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Study Of Serum Betatrophin Levels And It's Association With Insulin Resistance In Patients With Polycystic Ovary Syndrome.

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ABSTRACT: BACKGROUND- Betatrophin is a newly identified hormone produced in liver and adipose tissue that has been shown to be associated with insulin resistance and regulate glucose and lipid metabolism. Aim of this study was to investigate circulating betatrophin levels in women with PCOS and the correlations with insulin resistance and biochemical parameters in two age & sex matched groups.
MATERIALS & METHODS- 85 women with PCOS and 85 age-matched healthy women were recruited in a cross-sectional study. Serum concentrations of betatrophin were measured. Other biochemical parameters measured were- Fasting Serum Insulin, Fasting Serum Glucose ,Serum Cholesterol, Serum Triglycerides- Serum HDL Cholesterol, Serum LDL Cholesterol ,Serum VLDL Cholesterol .Insulin Resistance was calculated by using Homeostasis model & results were analyzed by multiple statistical methods.
RESULTS- Circulating betatrophin levels were found to be increased in patients with PCOS compared with those in the control group. Moreover, serum betatrophin levels were significantly positively associated with all other indexes.
CONCLUSION- This study suggests that Serum betatrophin may potentially serve as an independent predictor for the development of PCOS in women at-risk.
Keywords- PCOS, Betatrophin, Insulin resistance.

I. INTRODUCTION

The polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder that affects about one in fifteen women worldwide , with unclear pathogenesis. Hyperandrogenism is a principal characteristic of the syndrome, and it has been suggested that increased androgen synthesis plays a major role in the pathogenesis of PCOS . Insulin resistance (IR) is also frequently present in PCOS and appears to contribute to the increased steroidogenesis in these patients. For a confirmed diagnosis, 2 out of the following 3 clinical criteria are essential: oligomenorrhoea, hyperandrogenism or polycystic appearance of the ovaries on transvaginal ultrasonography. There are numerous metabolic consequences of PCOS in addition to the effects on the reproductive system. These include a higher risk of obesity, insulin resistance (IR), type 2 diabetes mellitus (T2DM) and premature arteriosclerosis [**1].**
However, the aetiology of PCOS is underpinned by both insulin resistance and hyperandrogenism, with insulin resistance exacerbating hyperandrogenism.

Insulin resistance occurs in approximately 80% of women with PCOS and occurs independently of obesity. Furthermore, women with PCOS are believed to be at and increased risk of developing type 2 diabetes mellitus (T2DM).

Thus, PCOS is a well-defined clinical model of insulin resistance and the pre-diabetic state [2].The deregulated production of adipokines in obese subjects appears to be implicated in the pathogenesis of IR, hyperandrogenism, and PCOS [**3].**

Betatrophin, also called refeeding-induced fat and liver protein (RIFL) , lipasin , or atypical angiopoietin-like protein 8 (ANGPLT8) , is a 22 kDa hormone. In mice, betatrophin is produced by the liver, white adipose tissue (WAT), and brown adipose tissue (BAT), while in humans, it is mainly produced by the liver . It has been shown that betatrophin has a dual role: it affects glucose homeostasis and lipid metabolism[**4**] .

ANGPTL8 was capable of inducing β-cell mass and improving glucose tolerance and potentially augmenting or replacing insulin injections , patients in comparison to normoglycemic subjects . It has also been observed that serum betatrophin concentration positively correlated with HbA1c and fasting plasma glucose in the group of patients with diabetes, prediabetic state, and metabolic syndrome

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and with fasting plasma glucose and HOMA-IR in T2D and non-diabetic subjects .

