificity no human serum protein was known to appreciably cross-react with the assay.

Percentage of non-protein-bound oestradiol

The percentage of oestradiol not bound to blood carrier proteins was determined by the centrifugal ultrafiltration dialysis method of Hammond *et al.* [10]. The validation of this method and reproducibility have already been described [10].

Statistics

All biochemical data were logarithmically transformed so that normal distribution statistics can be applied. These mean results are therefore expressed logarithmically to the base 10 plus or minus one standard deviation.

RESULTS

(a) *Premenopausal subjects*

Prolactin. No account has been taken of menstrual cycle status in comparing prolactin levels since the variation of prolactin over the menstrual cycle is trivial compared with the inter-person variations (see [11]). The mean prolactin levels (Table 2) are similar for all groups and there was no statistical difference between cancer and control levels within any one of the four centres. Neither was there any difference between centres even if a comparison were made using the combined patients and controls as a group.

Progesterone and oestradiol. The follicular and luteal phase levels of total blood progesterone and oestradiol were similar for all groups and therefore no statistically significant difference was observed

Table 2

	Guy's		Glasgow		Cairo		Tanta	
	N	Ca	N	Ca	N	Ca	N	Ca
Premenopausal								
Prolactin (ng/ml)	0.95 ± 0.23	0.82 ± 0.28	0.85 0.22	0.97 ± 0.24	1.01 ± 0.29	0.92 ± 0.36	0.85 ± 0.36	0.95 ± 0.32
SHBG (nmol/l)	1.82 ± 0.12	1.84 ± 0.15	1.75 ± 0.21	1.75 ± 0.25	1.79 ± 0.27	1.72 ± 0.21	1.91 ± 0.20	1.92 ± 0.23
Free E2 (%)	0.16 ± 0.06	0.16 ± 0.08	0.16 ± 0.08	0.16 ± 0.11	0.24 ± 0.10	0.24 ± 0.08	0.18 ± 0.07	0.19 ± 0.10
Number	21	21	19	14	27	23	25	25
Postmenopausal								
Oestradiol (pg/ml)	0.96 ± 0.27	0.99 ± 0.29	1.02 ± 0.29	1.06 ± 0.27	0.84 ± 0.16	0.95 ± 0.29	1.12 ± 0.19	1.10 ± 0.22
Progesterone (ng/ml)	− 0.40 ± 0.15	−0.41 ± 0.11	− 0.45 ± 0.10	− 0.39 ± 0.23	− 0.38 ± 0.20	− 0.42 ± 0.19	− 0.43 ± 0.16	− 0.39 ± 0.18
Prolactin (ng/ml)	0.72 ± 0.30	0.65 ± 0.34	0.62 ± 0.30	0.74 ± 0.30	0.58 ± 0.25	0.66 ± 0.42	0.74 ± 0.29	0.97 ± 0.45
SHBG (nmol/l)	1.75 ± 0.21	1.67 ± 0.25	1.85 ± 0.22	1.80 ± 0.24	1.70 ± 0.39	1.77 ± 0.25	1.80 ± 0.50	1.78 ± 0.20
Free E2 (%)	0.25 ± 0.09	0.30 ± 0.08	0.24 ± 0.08	0.27 ± 0.07	0.33 ± 0.09	0.29 ± 0.08	0.33 ± 0.08	0.28 ± 0.07
Number	20	20	20	20	3	19	5	15

All assay values are expressed as $\log_{10} \pm 1$ S.D.

between cancer and control groups within or between centres. To illustrate how similar the two racial groups are in terms of serum oestradiol and progesterone levels, Figs 1 and 2, respectively, show the accumulative percentage of the combined British or Egyptian volunteers with increasing steroid level.

Sex hormone binding globulin. There was no difference in SHBG levels between patients and controls within any one of the four centres.

