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Original Research Article

Polycystic Ovaries and Effects of Hormones: An Observational Study in Females of Southern Assam

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Conflict of interest: Nil

Abstract:

Introduction and Objective: Polycystic Ovarian Syndrome also called as PCOS or PCOD (Polycystic Ovarian Disorder) This disorder is an endocrinopathy, and that's why it should be referred to as PCOS, a syndrome rather than a disease. Amenorrhoea, weight gain, hirsutism, diabetes mellitus, hypertension, cardiovascular disease, cerebrovascular disease, breastmilk expression in absence of pregnancy and infertility are some of the clinical manifestations caused by PCOS. The objective of this study was to observe the various hormonal and biochemical factors that influence PCOS in the Barak valley region of Assam.

Methodology: A total number of 20 cases of reproductive age group of 18-38 yrs were selected for the study fulfilling the following criteria's: USG > 12 antral follicles and clinical complaints and findings with anovulation and hyperandrogenism Glucose estimation was done by GOD POD (glucose oxidase peroxidase). The tests were carried out in semiautomated biochemistry analyser (ERBA Transasia) HBA_1c estimation was done by Turbodyne SC Tulip and hormonal analysis was done by Classic radiance semiautomated CLIA plate analyser. Free testosterone was done from outsource.

Results: 7 cases are hypogonadotrophic and 13 cases are normogonadotrophic subjects who presented with polycystic ovaries. High TSH was found in 15.4% high LH/FSH ratio in 61.5% high prolactin in 69.2%, low prolactin in 15.4% high AMH in 84.6% and high insulin in 30.8% of cases The status of ANOVA shows a significant p value of <0.05 for LH and insulin. High AMH and high LH/FSH ratio were responsible for PCOS

Conclusion: Hormonal abnormalities hamper the normal folliculogenesis, hence needs corelation and correction.

Keywords: Polycystic Ovarian Syndrome, Insulin, LH/FSH ratio, anti-Mullerian hormone.

Introduction

Polycystic Ovarian Syndrome also called as PCOS or PCOD (Polycystic Ovarian Disorder) is a burning problem of the young females. It is a disorder which is very common and also the leading cause of female infertility. PCOS, also known as Stein-Leventhal Syndrome which is named after two doctors who first described it in 1935 [1].This disorder is an endocrinopathy, and that it should be referred to as PCOS, a syndrome rather than a disease [2]).It is a condition where women possesses many number of small cysts around the edge of their ovaries in most of the cases. Polycystic ovaries mean the ovaries which contain a large number of cysts which are not bigger than 8mm and also develop more follicles than normal every month. PCOS can also be hereditary, its observed from the studies that women with the history of polycystic ovaries are 50% more likely to develop PCOS. Its also seen that the common cause of menstrual irregularities is because of PCOS where the occurance is in 4-12% of female of reproductive age (12-45 yrs old) [1]. The majority of the women with PCOS have anovulation which results in infertility, amenorrhoea, but may be associated with dysfunctional bleeding and heavy bleeding which

results in anemia. Chronic unopposed estrogen leads to endometrial hyperplasia and potentially, to cancer [2] WHO has classified 3 catagories of anovulatory infertility of which PCOS falls under WHO Class-II [3]. Young females both married and unmarried reports with clinical complaints of amenorrhoea, weight gain, hirsutism, diabetes Mellitus, Hypertension, cardiovascular disease, cerebovascular disease, breast milk expression in absence of pregnancy, infertility, high blood pressure and hair loss or male pattern baldness [4]. Varying upon when it happens, impacts differ. PCOS amid youth and adulthood will cause decreased or no periods or menses, polycystic ovaries, obesity, and abundance of sex hormone levels. Though whenever related to age it causes diabetes, hypertension, unusual blood lipid for example cholesterol level likewise called as metabolic syndrome [5].

