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MCP-1 and fetuin A levels in patients with PCOS and/or obesity before and after metformin treatment

Research Article

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Abstract: Background/Aims: The aim of the study was to investigate MCP-1 and fetuin A levels in women with PCOS and/or obesity before and after metformin treatment. Materials/Methods: In the study consisted of 59 patients. Anthropometric measurements and biochemical tests, including MCP-1 and fetuin A measurement, were performed. For patients that were diagnosed with insulin resistance and started metformin treatment all the laboratory tests and anthropometric measurements were repeated after 6 months. Results: MCP-1 and fetuin A levels did not differ between patients with obesity with and without PCOS, between patients with PCOS with and without obesity, insulin resistance, arterial hypertension, dyslipidemia or menstrual disturbances. MCP-1 levels were significantly higher in patients with hyperandrogenemia than in patients without (456.3±141.1pmol/L vs. 372.5±108.5 pmol/L), while fetuin A levels were significantly higher in patients with metabolic syndrome (MetS) than in patients without MetS (278.5±41.1 mcg/ml vs. 240.0±42.0 mcg/ml). There was no significant change in MCP-1 and fetuin A levels after of metformin treatment. Conclusions: MCP-1 levels are higher in patients with hyperandrogenemia and fetiun A levels are higher in patients with metabolic syndrome. MCP-1 and fetuin A levels do not change significantly after metformin treatment.

Keywords: *Atherosclerosis • Cardiovascular risk • Metabolic syndrome*

1. Introduction

According to some studies, PCOS patients have an increased risk for diabetes mellitus [1,2] and often show an adverse cardiovascular risk profile – increased rate of arterial hypertension [3,4], dislipidemia [4-7] and subclinical inflammation and atherosclerosis [8-10].

Inflammation has a distinct role in the pathogenesis and progression of atherosclerosis. Monocyte chemotactic protein-1 (MCP-1) is a protein that plays a role in the recruitment of monocytes to sites of injury and infection. Vascular insults, such as hyperlipidaemia, cause the secretion of MCP-1 from endothelial cells and smooth muscle cells. There is a great deal of data about the key role of MCP-1 for the development of atherosclerosis [11-13].

Obesity and metabolic syndrome correlate with higher levels of MCP-1 [14]. Higher levels of MCP-1 were also found in PCOS patients [15,16] and they are directly linked to the NFκB and androstendione levels that suggest that proatherogenic inflammation could be related to the hyperandrogenemia in PCOS patients [16]. MCP-1 levels are high at baseline and do not change during treatment with the insulin sensitizing drug pioglitazone [17]. There is no data however about the effect of metformin on MCP-1 levels in PCOS patients.

Fetuin A (α2-HS-glycoprotein) is a glycoprotein that acts as a natural inhibitor of the insulin-receptor tyrosine kinase in the liver and skeletal muscles [18-21]. The circulating levels of fetuin A show negative correlation to the insulin sensitivity [22,23]. Its serum concentrations in humans are positively correlated to CRP levels, that are a marker of subclinical chronic inflammation [24,25]. There are controversies about the role of fetuin A in metabolic syndrome. Roos M. et al. do not find a correlation between fetuin A levels and the presence of metabolic syndrome [26]. On the other side, Ix JH. et al. show that increased fetuin A levels are positively correlated to the

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presence of metabolic syndrome and atherogenic lipid profile in patients with coronary heart disease [27].

There is a strong link between fetuin A levels and the risk for cardiovascular disease that is independent of the well known risk factors like arterial hypertension and smoking. High fetuin A levels are associated with 3.3 times higher risk for myocardial infarction and 3.8 higher risk for stroke than the lower levels [28]. These studies however included patients at higher mean age. There are no data about fetuin A levels in younger women with obesity and polycystic ovarian syndrome (PCOS) and their link to other markers cardiovascular risk.

2. Materials and methods

The aim of the present study is to investigate MCP-1 levels in premenopausal women with PCOS and/or obesity and to compare them to other classical cardiovascular risk factors before and after metformin treatment.
The present study includes patients with PCOS and/or obesity that met the inclusion and exclusion criteria.
Inclusion criteria:
- premenopausal women aged 18 to 45,
- PCOS, diagnosed by ESHRE-ASRM criteria [29], or
- obesity (BMI > 30 kg/m^2).

Exclusion criteria:
- Pregnancy,
- Serious illnesses as cardiac, renal or liver insufficiency,
- Other endocrine pathology like type 2 diabetes mellitus, adrenal tumors, hypothyroidism, pituitary tumors, hypogonadism.
- Insulin sensitizing medication (metformin or glitazones) or combined oral contraceptive (COC) use less than 4 months prior to the study.

The study was performed in adherence with the guidelines of the Declaration of Helsinki and was approved by the local ethics committee. All the patients signed an informed consent for participation in the study.

