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Original Article

Serum chemerin levels in Polycystic Ovary Syndrome after metformin therapy

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ABSTRACT

Objectives: The aim of this study was to study the effects of metformin therapy on serum chemerin levels in some phenotypes of polycystic ovarian syndrome cases, and to correlate chemerin levels with insulin resistance parameters and with hormonal profile.
Material and methods: This study was carried on 100 polycystic ovary cases and 70 control women. These cases were further subdivided into obese and normal weight cases. Fasting serum chemerin was measured by Enzyme-Linked Immunosorbent Assay method. Data were analyzed using SPSS for Windows 7.
Results: Before metformin therapy, the serum chemerin were significantly increased in PCOS cases as compared with the control cases. Also, a significantly higher chemerin levels were found in obese polycystic ovarian syndrome cases as compared with normal weight cases with polycystic ovarian syndrome. The serum chemerin levels were significantly positively correlated with glucose levels, insulin levels, and HOMA-IR in polycystic ovarian syndrome cases. After three months of metformin therapy, the serum chemerin, insulin, and HOMA-IR concentrations were significantly decreased in polycystic ovarian syndrome cases as compared with the levels before therapy.
Conclusion: The serum chemerin levels were significantly higher in cases of PCOS cases as compared with the controls. Metformin therapy resulted in a significant decrease in chemerin levels in polycystic ovarian syndrome cases.
The analysis of Receiver Operation Characteristic curves of serum chemerin suggested that serum chemerin levels may be of value to evaluate the polycystic ovarian syndrome cases under various methods of treatments.

1. Introduction

Poly-Cystic Ovary Syndrome (PCOS) is a syndrome characterized by menstrual dysfunction and hyperandrogenism, and is associated with insulin resistance [1].

Adipose tissue secretes various bioactive adipokines that play important roles in the regulation of many physiological processes [2]. In PCOS, visceral adipocytes have a role in the pathogenesis of metabolic disorders by interrupting insulin intracellular signaling in these cells resulting in impaired glucose intolerance and insulin resistance [3]. Thus, chemerin may contribute to the development of metabolic syndrome and PCOS [4].

Chemerin, an adipokine, is synthesized as an inactive precursor, prochemerin, which is rapidly converted to its active form by proteolytic cleavage. Chemerin plays a role in insulin sensitivity and insulin secretion [5], angiogenesis [6], and adipogenesis [7]. It was reported that serum chemerin concentrations are strongly associated with features of the metabolic syndrome (MS) such as obesity, and insulin resistance [8].

Tan et al. [3] found a significant increase in serum chemerin in patients with PCOS, suggesting that elevated chemerin levels in PCOS cases may be a compensatory mechanism to insulin resistance.

Metformin therapy in both lean and obese cases with PCOS was found to result in a significant decrease in serum testosterone and

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in LH levels, and an increase in FSH levels as well as normalization of the LH/FSH ratio, resulting in restoring menstrual cyclicity. Metformin therapy was effective in achieving ovulation either alone or when combined with clomiphene citrate [9,10], leading to increased rates of spontaneous ovulation [10].

1.1. Objectives

The aims of this study were to assess the serum chemerin levels in normal obese and thin women, to compare between serum levels of chemerin in normal and PCOCS patients, to study the effects of metformin therapy on the serum chemerin levels in obese and in normal weight cases with PCOS, and to correlate the chemerin levels with insulin resistance and with hormonal profile.

2. Material and methods

2.1. Participants

The present study was a cross sectional study carried on 100 PCOS cases and 70 control cases attending for management of primary infertility at the Department of Obstetrics and Gynecology, Faculty of Medicine, Mansoura University, Egypt, during the period between January 2016 and July 2018, in accordance with the inclusion/exclusion criteria.

Inclusion criteria: Women with PCOS were diagnosed according to the revised Rotterdam criteria [11], by the presence of two of the following three manifestations: (i) oligo-ovulation/or anovulation, (ii) clinical and/or biochemical hyperandrogenism, and (iii) polycystic ovaries on ultrasound.