Polycystic Ovarian Syndrome may be caused by abnormality of 4 endocrinologically active compartments - i)Hypothalamic pituitary compartment [6], ii) Ovarian compartment [7], iii) Adrenal compartment [8] and iv) Peripheral compartment (skin and adipose tissue)[9]. Hyperandrogenemia is the most standard hormonal adjustment of PCOS [10]. Biochemically, hyperandrogenism is usually assessed by assay of total testosterone (TT), free testosterone (fT), sex hormone binding globulin (SHBG), androstenedione (A), 17-hydroxy progesterone (17OHP) and dehydroepiandrosterone sulphate (DHEAS) in serum and by calculation of the free androgen index (FAI=(TT/SHBG)100). Women with PCOS often have high normal or higher than normal serum concentrations of all these androgens. Hyperandrogenism has a multifactorial origin attributed mostly to the ovaries with a substantial contribution from the adrenals and a minor contribution from fatty tissue [10]. The model of inheritance of PCOS has not yet been characterized. A few scientists have hypothesized autosomal prevailing transmission connected to a solitary hereditary deformity, however most creators characterize PCOS as a polygenic pathology. It is likewise conceivable that a specific quality in a given family may have a dominating impact, affecting the phenotypic appearances of the disorder. The primary competitor qualities are those encoding for variables associated with the combination, transport, guideline and impacts of androgens. Other competitor qualities are those encoding for variables associated with insulin digestion, for example, insulin receptors, flagging course proteins in charge of authoritative of insulin to its receptor, IGF framework, other development factors and the quality encoding for Calpain-10 chemical, in charge of insulin discharge and activity [11]. At last, recent proof of altered early gonadotropin-autonomous folliculogenesis in ladies with PCOS recommends that genes engaged with folliculogenesis may likewise be applicants in the etiopathogenesis of this syndrome. In the ovary, variations of LRH may decrease or upgrade pituitary LH incitement of ovarian theca and stroma cell T creation, ovarian follicle development, LH surge–induced ovulation, and corpus luteum function [12], whereas in adipocytes, LHR variations may change LH incitement of adipogenesis [13]. Variants in these multi-organ system genes could contribute to genetically determination of PCOS phenotypes for reproductive and metabolic pathophysiology [10]. In environmental factors in spite of the fact that the pervasiveness of PCOS is comparable in all nations, ethnic variables impact the phenotypic appearances of the disorder. The prevalence of PCOS among Caucasian ladies, differs from 4.7% in Alabama, to 6.5% in Spain and 6.8 % in Greece [14]. In the United Kingdom, PCOS and type II diabetes are more persistent in ladies of Asian origin [14]. These perceptions recommend the presence of various natural variables, for example, diet, physical movement and way of life by and large. The expanding impacts of metabolic issue in financially created nations have driven creators to propose that the pathogenic systems of these scatters are related with evolutionary advantages of interest as far as survival [14]. In endocrine factors ovarian folliculogenesis is managed by a fragile equilibrium among additional and intra-ovarian variables. Aggravation of this equilibrium may change and compromise follicular improvement and the production of matured/developed oocytes, prompting to infertility [10]. Extraovarian factors incorporate a progression of endocrine, paracrine and metabolic changes, which by causing variations from the norm in the follicular microenvironment, may modify folliculogenesis and oocyte improvement. These changes incorporate FSH deficiency, hypersecretion of LH, hyperandrogenemia of ovarian or adrenal root and hyperinsulinemia with insulin obstruction [15]. Anti-Müllerian hormone (AMH) is an important regulator of folliculogenesis in the ovaries [16]. It is secreted by granulosa cells of the ovarian follicles and its serum levels are elevated 2- to 3-fold in women with PCOS in comparison with normo-ovulatory women, consistent with the increased number of small antral follicles in PCOS [17,18]. However, it is unclear whether AMH is simply a marker which is increased in PCOS, or actually an important contributing factor to its pathophysiology.

Folliculogenesis and oogenesis likewise rely upon intraovarian factors, particularly follicular liquid variables (FFFs) [15] that are straight forwardly associated with their dimensions in plasma. Late examinations propose that FFFs embroiled in folliculogenesis of polycystic ovaries have a place with the group of development factors including cytokines and inhibins [15]. Nutrient D is a basic

controller of bone and mineral homeostasis. Late examinations have shown hypovitaminosis D is related with an improved probability of creating metabolic disarranges [19]. Vit. D lack has additionally been exhibited in patients with POCS [20]. Hefty patients with PCOS have been appeared to have lower serum dimensions of 25-OH-D than non-large ladies with PCOS and nutrient D insufficiency has been recommended to have a role in the improvement of insulin opposition (IR) and impeded glucose resilience in such patients [21]. PCOS / PCOD has gotten insufficient consideration in the social science literature.

The way to deal PCOS, first line of treatment is by Ayurveda, homeopathy or potentially allopathy. Indeed, even a blend of homeopathy and allopathy is embraced [1]. The various glands of human body like hypothalamus, pituitary, thyroid, pancreas, adrenal and ovaries secrete various hormones and biochemical substances which influence the growth of dominant ovarian follicle.

The main purpose of this study is to the observe the various hormonal and biochemical factors that influence PCOS female respondents in the Barak valley region of Southern Assam.

Materials and Methods:

The study was conducted in the premises of Ultra path Diagnostic and Research Centre. A total number of 20 cases of reproductive age group of 18-38 yrs were selected for the study fulfilling the following criteria's:-

1. USG > 12 antral follicles

2. Clinical complaints and findings consistent with anovulation and hyperandrogenism.

The parameters considered for evaluation of PCOD are as follows:

1. FBS, PPBS, HbA1c to rule out diabetes Mellitus.
2. T3,T4,FT4 and TSH to rule out thyroid and pituitary dysfunction
3. DHEA-S and Testosterone to rule out adrenal dysfunction
4. LH, FSH, LH: FSH ratio to rule out hypothalamopituitary dysfunction.
5. Insulin to rule out insulin resistance and pancreatic function
6. Testosterone and AMH secreted by granulosa cells to assess ovarian follicular function.
7. Serum total and free testosterone to rule out hyperandrogenism and androgenic tumors.

Sample Collection: For blood sugar estimation 2ml in sodium fluoride vial (ash top) For HbA1c, 2ml in K3 EDTA (purple top) For hormonal analysis 5ml in E/V or clot activator vial (red topped) Samples are centrifuged at 3000 rpm for 10-15 min and the serum / plasma are seperated in small aliquots for use and stored in refrigerator at 2-8°c.

Instruments and methods used: Glucose estimation was done by GOD POD (glucose oxidase peroxidase). The tests were carried out in semiautomated biochemistry analyser (ERBA Transasia) HBA_1c estimation was done by Turbodyne SC Tulip and hormonal analysis was done by Classic radiance semiautomated CLIA plate analyser. Free testosterone was done from outsource