Comparing the combined patient plus control groups, the geometric mean level of SHBG for women from Tanta was 79.7 nmol/l which was significantly higher than for those volunteers from Cairo (56.2 nmol/l; $P = 0.002$; $t = 3.23$) and Glasgow (56.1 nmol/l; $P = 0.003$; $t = 3.07$) and nearly so for London (67.6 nmol/l; $P = 0.056$; $t = 1.94$). There was a significant inverse linear correlation between body weight and SHBG levels in British ($P = 0.01$) and Egyptian ($P < 0.001$) subjects.

Percentage of non-protein bound oestradiol. On a national basis, Egyptian women (cancers plus normals) had a higher percentage of free oestradiol (1.61%) than British women (1.42%), a difference which is highly significant ($P < 0.001$). When the data were broken down for each of the centres there was no significant difference between patients and controls in the percentage of oestradiol which was not bound to blood proteins (see Table 2). Comparison of combined cancers and controls showed that there was a significant difference between the median percentage free oestradiol in women from Cairo (1.79%) and for subjects in Tanta (1.56%; $P= 0.004$), Glasgow (1.47%; $P = 0.0002$) or London (1.42%; $P = 0.0001$).

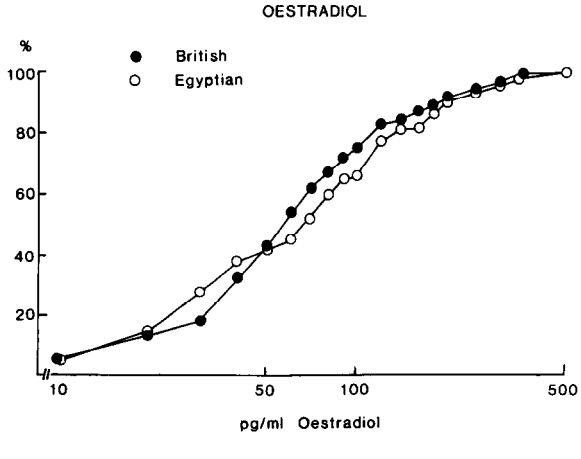

Fig. 1. Percentage of subjects with given amounts of total serum oestradiol. The graph depicts the proportion of British (●) or Egyptian (○) women (cancer plus controls) whose oestradiol levels were within a given limit.

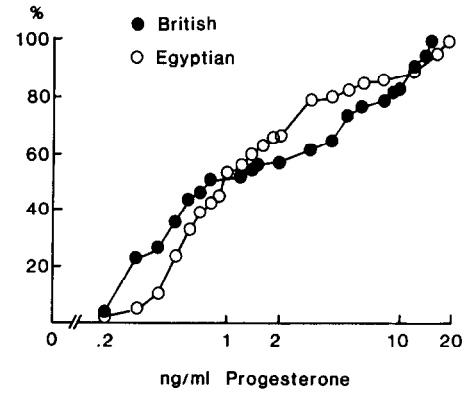

Fig. 2. Percentage of subjects with given amounts of serum progesterone. The graph depicts the proportion of British (●) or Egyptian (○) women (cancer plus controls) whose progesterone levels were within a given limit.

Correlation between SHBG and free oestradiol. There was a significant linear correlation between the percentage of free oestradiol and the amount of serum SHBG for controls or patients in all four centres. The significance levels ranged from 2% to 0.1% and the correlation equations for cancers and controls in any one centre were so similar that only the common regression lines are shown in Figs 3A and B, 4A and B. The relation between free oestradiol and SHBG concentrations did not differ for Tanta or Cairo (see Fig. 5) and therefore the data have been combined. For the same reason London and Glasgow (see Fig. 5) results have been aggregated.

A comparison of the British and Egyptian women showed that the latter have a highly significant difference ($F = 53.1$; $P < 0.001$) in the elevation of their regression line (see Fig. 5). Thus, for a given level of serum SHBG, the Egyptian women have a significantly raised percentage of free oestradiol compared with British women.