The age of the patients ranged between 21 & 26 years, the cases had primary infertility with duration of 2–3 years. PCOS cases were diagnosed during the infertility investigations, and the cases were without previous ovarian drilling or hormonal therapy.

The control cases had regular periods and normal findings on pelvic ultrasound scan.

The PCOS cases and control cases were further subdivided into obese and normal weight cases.

Exclusion criteria for the study included known cardiovascular disease, renal impairment, diabetes mellitus, thyroid disease, other endocrinopathies, neoplasms, current smoking, hypertension, and hormonal therapy or anti-hypertensive medication for at least 6 months before the study.

2.1.1. This clinical study involved the following groups

Group 1-before metformin therapy: 50 obese PCOS cases (BMI ≥30–<35 kg/m^2) before administration of metformin therapy.

Group 1-after metformin therapy: 33 of the studied obese PCOS cases were followed three months after administration of metformin therapy starting by one tablet (500 mg) once per day for one week, then two tablets/day for the second week, followed by 3 tablets/day to complete the three months. Seventeen cases discontinued during the second visit (after three months), five cases due to pregnancy (10%) and twelve cases due to gastrointestinal manifestations as nausea and vomiting (24%). The data of these 33 cases after 3 months of metformin therapy were compared with their data before administration of metformin for statistical analysis (paired *t*-test).

Group 2-before metformin therapy: 50 normal weight PCOS cases (BMI <25 kg/m^2) before administration of metformin therapy.

Group 2-after metformin therapy: 35 normal weight cases with PCOS (BMI <25 kg/m2) were followed at three months after administration of metformin therapy (as mentioned in group 1-before therapy). Fifteen cases discontinued during the second visit after three months due to pregnancy (six cases, 12%), or due to nausea and vomiting (nine cases, 18%).

The data of these cases after three months of metformin therapy were compared with their data at the start of the study for statistical analysis (paired *t*-test).

Group 3: consisted of 35 obese control women (BMI ≥30–<35 kg/m2).

Group 4: consisted 35 normal weight control women (BMI <25).

Obesity was defined as BMI ≥30–<35 kg/m^2, and normal weight as BMI <25 kg/m^2, according to the WHO criteria.

Sample size calculation was determined by using creative research systems, the survey software-system. The sample size was calculated by using the mean incidence of PCOS in cases with infertility as 55% [11], the confidence level of 95% (α) & confidence interval of 13.36; the sample size was found to be 35 cases in each group.

The power of the study was calculated to be 0.9.

2.2. Ethical approval

The study was approved by the IRB committee of Faculty of Medicine, Mansoura University, Egypt [code number: R/15.08.81, date: 24/1/2016].

Informed written consents were taken from the participants.

2.3. Sampling

All blood samples were obtained during the early follicular phase (day two to four of the cycle). Maternal venous blood (5 ml) was withdrawn after an overnight fast (10–12 h). All samples were collected and the serum was separated by centrifugation and was divided into two parts: the first part was used for assessment of fasting blood glucose, insulin and hormones; and the second part was stored at −80 °C till the time of assay of chemerin.

2.4. Methods of assay

- Serum levels of glucose were measured by the enzyme-calorimetric methods.
- Insulin was measured by electro-chemiluminescence immunoassay.
- Homeostasis Model of Assessment-Insulin Resistance (HOMA-IR) index for the assessment of insulin resistance was measured by calculation using the Matthews et al., equation [12].

$$\text{HOMA-IR} = [\text{glucose (mg/dl)} \times \text{insulin (mIU/ml)}] \div 405.$$

- Hormonal parameters such as LH, FSH, free testosterone, and total testosterone were analyzed by immune chemiluminescence method.
- Serum chemerin concentrations were measured with enzyme-linked immune-sorbent assay (ELIZA) by using kits supplied by BioVendor Research Company, with Cat. No. RD191136200R. This assay recognizes recombinant and natural human chemerin. The sensitivity of this assay, or lower limit of detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

2.5. Statistical analysis

Quantitative data were found to be normally distributed as confirmed by the Shapiro-Wilk test. Mean and standard deviation were used to describe data.