Test procedure

STEPS	T3	T4	FT4	TSH	LH	FSH	PRL	DHEA-s	Testo sterone	Insulin
Standard sample	25ul	13ul	13ul	25ul	25ul	25ul	13ul	5ul	5ul	25ul
Tracer reagent	25ul	25ul	25ul	50ul	50ul	50ul	50ul	25ul	25ul	50ul
Mix	1min	1min	1min	20-30sec	20-30 sec	20-30 sec		1min	1min	60sec
Biotin reagent	25ul	25ul	25ul	x	x	X	50ul	25ul	25ul	x
Mix	1min	1min	1min	x	x	X	60sec	1min	1min	x
Incubation	45min RT	45minRT	45min RT	45min RT	45min RT	45min RT	30min RT	30min RT	45min RT	60min RT
Wash	5times with wash buffer	5times with wash buffer	5times with wash buffer	5times with wash buffer	5times with wash buffer	5times with wash buffer	5times with wash buffer	5times with wash buffer	5times with wash buffer	5times with wash buffer
Signal reagent	50ul	50ul	50ul	50ul	50ul	50ul	50ul	50ul	50ul	50ul
Incubation	5 min RT	5min RT	5min RT	5 min RT	5 min RT	5 min RT	5Min RT	5min RT	5min RT	5min RT
Read	RLU	RLU	RLU	RLU	RLU	RLU	RLU	RLU	RLU	RLU

RLU –Relative light unit

Results: A total number of 20 cases of polycystic ovarian disease has been studied with the biochemical and hormonal status selected from complaints of anovulatory cycle, scanty and irregular menses and other clinical complaints like amenorrhoea, weight gain, hirsutism, diabetes mellitus, hypertension, cardiovascular disease, cerebrovascular disease, breast milk expression in absence of pregnancy and infertility. Table 1 depicts the overall biochemical and hormonal status of the studied subjects

Table 1: Overall biochemical and hormonal status of the studied patients

Case Number	AGE	FBS	PPBS	HbA1c	T3	T4	TSH	FT4	FSH	LH	LH/FSH Ratio	PRL	DHEA-S	INSULIN	Total Testosterone	Free Testosterone	AMH
Normal Values	→	<126 mg/dl	<200 mg/dl	4-6%	0.8-2.0 ng/ml	5.4-11.5 ug/dl	0.3-6.02 uIU/ml	0.7-2.0 ng/dl	2.8-35 mIU/ml	3.6-204 mIU/ml	<2:1	0-17 ng/ml	44-332 ug/dl	0-35 uU/ml	6-86 ng/dl	0.8-3.2 pg/ml	1.5-4.0 ng/ml
1	24	70.1	101.7	5.8	1.1	8.6	7.4	1.3	4.2	30.3	7.2	18.9	140	35.6	40	2	1.17
2	20	76.2	106.9	5.4	1.3	8.7	2.8	1.4	2.7	14.2	0.1	69.2	70	25.7	30	1.8	23.5
3	31	72.5	104.5	5.4	1.2	7.1	3.3	1.4	2.3	8.4	3.6	22.3	80	68.3	10	0.3	15.9
4	20	76	98.6	5.2	1.4	7.6	1.1	1.4	1.6	3.6	2.2	106	380.6	4.7	10	3.3	1.8
5	29	76.1	88.6	5.2	1.3	7.4	2.5	1.2	4.4	17.1	3.8	0.5	80	7.1	30	1.4	4.7
6	22	79.6	96.8	5.6	1.3	8.7	3	1.4	3.1	9.4	3	25.8	160	58.4	40	1.5	11.6
7	20	72.5	85	5.6	1.7	9	1.2	1	2.5	6.2	2.4	15.2	50	8.4	50	1.4	3.8
8	21	72.3	97.5	5.6	1.4	8.1	3.7	1.1	6	40.5	6.7	28.7	150	51.2	40	1.2	15.9
9	25	74	87.2	5.2	1.2	7.4	2.2	1.3	2.6	5.7	2.1	11	160	11.2	20	0.6	6.4
10	29	70.9	78	5.5	1.2	7.6	1.1	1.5	10.3	23	2.2	0.5	70	5.7	30	1.5	3.1
11	25	93.4	137.6	5.6	1.3	8.7	4.6	1.4	4.1	10.6	2.5	21.3	310	34.3	60	2.5	6.8
12	30	74.9	127.6	6.4	1.1	7.9	4.8	1	5.5	9.7	1.7	35.6	270	56.6	40	0.8	19.7
13	24	70.1	109.3	5.3	1.1	7.9	4.6	1.2	10.6	20.4	1.9	29.7	180	23.7	80	2.4	7.6
14	20	86.9	101.4	5.2	1.3	8.4	0.9	1.5	1.7	5.5	3.2	10.2	210	10.3	30	1.3	3.1
15	20	74	123.2	5.6	1.4	7.1	2.6	1.2	3.6	13.1	3.6	79.6	80	1.4	10	0.8	20.4
16	25	70.8	101.2	5.5	1.4	8.6	6.2	1.1	10.5	8.4	1.2	26.6	140	15.8	60	2.1	18.9
17	26	78.6	89.5	5.4	1.2	7.6	6	1.3	5.9	9.4	1.5	36	190	16.4	20	2.3	23.9
18	25	91.4	185.6	6.4	1.4	8.6	2.3	1.5	4.4	9.1	2	41.2	170	10.7	40	1.9	8.8
19	30	83.2	122.7	5.6	1.3	7.1	3.4	1	2.3	12.6	5.4	65.1	80	64.7	20	1.3	27.4
20	24	88.3	133.5	6.5	1.2	8.4	3.5	1.3	3.6	12.1	3.3	46.2	50	46.2	20	0.9	11.7

The mean of age and the various ranges of hormones, median, standard error of deviation, standard deviation, skewness and standard error of skewness has been shown.

