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Correlations of Serum Total and Free Testosterone with the Anthropometric, Diabetogenic, Atherogenic and Pro inflammatory Blood Parameters in Women with Functional Ovarian Hyperandrogenism.

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ABSTRACT:- Functional ovarian hyperandrogenism (FOH) is a form of polycystic ovary syndrome (PCOS) characterized by elevated circulating levels of androgens derived from the ovary. Chronic hyperandrogenism in women with FOH causes several secondary metabolic disturbances consequently leading to future diabetes mellitus, atherosclerosis and possibly CVD. The severity of androgen excess in general and free testosterone levels in particular is correlated with these secondary metabolic disturbances in women with FOH.

KEY WORDS:- FOH, PCOS, Hyperandrogenism, Diabetogenic, Atherogenic, Pro inflammatory, Pro coagulatory, Total and free testosterone.

INTRODUCTION

Polycystic ovary syndrome (PCOS) Is one of the common female endocrine disorders affecting approximately 4-8% of women of reproductive age and, is the main cause of female infertility. It affects women of all races and nationalities [2]. Hyperandrogenism or hyper-secretion of androgens is the most widespread biochemical feature in PCOS women, which accounts for 70-80% of PCOS [3]. Hyperandrogenism is characterized clinically by hirsutism, acne and androgen- dependent or male pattern alopecia [4]. PCOS is not a well-defined clinical entity. It represents a whole array of different disturbances leading to a similar structural change in the ovaries [5]. Several data suggest that PCOS is a form of functional ovarian hyperandrogenism (FOH).

Functional ovarian hyperandrogenism (FOH) is an ovarian dysfunction caused by excess androgens, which inhibit folliculogenesis and lead to polyfollicular morphology, which then disturbs the menstrual cycle and leads to anovulation [1]. On the basis of experimental observation, it was postulated that the clinical and biochemical features of FOH can arise as a consequence of hyper secretion of androgen by the ovary. Ovary is the primary source of androgens in functional ovarian

hyperandrogenism, driven by increased levels of leutinizing hormone (LH) as ovarian dysfunction causes LH insensitivity. FOH can result either from LH excess or from abnormal modulation of ovarian androgen responsiveness to LH [2]. FOH hence, represents the commonest clinical form of PCOS due to primary ovarian dysfunction [7].

Hyperandrogenism is associated with impaired glucose tolerance, increased risk of hypertension and dislipidemia, and elevated endothelial dysfunction [1], [4]. Women with FOH and PCOS have been reported to have markers of cardiovascular and endothelial disorders in addition to the familiar features of hirsutism, acne, and anovulatory infertility [2]. These metabolic derangements are known risk factors for the future development of type 2 diabetes and cardiovascular diseases in women with FOH [7], [8].

Although infertility is currently perceived as the main disturbance related to hyperandrogenism and PCOS, the need for monitoring role of insulin resistance in disturbing carbohydrate and lipid metabolism, on a long term basis in such women is largely neglected [7], [10]. The secondary metabolic disturbances in FOH women attributable to insulin resistance identifies the risk for potential metabolic and cardio vascular diseases in them, consequently leading to future diabetes mellitus, atherosclerosis and possibly CVD [9], [11]. So, the study, diagnosis and treatment of women with hyperandrogenism are important not only because it is one of the most common causes of infertility in women, but it also identifies the risk for potential metabolic and cardio vascular diseases in them.

METHODOLOGY

Design: - An observational, retrospective case-control design was adopted for the present study.

Setting: - Educare Institute of Dental Sciences, Malappuram, Kerala & Dianona Laboratories, Kottayam, Kerala.

Subjects: - The Request Letters for Participation were circulated among the out- patients of Abraham's Infertility Centre. Informed consent was obtained from the outpatient volunteers for the study and the Clinical Proforma of each participating person was completed and collected. After getting clearance from the Institutional Ethics Committee (IEC), the blood samples collected from the volunteers were processed and analyzed at Dianova Laboratories, an NABL accredited fully automated specialty clinical laboratory at Kottayam.

The Test and Control Group subjects were selected primarily based on the following inclusion-exclusion criteria.

Test Group: - The subjects in the Test Group were selected from among married woman in the age group of 20 to 33 years**,** visiting the infertility clinic for the first time. From them, women having elevated total testosterone levels (>0.8 ng /ml) were screened and selected. Women with total testosterone levels >2.0 ng /ml (ovarian or adrenal

tumours) were excluded. From the women screened as above, women with FOH were finally selected, using a five-step diagnostic work-up. Women with normal serum prolactin, DHEAS, T_3,T_4 and TSH levels and elevated LH:FSH ratio were identified as having FOH and were included in the Test Group (N=100). Women with normal prolactin levels but with a history of amenorrhoea (neuro-endocrine defects) were excluded.