Chi-square test (X^2) test was used to compare the clinical qualitative data.

Paired *t*-test (2-tailed) was used to test for significant change in quantitative data between cases before and three months after metformin therapy. Unpaired *t*-test was used for to test for significant change in quantitative data between study cases and controls.

Linear correlation relationship between quantitative variables was tested by Pearson product moment. p value < 0.05 was considered significant. Statistical analysis was performed by using Statistical Package for Social scientists (SPSS) for Windows 7 (SPSS Inc., Chicago, IL, USA) run on IBM personal computer.

3. Results

Table 1 represents the clinical features in group-1 cases and in group 2 cases before and after metformin therapy and the significance of changes was assessed by using Chi square test.

After metformin therapy, the menstrual cycles were found to be significantly regular after metformin therapy in group 1 and in group 2 ($p = 0.015$, & $p = 0.005$, respectively).

Also, there were a significant reduction in the numbers of cases with oligomenorrhea after metformin therapy as compared with the number before therapy in group 1 ($p = 0.002$) and in group 2 ($p = 0.049$).

Acne was not significantly affected after metformin therapy in group 1 and in group 2 ($p = 0.591$ & $p = 0.531$, respectively). Also, hirsutism was not affected in group 1 and in group 2 ($p = 0.193$ & $p = 102$, respectively).

After three months of metformin therapy, pregnancy occurred in 10% in group 1 and in 12% in group 2. There was no significant difference in pregnancy rates after 3 months of metformin therapy between the obese and normal weight cases with PCOS ($p = 0.709$).

Table 2 represents the chemerin levels, anthropometric and metabolic values in group 1 before metformin therapy as compared with group 1 after metformin therapy.

The obese PCOS cases after three months of metformin therapy (group 1-after therapy) had significantly lower chemerin levels as compared with the non-treated PCOS cases (group 1-before metformin therapy ($p = 0.009$).

In group 1 after metformin therapy there were a significant decrease in the values of WHR ($p = 0.017$), serum insulin ($p = 0.0001$), HOMA-IR ($p = 0.0001$), LH ($p = 0.0001$), LH/FSH ($p = 0.0001$), free testosterone ($p = 0.012$), and total testosterone ($p = 0.041$) as compared with the values in group 1 before therapy. The levels of FSH were significantly increased after metformin therapy ($p = 0.004$).

BMI was not significantly decreased in group 1 after metformin therapy ($p = 0.066$).

Table 3 represents the chemerin levels, anthropometric and metabolic values in group 2 before metformin therapy as compared with group 2 after metformin therapy.

The normal weight PCOS cases after three months of metformin therapy (group 2-after therapy) had a significantly lower chemerin levels as compared with group 2-before metformin therapy ($p < 0.0001$).

In group 2 after metformin therapy there were significantly lower values of fasting serum glucose ($p = 0.0001$), insulin ($p = 0.0001$), HOMA-IR ($p = 0.0001$), LH ($p = 0.0001$), LH/FSH ($p = 0.0001$), free testosterone ($p = 0.0304$), and total testosterone ($p = 0.043$) values as compared with the values in group 2 before metformin therapy. The levels of FSH were significantly increased after metformin therapy ($p = 0.005$).

BMI was not significantly decreased in group 2 after metformin therapy ($p = 0.094$).

Table 4 represents the chemerin levels, anthropometric and metabolic values in group 1 before metformin therapy versus control group 3.

Group 1 before metformin therapy had an increased chemerin levels, fasting blood glucose, insulin, HOMA-IR, LH, LH/FSH, free testosterone levels, total testosterone values as compared with the control group 3 values ($p < 0.0001$), but the levels of FSH were not significantly increased in group 1 before metformin therapy as compared with the control group 3 ($p = 0.621$).