Table 2: Summary statistics of the studied patients

FACTORS → STATISTICS ↓	AGE	FBS	PPBS	HbA1c	T3	T4	TSH	FT4	FSH	LH	LH_FSH	PRL	DHEA-S	INSULIN	Total Testosterone	Free Testosterone	AMH
No. of Samples	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Mean	24.50	77.59	108.82	5.60	1.29	8.03	3.36	1.28	4.60	13.47	2.98	34.48	151.03	27.82	34.00	1.57	11.81
Std. Error of Mean	0.84	1.62	5.48	0.09	0.03	0.14	0.40	0.04	0.63	2.03	0.39	6.08	20.03	4.99	4.13	0.16	1.85
Median	24.5	75.45	101.55	5.55	1.3	8	3.15	1.3	3.85	10.15	2.45	27.65	145	20.05	30	1.45	10.2
Std. Deviation	3.75	7.26	24.51	0.40	0.14	0.63	1.80	0.17	2.83	9.09	1.76	27.20	89.58	22.30	18.47	0.73	8.29
Skewness	0.31	1.04	1.71	1.35	1.07	-0.13	0.62	-0.37	1.31	1.78	1.10	1.19	1.10	0.59	0.79	0.46	0.41
Std. Error of Skewness	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51
Distribution	Symmetric	Positive Skewed	Positive Skewed	Positive Skewed	Positive Skewed	Symmetric	Positive Skewed	Symmetric	Positive Skewed	Positive Skewed	Positive Skewed	Positive Skewed	Positive Skewed	Positive Skewed	Positive Skewed	Symmetric	Symmetric

FBS Fasting Blood Sugar; **PPBS** Post prandial blood sugar; **T3** Triiodothyronine; **T4** Thyroxine; **TSH** Thyroid Stimulating Hormone; **FT4** Free Thyroxine; **FSH** Follicle Stimulating Hormone; **LH** Luteinizing Hormone; **LH_FSH** LH/FSH ratio; **PRL** Prolactin; **DHEA-S** Dehydroepiandrosterone sulphate; **AMH** Antimullerian hormone.

The statistics of the study subjects have shown a symmetric distribution of the various hormonal and biochemical factors in relation to age with T4 (thyroxine), FT4(free thyroxine), Free Testosterone and AMH (Antimullerian hormone) and a positive skewness or susceptibility with FBS(Fasting blood sugar), PPBS(Postprandial blood sugar), HbA_1c (Glycated haemoglobin), T3(Triiodothyronine), TSH (Thyroid Stimulating hormone), FSH (Follicle stimulating hormone), LH (luteinising hormone),

LH/FSH ratio, PRL (Prolactin), DHEA-S (Dihydroepiandrostenedione sulphate), Insulin to PCOS Anovulation is described under three classes WHO I, WHO II, WHO III depending upon the level of FSH. Out of 20 cases 7 cases are hypo gonadotrophic and 13 cases are normogonadotrophic and these subjects presented with polycystic ovaries and hyperprolactinaemic amenorrhoea.

Table 3: Status of the anovulation in the studied patients

Class	Hormones	No. of cases 20
WHO I	FSH low	7
WHO II	FSH Normal	13
WHOIII	FSH High	0

Therefore 35% of the subjects presented with low FSH and 65% of the patients presented with normal FSH, PCOS with major hormonal influence of the WHO type II study subjects, the hormonal status is as given below.

Table 4: Status of the hormones in the studied WHO - II

WHO TYPE II	13 cases
High TSH	2 cases (15.4%)
High LH/FSH ratio	8 cases (61.5%)
High Prolactin	9 cases (69.2%)
Low Prolactin	2 cases (15.4%)
High AMH	11 cases (84.6%)
High insulin	4 cases (30.8%)

High TSH was found in 15.4% of WHO Type II cases High LH/FSH ratio in 61.5% of subjects, High prolactin in 69.2% of cases, low prolactin in 15.4% of cases. High AMH in 84.6% of cases and high insulin in 30.8% of cases.

Table 5: The highlighted Pearson Correlation coefficient values are significant at the 0.05 level (2-tailed). Patients

FACTORS	FBS	PPBS	HbA1c	T3	T4	TSH	FT4	LH	FSH	LH_FSH	PRL	DHEA_S	INSULIN	Total Testosterone	Free Testosterone	AMH	AGE
FBS	1.000																
PPBS	**0.674**	1.000															
HbA1c	0.377	**0.702**	1.000														
T3	0.078	-0.009	-0.103	1.000													
T4	0.269	0.189	0.314	0.306	1.000												
TSH	-0.160	0.096	0.228	**-0.461**	0.142	1.000											
FT4	0.408	0.045	-0.153	0.289	0.199	**-0.558**	1.000										
LH	-0.359	-0.152	0.052	-0.217	0.013	0.320	-0.334	1.000									
FSH	-0.389	-0.120	0.021	-0.264	0.030	0.388	-0.411	0.396	1.000								
LH_FSH	-0.101	-0.061	-0.006	-0.062	-0.005	0.139	-0.013	**0.669**	-0.338	1.000							
PRL	0.099	0.313	0.091	0.175	-0.197	-0.091	-0.028	-0.210	-0.317	0.085	1.000						
DHEA_S	0.245	0.166	-0.074	-0.128	0.075	0.092	-0.101	-0.240	-0.060	-0.323	0.252	1.000					
INSULIN	0.090	0.167	0.344	-0.346	-0.031	0.358	-0.194	0.198	-0.162	0.390	-0.001	-0.071	1.000				
Total Testosterone	-0.053	0.100	0.065	0.016	**0.589**	0.364	-0.205	0.228	**0.592**	-0.204	-0.395	0.150	-0.015	1.000			
Free Testosterone	0.107	0.031	-0.222	0.103	0.314	0.190	0.148	-0.024	0.236	-0.195	0.304	**0.580**	-0.360	0.372	1.000		
AMH	-0.006	0.181	0.160	-0.060	-0.264	0.332	-0.362	-0.037	0.026	0.093	0.400	-0.235	0.428	-0.207	-0.246	1.000	
AGE	-0.032	0.070	0.170	**-0.508**	**-0.490**	0.253	-0.162	0.001	0.254	-0.131	-0.344	-0.137	0.393	-0.084	-0.301	0.207	1.000