Patients were divided into three groups – Obese (group 1), Lean PCOS (group 2) and Obese PCOS (group 3).
The following information for each patient was obtained: General information – name, age; Anthropometric data – height, weight, body mass index (BMI), waistcircumference, hip circumference, waist-to-hip ratio (WHR), waist-to-stature ratio (WSR); Obesity was diagnosed at BMI≥ 30 kg/m^2 [30]; Polycystic ovary syndrome was diagnosed according to the ESHRE-ASRM criteria– two out of the following: 1) oligo/amenorrhea; 2) clinical or biochemical hyperandrogenism and 3) polycystic ovaries at ultrasound examination when all other endocrine causes are excluded; Results form the oral glucose tolerance test (OGTT) – blood glucose (BG) and imunoreactive insulin (IRI) on 0, 60 and 120 min; Hormones (testosterone, androstendione, dehydroepiandrosteron sulphate (DHEAS), 17-OH-progesterone, estradiol, LH, FSH, TSH, prolactin); MCP-1 levels were measured by a enzyme-linked immunosorbent method. Blood samples for MCP-1 analysis were taken after overnight fast on Days 2–5 of a spontaneous or progestin-induced menstrual cycle, and the serum was separated and frozen; Fetuin A levels were measured by a sandwich enzyme immunoassay method. Blood samples for Fetuin A analysis were taken after overnight fast on Days 2–5 of a spontaneous or progestin-induced menstrual cycle, and the serum was separated and frozen; Metabolic syndrome (MetS) was was diagnosed according to the IDF and AHA/NHLBI criteria – 3 out of 5 risk factors – increased waist circumference (>80 cm), increased TG (>1.7 mmol/L), decreased HDL (<1.3 mmol/L), increased BP (>130/85 mmHg) and increased fasting blood glucose (>5.5 mmol/L).

The laboratory tests were performed in the Central Clinical Laboratory of The Alexandrovska University Hospital in Sofia, which is the reference laboratory for the country.

The patients that were diagnosed with insulin resistance based on fasting IRI (≥ 20 mU/L), IRI on 120 min (≥100 mU/L) or HOMA index (>2,0) started metformin treatment (1500-3000 mg/day) and were reevaluated after 6 months and all the laboratory test and anthropometric measurements were repeated.

2.1 Statistical methods

The data were processed using the statistical package SPSS 16.0. The level of significance for rejecting the null hypothesis was $p < 0.05$. The following statistical methods were applied: descriptive analysis, variation analysis, Kolmogorov– Smirnov's one sample non-parametric test, Student's t-test for two independent samples, Mann–Whitney's non-parametric test for two independent samples, one-way analysis of variance between-groups ANOVA, correlation analysis. Data are presented as mean±SD.

3. Results

In the present study we included 59 patients – 19 Obese, 27 Lean PCOS and 13 Obese PCOS women. Anthropometric characteristics of the three groups are shown on Table 1.

The groups were similar in age but obese and obese PCOS patients had significantly higher weight, waist circumference, hip circumference, WHR and WSR than lean PCOS subjects.

Table 1. Anthropometric characteristics of the groups.

	Group 1 Obese (n=19)	Group 2 Lean PCOS (n=27)	Group 3 Obese PCOS (n=13)
Age(wears)	31.2±7.4	27.0±4.6	26.3±3.5
BMI (kg/m²)	41.4±9.6*	23.3±3.6**	36.9±6.9
Waist (cm)	113.3±13.1*	72.5±10.2**	105.1±12.5
Hip (cm)	126.2±11.9*	98.8±10.4**	118.2±9.7
WHR	0.9±0.09*	0.8±0.07**	0.9±0.08
WSR	0.7±0.09*	0.5±0.07**	0.07±0.8

** <0,001 between group 2 and group 3;*
*** <0,001 between group 1 and group 2.*

The levels of MCP-1 and fetuin A did not differ significantly between the three groups (Table 2). There was however a statistical significant difference between MCP-1 levels in patients with (n=22) and without hyperandrogenemia (n=27) (Figure 1) and a statistical significant difference between fetuin A levels in patients with (n=9) and without metabolic syndrome (n=50) (Figure 2).

Table 2. MCP-1 and fetuin A levels.

	Group 1 Obese	Group 2 Lean PCOS	Group 3 Obese PCOS
MCP-1 pg/ml	392.5±151.4	431.7±157.9	402.0±71.9
Fetuin A mcg/ml	252.2±37.6	237.1±48.1	257.9±45.3

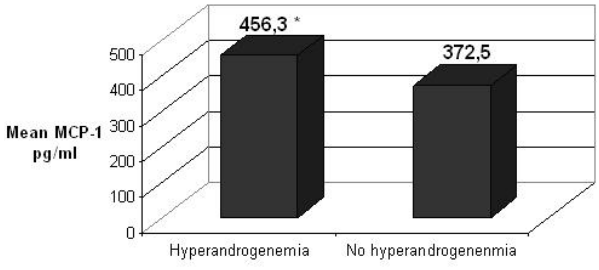

Figure 1. MCP-1 levels in patients with and without hyperandrigenemia.

