

Dipeptidyl Peptidase 4 in Women with Polycystic Ovarian Syndrome

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Abstract

Background: Polycystic ovary syndrome (PCOS) is the most prevalent reproductive endocrinopathy and currently considered to be a part of the metabolic syndrome. Women suffering from PCOS present with a group of symptoms related with menstrual disorder and androgen excess. Women with PCOS cluster risk factors associated with risk of insulin resistance, dyslipidemia, hyperandrogenemia, obesity, cardiovascular disease (CVD), infertility, and psychological disorders. **Objective :** The study was designed to determine the levels of DPP4 in PCOS patients and compared with control and study the correlation with biomarkers in serum of PCOS patients.

Subjects and Method: The study is case-control included 80 females and carried out from February 2019 till May 2019 for females in the reproductive age [18-45 years old], forty females were came from gynecological and obstetric hospital, in Karbala, and they all diagnosed by their physicians have polycystic ovarian syndrome and compared with forty control females {healthy females}. plasma activity of DPP4 and biochemical variables were performed.

Results: Results obtained in this study showed that there are significant differences ($p < 0.05$) in serum of DPP4, SHBG, DHEA-S, LH, FSH, free testosterone and total testosterone and there is positive significant correlation between DPP4 with free testosterone, SHBG, DHEA-S, LH and total testosterone.

Conclusion: Polycystic ovarian syndrome is a higher risk of type 2 diabetic mellitus, dyslipidemia and cardiovascular diseases, Dipeptidyl peptidase 4 (DPP4) was high in PCOS patient and had positive correlation with free testosterone, total testosterone, DHEA-S, SHBG and LH.

Keyword: Polycystic, Dipeptidyl, DPP4, (PCOS).

Introduction

Polycystic ovary syndrome (PCOS) is a female endocrine disorder featuring elevated concentrations of androgen, ovulatory dysfunction¹, its prevalence ranges from 9% to 18% in reproductive-aged women based on definitions and populations studied, it is associated

with reproductive, (menstrual irregularity, infertility and pregnancy complications), metabolic (metabolic syndrome, type 2 diabetes (T2D) and cardiovascular disease (CVD) and psychological (anxiety and depression),².

Dipeptidyl peptidase4 (DPP4) or (CD26), is a 110kDa glycoprotein expressed ubiquitously on the surface of different cell 3Soluble DPP4 (sDPP4) was regarded as a novel adipokine, It is involved in the catalytic degradation of glucagon like peptide-1 [GLP-1], which suggests main function in metabolism³, GLP-1 stimulates insulin secretion and suppresses glucagon secretion, GLP-1 also controls gastric emptying, body

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weight in relation to its impacts on glucose homeostasis. Inhibition of DPP4 results in increased concentrations of active GLP-1 and subsequent improvement of insulin release i.e. Vildagliptin increases insulin release rather than increase insulin sensitivity

Materials and Method

The case control study carried out from February 2019 till May 2019 of a total number of 80 females within the reproductive age (18 to 45 years old). forty cases females out of 80 were attended from gynecological and obstetric hospital in Karbala province, and they all diagnosed by their physicians as Polycystic Ovarian Syndrome, (depending on clinical, biochemical and Rotterdam criteria), and they were compared with forty healthy control women.

Inclusion Criterias: All PCOS patients have already been diagnosed and the diagnosis has been confirmed by the European human reproduction society and embryology and American society for reproductive medicine criteria.,

PCOS is diagnosed if there are any two of the following:

- Presence of PCOS on ultrasound examination.
- Menstrual disorders with an ovulation.
- Clinical or biochemical hyperandrogen.

Exclusion Criterias:

All Patients with history of medical condition

Other disorders that may affect menstrual regularity and hyperandrogenism such as thyroid dysfunction, and pituitary disease that related with hyperprolactinemia.