A two tailed Pearson correlation coefficient values among the clinical parameters shows Triodothyronine (T3) to have a significant negative correlation with TSH and age. TSH showed a significant negative correlation with free Thyroxine (FT4). Thyroxine (T4) showed a significant negative correlation with age and a positive correlation with Total Testosterone level. Serum LH showed a significant positive correlation with LH/FSH ratio. Serum FSH showed a positive correlation with total testosterone. DHEA –S showed a positive correlation with free testosterone. Age showed a negative correlation with T3(Triiodothyronine) and T4(Thyroxine)

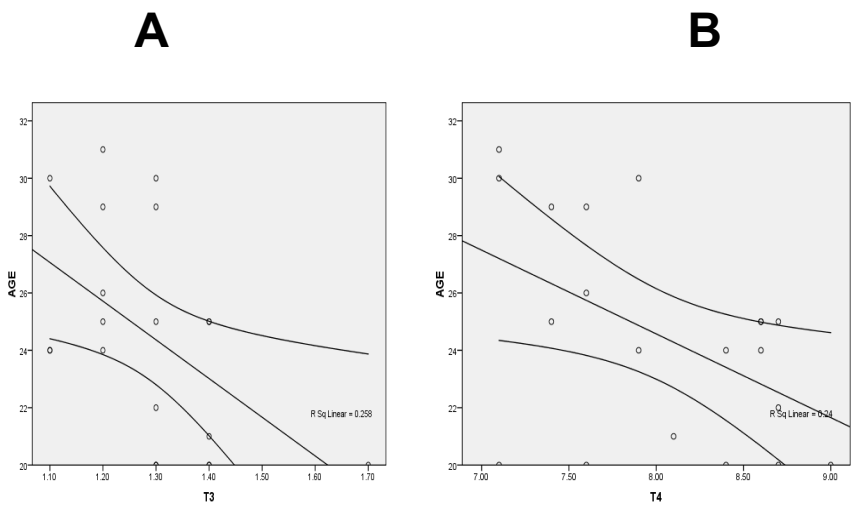

Figure 1A: Correlation Plots between (A) AGE vs T3 and (B) AGE vs T4

Correlation plot between age and T3 and T4 shows that with increasing age the level of thyroid hormones gradually decrease. Therefore there is a negative correlation of T3 and T4 with increase in age.

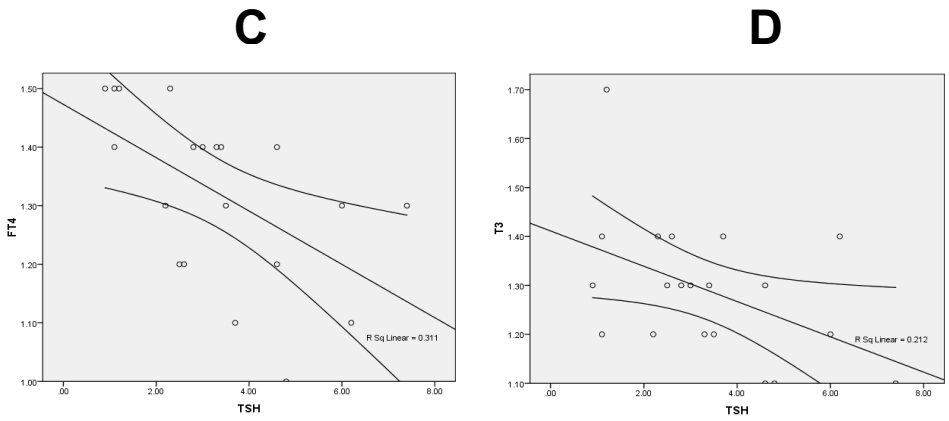

Figure 1B: Correlation Plots between (C) FT4 vs TSH and (D) T3 vs TSH

Correlation plot between FT4 and T3 also show a negative correlation with TSH. When FT4 and T3 are high, TSH is usually low. Therefore in hypothyroidism TSH is high while FT4 and T3 are low.

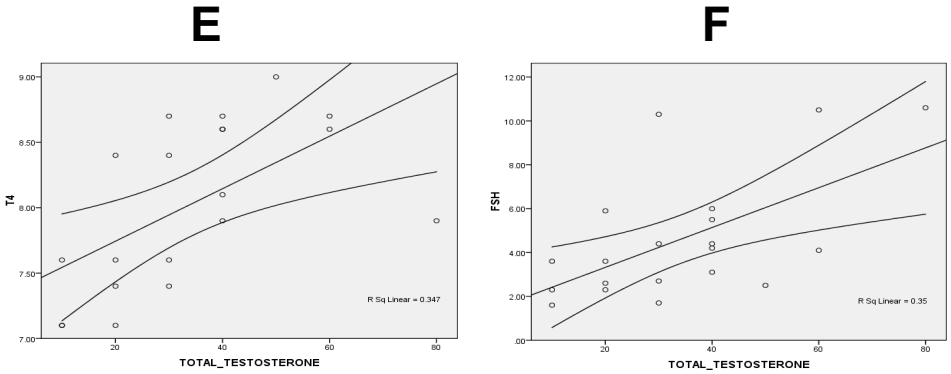

Figure 1C: Correlation Plots between (E) T4 vs Total Testosterone and (F) FSH vs Total Testosterone.