Control Group:-Subjects for the control group were selected from the female siblings of the patients and the hospital staff. Married non pregnant women in the age group of 20 to 33 years who had normal serum testosterone levels (<0.8 ng/ml), and who were not taking any oral contraceptives were included in the Control Group (N=50).

Methods: - Blood samples of all subjects were collected in the morning, on days 3 to 10 of their menstrual cycles after 12 hour fasting. Fasting samples were used for the determinations of blood glucose, insulin and lipid. For blood sugar estimation, blood collected with potassium oxalate and sodium fluoride (3:1) anticoagulant was used. The anthropometric data such as height, weight and waist circumference (WC) were collected, from which BMI and waist to height ratio (WHtR) were calculated (Table 10).

Determinations of fasting blood glucose (FBG), total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C) and triglycerides (TG) were carried out using Enzymatic End-point assay in Daytona Fully Automated Biochemistry Analyzer of M/s. Randox Diagnostics Ltd. Apo lipoprotein A_1 (Apo A_1) and apo lipoprotein B (Apo B) were carried out using immuno turbidometric assays in Orion Diagnostica Turbox Plus Analyzer. High sensitivity C-reactive protein (Hs-CRP) was carried out using sandwich-immuno metric assay in Nycocard Reader-11. Fibrinogen was determined using functional clotting assay method. Insulin, Prolactin (PRL), dehydro epi-androsterone sulphate (DHEAS), 17-hydroxy progesterone (17-OH PRG), tri-iodo thyronine (T_3), tetra-iodo thyronine (T_4), thyroid stimulating hormone (TSH), total testosterone (TT), free testosterone (FT), leutinizing hormone (LH) and follicle stimulating hormone (FSH) were assayed using Chemi luminescent immuno assay (CLIA) in Advia Centaur Fully Automated CLIA analyzer of M/s. Siemens Health Care Diagnostics India Ltd. QC was performed by participating in the BIO-RAD EQUAS international QC programmes.

The data from the above investigations were statistically analyzed by employing the Unpaired Student's t test and Multiple Pair-wise comparison procedures of One Way Anova (Holm-Sidak method). Pearson Product Moment Correlation as well as Linear Regression Analyses were also carried out. This study had an overall critical confidence level of 95% (α- 0.05 and β- 0.95) and hence, results with p values <0.05 were interpreted as statistically significant. All statistical tests were conducted using Sigma Stat 3.5 Version Software (M/s. Sigma-Aldrich Co., St. Louis, USA).

RESULTS

The results obtained from the present study are summarized in Tables1 to 13 shown below. Out of a total of 192 women with hyper-androgenemia (total testosterone >0.8 but not >2 ng/ml), 52.1% were due to functional ovarian causes. Adrenal causes (elevated DHEAS and 17-hydroxy progesterone levels) accounted for 21.3%, hypothyroidism (elevated TSH and lowered T_3 & T_4 levels) for 15.1% and hypothalamic-pituitary causes (elevated prolactin) for 11.5 % of hyper-androgenemia (Table 1).

Table1-Incidence of ovarian and non ovarian causes of hyperandrogenemia

Total (n=192)	FOH	Adrenal	Thyroid	HPA#
Incidence	100 (52.1%)	41 (21.3%)	29 (15.1%)	22 (11.5%)

#HPA –Hypothalamic-pituitary axis

The total study population comprised of 150 women, out which 50 were in the Control Group (Group I) and 100 were in the Test Group (Group II). The mean age of the study population was 26.5 ± 6.5 years and based on this, the subjects in both Control and Test Groups were sub divided into two groups viz. 20-26 years age group (Group I (a) & Group II (a) respectively) and 27-33 years age group (Group I (b) & Group II (b) respectively. Group I (a) had 22.1% (n=33) and Group II (a) had 11.3% (n=17) subjects, while Group I (b) and Group II (b) had respectively 45.3% (n=68) and 21.3% (n=32) women.

Table 2- Extra Ovarian Endocrine Markers of all Subjects (Mean ± SD)

Groups	PRL (ng/ml)	DHEAS (µg/ml)	17-OH PRG (ng/ml)	T_3 (ng/dl)	T_4 (µg/dl)	TSH (µIU/ml)
I (a)	13.73 ± 1.26	2.37 ± 0.41	1.94 ± 0.53	127.8 ±	7.04 ±	2.61 ± 0.45
I (b)	14.14 ± 1.33	2.09 ± 0.23	1.83 ± 0.48	122.4 ±	6.92 ±	2.56 ± 0.37
II (a)	14.02 ± 1.29	2.42 ± 0.45	1.89 ± 0.49	125.5 ±	6.97 ±	2.58 ± 0.40
II (b)	14.27 ± 1.36	2.23 ± 0.28	1.78 ± 0.45	119.8 ±	6.65 ±	2.49 ± 0.34