Collection of Sample: Blood specimens were collected; 5 ml venous blood samples were taking during 2nd – 5th day of the menstrual cycle (early follicular phase) for those of normal cycle. For patients with anovulation or oligomenorrhea blood sample were collected regardless of duration of the cycle. Blood samples were left for 20 minutes at room temperature. After coagulation, the serum was separated via centrifuge at (3000 run per minute) for five min., hemolysed samples were discarded. Sera were stored and frozen at - 40 °C until analysis.

Determination of Human Dipeptidyl Peptidase IV (DPP4) Principle: This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human DPP4.

Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody.

Then a biotinylated detection antibody specific for Human DPP4 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away.

The substrate solution is added to each well. Only those wells that contain Human DPP4, biotinylated detection enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow.

The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

The OD value is proportional to the concentration of Human DPP4. You can calculate the concentration of Human DPP4 in the samples by comparing the OD of the samples to the standard curve.

Detection range (0.31-20 ng/ml)

Results

A total number of 80 women were taken with age ranged between (18-45) years and divided into 40 infertile PCOS females with and 40 healthy control females, The diagnosis depends on biochemical features (raised LH, LH/FSH ratio and/or increased testosterone, decrease FSH); clinical features include irregular menstrual cycle, hirsutism, and obesity. PCOS has been confirmed by ultrasound

Comparison between polycystic ovary syndrome cases group and control group in the parameters measured:

The results obtained in this study showed that there is a no significant difference (P >0.05) in FSH and BMI while the results showed that there a significant difference (p< 0.05) in serum total testosterone, free testosterone, SHBG DHAE-S, LH and DPP4 as shown in table (1):

Table (1): Biochemical parameters in patients with polycystic ovary syndrome compared with control group.

Parameter	Patient group N= 40 Mean ± SD	Control group N= 40 Mean ± SD	P value
BMI (kg/m ²)	27.57 ± 3.73	27.8 ± 5.28	NS
DPP4 (ng/ml)	3.37 ± 0.55	1.93± 0.19	P<0.05
SHBG (pmol/ml)	30.58 ± 3.15	23.55 ± 1.51	P<0.05
DHEAS (ng/ml)	83.02 ± 9.87	61.73 ± 5.01	P<0.001
Total testosterone (ng/ml)	0.69 ± 0.021	0.26 ± 0.014	P<0.05
LH (m.lu/ml)	13.73 ± 0.63	9.93 ± 0.31	P<0.001
FSH (m.lu/ml)	6.81 ± 0.34	8.85 ± 0.69	NS
Free testosterone (pmol/ml)	30.51 ± 1.66	19.69 ± 0.89	P<0.05

Correlation between DPP4 with biochemical parameters in patient.

The results of linear regression analysis show significant positive correlation in serum DPP4 concentration with free testosterone, total testosterone, LH, SHBG and DHEA-S as shown in table (2):

Table (2): Correlation between DPP4, DHAЕ and SHBG with biochemical parameters in patient.

	DPP4
BMI (kg/m ²)	r = -0.003 p = 0.979
Free testosterone (pmol/ml)	r = 0.375** p = 0.01
Total testosterone (ng/ml)	r = 0.319** p = 0.04
LH (m.lu/ml)	r = 0.222* p = 0.04
FSH (m.lu/ml)	r = 0.038 p = 0.739
SHBG (pmol/ml)	r = -0.25* p = 0.02
DHEA-S (ng/ml)	r = 0.22* p = 0.04

