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ORIGINAL ARTICLE

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Selection criteria of normal controls to predict reliable cut-off values of various endocrine parameters in infertility

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OBJECTIVE(S) : To obtain unbiased normal control subjects from eumenorrheic non-PCOS healthy women based on their fertility potential.

METHOD(S) : Two hundred forty-nine eumenorrheic non-PCOS healthy subjects out of 1792 infertile women were looked into. They were classified on the basis of their male factor association as Group A (with male factor, n = 117) and Group B (without male factor, n = 132). IUI cycles with donor sperm in Group A and husband's sperm in Group B using clomiphen citrate and hCG protocol were studied.

RESULTS : Group A subjects showed a better pregnancy rate than group B (25.6% vs 13.27%). Serum insulin, serum hormone binding globulin, leptin and bioavailable estradiol in Group B showed significant rise as compared to those in Group A. 25.6% pregnancies in Group A being at par with accepted normal pregnancy rate, Group A can be considered as normal for endocrine evaluation.

CONCLUSION(S) : Eumenorrheic non-PCOS healthy infertile women with male factor (Group A) may be selected as ideal control subjects to obtain precise cut-off values of various endocrine and biochemical parameters in infertility.

Key words: normal control, free estrogen index, leptin, fertility potential, serum hormone binding globulin

Introduction

In the Indian sub-continent, determination of cut off values of various endocrine parameters in normal control subjects in reproductive age has unfortunately not yet been attempted seriously in the context of various menstrual aberrations, including PCOS. One of the major reasons for this is unavailability of volunteered eumenorrheic non-PCOS healthy women with proven fertility potential as control subjects and secondly, investigators in our region mostly refer to cut off values offered by workers in the western world [1-5]. Their control values have demonstrated contrasting differences, not only in a particular region of the population but also in the same population, due to the variation in selection criteria, genetic origin, race and habitat of control subjects. Hence, currently referred baseline cut off values of controls stand questionable and need reevaluation.

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To bring about homogeny in selection criteria of normal control subjects, significance of fertility potential in eumenorrheic non-PCOS healthy women, as a necessary measure was applied in the present study to obtain viable baseline cut-off values in the Indian context.

Material and Methods

Eumenorrheic non-PCOS infertile subjects (n = 474) were considered for participation in the study with their consent, from 1792 infertile women who came for treatment, from January 2000 to December 2003.

Their current and past medical, menstrual, and reproductive histories were recorded. Height (m) and weight (kg) of each subject was measured to calculate body mass index (BMI, kg / m²). Waist and hip girth (cm) were measured to calculate waist: hip ratio (WHR). Transvaginal ultrasound study (TVS) (Aloka 500 Japan) of each ovary was performed in three planes.

Inclusion criteria - Two hundred and forty nine subjects having cycle interval of 28 days ± 5 days and diagnosed as

non-PCOS on ultrasound on day 3 of the menstrual cycle were chosen for the study.

Exclusion criteria - Two hundred and twenty five subjects with acne, hirsutism, tubal factors (bilateral tubal block or bilateral hydrosalpynx), hyperandrogenism (serum testosterone > 80 ng / dL), hyperprolactinemia (serum protalin > 21 ng / mL) and abnormal thyroid stimulating hormone (serum TSH levels < 0.3 and > 5.2 mIU/mL) were excluded from the study.

The 249 subjects included in the study were further classified into two groups on the basis of presence or absence of male factor infertility.

Group A: Subjects associated with severe male factor (n = 117) out of 249 eumenorrheic non-PCOS subjects, were included in this group. The details of severe male factor as per WHO (1992) norms were — azoospermia: (n=67), severe oligospermia (n=39), severe asthenozoospermia (n=08), and necrospermia (n=03).

Group B: 132 out of 249 regularly menstruating non-PCOS subjects with their male partners having normospermia as per WHO norms (1992) comprised Group B.

Hormone assay

The fasting serum concentrations of estradiol (E2), follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P), 17- α hydroxyprogesterone (17- α OHP), prolactin (PRL), testosterone (T), thyroid stimulating hormone (TSH), sex hormone binding globulin (SHBG), insulin and leptin were measured on day 3 of the menstrual cycle by radio immunoassay (RIA) using commercially available Diagnostic Systems Laboratories (DSL, Texas – USA) kits.

Evaluation of free testosterone index (FTI) and free estradiol index (FEI)

The FTI was calculated from total T and SHBG as FTI = (100 x T)/SHBG (EqV), with both T and SHBG expressed in nanomoles per L. FEL, was calculated as [Estradiol (nmol / L) X 100 / SHBG] X 10 as suggested by Mathur et al [6].

Evaluation of fertility potential with IUI treatment cycles

Clomiphen citrate (50/100 mg)/ injection hCG 5000 IU protocol was used for ovulation induction. TVS monitoring of the follicular growth and endometrial development was carried out to determine the time of hCG injection. Semen sample produced by husband (Group B) or by donor (Group A) was processed by either swim up or direct layering sperm separation method randomly. Insemination was done within 36 to 42 hours of administration of hCG injection. Value of serum β–hCG > 25 mIU / mL was taken as a positive indicator of pregnancy on day 28 of the cycle. TVS on day 45 confirmed pregnancy clinically.

