



COMPARATIVE STUDY ON CARDIOVASCULAR RISK FACTORS IN NEWLY DIAGNOSED PATIENTS WITH POLYCYSTIC OVARY SYNDROME (PCOS) AND PCOS PATIENTS WHO ARE ON DRUG METFORMIN

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ABSTRACT

Case-control study was conducted to evaluate the effect of metformin on cardiovascular risk factors in women with polycystic ovary syndrome on three groups of women included: Thirty two patients newly diagnosed with PCOS without treatment, 30 patients with PCOS treated with metformin more than 4 months and 25 control subjects. Fasting serum samples were tested to measure the serum, Apolipoprotein C-I (ApoC-I), Free testosterone (FT), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), very low-density lipoprotein cholesterol (VLDL), high sensitive C-reactive protein (hs-CRP), Neopterin (Neo), Homocystein (Hcy), glucose, insulin, Homeostatic model assessment of insulin resistance (HOMA-IR), body mass index (BMI) and waist-to-hip ratio (WHR). Results revealed that newly diagnosed PCOS had higher levels of (TG), (VLDL), (ApoC-I), (hs-CRP), (Neo), (FT), Glucose, insulin, HOMA-IR and lower HDL-c compared with PCOS women who were on metformin treatment. Newly diagnosed women with PCOS had lower HDL-c and higher levels of all other parameters compared with control women.

KEY WORDS: Metformin cardiovascular risk factors polycystic ovary syndrome.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common metabolism and endocrine disorders among women, with prevalence of 5- 10% of female patients at reproductive age (1). It has become apparent that PCOS is associated with a broad range of cardiovascular risk factors. In particular, women with PCOS are at increased risk for dyslipidemia, insulin resistance, gestational and type 2 diabetes, systemic inflammation, and ultimately, cardiovascular morbidity (2-5). Different markers of cardiovascular disease (CVD) have been studied in women with PCOS. Inflammatory markers such as hs-CRP and Neopterin were elevated (6,7), hyperhomocysteinemia which is an early sign of cardiovascular diseases have been demonstrated (8). Dyslipidaemia is demonstrated in PCOS, it is characterized by elevated total cholesterol, TG, LDL-c, VLDL and decreased HDL-c (9). ApoC-I has been established to be elevated in PCOS (10). Experimental studies have shown that ApoC-I modulates lipid metabolism (11,12,13,14). Several data revealed that Insulin resistance is the common link between PCOS and cardiovascular diseases (15-17). These observations suggested the role of insulin sensitizing agents like metformin in improving these manifestations. The aim of the study is to evaluate the beneficial effects of metformin on the cardiovascular risk factors in treated PCOS women compared with newly diagnosed PCOS and control group.

MATERIALS AND METHODS

The study was conducted in the department of obstetrics and gynecology, Fallujah hospital, Al-Anbar governorate, Iraq. Thirty two newly diagnosed PCOS patients not

received treatment, 30 PCOS patients already on metformin for more than 4 months and 25 healthy control subjects were included in the study. The diagnosis of PCOS was based on Rotterdam conference criteria sponsored by ESHRE/ASRM which require for at least two of the three diagnostic features; (a) hyperandrogenism, (b) ovulatory dysfunction and (c) PCO morphology (19). This study excluded PCOS patients with The BMI was determined for all subjects as a marker of obesity where subjects with BMI >30 kg/m² are considered obese while subjects with BMI 25-29 kg/m² are considered overweight (20). Waist-to-hip ratio (WHR) was measured to observe the fat distribution as central or peripheral. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as marker of insulin resistance, HOMA-IR was calculated as fasting glucose (mmol/L) x fasting insulin (mIU/L)/22.5 (21). The 10 ml of venous blood was collected from all fasting subjects at 8:00 AM. The samples were centrifuged, the serum was separated and stored at -80°C. Serum levels of (TC), (TG), (HDL-c), (LDL-c), (VLDL) and glucose were measured by quantitative enzymatic colorimetric method. Serum levels of other parameters were measured by using enzyme linked immunosorbent assay (ELISA) technique. The ELISA kit of ApoC-I (Ucnk Company, China), FT (DRG Co., Germany), Hcy (CUSABIO Co., China), hs-CRP (Demeditec Co., Germany), Neo. (IBL Co., Germany) and insulin (DRG Co., Germany),

Statistical Analysis

The results obtained were analyzed by using SAS.2000. The variables were presented as mean ± standard error mean and compared using analysis of variance (ANOVA)

between groups. Correlation analysis (calculation of the Pearson coefficient) was used to assess the correlation

between variables, $P < 0.05$ was considered statistically significant.