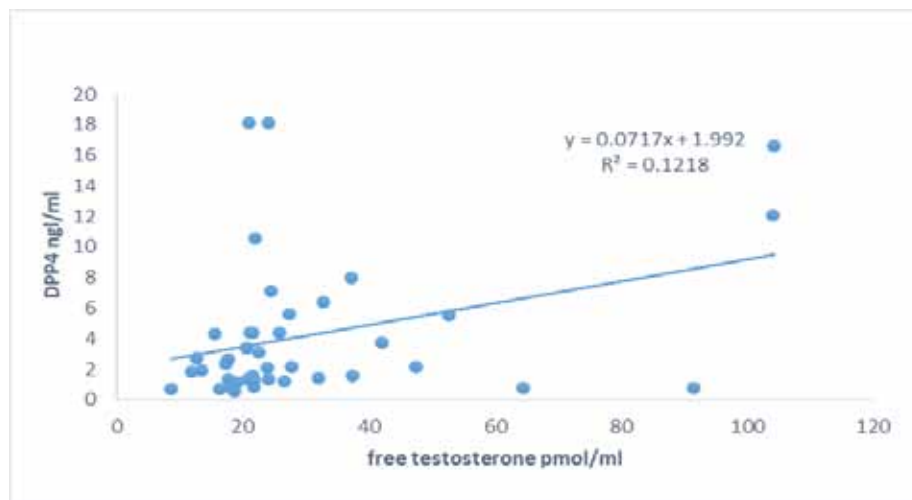


Figure (1): The correlation of serum levels of DPP4 (ng/ml) with free testosterone (pmol/ml) in the patient group.

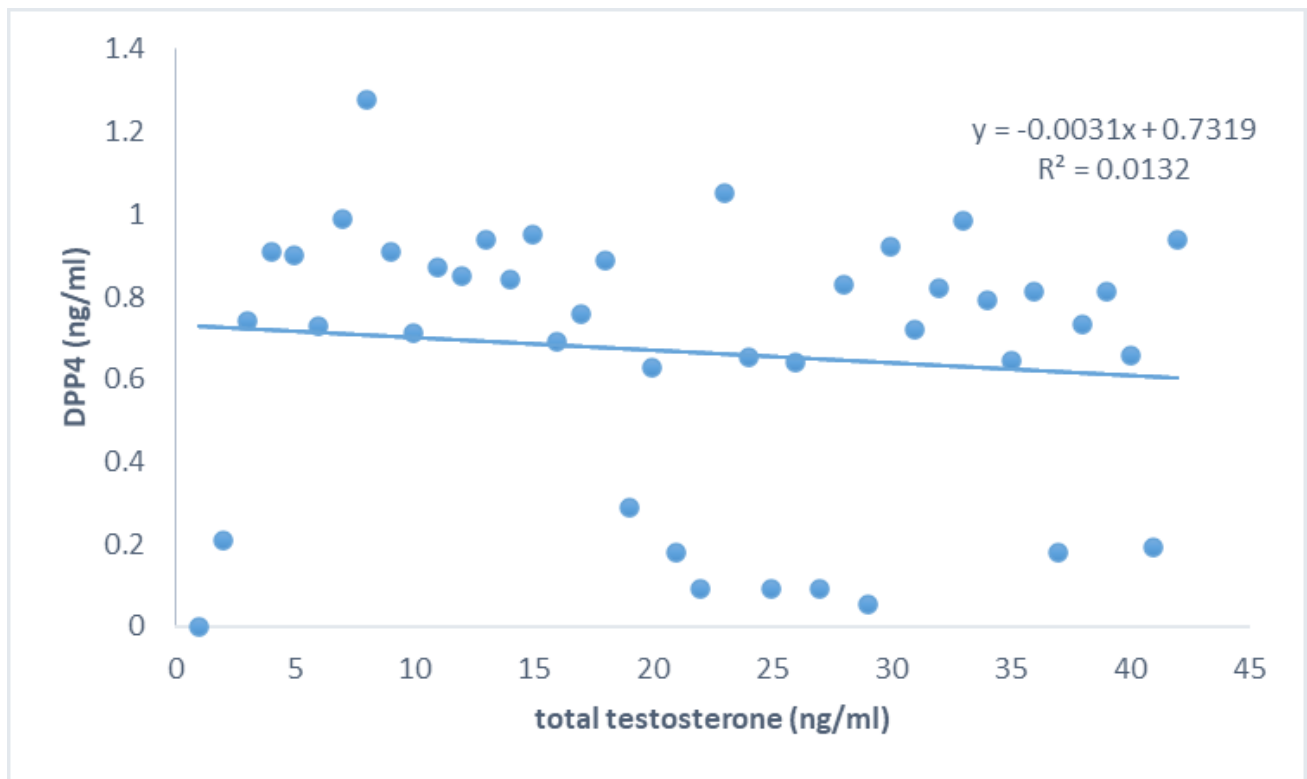


Figure (2) The correlation of serum levels of DPP4 (ng/ml) with total testosterone (ng/ml) in the patient group.

