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Evaluate The Level And Correlation Of Proinflammatory Marker Visfatin And Tumor Necrosis Factor Alpha (TNF-A) In Obese And Non-Obese PCOS Women

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ABSTRACT

PCO is an endocrinopathy disorder that is characterized by hyperandrogenism irregular period, hair growth and acne on face, irregular or complete absence of ovulation resulting difficulty in conceiving. PCO is more commonly encountered endocrine disorder among 20 % females of reproductive age in Pakistan. PSO is responsible for different morbidity in women.
Aim of study: *To evaluate the level of visfatin and TNF-α and correlate obesity with visfatin and TNF-α in obese and non-obese PCOS women with their respective healthy individual In best of our knowledge we are first how are reported correlation among visfatin and TNF-α and correlate these hormones in both lean and obese PCOS women.*
Methods: *A comparative cross-sectional study was conducted on obese and non-obese PCO women with their respective healthy induvial .30 in each group. biochemical estimation was done on Eliza machine.*
Statistical analysis: *Statistical analysis was performed by using SPSS 25. One way ANOVA test was used to compare the average of numeric data and chi-square was used to compare categorial data. Pearson correlation test was used to determine the [1]correlation among PCO, visfatin, TNF-α and obesity .value is ≤ 0.05 consider significant.*
Results*: We found a significant increase in serum visfatin and TNF-α in obese and non-obese PCOS women. We found positive correlation of among PCOS, visfatin, TNF-α and obesity.*
Conclusion*: We found the significant increase in both biochemical parameter visfatin and TNF-α in both obese and non-obese PCO women with their respective control. but when we compare obese and non-obsess PCO women, Obese PCO women have high level of these biochemical parameter. Its mean both group of PCO have intensity to produce PCO but obese people have high risk to develop PCO.By changing sedentary lief style and food consumption can be help full to cure the symptoms.*

Key words: *PCO, hyperandrogenism, Proinflammatory marker, Inflammation, BMI.*

INTRODUCTION

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PCO is very common and occurs in 20-25% of all women of childbearing age. Genetic, environmental factors, Obesity, sedentary life style junk food contribute to develop PCO but exact cause still unknown. When ovarian follicle greater than 12number and three centimeters in size term as ovarian cyst. High levels of androgens affect the signals from the brain and decrease or totally inhibit the release of egg from ovary. Hirsutism, acne and irregular periods is major symptoms in women with PCO due to Hyperandrogenism. (Nassar etal.,2018 , Rodriguez et al., 2019)

Overweight women at high risked to develop insulin resistance and result is glucosemia. hyperinsulinemia is another cause to synthesize more androgen and contribute to develop PCOS symptoms. (Rodriguez et al., 2019)

body mass index (BMI) 30 kg/m2) equal or above has great chance to develop syndetic health problems, including IR and diabetes (Meldrum et al., 2017)

Insulin exerts glucose homeostatic and anti-inflammatory effects. It decreases expression of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α to reduce inflammatory response. while insulin resistance in over weight and obese increase the release of these markers and exhibit inflammation. (Chang,Y.W., et al., 2021)vastatin and TNF-α have great interest for researcher in PCO.

Vastatin also known as Nicotinamide phosphoribol transferase (Nampt) and pre-B-cell colony boosting factor (Jha et al., 2023, Omer et al.,2018).

It possesses immunomodulatory and proinflammatory qualities. (Zaichko et al., 2024).

It stimulates the release of proinflammatory α -TNF from monocytes, macrophages, adipose tissues and theca cells of ovaries. (Wu X T yang et al 2018, kawai et al,,2021, wurfel et al., 2023).

The aim of present research is evaluating the level of visfatin and TNF-α in obese and non-obese PCOS women and corelate vastatin and TNF-α in obese and non-obese PCOS women with their respective healthy controls. In best of our knowledge, we are the first how are reporting the correlation of these hormones in both lean and obese PCOS women with their respective controls.

MATERIAL & METHODS

It was comparative cross-sectional study and the patients diagnosed by the gynecologist of obstetrics and gynecology department by ultrasound were collected from sheikh Zayid Hospital Lahore.

Sample size calculation

The sample size was calculated by WHO calculator. Value for calculation were taken from (Cardoso et al.,2020)

Total number of subjects is 120,30 in each group. Detail of groups are as follow: Group A: non-obese PCOS women with BMI (18.5-24.99kg/m2). Group B: obese PCOS women with BMI (25-39.99 Kg/m^2), Group C: non-obese healthy control women with BMI (18.5-24.99 Kg/m^2). Group D: obese healthy control women with BMI (25-39.99 Kg/m^2).