TABLE 1. Demographic data of PCOS women

Factors	No. newly diagnosed PCOS women	No. PCOS Patients on metformin	Controls
Obese	25 (78.1%)	12(40%)	8(32%)
Overweight	7 (21.9%)	9 (30%)	17(68%)
Positive family history	6 (18.7%)	7 (23.3%)	-
Insulin resistance	24 (75%)	10 (33.3%)	-
Hyperandrogenemia(↑Free testosterone)	21 (65.6%)	14(46.6%)	-

RESULTS

Most newly diagnosed PCOS patients were obese (78.1%), other were overweight women (21.9%), (18.7%) have positive family history, (75%) have insulin resistance and (65.6%) have hyperandrogenesim. The serum levels of insulin , glucose , TC ,TG , LDL-c , VLDL, ApoC-I , Hcy, hs-CRP , Neo and also BMI ,WHR , HOMA-IR are all significantly higher in newly diagnosed PCOS patients than control subjects($p < 0.05$), (table 2 and figure 1). The serum levels of HDL-c are significantly lower in newly diagnosed PCOS patients than controls,(figure 1). The PCOS patients who were on metformin (group 2) demonstrated a significant reduction in BMI , WHR

,HOMA-IR , serum levels of insulin , glucose ,FT, ApoC-I ,hs-CRP and Neo in comparison with newly diagnosed PCOS patients($p < 0.05$),(table 2). There are no significant differences in serum levels of Hcy , TC , LDL-c between PCOS patients on metformin course. and newly diagnosed PCOS women ($p < 0.05$),(table 2 and figure 1). However, There is improvement in the serum levels of HDL-c in PCOS patients taking metformin ($p < 0.05$),(figure 1). The PCOS women who were on metformin revealed a significant differences in Hcy , FT , TC ,LDL-c, VLDL and WHR in comparison with control women(Table 2 , Figure 1).

TABLE 2. Clinical , metabolic and hormonal Characteristics in three groups of women with PCOS without treatment , on treatment and control group.

Characteristics	Newly diagnosed women with PCOS (Group1)	PCOS patients on treatment (Group2)	Control (Group3)
Ages(years)	28.4±4.2	28.1±3.9	27.9±4.5
BMI (kg/m ²)	31.30± 0.57 a	29.02 ± 0.61 b	28.33±0.65
WHR	0.85 ± 0.08 a	0.81 ± 0.01 b	0.77 ± 0.01 c
HOMA-IR	4.11 ± 0.50 a	2.07 ± 0.19 b	1.79 ± 0.15
Glucose(mmol/L)	4.91 ± 0.09 a	3.98 ± 0.12 b	3.66± 0.16
Insulin (μIU/L)	18.87 ± 2.39 a	12.03 ± 1.00 b	11.00± 0.81
TC(mmol/L)	5.36 ± 0.13 a	5.06 ± 0.19	3.99± 0.19 c
TG(mmol/L)	1.42 ± 0.11 a	1.07 ± 0.05 b	1.06± 0.07
LDL-C(mmol/L)	3.43 ± 0.12 a	3.24 ± 0.14	2.53± 0.18 c
HDL-C(mmol/L)	1.16 ± 0.04 a	1.42 ± 0.05 b	1.47± 0.07
VLDL(mmol/L)	0.78 ± 0.02 a	0.62 ± 0.02 b	0.57 ± 0.02 c
ApoC-I(μg/ml)	5.55 ± 0.54 a	3.27 ± 0.33 b	2.80± 0.26
Homocystein (μmol/L)	11.80 ± 0.50 a	11.49 ± 0.52	9.14 ± 0.63 c
Neopterin (mg/dl)	8.71 ± 0.78 a	5.66 ± 0.55 b	5.32± 0.55
hs-CRP(mg/dl)	4.26 ± 0.53 a	2.58 ± 0.28 b	1.88± 0.21
FT(pg/ml)	3.93 ± 0.29 a	2.70 ± 0.19 b	2.29 ± 0.16

Values represent means± Standard error mean(SEM)

Notes : TC=Total cholesterol , TG= Triglyceride , LDL-C = Low density lipoprotein , HDL-C = High density lipoprotein , VLDL=Very low density lipoprotein , ApoC-I= Apolipoprotein C-I , hs-CRP = High sensitive C-reactive protein , FT = Free testosterone , BMI = Body mass index , WHR = Waist-to-hip ratio , HOMA-IR = Homeostatic model assessment of insulin resistance.