Table 5 represents the chemerin levels, anthropometric and metabolic values in group 2 before metformin therapy as compared with control group 4.

Group 2 before metformin therapy had a significantly increased values of chemerin levels, fasting blood glucose, insulin, HOMA-IR, LH, LH/FSH, total testosterone values as compared with the control group 4 values ($p < 0.0001$), but the levels of FSH were not significantly increased in group 2 before metformin therapy as compared with the control group 4 ($p = 0.469$). The free testosterone levels were significantly increased in group 2 before metformin therapy as compared with the control group 4 values ($p = 0.015$).

Table 6 represents the correlation between fasting serum chemerin levels and other parameters studied.

In obese PCOS cases before metformin therapy (group 1 before therapy), chemerin levels were significantly positively correlated with BMI ($p = 0.044$), fasting serum glucose levels ($p < 0.0001$), insulin levels ($p < 0.0001$), HOMA-IR values ($p = 0.002$), and LH levels ($p = 0.016$).

In the normal weight PCOS cases before metformin therapy

Table 1
Chi square test for qualitative data before and after metformin therapy.

		Before therapy		After therapy			
		Number	%	Number	%	χ^2	p value
Regular cycles	Group 1[a]	14/50	28%	18/33	60.06%	5.913	0.015
	Group 2[b]	16/50	32%	22/35	2.86%	7.93	0.005
Oligo-menorrhea	Group 1[a]	34/50	68%	11/33	33.33%	9.62***5***	0.002
	Group 2[b]	29/50	58%	10/35	28.57%	3.89	0.049
Hirsutism	Group 1[a]	30/50	60%	15/33	45.45%	1.69	0.193 (***NS***)
	Group 2[b]	30/50	60%	10/35	28.57%	2.679	0.102 (***NS***)
Acne	Group 1[a]	18/50	36%	10/33	30.30%	0.289	0.591 (***NS***)
	Group 2[b]	16/50	32%	9/35	25.71%	0.392	0.531 (***NS***)
Pregnancy	Group 1[a]	–	–	5/50	10.0%	0.102	0.749 (***NS***)
	Group 2[b]	–	–	6/50	12.0%		

The number of pregnancies was calculated in relation to the total number of cases at the start of study (50 cases).
(***NS***): p value > 0.05: is not statistically significant.
[a] Group 1 before metformin therapy: 50 cases & Group 1 after therapy: 33 cases.
[b] Group 2 before metformin therapy: 50 cases & Group 2 after therapy 35 cases.

Table 2
Serum chemerin levels, anthropometric and metabolic values (mean ± SD) in group 1 before and after metformin therapy.

	Group 1 before metformin therapy (33 cases)	Group 1 after metformin therapy (33 cases)	*p* value (paired *t*-test)
Chemerin (ng/ml)	294.66 ± 55.82	263.63 ± 38.76	=0.009
BMI	31.13 ± 1.09	30.59 ± 0.71	0.066 (***NS***)
WHR	0.882 ± 0.028	0.866 ± 0.026	=0.017
FBGL (mg/dl)	103.17 ± 6.424	102.914 ± 4.83	=0.850 (***NS***)
Insulin (mIU/ml)	15.543 ± 1.569	10.829 ± 1.404	<0.0001
HOMA-IR	4.065 ± 0.745	2.761 ± 0.431	<0.0001
FSH (mIU/ml)	4.04 ± 0.343	4.269 ± 0.305	=0.004
LH (mIU/ml)	8.33 ± 0.77	5.26 ± 0.79	<0.0001
LH/FSH	2.099 ± 0.152	1.26 ± 0.199	<0.0001
F. testo (pg/ml)	4.5 ± 1.098	3.854 ± 0.989	=0.012
T. testo (ng/ml)	0.709 ± 0.131	0.628 ± 0.181	=0.041

FBGL: fasting blood glucose levels, BMI: Body Mass Index, WHR: Wist-Hip Ratio.
HOMA-IR: Homeostasis Model of Assessment-Insulin Resistance Index.
FSH: follicle stimulating hormone, LH: luteinizing hormone.
LH/FSH: luteinizing hormone: follicle stimulating hormone ratio.
F. testo.: free testosterone, & T. testo.: total testosterone.
(***NS***): *p* value > 0.05: is not statistically significant.