Apart from its role in glucose homeostasis, betatrophin has an impact on lipid metabolism. In experimental data, betatrophin suppressed adipose triglyceride lipase activity and increased TG accumulation in hepatocytes, adipocytes, and beta cell. In the fasted state, similar serum TG levels in ANGPTL8 mice and wild-type animals were noticed betatrophin could be involved in glucose and lipid metabolism, which is often impaired in women with PCOS. [**5**]

II. MATERIALS & METHODS-

Study participants-

The study has been conducted on two age & sex matched group of participants attending gynecology O.P.D. of J.L.N. Medical college and associated group of Hospitals, Ajmer (Rajasthan). Diagnosis of poly cystic ovary syndrome (PCOS) was based upon the diagnostic criteria of **Rotterdam PCOS Consensus Criteria**. Two out of the following three criterias are required:

1. Oligo/ anovulation
2. Hyperandrogenism

Clinical (hirsutism or less commonly male pattern alopecia)

Or

Biochemical (raised FAI or free testosterone)

3. Polycystic ovaries on ultrasound

Total 170 women were enrolled and divided in two groups. Cases comprised of 85 diagnosed cases of polycystic ovary syndrome (PCOS) & Controls were group of 85 healthy women.

Inclusion criteria-

Women between the age group 18 – 45 years.

Exclusion criteria-

Patients with Diabetes Mellitus.

2. Patients with Hepatic and Renal dysfunction.
3. Patients with Thyroid dysfunctions.
4. Pregnant women.
5. Smokers and Alcoholics.

This study was reviewed by the ethical committee. Informed & written consent was taken from all the subjects at the beginning of study.

Anthropometric and laboratory measurements-

Participants were weighed barefoot and in light clothing, height was measured by using measuring tape. BMI was calculated as weight (Kg)/ height (m^2) Blood pressure will be measured using a mercury sphygmomanometer in sitting position after a rest of 10 minutes.Venous blood sample was collected after an overnight fasting(10-12 hrs) under aseptic precautions. The plasma/serum was subjected to measurement of the following Biochemical Parameters-

1. Serum Betatrophin- ELISA method
2. Fasting Serum Insulin- Chemileuminesence Immunometic Assay
3. Fasting Serum Glucose- Enzymatic GOD-POD, end point method (Trinder P; 1969)
4. Serum Cholesterol- Enzymatic CHOD-POD, end point method (Allian CC; 1974)
5. Serum Triglycerides- Enzymatic CHOD-POD, end point method (Fossati P; 1982)
6. Serum HDL Cholesterol- Phosphotungstic acid, end point method (Finley PR; 1978)
7. Serum LDL Cholesterol – Calculated from the Friedewald's Formula (Friedewald WT; 1972)
8. Serum VLDL Cholesterol- Calculated from the Friedewald's Formula (Friedewald WT; 1972)
9. Insulin Resistance was calculated by using Homeostasis model

Fasting plasma glucose (mmol/Lt) X Fasting insulin (µU/ml) / 22.5 (Mathews et al; 1985)

Statistical analysis-

Data was analysed by SPSS Software and p-value < 0.05 was considered significant. The vitamin D levels among the two groups were compared by unpaired student t test.

Table 1 : BMI in cases and controls

	N	Mean	Std. Deviation	Minimum	Maximum
CASE	85	26.62	2.02	23.20	30.30
CONTROL	85	22.70	1.82	20.10	25.60

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Result (p value)		p<0.001 (S)

S=Significant ; NS = Non Significant

Table no.1 shows that mean of BMI among cases is 26.62 with standard deviation of 2.02 and mean of BMI among controls is 22.70 with standard deviation of 1.82, it shows that BMI is high in pcos cases significantly (p value= 0.001)

Table 2: Serum Glucose [mg/dl] in case and control (fasting)

	N	Mean	Std. Deviation	Minimum	Maximum
CASE	85	90.09	6.87	78.30	102.70
CONTROL	85	93.09	14.18	72.30	122.50
Result (p value)		0.080 (NS)			

S= Significant ; NS = Non Significant

Table no.2 shows that mean of serum glucose level among cases is 90.09 mg/dl with standard deviation of 6.87 mg/dl and mean of serum glucose level among controls is 93.09 mg/dl with standard deviation of 14.18 mg/dl , it shows that serum glucose level is not significant in pcos (p value= 0.08)

Table 3 :Serum level of Cholesterol (mg/dl) in cases and control

	N	Mean	Std. Deviation	Minimum	Maximum
CASE	85	179.34	21.89	143.36	232.02
CONTROL	85	156.37	22.79	115.10	195.40
Result (p value)		p<0.001 (S)			

S= Significant ; NS = Non Significant

Table no.3 shows that mean of serum cholesterol level among cases is 179.34 mg/dl with standard deviation of 21.89 mg/dl and mean of serum cholesterol among controls is 156.37 mg/dl with standard deviation of 22.79 mg/dl, it shows that cholesterol level is high in pcos cases significantly (p value= 0.001).