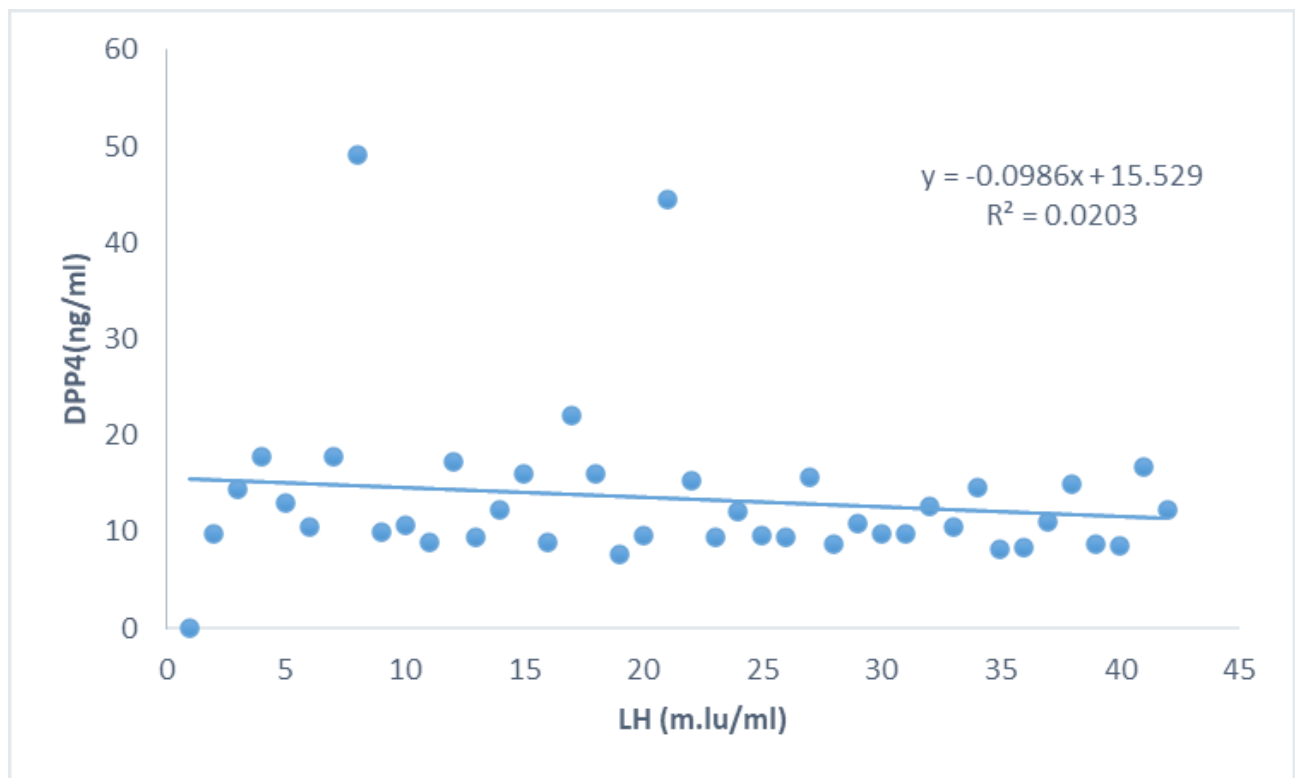


Figure (3) The correlation of serum levels of DPP4 (ng/ml) with LH (m.lu/ml) in the patient group.