Table 3- Statistical analysis of Extra Ovarian Endocrine Markers

Comparison	DF	PRL	DHEAS	17-OH PRG	T_3	T_4	TSH
I (a) vs. II	99	0.288	0.591	0.641	0.322	0.736	0.735
I (b) vs. II	47	0.750	0.164	0.719	0.429	0.341	0.509
II (a) vs. II	98	0.376	< 0.05*	0.285	0.053	0.115	0.271
I (a) vs. I	48	0.290	< 0.05*	0.477	0.117	0.693	0.695

DF-Degrees of freedom. Statistically significant p values are indicated by * mark

Table 4- Endocrine Markers of Ovarian Function of all Subjects (Mean ± SD)

Groups	TT (ng/ml)	FT (pg/ml)	LH (µIU/ml)	FSH (µIU/ml)	LH:FSH
I (a)	0.58 ±	1.46 ±	4.29 ± 0.47	6.99 ± 1.76	0.61 ±

	0.14	0.57			0.27
I (b)	0.63 ± 0.15	1.53 ± 0.66	4.41 ± 0.56	7.13 ± 1.82	0.62 ± 0.31
II (a)	1.33 ± 0.35	3.63 ± 0.48	8.57 ± 1.23	3.46 ± 0.35	2.38 ± 0.04
II (b)	1.37 ± 0.38	3.72 ± 0.54	8.82 ± 1.45	3.62 ± 0.41	2.41 ± 0.05

TT-Total Testosterone, FT-Free Testosterone

Table 5- Statistical analysis of Endocrine Markers of Ovarian Function

Compariso	TT	FT	LH	FSH	LH:FSH
I (a) vs. II	<0.001	<0.001*	<0.001*	<0.001*	<0.001*
I (b) vs. II	<0.001	< 0.001*	< 0.001*	< 0.001*	< 0.001*
II (a) vs. II	0.544	0.434	0.293	0.484	0.402
I (a) vs. I	0.249	0.698	0.427	0.793	0.907

Statistically significant p values are indicated by an asterisk mark (*)

Extra ovarian endocrine parameters in Tables 2-3 are not statistically different between the Test and Control Groups. Tables 4-5 show that the ovarian endocrine markers are varied significantly between two input groups.

Table 6- Incidence of biochemical PCOS in the Test Group Subjects

Groups	↑ LH:FSH ratio (PCOS)	↔ LH:FSH ratio (No PCOS)
II(a) (n=68)	46 (67.7%)	22 (32.3%)
II (b) (n=32)	25 (78.1%)	7 (21.9%)
Total	71(71.0%)	29 (29.0%)

Table 6 shows that a great majority of Test Group subjects (71.0%) have biochemical PCOS with a higher preponderance in the older (78.1%) than in the younger (67.7%) age group.

Table 7- Anthropometric data of all Subjects (Mean ± SD)

Groups	Height (cm)	Weight (kg)	BMI (kg/m^2)	WC (cm)	WHtR
I (a)	161.63 ± 7.12	54.78 ± 5.04	20.85 ± 1.73	71.25 ±	±
I (b)	161.47 ± 7.09	55.84 ± 5.11	21.50 ± 1.81	72.84 ±	0.459 ±
II (a)	161.81 ± 7.17	61.65 ± 6.90	23.51 ± 2.87	76.83 ±	0.472 ±
II (b)	161.59 ± 7.07	62.73 ± 7.2	24.04 ± 3.16	78.21 ±	0.488 ±

Table 8- Statistical analysis of Anthropometric data

Comparis	Height	Weight	BMI	WC	WHtR
I (a) vs. II	0.906	<0.001*	<0.001*	< 0.001*	< 0.001*
I (b) vs. II	0.955	< 0.01*	< 0.01*	< 0.01*	< 0.05*
II (a) vs. II	0.886	0.473	0.406	0.266	0.092
I (a) vs. I	0.940	0.485	0.221	0.083	0.083

Table 9- FBG and Lipid Profile of all Subjects (Mean ± SD)

Groups	FBG (mg/dl)	TC (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	TG (mg/dl)
I (a)	84.46 ± 6.15	147.25± 20.61	78.12 ± 12.63	53.11 ± 9.74	119.85 ± 23.42
I (b)	85.22 ± 6.34	154.42± 22.53	82.89± 16.74	51.35 ± 8.66	124.67± 26.17
II (a)	92.81 ± 8.55	176.88± 25.93	92.56 ± 17.95	47.34 ± 7.78	139.97 ± 27.22
II (b)	95.43 ± 9.76	189.14± 27.75	100.87 ± 19.84	45.46 ± 6.51	147.38 ± 29.57

