ORIGINAL ARTICLE

FEMALE HYPERANDROGENEMIA AND NORMAL SERUM LEVELS OF TESTOSTERONE AND SEX HORMONE BINDING GLOBULIN

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Abstract   It is well known that the reference values usually employed for endocrine biochemical measurements are those suggested by the suppliers of commercial kits despite their advice that each laboratory should set its own reference values. Our objectives were to (i) determine reference ranges for serum testosterone (T) and sex hormone binding globulin (SHBG) appropriate to our laboratory and population, and (ii) to analyze their influence on evaluating hyperandrogenemia. SHBG and T were measured, and free and bioavailable testosterone calculated, in (a) 30 selected non-hyperandrogenic women, (b) 87 non-selected healthy female blood donors, (c) 53 women with hyperandrogenism, and (d) 38 women with hyperandrogenic disorders but without biochemical hyperandrogenemia according to normal ranges suggested by the kit manufacturer. Mean serum SHBG concentrations were significantly different among all four groups. SHBG levels were significantly higher in selected normal women (group a). Using our results for this selected control group as new reference values, 12 out of 38 (31.6%) women with hyperandrogenic disorders without apparent hyperandrogenemia (group d) were recategorized as hyperandrogenemic. Similarly, 4 out of 63 (6.4%) non-selected, normal weight, women (group b), were recategorized as hyperandrogenic. Therefore, the diagnosis of hyperandrogenemia would improve accuracy by using customized reference SHBG values instead of those suggested by the suppliers.

Key words: hyperandrogenemia, hyperandrogenism, sex hormone binding globulin.

Resumen   ***Hiperandrogenemia femenina y niveles séricos normales de testosterona y globulina ligadora de esteroides sexuales.*** Con frecuencia los valores de referencia utilizados para las evaluaciones bioquímicas endocrinológicas son los sugeridos por los kits utilizados, a pesar de las recomendaciones de que cada laboratorio debiera obtener sus propios valores de normalidad. Nuestros objetivos fueron (i) analizar los rangos de referencia para testosterona (T) y globulina ligadora de esteroides sexuales (SHBG) apropiados para nuestro laboratorio y población, y (ii) analizar su influencia en la evaluación de la hiperandrogenemia. Se midió T y SHBG y se calculó testosterona libre y biodisponible en un grupo (a) control de 30 mujeres no hiperandro-génicas, (b) 87 mujeres no seleccionadas donantes de sangre, (c) 53 mujeres con hiperandrogenismo, y (d) 38 mujeres con desórdenes hiperandrogénicos pero sin hiperandrogenemia de acuerdo a los rangos de normalidad sugeridos por el kit. La concentración media de SHBG fue significativamente diferente entre los cuatro grupos. Los niveles de SHBG fueron significativamente más altos en las mujeres controles seleccionadas (grupo a). Tomando en consideración los resultados obtenidos en este grupo y estableciendo los rangos de referencia adecuados, 12 de 38 mujeres (31.6%) hiperandrogénicas sin hiperandrogenemia (grupo d) fueron recategorizadas como con exceso androgénico bioquímico. De igual manera, al analizar mujeres normopesas no seleccionadas, en edad reproductiva (grupo b), 4 de 63 (6.4%) pudieron ser definidas como hiperandrogénicas. Utilizando valores adecuados de referencia para SHBG, se mejora la precisión del diagnóstico de exceso androgénico.

Palabras clave: hiperandrogenemia, hiperandrogenismo, globulina ligadora de esteroides sexuales

The measurement of sex hormone binding globulin (SHBG) allows, together with that of total testosterone (T), the calculation of free and bioavailable fractions of this hormone, which are sensitive markers of androgen excess[1]. However, the reliability of those measurements depends not only on their technical characteristics but also on the definition of the "normal" reference range used to compare the patients' observed values. It is well known that reference values usually employed are those suggested by the suppliers of commercial kits despite their advice that each laboratory should obtain its own reference values.

In the present work, we aimed to analyze the normal reference ranges for T and SHBG of our population in our laboratory. We studied two different "normal" female control populations and compared the values obtained with the ranges of the commercial kits employed in the characterization of hyperandrogenemia.

Materials and Methods

This prospective cohort study included four groups of women in fertile age: (a) 30 carefully selected healthy, eumenorrheic, women with normal weight (BMI $\geq 19 \leq 25$) and without any clinical evidence of androgen excess; (b) 87 non-selected female blood donors; (c) 53 hyperandrogenic women of reproductive age with clinical evidence of hirsutism, acne, seborrhea, and/or irregular menstrual cycles with hyperandrogenemia as defined through the kit reference values (see below); and (d) 38 women of reproductive age with clinical evidence of hirsutism, acne, seborrhea and/or irregular menstrual cycles without hyperandrogenemia as defined by the reference values of the same kit. That is to say that women in group (d) had clinical signs of androgen excess in spite of normal androgen serum levels. A single endocrinologist performed a thorough clinical examination of women in groups (a), (c) and (d). Hirsutism was defined as the existence of a Ferriman & Gallwey index of at least 8[2]. Irregular menstrual cycles were defined as those with an interval smaller than 21 days or longer than 35 days. Acne and seborrhea were defined as present or absent.