Table 3
Serum chemerin levels, anthropometric and metabolic values (mean ± SD) in group 2 before and after metformin therapy.

	Group 2 before metformin therapy (35 cases)	Group 2 after metformin therapy (35 cases)	P value (paired *t*-test)
Chemerin (ng/ml)	253.57 ± 38.70	183.057 ± 24.397	<0.0001
BMI	24.657 ± 0.328	24.47 ± 0.248	=0.094 (***NS***)
WHR	0.839 ± 0.03	0.829 ± 0.013	=0.076 (***NS***)
FBGL (mg/dl)	109.2 ± 7.677	101.886 ± 5.362	<0.0001
Insulin (mIU/ml)	12.572 ± 2.536	10.329 ± 0.581	<0.0001
HOMA-IR	3.426 ± 0.892	2.628 ± 0.357	<0.0001
FSH (mIU/ml)	4.109 ± 0.35	4.369 ± 0.399	=0.005
LH (mIU/ml)	8.646 ± 0.984	5.063 ± 0.851	<0.0001
LH/FSH	2.08 ± 0.02	1.436 ± 0.233	<0.0001
F. testo (pg/ml)	4.4 ± 0.72	4.06 ± 0.547	=0.0304
T. testo (ng/ml)	0.623 ± 0.089	0.592 ± 0.043	=0.043

FBGL: fasting blood glucose levels, BMI: Body Mass Index WHR: Wist-Hip Ratio.
HOMA-IR: Homeostasis Model of Assessment-Insulin Resistance Index.
FSH: follicle stimulating hormone, LH: luteinizing hormone.
LH/FSH: luteinizing hormone: follicle stimulating hormone ratio.
F. testo.: free testosterone, & T. testo.: total testosterone.
(***NS***): *p* value > 0.05: is not statistically significant.

Table 4
Serum chemerin levels, anthropometric and metabolic values (mean ± SD) in group 1 before metformin therapy as compared with the control groups 3.

	Group 1 before metformin therapy (50 cases)	Group 3 (35 cases)	P1 (Group 1 before Vs gr 3)
Chemerin (ng/ml)	296.79 ± 50.84	231.6 ± 36.81	<0.0001
BMI	31.15 ± 1.11	31.43 ± 1.209	=0.256 (***NS***)
WHR	0.89 ± 0.03	0.881 ± 0.027	=0.143 (***NS***)
FBGL (mg/dl)	104.0 ± 5.82	92.8 ± 5.589	<0.0001
Insulin (mIU/ml)	15.62 ± 1.569	10.786 ± 1.70	<0.0001
HOMA-IR	4.12 ± 0.72	2.52 ± 0.527	<0.0001
FSH (mIU/ml)	4.03 ± 0.41	4.011 ± 0.415	=0.621 (***NS***)
LH (mIU/ml)	8.29 ± 0.76	4.74 ± 0.58	<0.0001
LH/FSH	2.1 ± 0.16	1.212 ± 0.199	<0.0001
F. testo (pg/ml)	4.48 ± 0.74	2.48 ± 0.47	<0.0001
T. testo (ng/ml)	0.856 ± 0.093	0.489 ± 0.078	<0.0001

FBGL: fasting blood glucose levels, BMI: Body Mass, Index WHR: Wist-Hip Ratio.
HOMA-IR: Homeostasis Model of Assessment-Insulin Resistance Index.
FSH: follicle stimulating hormone, LH: luteinizing hormone.
LH/FSH: luteinizing hormone: follicle stimulating hormone ratio.
F. testo.: free testosterone, & T. testo.: total testosterone.
(***NS***): *p* value > 0.05: is not statistically significant.