Data were represented as mean ± SEM. For continuous variables, the mean values of subjects having male factor (Group A) and of subjects having no male factor (Group B) were compared by the one-way ANOVA. P value was calculated by using student's '*t*' test. $P < 0.05$ was considered as statistically significant. Statistical analysis was done by using 'Graph Pad Prism' software.

Results

One hundred and seventy eight out of the 249 proposed normal control subjects were subjected to intrauterine insemination (IUI) — with husband's sperm in case of 95 women (Group B) and with donor sperm in case of 83 women (Group A). Pregnancies per 100 patients and pregnancies per 100 cycles were 1.6 folds (50.6 % vs 31,6%) and 1.93 folds (25.6% vs 13.27%) respectively in Group A as compared to those in Group B. Also, the number of IUI cycles required for achieving pregnancy in Group A was relatively low (1.98 vs 2.38). (Table 1).

The comparative representation of hormonal data of various parameters between these two groups under study are presented in Table 2. It is evident that, serum FSH and LH values showed no difference in Group A and Group B. Values of ovarian steroids (estradiol and testosterone) also showed no significant variation. Serum progesterone and 17- α OHP levels also remained almost the same. However, insulin concentration in Group B was significantly higher than that in Group A (12.3 ± 0.6 vs 9.2 ± 0.8 μIU/ml, $P < 0.01$). At the same time, SHBG levels exhibited significant decrease in Group B as compared to that in Group A (41.8 ± 1.1 vs 52.3 ± 1.3 nmol/L, $P< 0.0001$). Free estrogen index (FEI) in Group A was significantly low as compared to that in Group B (2.88 ± 0.15 vs 4.77 ± 0.47, $p < 0.0001$), though free testosterone index (FTI) was not significantly different in the two groups (Table 2). Concentration of leptin was significantly elevated in Group B as compared to that in Group A (16.4 ± 1.2 vs 12.5 ± 1.0 ng/mL, $P <0.01$). Mean body mass index (BMI) and waist: hip ratio (WHR) in both the groups remained unchanged, however, waist girth in Group B was found to be raised as compared to that in Group A (81.2 ± 2.2 vs 76.2 ± 2.5 cm, $P < 0.01$).

Discussion

It is evident from Table 1 that eumenorrheic non-PCOS healthy women with male factor (Group A), had a pregnancy rate (PR) of 25.60 % which was at par with

Table 1. Results of pregnancy outcome in IUI treatment cycles in possible normal control subjects

Group	No. of patients	No. Of IUI cycles	Cycles per patient	Number of pregnancies / cycles	Number of pregnancies / patients
Group A	83	164	1.98	42 /164 (25.60%)	42/83 (50.60%)
Group B	95	226	2.38	30 /226 (13.27%)	30/95 (31.60%)

Note : Subjects in Group A were inseminated with donor sperm and subjects in Group B were inseminated with normospermic husband's sperm. In Group A cycle wise preganncy rate was 1.93 times that in Group B and patient wise it was 1.60 times that in Group B

Table 2. Comparison of various endocrine and clinical parameters in possible normal control subjects with / without male factor.

Parameters	Group A (n=117) (With male factor) Mean (± SEM)	Group B (n =132) (Without male factor) Mean (± SEM)	P value
Age (years)	27.4 ± 0.6	28.1 ± 0.5	NS
BMI	23.2 ± 0.4	23.0 ± 0.4	NS
Waist : Hip ratio	0.84 ± 0.01	0.85 ± 0.01	NS
Waist girth ((cm)	76.2 ± 2.5	81.2 ± 2.2	<0.01
Ovarian volume (mL)	6.0 ± 0.4	5.4 ± 0.4	NS
FSH (mIU/mL)	5.0 ± 0.2	5.6 ± 0.3	NS
LH (mIU/mL)	4.1 ± 0.2	4.2 ± 0.3	NS
FSH: LH ratio	1.22 ± 0.04	1.33 ± 0.07	NS
Estradiol (pg/mL)	41.0 ± 2.2	48.2 ± 4.1	NS
Testosterone (nmol/L)	1.23 ± 0.08	1.15 ± 0.06	NS
SHBG (nmol / L)	52.3 ± 1.3	41.8 ± 1.1	< 0.0001
Free Estrogen Index	2.88 ± 0.15	4.77 ± 0.47	< 0.0001
Free Testosterone Index	2.19 ± 0.13	2.72 ± 0.4	NS
Insulin (μIU/mL)	9.2 ± 0.8	12.3 ± 0.6	< 0.01
Leptin (ng/mL)	12.5 ± 1.0	16.4 ± 1.2	< 0.01
Progesterone (ng/mL)	1.1 ± 0.1	1.1 ± 0.2	NS
17- α OHP (ng/mL)	0.8 ± 0.1	1.0 ± 0.2	NS

Note : $P < 0.05$ is considered statistically significant. NS= non significant. P values are determined with the use of the 'Graph Pad Prism' software.