Inclusion criteria

PCOS women Diagnosed case of by clinician without treatment, Women of reproductive age both obese (BMI 25-39.99 kg/m2) and non-obese (BMI 18.5-24.99 Kg/m^2) PCOS women and age of 14 to 40 years

Exclusion criteria for non-obese and obese PCOS women and healthy control

Diabetes mellitus, Hypertension. Hypothyroidism. hypothalamic or pituitary dysfunction, Liver, kidney or thyroid diseases, Hyperprolactinemia, Cushing's syndrome ovarian and

pituitary disorders, Underweight PCOS women BMI < 18.49 Kg/m^2 , Morbid obesity BMI > 40 Kg/m^2 .

Data Collection Procedure
Written informed consent from the individuals were taken and demographic details of individuals i-e patient ID, age, marital status, history of menstrual disturbance, miscarriages, Infertility, weight gain, acne, hirsutism, and pigmentation were taken.

Sample collection and proceeding
5 ml blood was be obtained by venous venipuncture and collected in serum separation tubes. After clotting the blood will be centrifuged at 3000 rpm (revolution per minute) for 10 minutes. The serum will be transferred in properly labeled autoclaved Eppendorf tubes and stored at -70℃ for subsequent biochemical analysis. Serum vastatin and TNF-α was measured by using ELIZA kits. (Nida etal.,2021).

Statistical Analysis
The data was entered and analyzed in SPSS 25. Shapiro. Numeric data i.e., age, serum visfatin, and TNF-α was presented in Mean ± SD. Categorical data i.e., marital status, history of menstrual disturbance, miscarriages, Infertility, weight gain, acne, hirsutism, and pigmentation were presented in frequency and percentage. One-way ANOVA test was used to compare the average of numeric data among four groups. Chi-square and Fisher's exact test were used to compare the categorical data among the groups. Pearson's and Spearman Rho correlation test was used to determine the correlation of visfatin with TNF-α in obese, non-obese PCOS women and healthy control. A p-value ≤ 0.05 was considered significant.

RESULT

Demographic Characteristics of PCOS And Healthy Control Women:
Significant difference were found in body mass, irregularity of menstrual cycle, conception of pregnancy, fatty food, exercise, miscarriage, weight gain , Hirsutism, Acne, while nine significant result found in age (table 3)

Biochemical Estimation
Serum Visfatin and TNF-α Levels.
Significant increase of vasfitin level in obese PCO and non-obese PCO women as compare to their respective control (p value <0.001, table 1) Significant increase of α TNF level in obese PCO and non-obese PCO women as compare to their respective control (p value <0.001, table 1)

Correlation of serum visfatin levels with TNF-α, and BMI
The results revealed that serum visfatin levels had a significant positive correlation with TNF-α (r = 0.454; r = 0.442), and BMI (r = 0.400; r = 0.508) in Obese PCOS and Obese Healthy Control. However, no significant correlation was observed in Non-Obese PCOS (TNF-α, r = 0.104; BMI, r = 0.123), and Non-Obese Healthy Control (TNF-α, r = 0.274; BMI, r = 0.229). (Table 2).

DISCUSSION
Obesity is well established risk factor for PCOS. Newly discovered visfatin have great focus for researcher in PCO release from monocytes macrophages, lymphocytes and adipose tissues.(Jha et al., 2023).

In our research we found significant increased serum visfatin level in obese women with PCO as compared to obese women without PCO (11.5 %, table 1). level of visfatin in blood is directly proportional to BMI as it is release from adipose tissus. (Rajasekar et al., 2023, Rostamtabar et al.,2021)

Numbers of researchers demonstrate that obese PCOS women have high serum visfatin level than obese healthy control. (Hussaien et al.,.2015,Bannigida etal.,2020),

In obese PCO women increase level of visfatin develop insulin resistance by producing antireceptor antibodies that cause changes in 3D structure of insulin receptor which inhibit the binding of insulin with receptors that cause hyperinsulinemia which leads to decrease production of sex hormone binding globulin and insulin like growth factor 1(IGF-1) in liver. (Ajmal et al.,2019, Rosenfield et al., 2016, saddick et al., 2020, Tawfeek et al., 2017)

Decrease level of sex hormone binding globulin (SHBG) leads to decrease binding of testosterone with receptors in liver which cause increase level of testosterone. (Singet et al.,2023, Sadick et al.,2020, Kumar et al.,2022).

Up regulation of hypothalamic pituitary ovarian axis produces high concentration of luteinizing hormone (LH)

and decrease production of Follicle-stimulating hormone (FSH) which also leads to hyperandrogenemia. (Singh et al.,2023) develop acne, weight gain, alopecia and hirsutism. (Ashraf et al., 2021).