(b) *Postmenopausal subjects*

It is clear that the small number of Egyptian control women render an in-depth comparison invalid. However, there are sufficient numbers to compare Egyptian and British patients and it can be seen that there is no difference in any of the hormonal measurements. In addition there was no difference between British controls and cancer patients. In fact, the only difference which occurred between the British and Egyptian cancer patients was the relationship between body weight and SHBG concentration. The linear regressions were similar to those found in the premenopausal situation and the elevation of the lines were significantly different ($F = 37.1$; $P < 0.001$). Thus in both pre- and postmenopausal Egyptian women there was more SHBG than British women when standardized for body weight.

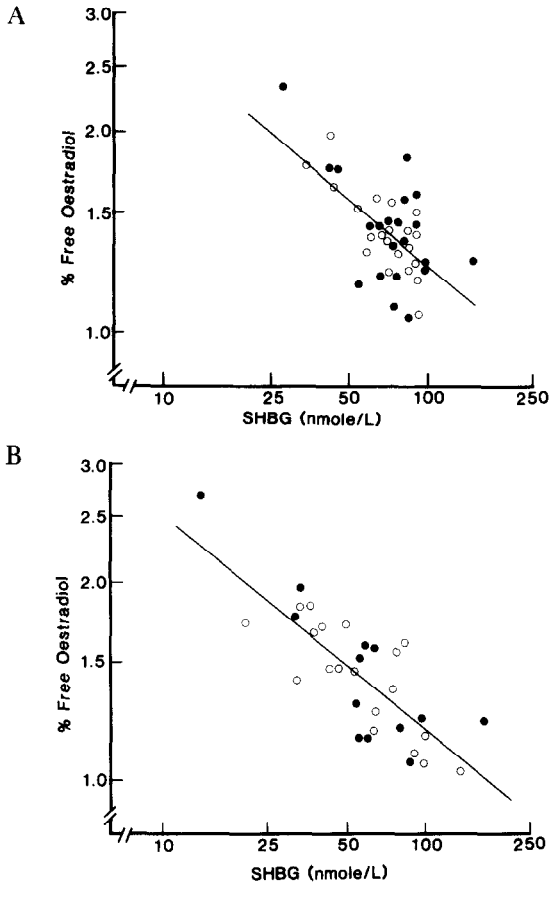

Fig. 3A and B. Linear correlation between percentage free oestradiol and serum SHBG levels in British subjects. Patients and controls from Guy's (Fig. 3A) and Glasgow (Fig. 3B) are denoted by ● and ○, respectively. The equation of the linear regression line for the combined patients and controls in London is y = 0.789 − 0.345x (r = −0.65; n = 42; P < 0.001) *and in Glasgow is* y = 0.736 − 0.330x (r = −0.68; n = 32; P < 0.001) *where* y *is the percentage free oestradiol and* x *is the concentration of SHBG* (nmole/l); *both expressed as* log_{10}. *Thereis no significant difference between the lines.*