Table 3. Comparison of cut off values of normal control (NC) reported by various authors and in our study

Authors and normal control (n)	FSH: LH	Estradiol pg / mL	Testosterone ng / dL	SHBG nmol /L	Free Estradiol Index	Free Testosterane Index
Arroyo et al [1] (n=12)	0.90	19.7 ± 5.1	6.1 ± 4.0	40.0 ± 3.0	4.4 ± 0.7	2.0 ± 0.2
Villa et al [2] (n = 7)	0.93	22.3 ± 2.2	29.7 ± 14.4	54.5 ± 1.5	0.41	4.9 ± 3.1
Petermann et al [3] (n=6)	0.7-1.31	47.5 - 141.4	13 - 59	24.8-145.8	3.44	0.31 - 3.8
Genarelli et al [16] (n=32)	1.23	31.8 ± 20.3	46.1 ± 4.4	30.5 ± 2.1	1.39 ± 6.32	6.0 ± 2.8
Our study (n = 117)	1.22	41.0 ± 2.2	42.7 ± 2.8	52.3 ± 1.3	2.88	2.2 ± 0.3

Note : Values are depicted as either mean ± SEM or range or median

Table 4. Comparison of insulin and leptin cut off values as normal control reported by various authors

Authors and normal controls (n)	Insulin mIU/mL	Leptin ng/mL
Spritzer et al [4] (n=13)	16.04 ± 4.66	9.52 ± 1.90
Rouru et al [5] (n = 19)	9.8 ± 9.4	12.50 ± 10.40
Petermann et al [3] (n = 6)	3.6 - 24.7	6.4 - 35.7
Genarelli et al [16] (n=32)	6.4 ± 4.5	14.0 ± 8.0
Our study (n = 117)	9.2 ± 0.8	12.3 ± 0.6

Note : Values are depicted as either mean ± SEM or range.

the accepted normal pregnancy rate of any normal ovulating non-PCOS woman [7] and they stand as an ideal group for consideration as normal control.

Follicle progresses in development only when FSH is elevated and LH is low. An environment, in which aromatization in the granulosa cells prevail, favors the follicle, arising at the end of luteal phase or in early subsequent cycle and its success depends on its ability to convert an androgen-dominated microenvironment to an estrogen-dominated microenviornment [8]. It is apparent from Table 2 that, FSH levels in both the groups were higher than LH levels, indicating a favorable estrogenic microenvironment for the follicle. Therefore, prima facie, there did not seem to be any difference in these parameters in both the groups.

SHBG is regulated by insulin. Table 2, shows the inverse correlation of insulin with SHBG. Increased insulin level inhibits hepatic synthesis of SHBG independent of any effect on sex steroids [9]. The results seen in Table 2 suggest mild hyperinsulinemia in Group B with corresponding lowering of SHBG. These results corroborated the view of inverse relationship of insulin with SHBG as pointed by other recent workers in the field [10,11]. SHBG production is stimulated by estrogen and it is a marker for the endogenous estrogen changes that occur in normal ovulating women [12]. A decrease in SHBG allows more androgen and estrogen to be bioavailable. Findings in the present study indicated that Group B suffered from inadequate estrogenic microenvironment during the menstrual cycle. Findings of Vermeulen et al [13] coincided with this contention. Therefore, lower FEI in Group A indicated a better estrogenic microenvironment on account of higher aromatisation of low levels of testosterone to estradiol. It may be recalled that Erikson et al [14] reported that a low concentration of androgen supports aromatization to estrogens.

Increased waist girth in Group B indicated its positive co-relationship with raised levels of insulin and leptin. Waist girth, an expression of degree of central fat deposition might have brought about corresponding changes in the levels of leptin, a peptide secreted by adipose tissue, expressed by ob gene. In humans, leptin resistance is associated with adiposity-induced hyperinsulinemia and is closely related to the metabolism of insulin and glucose [15]. The results givien in Table 2 suggest the presence of mild hyperinsulinemia probably due to hyperleptinemia in Group B. Hence, Group A appeared to be an ideal normal control since, unlike group B, it possessed the prerequisite central body fat distribution and the other relevant parameters in order.

The comparative results given in Table 3 and 4 clearly show handful numbers of control subjects demonstrating a wide range with ambiguity and contrasting differences in control values amongst western investigators against a backdrop of a good number of control subjects (n = 117)

with unbiased precise control values of various endocrine parameters in our study.

Group A subjects with male factor were found to be infallible as normal control when compared to Group B subjects (without male factor). Hence, we conclude that precise cut off values of most of the endocrine and biochemical parameters in regularly menstruating non-PCO women with male factor may be considered as ideal control in the Indian context.

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