In our research non-obese PCOS women have high serum Visfatin level than non-obese healthy control. (41.6% table 1). Similar to our study a researcher reported increase visfatin level in lean PCOS women (Lin et al.2021). While other researchers reported no significant findings. (Kim et al.2018, Teama et al.,2018).

The lean person has normal range of BMI but they have higher serum visfatin as compared to their respective control.

In non-obese oxidative stress and hyperandrogenism promote inflammation that cause tissue damage and apoptosis that cause release of cytokines from lymphocytes and macrophages that initiate the damage of granulosa cells and oocytes with in ovarian follicles that leads to formation of cyst if it is not control then continuously upregulation of this system produce the polycystic ovary. (Zeber et al.,2023, Lu.J. Wang et al.,2018).

The second parameter of our study is TNF-α obese PCO women exhibit significant increase (51.0% table 1) serum TNF-α as compared to obese women without PCO in our research.

Increase TNF-α was reported in PCOS women with higher BMI. (Ihsan et al.2018, Cardoso et al., 2020). It is cleared that in obese increased level of TNF-α is due to BMI. (Omear et al., 2023)

Non-obese PCO exhibit significant increased serum TNF-α (80.4% table 1) as compared to non-obese women without PCO in our research. TNF-α is a proinflammatory cytokines and its concertation reflect the level of inflammation.in PCO. (Zhang c et al., 2024)

Increase concentration of serum TNF-α was reported in PCO women with normal range of BMI (Ozegowska et al., 2020).

The lean or non-obese person have normal range of BMI but they have higher serum TNF-α as compared to their respective control. In obese PCO women as discuss above paragraph there is two mechanisms of hyperandrogenism while in lean PCO women there is only one upregulation of hypothalamic pituitary ovarian axis that leads to hyperandrogenism. (Singh et al.2023, walters et al.,2018)

Inflammation is the leading cause of PCO in both overweight and lean women. (Nehir et al.,2016) Hyperandrogenism and insulin resistance cause oxidative stress and inflammation which signal to body immune system and macrophages to become activate and release inflammatory molecules such as TNF-α.(Shabir et al., 2023).In PCO women insulin resistance cause increase level of glucose increase the release of TNF-α from mononuclear cells which

also increase the reactive oxygen species (ROS) synthesis and cause inflammation.(Malin et al., 2014, Lu et al., 2018).
Main focus of our research is to find out correlation among serum level of visfatin and TNF-α and to compare these hormones in both lean and obese PCOS women. In best of our knowledge, we are first how are reported correlation among visfatin and TNF-α and correlate these hormones in both lean and obese PCOS women.

Statistical analysis of our result show significant positive correlation of visfatin , TNF-α and obesity in obese PCOS women and obese healthy control. Our results are consistent with the previous studies which also found positive correlation of visfatin and TNF-α with obesity (Hetta et al., 2018, Rajaskar et al., 2023, yin et al., 2019).
Abiad and coworker. (2018) reported that PCO is an independent of obesity.
In our study there is significant positive correlation of serum visfatin , TNF-α in obese PCO and obese healthy control.Other researches demonstrate positive correlation between serum visfatin and α TNF-α(Yin et al., 2019) and TNF and BMI (.Rajkovic et al.,2014).
While there is no significant result were found in non-obese PCOS and their control. Many researchers reported negative correlation of visfatin,TNF-α with BMI (Ugu,et al., 2022, Ben-showornek et al.,2019).

In our research we find increase level of visfatin in both obese and lean PCO women but when we compare the level of visfatin in obese and non-obese PCO women we observe the higher level of visfatin in obese PCO women.(tabel 1).Same for TNF-α.Obese people are more susceptible to develop PCO because they have high risk of inflammation and visfatin level to enchance TNF-α as compared to lean but we cannot ignore that lean who have hormonal disturbance have the risk to produce PCOS .
Same for TNF-α. Obese women are more susceptible to develop PCO but we cannot ignore that lean who have hormonal disturbance have same risk to produce PCOS.
The BMI of group obese PCOS women and obese healthy control was significantly higher as compared to non-obese PCOS women and non-obese healthy control. There is significant difference in BMI among obese and non-obese PCOS and their respective healthy control. In contrast to our study numerous researchers found insignificant difference in BMI (Cardoso et al.,2020)
The proportion of irregular menstrual cycle, fatty food, exercise, miscarriages, weight gain, hirsutism, acne and pigmentation were significantly higher in both obese and non-obese PCOS patient as compared to obese and non-obese healthy control.
Similar to our study previous research also found the proportion of irregular menstural cycle,hirsutism,acne higher in PCOS as compared to healthy control(omer et al.,2018)
Similar to our study a study conducted which showed that weight gain is higher in obese PCOS women. (Gurbuz et al 2023).