(group 2 before therapy), chemerin levels were significantly positively correlated with fasting serum glucose ($p = 0.016$), fasting serum insulin ($p = 0.002$), and HOMA-IR values ($p = 0.001$).

Figs. 1 and 2 represent ROC curve analysis of serum chemerin in obese and in normal weight PCOS cases before metformin therapy.

The Receiver Operation Characteristic (ROC) curve analysis of serum chemerin in group 1 before metformin therapy versus control group 3 shows that the area under curve was 0.834, sensitivity was 74.3%, and specificity was 91.4%, at a cut off levels of 269 ng/ml.

ROC curve analysis of serum chemerin in group 2 before metformin therapy versus control group 4 shows that the area under

Table 5
Serum chemerin levels, anthropometric and metabolic values (mean ± SD) in group 2 before metformin therapy as compared with the control groups 4.

	Group 2 before metformin therapy (50 cases)	Group 4 (35 cases)	P1 (Group 2 before Vs gr 4)
Chemerin (ng/ml)	256.4 ± 51.29	109.743 ± 1.8	<0.0001
BMI	24.64 ± 0.36	23.914 ± 0.55	=0.342 (***NS***)
WHR	0.83 ± 0.04	0.722 ± 0.02	=0.869 (***NS***)
FBGL (mg/dl)	108.02 ± 7.87	84.229 ± 6.53	<0.0001
Insulin (mIU/ml)	12.36 ± 2.43	8.543 ± 0.95	<0.0001
HOMA-IR	3.33 ± 0.87	1.787 ± 0.318	<0.0001
FSH (mIU/ml)	4.2 ± 0.41	4.12 ± 0.586	0.469
LH (mIU/ml)	8.67 ± 0.92	5.254 ± 0.781	<0.0001
LH/FSH	2.19 ± 0.6 2	1.281 ± 0.307	<0.0001
F. testo (pg/ml)	4.62 ± 0.92	3.397 ± 0.692	=0.015
T. testo (ng/ml)	0.823 ± 0.086	0.523 ± 0.051	<0.0001

FBGL: fasting blood glucose levels, BMI: Body Mass Index, WHR: Wist-Hip Ratio.
HOMA-IR: Homeostasis Model of Assessment-Insulin Resistance Index.
FSH: follicle stimulating hormone, LH: luteinizing hormone.
LH/FSH: luteinizing hormone: follicle stimulating hormone ratio.
F. testo.: free testosterone, & T. testo.: total testosterone.
(***NS***): p value > 0.05: is not statistically significant.

Table 6
Correlation between chemerin levels and studied variables.

	Gr 1-before		Gr-1-after		Gr 2-before		Gr 2-after	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
BMI	0.343	0.044*	0.095	0.687	0.035	0.841	0.294	0.087
WHR	0.024	0.928	0.024	0.892	0.128	0.463	0.226	0.193
Glucose	0.641	0.0001*	0.346	0.009*	0.406	0.016*	0.197	0.256
Insulin	0.961	0.0001*	0.071	0.686	0.641	0.002*	0.264	0.125
HOMA-IR	0.504	0.002*	0.07	0.687	0.637	0.001*	0.121	0.489
FSH	0.021	0.904	0.075	0.669	0.23	0.169	0.094	0.587
LH	0.406	0.016*	0.050	0.774	0.011	0.951	0.011	0.951
LH/FSH	0.252	0.146	0.087	0.619	0.005	0.977	0.137	0.433
Free Testo	0.074	0.673	0.266	0.123	0.240	0.165	0.219	0.206
Total Testo	0.106	0.545	0.303	0.076	0.282	0.144	−0.317	0.064

*$p < 0.05$ is statistically significant.

curve was 0.997, sensitivity was 100%, and specificity of 94.3% at a cut off levels of 156 ng/ml.