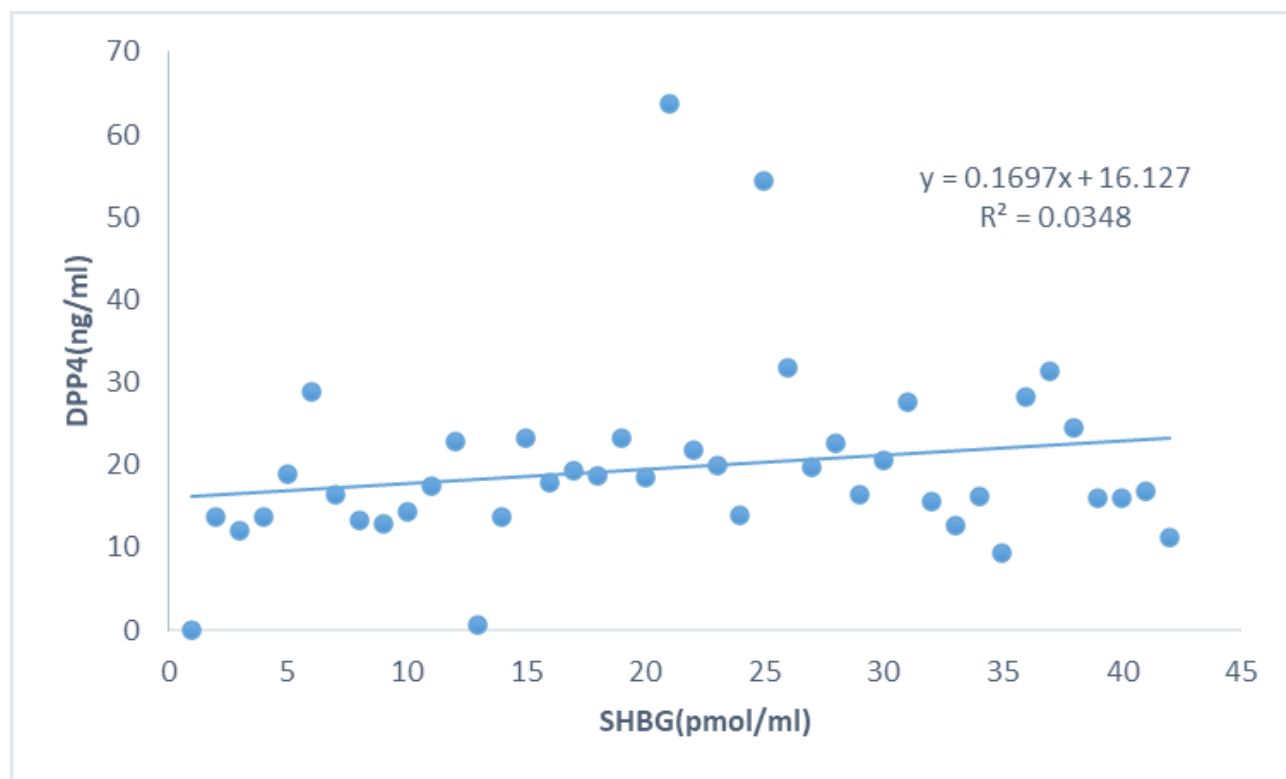


Figure (4) The correlation of serum levels of DPP4 (ng/ml) with SHBG (pmol/ml) in the patient group

Discussion

Polycystic ovarian syndrome (PCOS) is the most prevalent endocrinopathy of females of reproductive age (PCOS is a heterogeneous disease it impacts at 7 percent of females, According to the National Institutes of Health Office for Disease Prevention 4).

The three many prevalent factors connected with PCOS involve ovulation irregularities, enhanced concentrations of androgen, and cystic ovaries, ovulation Problems and high levels of androgen happen in the majority of females with PCOS, in addition, hirsutism, menstrual irregularity, acne, and alopecia are immediately associated with high androgen levels, and the incidence of polycystic ovaries in pelvic ultrasound exceeds 70% in PCOS patients⁴.

In this present study, there was a significant increase in the mean of DPP4 when compared patient with control groups at $p < 0.05$, this results is agreement with the result of⁵.