Women were excluded from the analysis if they reported oral contraceptive use within the past six months or any other medication that could affect the endocrine evaluation. Ethical approval was obtained, and all participants gave written informed consent for participating in the study.

Blood samples were obtained between 0800 and 1000h in the morning, after an overnight fast, in the early follicular phase in those with regular menstrual cycles. T was analyzed directly by RIA (Testosterone RIA; Diagnostic Systems Laboratories, Inc., Texas, USA) with a sensitivity of 0.05 ng/ml, an intra-assay coefficient of variation (CV) of 4.1%, and an inter-assay CV of 5.5%, with a suggested median normal value of 24 ng/dl (0 to 80, 95% range). SHBG was measured by a chemiluminescent enzyme immunometric assay (ICMA) (SHBG; Immulite 1000 systems, Siemens, UK) with a sensitivity of 0.2 nmol/l, CV intra and inter-assay of 6.5 y 8.7%, respectively, with a suggested median normal value of 51 nmol/l (18 to 114, 95% range). Free (fT) and bioavailable (bT) testosterone were calculated according to the method of Sodegard[3]. from T and SHBG with the following normal reference values: fT 0.21-0.56 ng/dl and bT 5-13 ng/dl. All the assays were done in duplicate and the mean of two values was used.

Data are given as mean ± SD (95% CI). Between-group comparisons of continuous data were analyzed by ANOVA. In all cases a difference was considered significant when *p* < 0.05.

Results

The demographic and clinical data of the four groups are described in Table 1. Age and BMI were significantly lower in group (a) compared to group (b) (p = 0.004 and p < 0.001 respectively). Age was significantly lower in group (c) compared to groups (a), (b) and (d) (p < 0.001, p = 0.001 and p = 0.003, respectively). Biochemical data is shown in Table 2. Mean serum SHBG concentrations were significantly different among all the groups. SHBG level was significantly higher in group (a) compared to group (b) (p < 0.001), with a mean age and BMI lower in group (a) (p = 0.004 and p < 0.001 respectively). Serum T levels and fractions were similar in both groups.

Mean serum SHBG concentration was significantly lower in group (c) than in group (a) (p < 0.001), with significantly higher levels of T, fT and bT (p < 0.001). When group (c) was compared to group (b), there were still differences in T, fT and bT (p < 0,001), but without differences in SHBG.

When groups (c) and (d) were compared, SHBG was higher in group (d) (p = 0.001), with higher levels of T, fT and bT in group (c) (p < 0.001). SHBG levels were significantly lower in group (d) than in group (a) (p < 0.001). From women of group (b), we selected those in reproductive age with a normal weight, n = 63, age 31.2 ± 7.9 years, BMI < 26. The biochemical evaluation was SHBG 57.4 ± 26.9 nmol/l, T 29.9 ± 15.6 ng/dl, fT 0.25 ± 0.15 ng/dl, bT 5.9 ± 3.6 ng/dl. There were no statistical differences between this subgroup and the total of women in group (b). Again, SHBG level was significantly lower in this subgroup compared to group (a) (p = 0.001).

Manufacturer's "normal" population for T and SHBG shows an asymmetric distribution skewed to the right, with a non normal distribution of values. This shows that the population sampled by the manufacturer is taken from the general population, without previous clinical examination, similar to our group (b).

TABLE 1.– *Demographic and clinical data of the four groups studied*

	Group (a) n = 30	Group (b) n = 87	Group (c) n = 53	Group (d) n = 38
Age*	29.4 ± 3.9 (range 19-38)	35.5 ± 10.5 (range 19-57)	26.9 ± 5.6 (range 17-42)	31.6 ± 8.3 (range 21-45)
BMI in kg/m²* (mean ± SD)	21.2 ± 1.5	24 ± 3.6	23.4 ± 4.6	22.7 ± 2.6
Irregular cycles (%)	0	NA	35.8	21.0
Acne (%)	0	NA	54.7	55.3
Seborrhea (%)	0	NA	42.3	26.3
Alopecia (%)	0	NA	28.3	31.6
Hirsutism (%)	0	NA	60.4	55.3

*Mean ± SD

TABLE 2.– *Biochemical results of serum sex hormone binding globulin (SHBG), total testosterone (tT), bioavailable testosterone (bT), and free testosterone (fT) in all four groups studied*

	Group (a) Normal selected n: 30	Group (b) Normal Random n: 87	Group (c) Hyperandrogenism with hyperandrogenemia n: 53	Group (d) Hyperandrogenism without hyperandrogenemia n:38
SHBG(nmol/l)	78.5 ± 16.6* (45.3-111.7)	53.8 ± 24.8*	41.9 ± 14.8*	56.3 ± 19.5*
T(ng/dl)	30.7 ± 14.5 (1.7-59.7)	26.4 ± 15.2	66.5 ± 25.1¶	36.4 ± 13.4
bTb(ng/dl)	5.0 ± 2.5 (0.0-10.0)	5.7 ± 3.5	19.3 ± 10.3¶	7.9 ± 2.8
fT(ng/dl)	0.21 ± 0.10 (0.01-0.41)	0.24 ± 0.15	0.84 ± 0.45¶	0.33 ± 0.12

Data expressed as mean ± SD (IC95).
* p < 0.001 between group (a) and all the other groups.
¶ p < 0.001 between group (c) and the rest of the groups.