Correlation plot between total testosterone vs T4 and FSH shows a positive synergistic effect i.e when T4 and FSH are high, testosterone level is raised i.e with hyperthyroidism and hypergonadotropism; there is a rise in androgenic hormones.

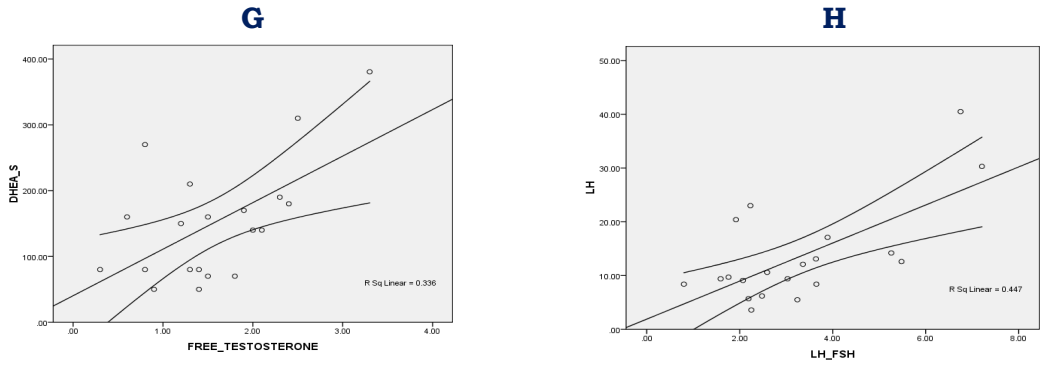

Figure 2: Correlation Plots between (G) DHEA-S vs Free Testosterone and (H) LH vs LH/FSH ratio

Free testosterone showed a positive corelation with adrenal hormone DHEA-S. LH and Insulin may be associated with total testosterone and total testosterone may be associated with prolactin level in the occurrence of polycystic changes in the ovary.

Table 6: Status of ANOVA

FACTORS		Sum of	df	Mean	F	p-value
FBS	Between Groups	196.40	8	24.550	0.336	0.934
	Within Groups	804.74	11	73.158		
	Total	1001.14	19			
PPBS	Between Groups	4233.29	8	529.162	0.811	0.608
	Within Groups	7181.66	11	652.878		
	Total	11414.95	19			
HbA1c	Between Groups	0.96	8	0.120	0.648	0.725
	Within Groups	2.04	11	0.185		
	Total	3.00	19			
T3	Between Groups	0.21	8	0.026	1.734	0.196
	Within Groups	0.17	11	0.015		
	Total	0.38	19			
T4	Between Groups	3.28	8	0.410	1.063	0.450
	Within Groups	4.24	11	0.385		
	Total	7.52	19			
TSH	Between Groups	37.29	8	4.661	2.091	0.128
	Within Groups	24.52	11	2.229		
	Total	61.81	19			
FT4	Between Groups	0.13	8	0.017	0.654	0.721
	Within Groups	0.28	11	0.025		
	Total	0.41	19			
LH	Between Groups	1277.23	8	159.654	6.010	**0.004**
	Within Groups	292.22	11	26.565		
	Total	1569.45	19			
FSH	Between Groups	60.67	8	7.584	0.908	0.543
	Within Groups	91.84	11	8.349		
	Total	152.51	19			
LH_FSH	Between Groups	25.29	8	3.161	1.130	0.414
	Within Groups	30.76	11	2.797		
	Total	56.05	19			
PRL	Between Groups	5779.05	8	722.381	0.960	0.510
	Within Groups	8281.34	11	752.849		
	Total	14060.39	19			
DHEA_S	Between Groups	29641.79	8	3705.223	0.332	0.936
	Within Groups	122813.36	11	11164.850		
	Total	152455.14	19			
INSULIN	Between Groups	8441.70	8	1055.213	11.511	**< 0.001**
	Within Groups	1008.39	11	91.672		
	Total	9450.09	19			
Total	Between Groups	2193.33	8	274.167	0.704	0.684
	Within Groups	4286.67	11	389.697		
	Total	6480.00	19			
Free	Between Groups	3.25	8	0.407	0.640	0.731
	Within Groups	6.99	11	0.636		
	Total	10.25	19			
AMH	Between Groups	673.43	8	84.179	1.462	0.274
	Within Groups	633.27	11	57.570		
	Total	1306.70	19			

The status of ANOVA shows a significant p value of <0.05 for LH and insulin. This implies that the hypothalamus pituitary compartment and the ovarian compartment plays a significant role in PCOS.

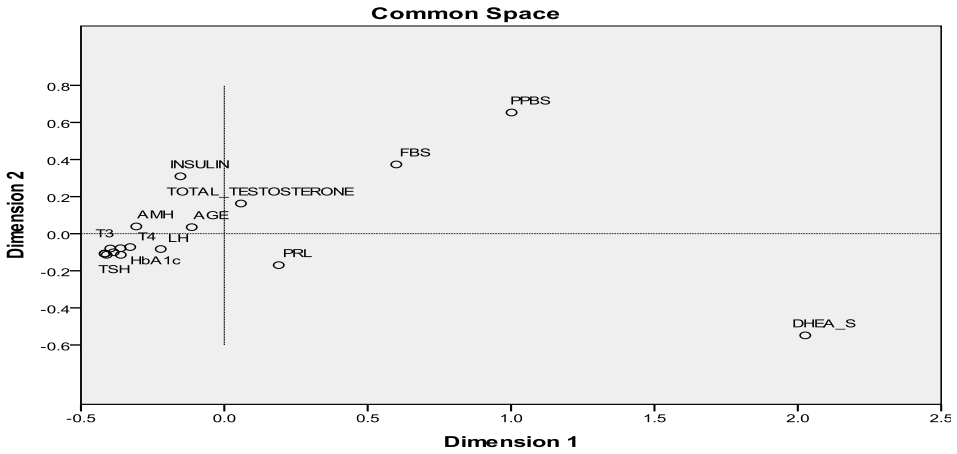

Figure 2(A): Multidimensional Scaling (MDS) Plot for the studied clinical parameter

In MDS scaling plot for the studied biochemical and hormonal parameters. DHEAS is found to be far away from influencing PCOS. It is an independent factor that is usually associated with low FSH. FBS and PPBS, prolactin and total testosterone and independently may exert a significant role accountable for PCOS.