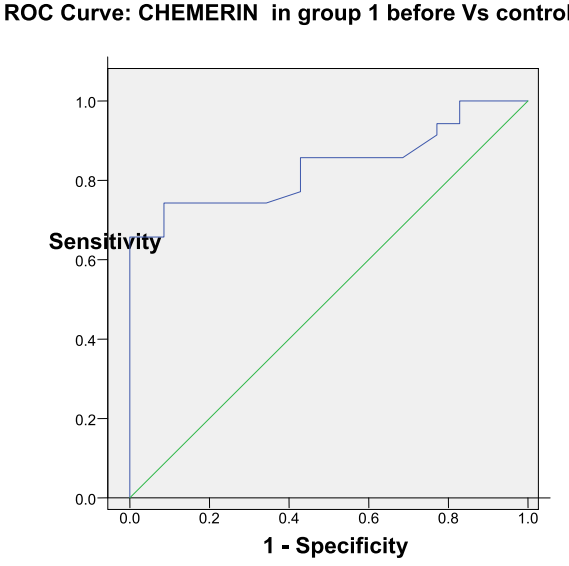

Fig. 1. ROC curve of chemerin in group 1 before metformin therapy.

4. Discussion

In the present study, the menstrual cycles were found to be regular after metformin therapy. Also, BMI and WHR were significantly decreased in group 1 after metformin therapy. These results are in agreement with the results of Fleming et al. [9]. However, most large randomized controlled trials have failed to confirm this [13].

It was reported that PCOS have an increased incidence of insulin resistance. High levels of insulin, HOMA-IR, and testosterone were reported in normal weight PCOS cases [14]. Additionally, in both lean and obese PCOS patients, insulin and HOMA-IR levels were also higher than in normal healthy controls [15].

The plasma chemerin levels were reported to be significantly higher in obese subjects than those with normal weight cases [8].

The results of the present study as regards the increased serum chemerin levels in obese control cases as compared with normal weight control cases are in agreement with previous report [8]. The increased chemerin levels in PCOS cases were found to be associated with insulin resistance, and this finding is in agreement with previous findings [15–17].

It was reported that chemerin can contribute to the pathogenesis of PCOS by negatively regulating the FSH-induced follicular steroidogenesis [18]. In vitro, chemerin suppressed FSH-induced progesterone and estradiol secretion in cultured preantral follicles and granulosa cells [18]. It was found that recombinant chemerin promoted the formation of new blood vessels, indicating a new role for chemerin in PCOS [19].

In the present study, serum chemerin levels, serum glucose and insulin levels were significantly higher in patients with polycystic ovarian syndrome than in the control group. This is in agreement with the findings that insulin and HOMA-IR levels were higher in non-obese PCOS patients as compared with the normal healthy controls cases [15].

In the present study, we found high levels of total testosterone and free testosterone in obese and in normal weight cases with PCOS. In obese PCOS patients, free testosterone levels were significantly higher than in normal weight PCOS patients. This is in agreement with previous results reporting that PCOS is associated with high chemerin levels [20], metabolic, endocrine and dyslipidaemic disorders [14,21].

In the present study, the increased levels of chemerin observed in PCOS women may be due to the altered gonadal and adrenal steroids [17]. Our findings are in agreement with that of Sepideh et al. [22], suggesting that changes in serum chemerin levels could be considered as a criterion for polycystic ovarian syndrome.

In the present study, the cause of the decrease in chemerin levels after metformin therapy may be due to a decrease in gonadal