^a Statistically significant difference between newly diagnosed PCOS women and control women. ^b Statistically significant difference between newly diagnosed PCOS women and PCOS women on metformin. ^c Statistically significant difference between PCOS women on metformin and controls . Conversion factors from SI units to US units for TC ,LDL-c,VLDL,HDL-c was(x 38.7) , for TG (x 88.6) , Glucose (÷0.0555) , Hcy(÷7.39) .

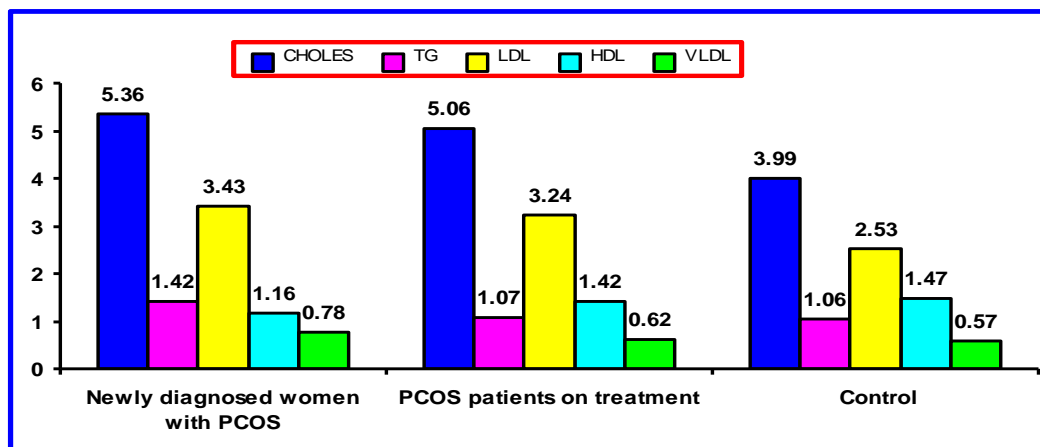


FIGURE 1. Lipid profile

Group 1 : Newly diagnosed women with PCOS and without treatment.

Group 2 : PCOS women taking metformin for more than 4 months.

Group 3 : Control women.

DISCUSSION

In the present study, newly diagnosed patients with PCOS have elevated levels of cardiovascular risk markers in comparison with controls (Table 2).

The serum levels of hs-CRP and neopterin were significantly increased in newly diagnosed PCOS women, this in line with previous study(7). Neopterin is a specific marker of inflammation, it is synthesized and released solely by activated macrophages and monocytes. It has been shown that in obese subjects, adipose tissue contains an increased number of resident macrophages (even greater than 40% of the total cell population in some circumstances)(22)These inflammatory markers (hs-CRP and neopterin) are positively correlated with BMI and WHR respectively(Table 3). Both markers also positively with insulin resistance. In the present study, The BMI and WHR were reduced in PCOS patients on metformin treatment which may resulted in reduction of hs-CRP and Neo levels. Several studies have demonstrated reduction in CRP with weight reduction (23,24). The proposed mechanisms which mediated the anti-inflammatory effect of metformin are either by reducing the insulin resistance and /or in fact, metformin can exert a direct vascular anti-inflammatory effect by inhibiting the releasing of the proinflammatory cytokines in endothelial cells, vascular smooth muscle cells, and macrophages.

There are no significant differences in hs-CRP, Neo levels between PCOS women on metformin and control women(Table 2). The present data illustrated an elevated glucose, insulin levels and higher HOMA-IR as compared with controls which could indicate insulin resistance in these patients. In the current study, PCOS patients taking metformin showed an improvement (reduction) in serum glucose, insulin and HOMA-IR which reflect the improvement in insulin sensitivity. There are no significant changes in these parameters between PCOS patients on metformin and controls.