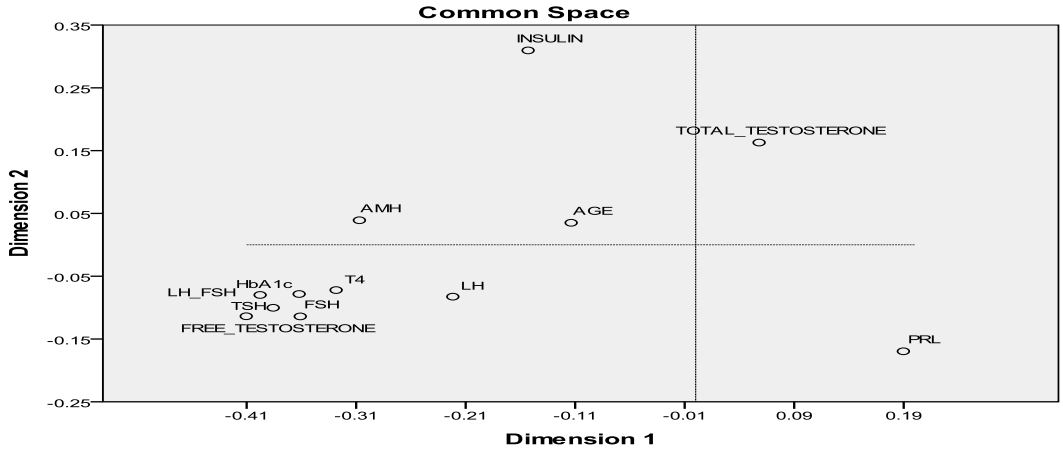

Figure 2 (B): Multidimensional Scaling (MDS) Plot for the studied clinical parameters

T4, TSH, LH / FSH ratio, HbA1c, Free testosterone. FSH are redundant factors, forming a cluster of dependence on each other for the manifestation of PCOS. Insulin, AMH and age factors may influence PCOS independently or may remain in close association with other variables.

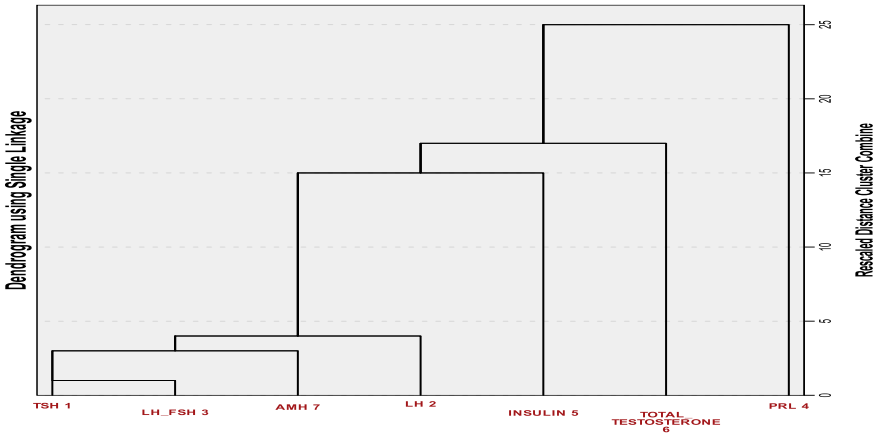

Figure 3: Hierarchical Cluster Analysis of the major clinical parameters obtained from MDS Plot

The hierarchial cluster analysis of the major parameters obtained from MDS plot shows the major influence of TSH and LH / FSH ratio followed by AMH, LH and Insulin levels. The total

testosterone and prolactin are independent factors that may remain associated with the other factors.

Discussion

PCOS is a common endocrinopathy which can be characterized by elevated circulating androgens. According to study done by Giudice, 2006, it was found that there are certain effects in women. In our study we have found significant influence of Luteinising hormone and Insulin on the respondents It was reported that the endocrinologic and metabolic abnormalities in PCOS may have complex effects on the endometrium, contributing to the infertility and endometrial disorders observed in women with PCOS [22](Giudice et al., 2006). The endometrium is also a target for insulin, the receptor for which is cyclically regulated in normo-ovulatory women in addition to being responsive to the steroid hormones estradiol, progesterone, and androgens.

Thus, it was found that elevated estrogen, hyperinsulinemia, elevated free IGF-I and androgens, and obesity all likely contribute to endometrial dysfunction, infertility, increased miscarriage rate, endometrial hyperplasia, and endometrial cancer common in women with PCOS. Based on the census done by Rotterdam in 2003, it was found that PCOS is a syndrome of ovarian dysfunction along with some cardinal features such as hyperandrogenism and polycystic ovary morphology. And also, no specific diagnosis criteria are enough for the diagnosis of PCOS. It can also be associated with an increased risk of type 2 diabetes and cardiovascular diseases. Its effects include menstrual irregularities, signs of androgen excess, and obesity [23](Rotterdam et al., 2003).