International Journal Dental and Medical Sciences Research
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Table 4: Serum level of Triglycerides [mg/dl] in cases and controls

	N	Mean	Std. Deviation	Minimum	Maximum
CASE	85	112.39	15.58	70.2	141.1
CONTROL	85	84.69	16.25	55.80	110.30
Result (p value)		p<0.001 (S)			

S=Significant ; NS = Non Significant

Table no.4 shows that mean of serum triglycerides level among cases is 112.39 mg/dl with standard deviation of 15.58 mg/dl and mean of serum triglycerides among controls is 84.69 mg/dl with standard deviation of 16.25 mg/dl, it shows that triglycerides level is high in pcos cases significantly (p value= 0.001)

Table 5 : Serum VLDL (mg/dl) level in cases and control

	N	Mean	Std. Deviation	Minimum	Maximum
CASE	85	22.45	8.25	14.20	28.20
CONTROL	85	39.93	14.83	11.10	60.10
Result (p value)		p<0.001 (S)			

S= Significant ; NS = Non Significant

Table no.5 shows that mean of serum VLDL level among cases is 22.45 mg/dl with standard deviation of 8.25 mg/dl and mean of serum VLDL among controls is 39.93 mg/dl with standard deviation of 14.83 mg/dl, it shows that VLDL level is high in pcos cases significantly (p value= 0.001).

Table 6: Serum HDL (mg/dl) level in cases and controls

	N	Mean	Std. Deviation	Minimum	Maximum
CASE	85	45.87	6.44	34.7	59.3
CONTROL	85	24.14	12.66	11.10	55.60
Result (p value)		p<0.001 (S)			

S= Significant ; NS = Non Significant

Table no.6 shows that mean of serum HDL level among cases is 45.87 mg/dl with standard deviation of 6.44 mg/dl and mean of serum HDL among controls is 24.14 mg/dl with standard deviation of 12.66 mg/dl, it shows that HDL level is high in pcos cases significantly (p value= 0.001)

International Journal Dental and Medical Sciences Research
Volume 3, Issue 1, Jan-Feb 2021 pp 1029-1037 www.ijdmsrjournal.com ISSN: 2582-6018

Table 7 : Serum LDL (mg/dl) level in cases and controls

	N	Mean	Std. Deviation	Minimum	Maximum
CASE	85	110.95	23.01	72.80	178.81
CONTROL	85	93.64	22.30	51.70	138.80
Result (p value)		p<0.001 (S)			

S= Significant ; NS = Non Significant

Table no.7 shows that mean of serum LDL level among cases is 110.95 mg/dl with standard deviation of 23.01 mg/dl and mean of serum LDL among controls is 93.64 mg/dl with standard deviation of 22.30 mg/dl, it shows that LDL level is high in pcos cases significantly (p value= 0.001)

Table 8 : HOMA-IR in cases and controls

	N	Mean	Std. Deviation	Minimum	Maximum
CASE	85	5.02	1.46	2.21	7.71
CONTROL	85	2.72	0.46	1.12	3.28
Result (p value)		p<0.001 (S)			

S=Significant ; NS = Non Significant

Table no.8 shows that mean of HOMA-IR among cases is 5.02 with standard deviation of 1.46 and mean of HOMA-IR among controls is 2.72 with standard deviation of 0.46, it shows that HOMA-IR is high in pcos cases significantly (p value= 0.001).