The serum levels of FT were higher in newly diagnosed women with PCOS as compared with control women. The levels of FT were positively correlated with BMI. The present data also demonstrated that PCOS women who were on metformin had significantly lower FT levels than newly diagnosed women with PCOS. The reduction in

BMI could lead to decreasing FT levels. Some studies revealed that weight reduction in PCOS is also associated with improvements in androgens(26)

Newly diagnosed women with PCOS displayed an unfavorable lipid profile with higher cholesterol, LDL-c, VLDL, TG and lower HDL-c levels than the controls(Figure 1), which is also reported previously(9,27). The PCOS women who were on metformin revealed a significant decrement in TG and VLDL, significant increment in HDL-c and non significant reduction in TC and LDL-c compared with newly diagnosed PCOS women. There are a significant changes between PCOS women on metformin and control women in TC, LDL-c, VLDL and non significant changes in TG and HDL-c.

This study showed that (Hcy) levels are elevated in PCOS women compared with controls(Table 2), which is reported in other studies (28), in contrast to other studies who showed that there is no difference in Hcy levels between newly diagnosed PCOS patients and control subjects(29). There was a correlation between insulin levels and (Hcy) levels which also confirmed previously(28). Elevated plasma (Hcy) had been reported to be a risk factor for cardiovascular disease (30).

The present data demonstrated a non significant decrease in homocystein levels of PCOS patients taking metformin in comparison with newly diagnosed PCOS women, in line with previous study(31). This mean that there are other factors rather than insulin may cause increased (Hcy) levels. Some studies showed that metformin increase Hcy levels(32), other data illustrated that PCOS patients taking metformin for 6-16 weeks revealed lower (Hcy) levels than baseline (33). Moreover there are a significant differences in (Hcy) levels between PCOS women on metformin and controls. The present data demonstrate an elevated levels of ApoC-I in newly diagnosed PCOS women compared with controls, in agreement with other study(10). In the current study, PCOS patients taking metformin have lower ApoC-I levels in comparison with newly diagnosed PCOS patients. ApoC-I is mainly secreted by the liver as a component of VLDLs which is triglyceride rich lipoprotein (34). ApoC-I is positively correlated with TG levels and the improvement of TG

levels in the this study with reduction in VLDL production could resulted in decreased ApoC-I levels. Additionally no significant differences were noticed in ApoC-I levels between PCOS women on metformin and control women.

In conclusion, newly diagnosed PCOS women showed high including cardiovascular risk. Dyslipidemia, low grade chronicinflammation, hyperandrogenism, hyperhomocysteinemia, obesity and insulin resistance compared to treated PCOS women and controls.

Metformin had a beneficial effects on most cardiovascular risk factors . Reduction of BMI, WHR and improvement of insulin resistance may be the attributable mechanisms mediating these effects.