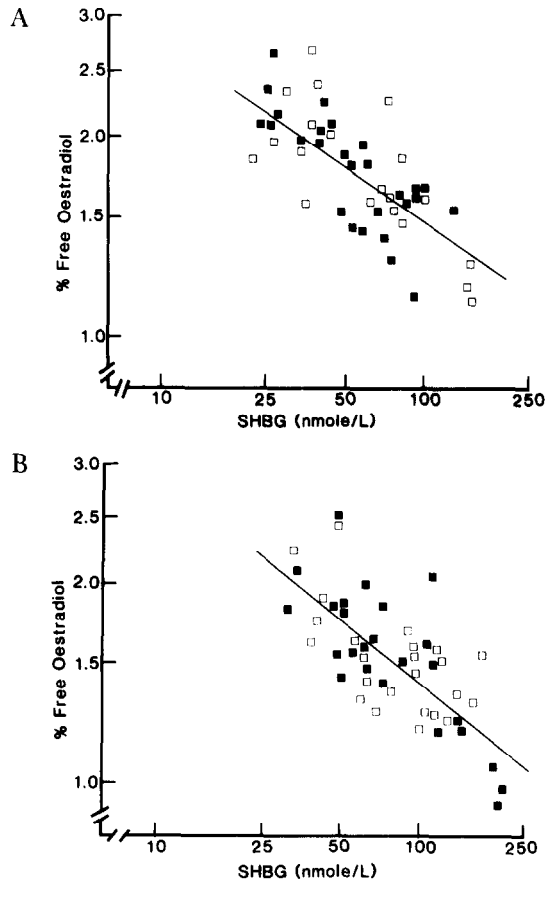

Fig. 4A and B. Linear correlation between percentage free oestradiol and serum SHBG levels in Egyptian subjects. Patients and controls from Cairo and Tanta are denoted by ● and ○, respectively. The equation of the linear regression line for the combined patients and controls in Cairo is y = 0.758 − 0.296x (r = −0.752; n = 47; P < 0.001) *and in Tanta is* y = 0.758 − 0.299x (r = −0.714; n = 49; P < 0.001), *where* y *is the percentage free oestradiol and* x *is the concentration of SHBG* (nmole/l); *both expressed as* log_{10}. *There is no significant difference between the lines.*

DISCUSSION

The purpose of this study was to address two questions. These were whether comparisons of blood hormone levels in Egyptian and British women could explain the difference in (a) incidence of breast cancer and (b) the clinical course of the disease.

The results obtained in this study showed that within any given centre there was no difference in any of the biochemical variables between cancers and respective controls; as far as free oestradiol is concerned this is contrary to previous results [1, 2]. However, the situation could be that women in different countries could have hormonal levels which are related to their own national breast cancer rates without necessarily there being a difference between the levels in individuals within each country. As an example of this concept a group of women on the *same* dose of stilboestrol would be at an increased risk of endometrial cancer without all of the women necessarily developing cancer. A case-control study of such a group would find no difference in stilboestrol levels. Thus, the hormonal milieu could be important in determining the overall national risk but the initiation of the disease within an individual could depend on other factors. In the two stage aetiological model of breast cancer [12] hormonal factors might be involved in determining risk by affecting the late stages of disease progression. For these reasons, and the fact that the hormone levels between cancers and controls in Egypt or Britain were indistinguishable, it was thought justifiable to combine these data to address the question whether the national difference in breast cancer incidence is associated with a difference in hormonal milieu. The only difference was the higher proportion of free oestradiol found in premenopausal Egyptian women compared with

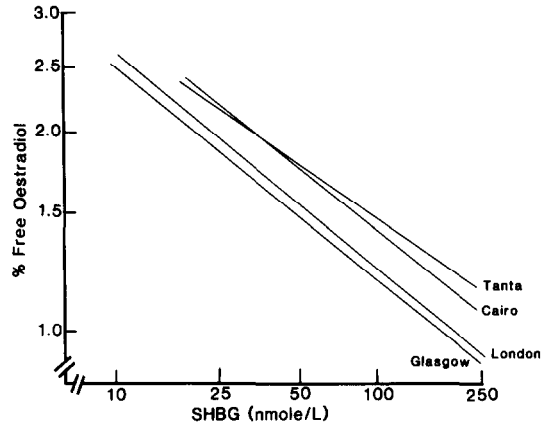

Fig. 5. Linear correlation between the percentage of free oestradiol and serum SHBG in Egyptian and British women. The figure shows the linear regression lines for the four populations as shown in Fig. 3A, B, 4A and B. The equation of the linear regression for the Egyptian population is $y = 0.765 - 0.302x$ ($r = -0.76$; $n = 96$; $P < 0.001$) *and the British population* $y = 0.733 - 0.320x$ ($r = -0.81$; $n = 74$; $P < 0.001$) *where* y *is the percentage free oestradiol and* x *is the concentration of SHBG* (nmole/l); *both expressed as* $\log_{10}$. *There is no significant difference between the slopes of the two regression lines although there is a highly significant difference in elevation* ($F = 52.0$; $P < 0.001$).