In our study there is significant difference in mean of age and marital status among obese PCOS women. High level of androgen in PCO women increase LH/FSH ratio with increase number of follicular count lead anovulation and menstrual disorders. (Yousuf& Rehman..2024).

TABLE 1 Mean and percentages differences of serum visfatin and TNF-α among groups

Variables	Obese PCOS	Obese Healthy Control	% Increased or Decreased	P value

Visfatin	53±9.8	47.6±11.4	11.5	0.001
Tnf-α	17.8±2.1	8.7±2.3	51.0	0.001
	Non-obese PCOS	Non-obese healthy control	% increased or decreased	
Visfatin	40.1±7.1	23.4±4.3	41.6	0.001
TNF-α	11.0±2.6	2.2±0.4	80.4	0.001

TABLE 2 Correlation of serum visfatin with TNF-α and BMI

	Serum Visfatin Levels	TNF-α	BMI
Non-obese PCOS	Correlation Coefficient (r)	0.104	0.123
	p-value	0.583	0.519
Obese PCOS	Correlation Coefficient (r)	0.454	0.400
	p-value	0.012	0.028
Non-obese healthy control	Correlation Coefficient (r)	0.274	0.229
	p-value	0.143	0.224
Obese healthy control	Correlation Coefficient (r)	0.442	0.508
	p-value	0.014	0.004

TABLE 3 Comparison of demographic data among groups

		Group A	**Group B**	**Group C**	**Group D**	**p-value**
Age	**(Mean + SD)**	**25.8 ± 4.1**	**24.3 ± 3.1**	**25.0 ± 5.0**	**25.0 ± 5.1**	**0.599**
BMI	**(Mean + SD)**	**22.2 ± 1.7**	**35.3 ± 2.9**	**20.1 ± 2.0**	**34.5 ± 3.1**	**0.000**
Marital Status	**Married**	**17 (56.7%)**	**20 (66.7%)**	**12 (40.0%)**	**14 (46.7%)**	**0.178**
	Unmarried	**13 (43.3%)**	**10 (33.3%)**	**18 (60.0%)**	**16 (53.3%)**	
Regularity of	**Regular**	**9 (30.0%)**	**10 (33.3%)**	**30 (100.0%)**	**30 (100.0%)**	**0.000**

Menstrual Cycle	**Irregular**	**21 (70.0%)**	**20 (66.7%)**	**0 (0.0%)**	**0 (0.0%)**	
Conception of Pregnancy	**Yes**	**10 (33.3%)**	**4 (13.3%)**	**30 (100.0%)**	**30 (100.0%)**	**0.000**
	No	**20 (66.7%)**	**26 (86.7%)**	**0 (0.0%)**	**0 (0.0%)**	
Fatty Food	**Yes**	**17 (56.7%)**	**19 (63.3%)**	**7 (23.3%)**	**4 (13.3%)**	**0.000**
	No	**13 (43.3%)**	**11 (36.7%)**	**23 (76.7%)**	**26 (86.7%)**	

Variables		Group A	Group B	Group C	Group D	p-value#
Exercise	Yes	4 (13.3%)	2 (6.7%)	10 (33.3%)	12 (40.0%)	0.005
	No	26 (86.7%)	28 (93.9%)	20 (66.7%)	18 (60.0%)	
Miscarriages	Yes	6 (20.0%)	4 (13.3%)	0 (0.0%)	1(3.3%)	0.028
	No	24 (80.0%)	26 (86.7%)	30 (100.0%)	29 (96.7%)	
Weight Gain	Yes	21 (70.0%)	26 (86.7%)	4 (13.3%)	3 (10.0%)	0.000
	No	9 (30.0%)	4 (13.3%)	26 (86.7%)	27 (90.0%)	
Hirsutism	Yes	25 (83.3%)	21 (70.0%)	1 (3.3%)	3 (10.0%)	0.000
	No	6 (16.7%)	9 (30.0%)	29 (96.7%)	27 (90.0%)	
Acne	Yes	25 (83.3%)	23 (76.7%)	3 (10.0%)	7 (23.3%)	0.000
	No	6 (16.7%)	7 (23.3%)	27 (90.0%)	23 (76.7%)	
Pigmentation	Yes	17 (56.7%)	14 (46.7%)	1 (3.3%)	2 (6.7%)	0.000
	No	13 (43.3%)	16 (53.3%)	29 (96.7%)	28 (93.3%)	

CONCLUSION:

We can conclude that PCO not related to obesity. both non-obese or lean women also develop PCO. But the level of proinflammatory marker visfatin and α TNF are higher in obese women. its mean obese women are suspectable to produce cyst and develop PSCOS and related disorders. These markers can be use as early diagnostic marker and its antagonist can be used for prognosis. symptom cane be cure by exercise and avoiding junk food.

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