Table 10- Statistical analysis of FBG and Lipid Profile

Comparison	FBG	TC	LDL-C	HDL-C	TG
I (a) vs. II (b)	<0.001*	<0.001*	<0.001*	<0.01*	<0.001*
I (b) vs. II (a)	<0.001*	<0.001*	<0.001*	< 0.05*	<0.01*
II (a) vs. II (b)	< 0.05*	<0.05*	<0.05*	0.281	0.20
I (a) vs. I (b)	0.684	0.264	0.264	0.533	0.517

Statistically significant p values are indicated by an asterisk mark (*)

Table 11- Atherogenic and Pro inflammatory Markers of all Subjects (Mean ± SD)

Groups	Apo A	Apo B	Hs-CRP	Fibr.
I (a)	1.67 ±	1.21 ±	0.59 ± 0.04	194.6 ±
I (b)	1.59 ±	1.28 ±	0.72 ± 0.07	198.7 ±
II (a)	1.08 ±	1.92 ±	1.57 ± 0.18	226.5 ±
II (b)	1.02 ±	2.03 ±	1.65 ± 0.21	239.3 ±

Fibr. – Fibrinogen

Table 12- Statistical analysis of Atherogenic and Pro inflammatory Markers

Comparison	Apo A	Apo B	Hs-CRP	Fibrinogen
I (a) vs. II (a)	<0.001*	<0.001*	<0.001*	<0.001*
I (b) vs. II (b)	<0.001*	< 0.001*	< 0.001*	< 0.001*
II (a) vs. II (b)	<0.05*	< 0.01*	<0.05*	< 0.01*
I (a) vs. I (b)	0.117	0.079	<0.05*	0.233

Statistically significant p values are indicated by an asterisk mark (*)

A statistically significant increase in weight, BMI, WC, WHtR, FBG, TC, LDL-C, TG, Apo B, hs-CRP and fibrinogen as well as a decrease in HDL-C and Apo A were observed in the Test compared to the Control Group (Tables 7-12).

Table 13- Correlations of TT and FT with the endocrine markers of FOH

		FT	LH	FSH	LH:FS
TT	r	1.00#	1.00#	-0.995	0.999
	p	<0.001	<0.001**	<0.01*	<0.01*
FT	r	-	1.00#	-0.997	1.00#
	p	-	<0.001**	<0.01	<0.001

DISCUSSION

FOH accounts for the majority of hyperandrogenemia, and is more frequent in women of younger age. This pattern of FOH has been reported earlier [1]. The endocrine alterations in women with functional ovarian

hyperandrogenism such as the elevations of total and free testosterone (TT and FT), leutinizing hormone (LH) and the decrease in follicle stimulating hormone (FSH) had been reported [2], [3]. The results of our study are well in agreement with this. The present study also showed that other endocrine parameters such as prolactin DHEAS, 17-hydroxy progesterone, TSH, total T_3 and T_4 were not markedly varied between women with and without FOH. This is consistent with previous studies reporting similar endocrine changes in FOH [4], [5]. Concordant with earlier reports, this study showed that body weight, BMI, WC and WHtR increased significantly in women with ovarian hyperandrogenism [6]. The diabetogenic, pro atherogenic, pro inflammatory and pro coagulatory markers in blood were comparatively higher in women with FOH, as shown by the present study. Results concordant with ours were reported by several authors [7], [8], [9]. This study also showed comparable results to previous reports of the correlations of hyperandrogenemia with elevated LH: FSH ratio [3], [4], [5].

CONCLUSIONS

Women with FOH showed significant elevations in anthropometric indices as well as in blood levels of diabetogenic, atherogenic, proinflammatory and pro coagulatory markers as compared to their age matched controls. One of the prominent outcomes of this study is that the present study could establish that LH rather than FSH showed the strongest positive correlation with total and free testosterone in women with FOH. Moreover, both total and free testosterone levels positively correlate with LH: FSH ratio, but the latter has the highest correlation with this determining hormonal ratio in women with FOH. The implications of this finding is that although both total and free testosterone levels are recognized as important etiological factors in the pathogenesis of FOH, free testosterone levels are better predictors of the development of biochemical PCOS than total testosterone levels in women with FOH. Since the diabetogenic, atherogenic, proinflammatory and pro coagulatory changes are related to hyperandrogenism, the extent of hyperandrogenism is a potential risk factor for the development of future type 2 diabetes and CVD.

SUMMARY

From the present study it could be summarized that elevations in total testosterone, free testosterone and LH accompanied by a low normal FSH leading to higher LH:FSH ratio are the prominent endocrine changes seen in women with FOH. The alterations in LH, FSH and LH: FSH ratio is correlated to both total and free testosterone levels but the latter is a better predictor of biochemical PCOS and future health risks in these women.

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