Table 9 : Serum Betatrophin[pg/ml] in cases and control

	N	Mean	Std. Deviation	Minimum	Maximum
CASE	85	764.39	23.69	730.20	814.20
CONTROL	85	686.26	16.76	652.10	713.30
Result (p value)		p<0.001 (S)			

S= Significant ; NS = Non Significant

Table no.9 shows that mean of serum Betatrophin level among cases is 764.39 pg/ml with standard deviation of 23.69 pg/ml and mean of serum Betatrophin level among controls is 686.26 pg/ml with standard deviation of 16.76 pg/ml, it shows that betatrophin level is significantly high in pcos than normal (p value<0.001).

International Journal Dental and Medical Sciences Research
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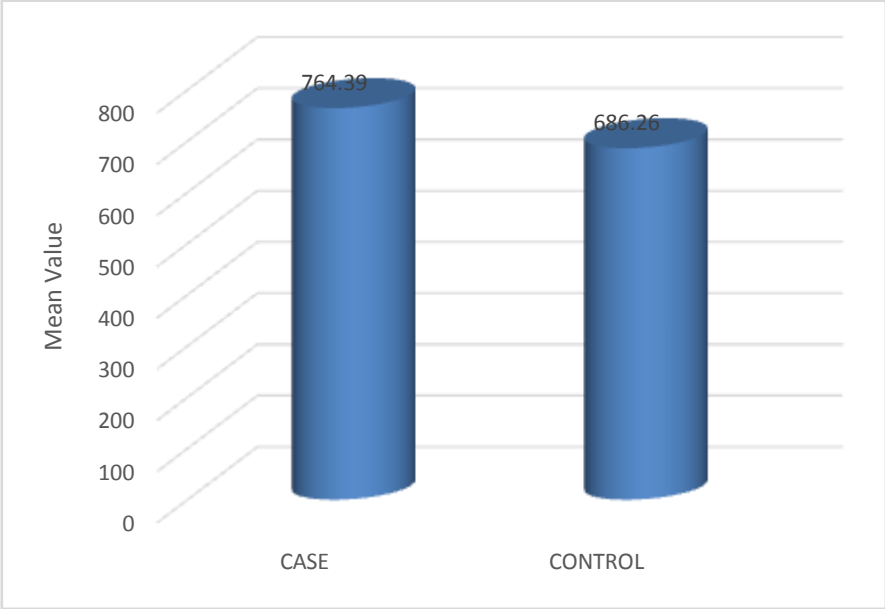

FIG 1- Comparison of Serum Betatrophin[pg/ml] in cases and control.

Table 2-Correlation between Betatrophin and Insulin

Correlations			
		Betatrophin	**Insulin**
Betatrophin	Pearson Correlation	1	.110
	Sig. (2-tailed)		.314
	N	85	85
Insulin	Pearson Correlation	.110	1
	Sig. (2-tailed)	.314	
	N	85	85