These result indicated that a deregulation of level of DPP4 might be extra feature of the metabolic inequalities related with PCOS, Although PCOS was well defined

as IR and hyperandrogen, DPP4 was not different in this group. However, a connection between DPP4 and markers of IR were found.⁶

Dipeptidyl peptidase 4 inactivates both incretin hormone (GIP, GLP-1), leading to high blood glucose and insulin resistance therefore DPP4 inhibitors are used in the treatment of type 2 diabetes several previous studies demonstrating strong association of the syndrome with IR, more than half of patients with PCOS had IR.

DPP4 inhibitors can help patients with diabetes decrease IR, reduce glycosylated hemoglobin androgen levels also correlate positively with IR in PCOS patients because hyperandrogenism induces IR by reducing insulin clearance and increasing lipoprotein lipase activity and triacylglycerol release Compensatory hyperinsulinemia in turn further aggravates hyperandrogenism SHBG lowers free androgen levels by binding free androgens to alleviate hyperandrogenism and IR⁷.

The presented study indicate that serum SHBG was a significant decrease in the mean SHBG when compared patients with control group at $p < 0.05$.

Our results are in accordance with the previous

work done by ⁸⁻¹⁰. Who showed that hyperandrogenism carried a significant risk of hyperinsulinemia due to stimulation of ovarian androgen secretion and inhibition of hepatic SHBG production and this results agree with inverse positive correlation between DPP4 and SHBG, figure (4).

The results in table (1) show a significant increase differences in DHES when compared patients with control group compared at $p < 0.001$.

These results were in agreement with some previous studies¹¹⁻¹³

Who showed the abnormally high levels of DHEA-S occur in about 20-30% of women with PCOS.

One explanation for elevated DHEAS levels in PCOS might be found in an altered cortisol metabolism. In particular, it has been shown that the peripheral metabolism of cortisol is increased in PCOS due to the enhanced inactivation of cortisol by 5 α -reductase or impaired reactivation of cortisol from cortisone by 11 β -hydroxysteroid dehydrogenase type 1. This could lead to a decreased negative feedback on ACTH, resulting in increased pituitary adrenal axis activity, including androgen synthesis to maintain normal cortisol levels. Moreover, increased peripheral sulfatase activity has been observed in women with PCOS, which might increase circulating DHEAS levels

The results of the present study showed a statistically significant positive correlation between DPP4 with DHEA-S.

The results in table (1) show a significant increase differences in total testosterone and free testosterone when compared patients with control group compared at $p < 0.05$.

Our results are in accordance with the previous work done by ¹⁴⁻¹⁷

Several Studies showed that women with diabetes had higher level of free testosterone comparing women without diabetes. therefore, reduction in the sex hormone binding globulin (SHBG) and high levels of free testosterone are accepted for the occurrence of diabetes type 2 and highlights the relationship between androgens and insulin sensitivity

Hyperinsulinemia could result in hyperandrogenism by increasing androgen production, decreasing androgen

catabolism, or increasing the tissue availability of T by decreasing serum SHBG levels ¹⁸

The results of the present study showed a statistically significant positive correlation between DPP4 with total testosterone figure (1), this is agreement with the finding of studies, and between DPP4 with free testosterone, figure (2). This is in agreement with In this study on case groups when compared with the control groups, found a significant difference in the concentrations of LH hormone $p < 0.05$ and no significant with FSH ^{.19} observed that elevated LH and LH to FSH proportion, 75% of PCOS females had an increased LH concentration and 94% increased LH to FSH proportion. These finding is similar to finding of ²⁰ indicated the plasma LH concentration for females with PCOS is increased. Similar results were found in a study done by ²¹ who showed that PCOS females had greater concentration of serum LH than normal females.

The data of the current study showed that there is positive significant correlations between serum DPP4 with LH, figure(3),this result agreement with 6 therefore several studies demonstrated that DPP4 inhibited androgen production by theca cells and also suggested that DPP4 inhibitors reduces pituitary luteinizing hormone and increases the production of SHBG

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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