REFERENCES

- 1- Lindholm A, Andersson L, Eliasson M, Bixo M, Sundstrom-Poromaa I. Prevalence of symptoms associated with polycystic ovary syndrome. *Int J Gynaecol Obstet* 2008;102:39–43.
2. Lo JC, Feigenbaum SL, Yang J, Pressman AR, Selby JV, Go AS Epidemiology and adverse cardiovascular risk profile of diagnosed polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006; 91:1357–1363
3. Dunaif A, Segal KR, Futterweit W, Dobrjansky A Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes* 1989; 38:1165–1174
4. Wild RA, Applebaum-Bowden D, Demers LM, Bartholomew M, Landis JR, Hazzard WR, Santen RJ Lipoprotein lipids in women with androgen excess: independent associations with increased insulin and androgens. *Clin Chem* 1990; 36:283–289
5. Kelly CC, Lyall H, Petrie JR, Gould GW, Connell JM, Sattar N Low grade chronic inflammation in women with polycystic ovarian syndrome. *J Clin Endocrinol Metab* 2001;86:2453–2455
6. Boulman N, Levy Y, Leiba R, Shachar S, Linn R, Zinder O, Blumenfeld Z Increased C-reactive protein levels in the polycystic ovary syndrome: a marker of cardiovascular disease. *J Clin Endocrinol Metab* 2004; 89:2160–2165.
- 7- Burcu Barutcuoglu, A. Erkin Bozdemir, Didem Dereli Zuhail Parildar, M. Isil Mutf, Dilek Ozmen, and Oya Bayindir. Increased Serum Neopterin Levels in Women with Polycystic Ovary Syndrome. *Annals of Clinical & Laboratory Science* 2006;36:267-272.
- 8-Saghar Salehpour, Ozra Manzoor-al-ajdad, Elham Neisani Samani, Alireza Abadi: Evaluation of Homocysteine Levels in Patients with Polycystic Ovarian Syndrome. *International Journal of Fertility and Sterility* 2011;4: 168-171
- 9- Bratati Singh , Suchismita Panda , Rachita Nanda , Sanghamitra Pati , Manaswini Mangaraj , Pratima Kumari Sahu, Prakash Chandra Mohapatra: Effect of Metformin on Hormonal and Biochemical Profile in PCOS Before and After Therapy. *Ind J Clin Biochem* 2010 ;25:367–370
- 10- Shuo Huang, Jie Qiao, Rong Li, Lina Wang, and Meizhi Li. Can serum apolipoprotein C-I demonstrate metabolic abnormality early in women with polycystic ovary syndrome?. *Fertility and Sterility* 2010; 94:205-210.
- 11- M. Westerterp, W. De Haan, J. F. P. Berb'ee, L. M. Havekes, and P. C. N. Rensen, "Endogenous apoC-I increases hyperlipidemia in apoE-knockout mice by stimulating VLDL production and inhibiting LPL," *Journal of Lipid Research* 2006;47:1203–1211.
- 12- J. F. P. Berb'ee, C. C. Van Der Hoogt, D. Sundararaman, L. M. Havekes, and P. C. N. Rensen, "Severe hypertriglyceridemia in human APOC1 transgenic mice is caused by apoC-I-induced inhibition of LPL," *Journal of Lipid Research* 2005;46: 297–306.
- 13- E. Sehayek and S. Eisenberg, "Mechanisms of inhibition by apolipoprotein C of apolipoprotein E-dependent cellular metabolism of human triglyceride-rich lipoproteins through the low density lipoprotein receptor pathway," *Journal of Biological Chemistry* 1991; 266: 18259–18267.
- 14- L. Dumont, T. Gautier, J. P. Pais De Barros et al., "Molecular mechanism of the blockade of plasma cholesteryl ester transfer protein by its physiological inhibitor apolipoprotein CI," *Journal of Biological Chemistry* 2005; 280: 38108– 38116,.
- 15- Lo, JC, Feigenbaum, SL, Yang, J, Pressman, AR, Selby, JV, Go, AS Epidemiology and adverse cardiovascular risk profile of diagnosed polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006; 91: 1357–63.
- 16- Legro, RS, Kunesman, AR, Dodson, WC, Dunaif, A Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J Clin Endocrinol Metab* 1999 ;84: 165–69.
- 17- Ehrmann, DA, Barnes, RB, Rosenfield, RL, Cavaghan, MK, Imperial, Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care* 1999; 22: 141–46.
- 18- Lord, JM, Flight, IH, Norman, RJ Metformin in polycystic ovary syndrome: systematic review and meta-analysis. *BMJ* 2003 ;327: 951–53.
- 19- The Rotterdam ESHRE/ASRM sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS) . *Hum Reprod* 2004; 19:41-47