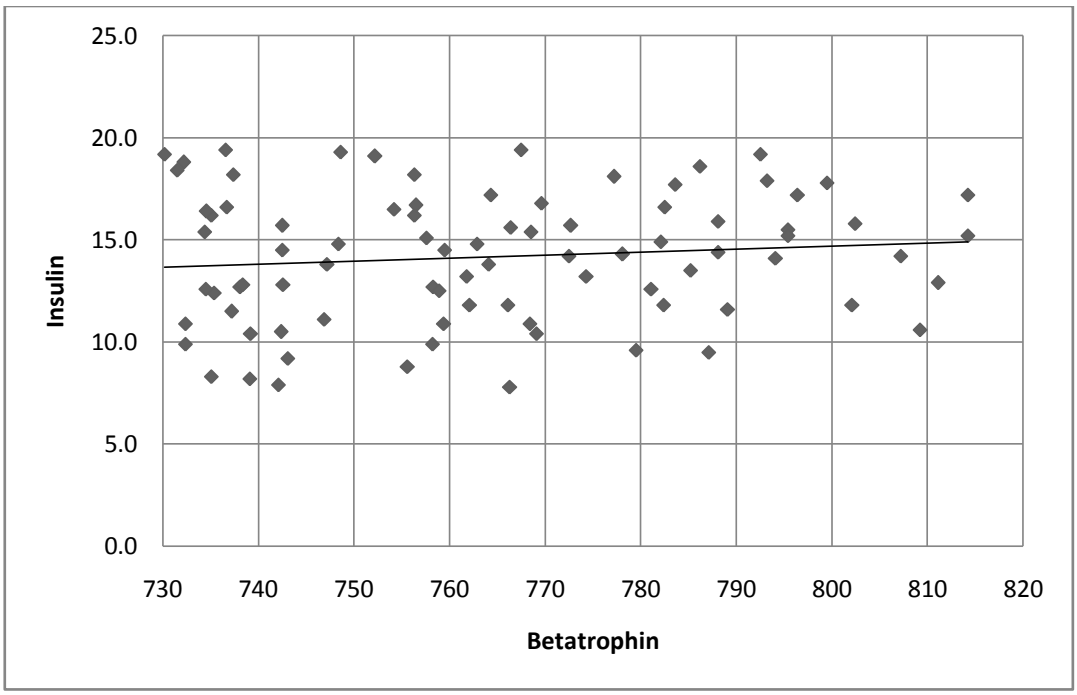

FIG 2- Correlation graph between Betatrophin and Insulin

International Journal Dental and Medical Sciences Research
Volume 3, Issue 1, Jan-Feb 2021 pp 1029-1037 www.ijdmsrjournal.com ISSN: 2582-6018

Table 3- Correlation between Betatropin and BMI

Correlations			
		Betatrophin	**BMI**
Betatrophin	Pearson Correlation	1	.002
	Sig. (2-tailed)		.984
	N	85	85
BMI	Pearson Correlation	.002	1
	Sig. (2-tailed)	.984	
	N	85	85

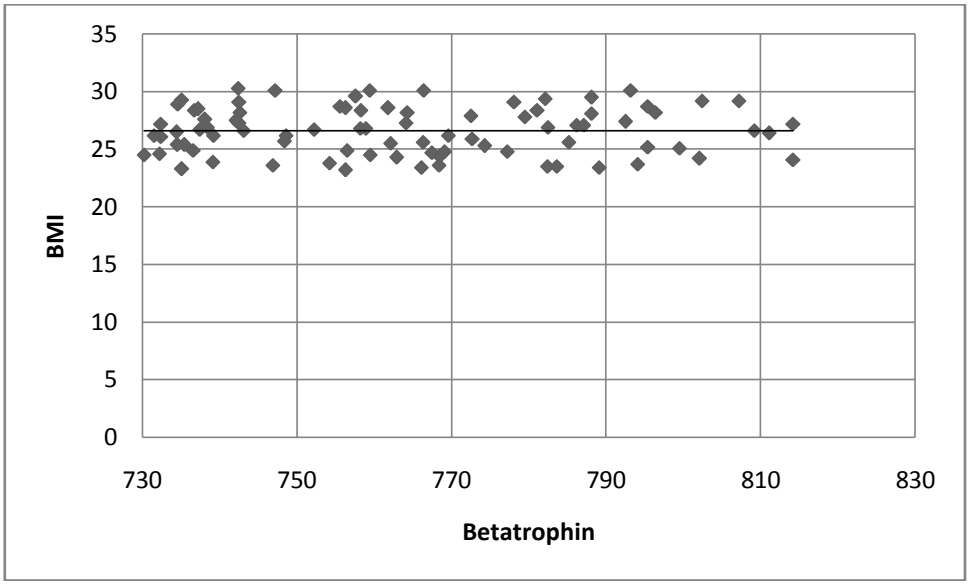

FIG 3- Correlation graph between Betatropin and BMI

Table 4- Correlation between Betatropin and HOMA-IR

Correlations			
		Betatrophin	**HOMAIR**
Betatrophin	Pearson Correlation	1	.041
	Sig. (2-tailed)		.706
	N	85	85
HOMA-IR	Pearson Correlation	.041	1
	Sig. (2-tailed)	.706	
	N	85	85