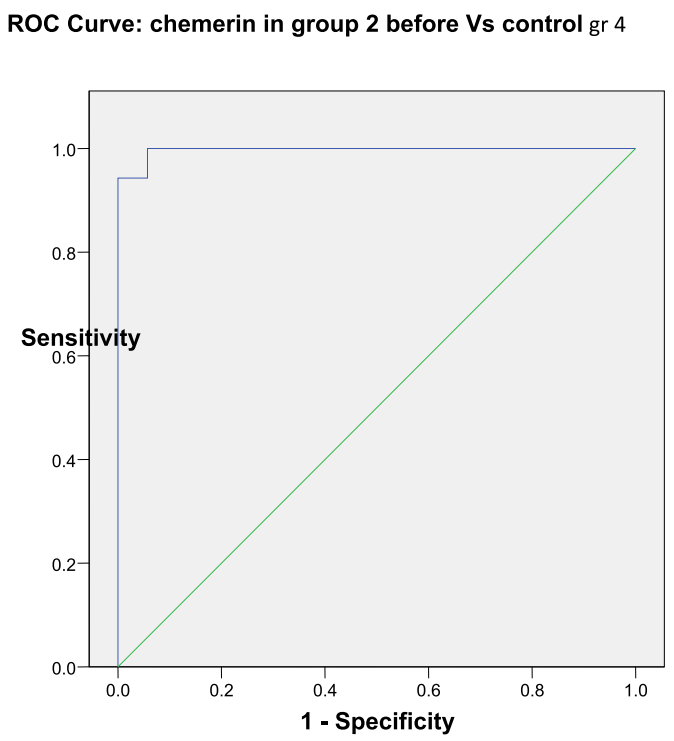

Fig. 2. ROC curve of chemerin in group 2 before metformin therapy.

steroid levels, because metformin acts as an inhibitor of aromatase enzyme [18].

In the present study, the serum chemerin levels in obese PCOS cases before receiving metformin therapy were significantly positively correlated with BMI, fasting serum glucose levels, fasting serum insulin levels, HOMA-IR, and LH levels. After metformin therapy the levels of chemerin decreases with improvement of the insulin resistance parameter (HOMA-IR). Our results are in agreement with the findings of Tan et al. [3], Bozaoglu et al. [23] and Jialal et al. [24]. On the other hands, this association was not supported by most large recent randomized controlled trials [13].

The Receiver Operation Characteristic (ROC) curves were used to calculate the sensitivity of this marker for discriminating cases with PCOS from healthy controls. The analysis of ROC curves may suggest that serum chemerin may be a valuable biomarker for diagnosis and for monitoring cases with PCOS during treatment.

Strengths and weakness: The results of the present study were strengthened by:

(1) The results of Fleming et al. [9] support our findings that the menstrual cycles were found to be regular after metformin therapy. Also, BMI and WHR were significantly decreased in group 1 after metformin therapy.
However, most large randomized controlled trials have failed to confirm these significant clinical improvements [13].
(2) The increased serum chemerin levels in PCOS, especially in obese cases was reported by many authors [3,7,15–18,20,22,24]. Chemerin can contribute to the pathogenesis of PCOS by negatively regulating the FSH-induced follicular steroidogenesis [18], and by promoting the formation of new blood vessels [19].
(3) Increased insulin and HOMA-IR levels in non-obese PCOS patients as compared with the normal healthy control women [15].
(4) Our finding of increased free and total testosterone levels in PCOS are supported by previous results reporting that PCOS is associated with metabolic, endocrine and dyslipidaemic disorders [14].
(5) The cause of the decrease in chemerin levels after metformin therapy may be due to a decrease in gonadal steroid levels, because metformin acts as an inhibitor of aromatase enzyme [18].
(6) The findings of Bozaoglu et al. [23] and Jialal et al. [24] support our findings that there was significant correlation between chemerin and body mass index, serum levels of insulin, glucose, HOMA-IR, and LH in obese PCOS cases.

4.1. Unanswered questions

(1) Does the ovary in PCOS cases secrete chemerin?
(2) What are the structures in the ovaries secreting chemerin?

4.2. Future research

- Experimental immuno-histochemical studies on ovarian biopsies (before and after metformin therapy) to determine the site of chemerin expression.

5. Conclusion

The serum chemerin levels were significantly positively associated with WHR, glucose levels, insulin levels, and HOMA-IR in PCOS cases. The plasma chemerin levels were significantly higher in obese PCOS subjects than the normal weight cases. Metformin therapy resulted in a significant decrease in chemerin levels in PCOS cases.

The analysis of Receiver Operation Characteristic (ROC) curves of serum chemerin suggested that serum chemerin levels may be of value to evaluate the PCOS cases under various methods of treatments.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2019.01.050.

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