The study done by E. Carmina, 2006 to determine the effects of insulin resistance and elevated adipocytokines on abnormal carotid intima-media thickness (IMT) and brachial flow-mediated dilatation (FMD) in young women with PCOS [24]. The study was done on 50 young women with PCOS and 50 matched ovulatory controls. Carotid IMT, brachial FMD, and blood for fasting glucose, insulin, leptin, adiponectin and resistin were measured. It was found that, IMT was increased (P .01), FMD was decreased (P .01), fasting insulin was increased (P .01), and adiponectin was lower (P .05), whereas leptin and resistin were not different compared with matched controls. Thus, it was reported that endothelial function is correlated with insulin resistance and lower adiponectin (E. Carmina et al., 2006).[24] Polycystic ovary syndrome (PCOS) is associated with the metabolic syndrome and, consequently, with a potentially increased risk of cardiovascular disease (CVD). Schmidt examined postmenopausal PCOS women to check whether they differ from controls regarding cardiovascular risk factors, myocardial infarction (MI), stroke and mortality. They conducted this study on 35 PCOS women (61-79 yrs) and 120 age-matched controls. Blood pressure, glucose, insulin, triglycerides, total cholesterol, highand low-density lipoprotein, apolipoprotein A1 and B, fibrinogen, and plasminogen activator inhibitor antigen were studied. It was found that stroke, diabetes, cancer, and mortality prevalence was similar to controls but PCOS women had a higher prevalence of hypertension and higher triglyceride levels than controls [25] (Schmidt, Johanna, et al.2011). Women with PCOS have a 2.7-fold increased risk for developing endometrial cancer. A major factor for this increased malignancy risk is prolonged exposure of the endometrium to unopposed estrogen that results from anovulation.

Transvaginal ultrasound or endometrial biopsy is recommended for women with PCOS who have thickened endometrium, prolonged amenorrhea, unopposed estrogen exposure or abnormal vaginal bleeding. Dumesic reported that the reduced risk for endometrial cancer is about 50% and 70% for 4 and 12 years of oral contraceptive use, respectively. After stopping oral contraception, the risk of endometrial cancer rises from being reduced. Also, there is no sufficient data to associate PCOS with breast cancer [26] (Dumesic et al., 2016).World health organization (WHO) has provided a classification of ovulation disorders. Based on the study done by ESHRE Capri Workshop Group which was solely based on WHO group 2 anovulation. Bioinformatics online tools and databases were used for this review from Pubmed and EMBASE. The disorders resulting in ovulatory disturbances are main cause of infertility and they are frequent in WHO group 2 anovulation PCOS. It was found that women with this syndrome take more time to pregnancy. Clomi-phene citrate with gonadotrophins and laparoscopic ovarian surgery can be used for treatment in sub fertile anovulatory patients with PCOS. Patients with PCOS need to be monitored regularly as there is a chance of cardiovascular risk in PCOS patients.

In our observational study of the case series of PCOS, we found a number of hormonal factors contributing to PCOS of which the hypothalamo-pituitary compartment and the ovarian compartment are observed to play a major role. There is a symmetric age distribution of females and symmetric hormonal distribution with anti-Mullerian hormone, free testosterone, thyroxine and free thyroxine among the respondents. The rest of the biochemical and hormonal parameters showed a positive skewness with a tendency in contributing to the disease. The ANOVA study has shown significant p-value of <0.05 for Insulin and LH (luteinising hormone). In the hypothalamo-

pituitary compartment, there is relative elevation of LH along with LH/FSH ratio.

FSH is not increased with LH due to negative synergistic feedback of elevated oestrogen level and follicular inhibition. About 75% of patients showed mild elevated prolactin levels contributing to hyperprolactinaemic amenorrhoea. In our observation there was a relative rise of LH in relation to FSH. About 61.5% of cases showed high LH:FSH ratio of more than or equal to 2:1. About 69.2% of cases presented with high prolactin and 15.4% with low prolactin levels.

High serum AMH (Antimullerian hormone) is associated with about 84.6% of cases and indicates a good ovarian reserve promoting follicular growth but with a relative low FSH, it exerts no significant effect in growth of a dominant follicle and hence multiple uniform sized follicles causing polycystic changes and anovulation . In the ovarian compartment, increased synthesis of androgens by ovary due to stimulation of theca cells by high LH and Insulin like growth factor have contributed to PCOS and 30.8% have shown association with high serum insulin level.

Conclusion

The PCOS are a major problem of this generation causing obesity and subsequent infertility in females due to non-maturation of the antral follicles to produce ovum. Women with PCOS are often insulin resistant. The ovaries and adrenals are sensitive to insulin. Hyperinsulinaemia promotes ovarian androgen synthesis. Sex hormones and androgens are synthesised mainly from the gonads and adrenal cortex from lipid cholesterol. Hence obesity and fat containing food habits encourage increase in testosterone and dehydroepiendosterone to the higher range of normal.

In spite of a good ovarian reserve provided by high AMH, the support of normal LH/FSH ratio and a relatively high FSH for the development of a dominant follicle is necessary to promote folliculogenesis and ovulation. Serum Prolactin levels act independently causing hyperprolactinaemic amenorrhoea or breast milk expression without pregnancy and sometimes associated with high TSH. Hypothyroidism is associated with obesity. The biochemical and hormonal profile for suspected PCOS should be sincerely done and necessary corrections of the abnormal biochemical and hormonal parameters to be made to prevent infertility. Further case control studies with vitamin D correlation with PCOS will add more insight into the problem.

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