- 20- Centers for Disease Control and Prevention (CDC): Body mass index. Atlanta , U.S.A. 2007.
- 21- Matthews , D.f. ; Hosker,j.p.;Rudenski,A.S.; Naylor , B. A. ; Treacher , D. F. and Turner , R. C.: Homeostasis model assessment : insulin resistance and β cell function from fasting plasma glucose and insulin concentration in man . *Diabetologia* .1985;28:412-419.
- 22-Neels JG, Olefsky JM. Inflamed fat: what starts the fire? *J Clin Invest* 2006;116:33–5.
23. Jae SY, Fernhall B, Heffernan KS, Jeong M, Chun EM, Sung J, Lee SH, Lim YJ, Park WH Effects of lifestyle modifications on C-reactive protein: contribution of weight loss and improved aerobic capacity. *Metabolism* 2006 55:825–831.
- 24- Morin-Papunen L., Rautio K., Ruokonen A., Hedberg P., Puukka M., Tapanainen J.S. Metformin reduces serum C-reactive protein levels in women with polycystic ovary syndrome. *J. Chn. Endocri-nol. Metab.*2003; 88:4649-4654.
- 25- Zmijewski JW, Lorne E, Zhao X, Tsuruta Y, Sha Y, Liu G, Siegal GP, Abraham E Mitochondrial respiratory complex I egulates neutrophil activation and severity of lung injury. *Am J Respir Crit Care Med*2008; 178:168–179
- 26-Hoeger KM Role of lifestyle modification in the management of polycystic ovary syndrome. *Best Pract Res Clin Endocrinol Metab* 2006;20: 293–310
- 27- Apridonidze T, Essah PA, Iuorno MJ, et al. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2005;90:1929-1935.
- 28- Badawy A, State O, El Gawad SSh, El Aziz OA. Plasma homocysteine and PCOS: the missed link. *Eur Obstet Gynecol Reprod Biol.* 2007; 131: 68-72.
- 29- Mancini F, Cianciosi A, Reggiani GM, Facchinetti F, Battaglia C, de Aloysio D. Endothelial function and its relationship to leptin, homocysteine, and insulin resistance in lean and overweight eumenorrheic women and PCOS patients: a pilot study.*Fertil Steril.* 2009; 91: 2537-2544.
- 30-Knekt P, Alfthan G, Aromaa A, Heliövaara M, Marniemi J, Rissanen H, et al. Homocysteine and major coronary events. *J Intern Med.* 2001; 249: 461-465.
- 31- Murat Yilmaz, Neslihan Bukan, Göksun Ayyaz, Ayhan Karakoç, Füsun Törüner, Nuri Çakir , Metin Arslan : The effects of rosiglitazone and metformin on oxidative stress and homocysteine levels in lean patients with polycystic ovary syndrome. *Human Reproduction* 2005;20: 3333–3340,
- 32- Esra Bulgan Kilicdag, Tayfun Bagis, Ebru Tarim, Erdogan Aslan, Serkan Erkanli, Erhan Simsek, Bulent Haydardedeoglu and Esra Kuscü. Administration of B-group vitamins reduces circulating homocysteine in polycystic ovarian syndrome patients treated with metformin: a randomized trial. *Human Reproduction* 2005;20:1521–1528,
- 33- Morey Schachter, Arieah Raziell, Devorah Strassburger, Carmela Rotem , Prospective, randomized trial of metformin and vitamins for the reduction of plasma homocysteine in insulin-resistant polycystic ovary syndrome. *Fertility and Sterility* 2007;88: 227-230
- 34- Cohn J. S., Tremblay M., Batal R., Jacques H., Veilleux L., Rodriguez C., Bernier L., Mamer O., Davignon J. Plasma kinetics of VLDL and HDL apoC-I in normolipidemic and hypertriglyceridemic subjects. *J. Lipid Res*2002.;43: 1680–1687

TABLE 3. Correlation analysis between all parameters in newly diagnosed women with PCOS

	Hcy	HOMA-IR	BMI	GLU	FT	hsCRP	Insul	Neo	ApoC-I	HDL	LDL	VLDL	WHR	TG	TC
Hcy	----	0.08	0.04	-0.17	-0.09	0.32	0.11*	0.36*	0.15	0.28	-0.17	0.13	-0.03	0.10	0.06
HOMA			-0.26	0.12	-0.17	0.44*	0.97**	0.41*	0.69**	0.32	0.36*	-0.12	-0.16	-0.19	0.22
BMI				-0.26	0.36*	0.11	-0.19	-0.06	-0.15	0.09	-0.15	0.28	-0.002	0.30	-0.12
GLU					0.001	-0.02	-0.04	0.007	0.19	0.21	0.36*	-0.15	-0.11	-0.29	0.34
FT						-0.006	-0.16	-0.05	-0.08	-0.13	0.28	0.14	-0.20	0.005	0.26
hsCRP							0.47**	0.21	0.57**	-0.37*	0.07	0.05	0.22*	-0.01	-0.006
Insul								0.41*	0.63	0.29	0.31	-0.06	-0.13	-0.15	0.15
Neo									0.28	0.28	-0.17	-0.03	-0.15	-0.11	-0.17
ApoC-I										0.38	0.05	-0.23	-0.31	0.23*	0.07
HDL											-0.06	-0.24	-0.007	-0.21	0.28
LDL												0.12	0.09	-0.003	0.83**
VLDL													-0.02	0.07	0.07
WHR														0.32	0.18
TG															0.16

*significantly difference $p < 0.05$ ** significantly difference $p < 0.01$ Hcy=Homocystein , HOMA-IR = Homeostatic model assessment of insulin resistance , BMI = Body mass index, GLU=Glucose , FT = Free testosterone , hs -CRP = High sensitive C-reactive protein ,Insul=Insulin , Neo = Neopterin , ApoC-I= Apolipoprotein C-I HDL-c= High density lipoprotein , LDL-c = Low density lipoprotein , VLDL=Very low density lipoprotein , WHR = Waist-to-hip ratio , TG= Triglyceride , TC=Total cholesterol															