British women. It has recently been reported that the amount of oestradiol not bound to carrier protein is a better index of oestrogen action than the concentration of total oestradiol in blood [13]. If the concept that the higher the concentration of free oestradiol the greater will be the degree of oestrogenic stimulation [3] is true then it would be expected that breast cancer incidence would be higher in Egypt than in Britain. Such statistics that exist for Egyptian women do not bear out this prediction since they indicate a premenopausal rate similar to that in the United Kingdom but that in postmenopausal women the rate in Egyptian women is much less [4, 14]. Therefore, there is no obvious hormonal explanation for the differences in breast cancer incidence rate.

Since the percentage of free oestradiol is inversely related to SHBG one would expect that SHBG levels would be lower in Egyptian compared to British women. This expectation is reinforced by the fact that Egyptian women are significantly heavier than British women and that weight, like free oestradiol, is also inversely related to SHBG levels. Thus the finding that SHBG levels are similar, or even higher, is unexpected and results from there being a different relationship between free oestradiol and serum SHBG in pre- and postmenopausal Egyptian and British women. Thus for a given amount of SHBG there is, on average, a higher proportion of free oestradiol in Egyptians.

Moore *et al.* [3] have reported that difference in weight could not explain why Japanese women have a lower percentage of free oestradiol than British women. In Fig. 6 the linear regression lines for body weight and serum levels of SHBG (neither variable being log transformed) are shown for the Japanese and British subjects studied by Moore *et al.* [3] and for the Egyptian and British women in the present study. It is clear that the regression lines for the British women in the two studies are essentially the same. It is also apparent that each country has a different relationship between weight and SHBG levels. Thus for a given weight Japanese women would have the least amount of serum SHBG whilst Egyptians would have the most. However, when the percentage of free oestradiol is considered, the order is the same even though there is an inverse relationship between percentage of free oestradiol and SHBG levels. This implies that the mechanism governing the relationship between the binding of oestradiol and its binding protein varies according to geographical location. It also means that the interpretation of international comparisons of hormone levels is rendered extremely difficult. It would be interesting to know whether migration would affect these relationships.

In addressing the question concerning clinical course of the disease, the present results would suggest that breast tumours of premenopausal Egyptian women, relative to British, are in a hyperoestrogenized milieu. It is conceivable therefore that such tumours would have a faster growth rate and this would be in keeping with the suggestion that breast cancers in Egyptian patients are more aggressive than those in British subjects [5, 6].

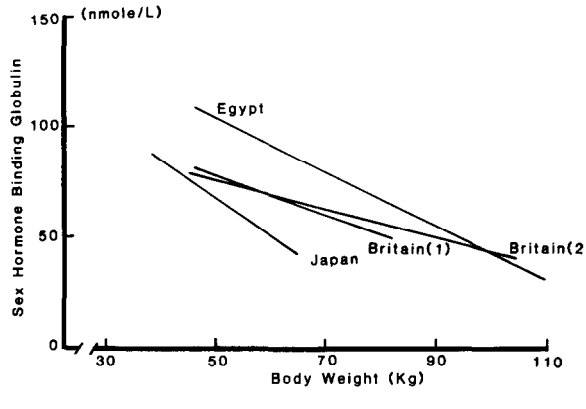

Fig. 6. Linear regression lines for concentration of SHBG and body weight The regression lines of the women from Japan ($y = 152 \pm 1.7x$; $r = -0.27$; $n = 51$; $P = 0.05$) *and Britain* (1) ($y = 121 - 0.9x$; $r = -0.35$; $n = 64$; $P < 0.01$) *are taken from Moore* et al. [3]. *The regression lines of the women from Egypt* ($y = 156 - 1.10x$; $r = -0.35$; $n = 95$; $P < 0.001$) *and Britain* (2) ($y = 110 - 0.68x$; $r = -0.29$; $n = 76$; $P = 0.01$) *are derived from the present study, where* y *and* x *refer to the concentration of SHBG* (nmole/l) *and weight* (kg), *respectively. The length of the regression lines correspond to the weight range of the appropriate populations.*

Acknowledgements—This investigation is part of the Anglo-Egyptian Health Agreement between the Governments of The United Kingdom and The Arab Republic of Egypt. The authors are indebted to Mrs. Maureen Cobbing in preparing this manuscript.

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