International Journal Dental and Medical Sciences Research
Volume 3, Issue 1, Jan-Feb 2021 pp 1029-1037 www.ijdmsrjournal.com ISSN: 2582-6018

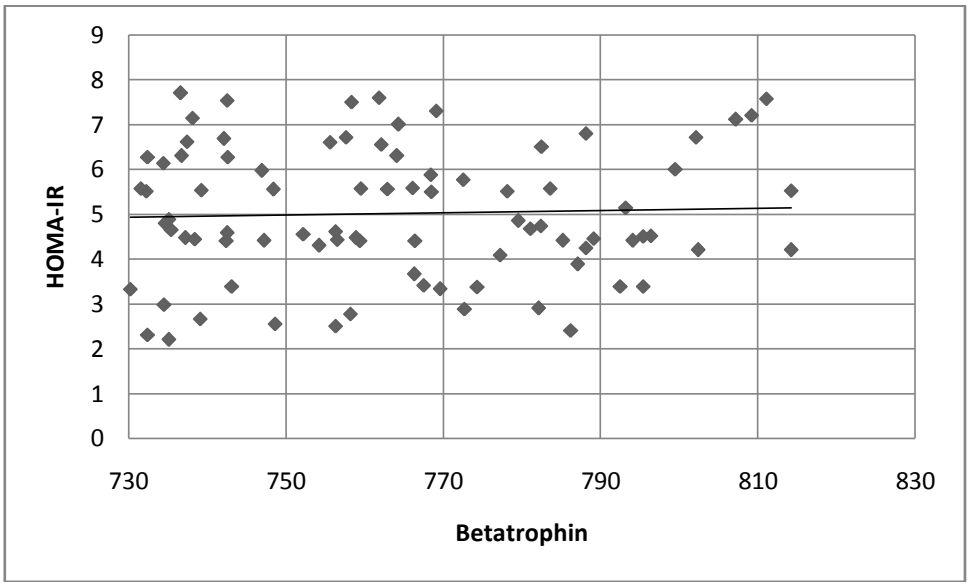

FIG 4- Correlation between Betatropin and HOMA-IR.

III. DISCUSSION-

Women with PCOS and insulin resistance exhibited higher betatrophin concentrations. We found that both fasting insulin levels and PCOS diagnosis correlated with betatrophin levels. Furthermore, betatrophin levels were significantly correlated with fasting insulin levels and HOMA-IR only in patients with PCOS. Betatrophin has recently been introduced as a novel potent stimulator of b-cell replication and improved glucose tolerance by increasing the β-cell division rate .There is evidence suggesting that betatrophin expression can be induced by a high-fat diet and insulin, resulting in increased serum triglyceride levels and insulin resistance instead of improved glucose metabolism. However, several reports have indicated that betatrophin was increased in T2DM and type 1 diabetes mellitus, indicating that betatrophin could be a potent diagnostic marker for metabolic syndrome.

As indicated in this study, we determined that circulating betatrophin levels were markedly increased in patients with PCOS compared with those in the control group. Moreover, serum betatrophin levels were significantly positively associated with indexes of insulin resistance, including fasting insulin levels and HOMA-IR. These findings corroborate those of a previous population-based study that indicated that serum betatrophin levels were elevated in patients with T2DM and associated with insulin resistance. However, it is unclear whether increased betatrophin expression is a compensatory response or only a marker of insulin resistance in PCOS. Notably, increased circulating betatrophin levels were identified in women with PCOS and insulin resistance but not in control women. Additionally, it is seen that betatrophin as a novel hormone may be involved in the generation of an atherogenic lipid profile.

IV. SUMMARY-

It is thus concluded from our study that obesity leads to metabolic derrangements and PCOS is also one of them. Obese patients have high BMI so prone to high HOMA-IR and dyslipidemia. Obesity leads to alteration in biomarkers value. Serum Betatrophin level significantly increases in pcos patients than controls. Thus our study shows that measurement of biomarkers like Betatrophin in PCOS patients may be helpful in assessing the risk. Further studies need to be conducted on this matter to prove the usefulness and constraints in using serum Betatrophin as biomarker in the progression of PCOS.

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