Taking into consideration the results obtained in group (a), the suggested reference values (mean ± 2SD) for our laboratory would be: SHBG 45.3 to 111.7 nmol/l - T 1.7 to 59.7 ng/dl – fT 0.01 to 0.41 ng/ml - bT 0 to 10 ng/ml. The suggested normal value of SHBG set by the kit is 18 to 114, and for T, ND to 80, and calculated fT and bT: 0.21-0.56 ng/dl and bT 5-13 ng/dl respectively. When analyzing group (d), 12 out of 38 (31.6%) would have been categorized as hyperandrogenic taking into consideration the reference values obtained from group (a), and when analyzing group (b) (normal weight in reproductive years), 4 out of 63 (6.4%) would have been categorized as hyperandrogenic taking into consideration the reference values obtained from group (a).

Discussion

Polycystic ovary syndrome (PCOS) is a frequent endocrine problem, with an estimated prevalence of 4.6% to 9%[4]. PCOS is the most frequent cause of female hyperandrogenism. It is characterized by androgen excess and anovulation. Abnormalities in serum androgen concentration are the cornerstone for the diagnosis and management of this condition. The serum concentration of SHBG is specially diminished due to androgen excess and insulin resistance[5-8].

It has been rightly suggested that each laboratory should obtain its own reference-hormone values for the local population. We decided to measure the SHBG and T concentrations in different groups of women in Buenos Aires in order to establish the local reference values in a group of women of Buenos Aires in order to increase the accuracy of the detection of this prevalent condition. Other laboratories are encouraged to design their appropriate reference ranges for their population.

The hepatic production of SHBG is modified by age, weight, diet, and hormones among other factors. In a previous study we found the lowest SHBG levels in adolescent hirsute girls, even before the appearance of high androgen values[9]. Those results suggested an important role of SHBG decrease in adolescence versus a more accentuated testosterone increase in adults, as factors conditioning the development of hirsutism in these two different periods of life. In postmenopausal women low SHBG levels have been linked to an increased risk of diabetes mellitus and cardiovascular disease[7]. Moreover, a low serum SHBG concentration has also been associated to a greater risk of breast carcinoma in postmenopausal women, probably due to an increase of free estrogens[8].

Statistically significant differences between strictly and non-strictly selected women were detected in mean SHBG and T serum concentrations. We observed that when we carefully selected the group of women defined as normal, taking mostly in consideration weight and absence of physical signs of androgen excess, the normal reference range of serum SHBG narrowed. By using this customized SHBG range, patients defined previously as normal because they showed "normal" androgen levels, were redefined as hyperandrogenic. The difficulties in measuring androgens are very well described in the literature[10, 11]. In this study we emphasize the importance of a precise definition of the SHBG reference range in the evaluation of women with androgen excess.

The Rotterdam PCOS Consensus Workshop Group 2003, included in this syndrome women with anovulation and polycystic ovaries as defined by ultrasonography without androgen excess[12]. This has been a point of intense

debate, since polycystic ovaries by ultrasonography are highly non-specific. Moreover, low SHBG levels have been found in this group as well as high insulin levels[13].

Different studies have shown insulin resistance as a common issue in PCOS, independent of obesity. Insulin levels correlate with androgen levels. A risk of developing metabolic or cardiovascular abnormalities has been described in PCOS[14, 15]. Low SHBG levels have been postulated as predictors of metabolic risk. This correlation is so strong that a low SHBG is a predictor of type 2 diabetes mellitus[16]. The diagnosis of PCOS should be made if two of the three following criteria are met: androgen excess, ovulatory dysfunction, and polycystic ovaries, whereas disorders that mimic PCOS should be excluded[17]. Biochemical hyperandrogenism refers to an elevated serum androgen level and typically include an elevated total, bioavailable or free serum T level. Legro and col.[17] emphasize that given the variability in T levels and the poor standardization of assays, it is difficult to define an absolute level diagnostic of PCOS, and the Task Force recommends familiarity with local assays as we have mentioned.

In this observational study we show reference SHBG and T levels for a population of premenopausal women from Buenos Aires. We emphasize that hyperandrogenemia is better defined by establishing the reference ranges in a carefully selected population. This is particularly important in regard of SHBG levels. By using these suggested values, the diagnosis of PCOS could be more accurate.

Conflict of interest: The authors have none to declare.

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No grand idea was ever born in a conference, but a lot of foolish ideas have died there.

Ninguna gran idea nació jamás en una conferencia, pero un montón de ideas tontas han muerto en ellas.

F. Scott Fitzgerald (1896-1940)

The Crack-Up. Edited by Edmund Wilson. New York: New Directions, 1945. The Note-Books: E. Epigrams, wisecracks and jokes. p 123