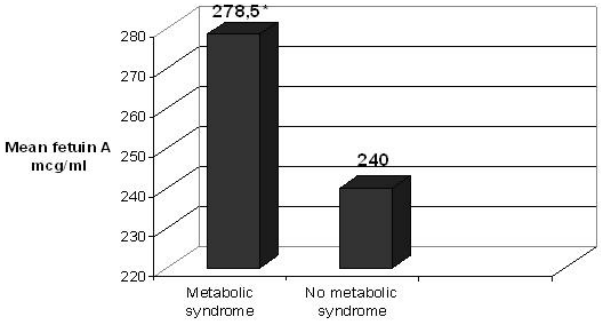

Figure 2. Mean Fetuin A levels in patients with and without metabolic syndrome.

There were no differences in MCP-1 and fetuin A levels between patients with different PCOS phenotype, with and without visceral obesity or insulin resistance based on variety of criteria, with and without arterial hypertension, dyslipidemia, menstrual disturbances and obstructive sleep apnea. MCP-1 levels correlated positively with testosterone levels (r=0.4, p=0.002) but not with other androgens.

Of the 59 included in the study patients insulin resistance was diagnosed in 31 (52,5%) and they started metformin treatment. 2 of them became pregnant, 6 stopped their treatment because of side effects and 23 came back for reevaluation. The data before and after metformin treatment are shown on Table 3.

Table 3. Results before and after metformin treatment.

	Before treatment (n=23)	After treatment (n=23)
Body weight (kg)	82.9±20.6	81.5±21.3
BMI(kg/m²)	31.7±7.7	31.2±7.8
IRI OGTT 0 min (mU/l)	18.2±9.8	12.3±6.9**
IRI OGTT 60 min (mU/l)	133.1±84.8	105.5±72.7*
IRI OGTT 120 min (mU/l)	92.4±59.2	79.0±63.4
HOMA index	3.5±1.9	2.4±1.4*
ESR	21.2±6.3	8.7±5.6***
MCP-1 pg/ml	400.3±136.9	379.0±124.9
Fetuin A mcg/ml	240.2±56.3	241.3±39.2

** <0,05; ** <0,01; *** <0,001*

After 6 months of metformin treatment there was a reduction in mean weight and BMI that was however not statistically significant. Significant reduction was observed in IRI levels on 0 and 60 min of OGTT and HOMA index and ESR. Serum concentrations of MCP-1 and fetuin A did not significantly change before and after metformin treatment (p=0.472) probably because of the small number of the patients. There was no correlation

Table 4a. Results before and after metformin treatment – obese (n=5).

	Before treatment	After treatment
Body weight (kg)	96.5±7.2	95.2±5.7
BMI(kg/m²)	37.4±5.3	36.7±4.8
IRI OGTT 0 min (mU/L)	23.82±7.9	14.4±9.2*
IRI OGTT 60 min (mU/L)	217.9±124.9	145.0±110.4
IRI OGTT 120 min (mU/L)	107.5±71.2	69.9±52.1
HOMA index	4.9±1.1	2.4±1.4*
ESR	17.6±10.8	11.0±2.5*
MCP-1 pg/ml	337.3±204.2	288.7±174.7
Fetuin A mcg/ml	274.1±30.5	230.5±36.5

**- <0,05*

Table 4b. Results before and after metformin treatment – lean PCOS (n=12).

	Before treatment	After treatment
Body weight (kg)	66.8±20.6	64.2±11.1*
BMI(kg/m^2)	25.6±3.7	24.8±3.8
IRI OGTT 0 min (mU/L)	11.4±3.8	8.9±4.8*
IRI OGTT 60 min (mU/L)	89.6±37.4	73.1±34.3
IRI OGTT 120 min (mU/L)	71.8±37.6	51.5±33.9
HOMA index	1.9±0.6	1.8±1.1
ESR	11.9±5.8	7.5±6.9*
MCP-1 pg/ml	454.4±152.7	372.9±113.6
Fetuin A mcg/ml	217.4±68.5	234.3±29.6

*- *<0,05*

Table 4c. Results before and after metformin treatment – obese PCOS (n=6).

	Before treatment	After treatment
Body weight (kg)	97.8±23.6	95.7±22.6
BMI(kg/m^2)	36.7±7.9	35.9±7.5
IRI OGTT 0 min (mU/L)	24.2±12.1	16.2±5.8
IRI OGTT 60 min (mU/L)	144.3±56.3	131.4±71.3
IRI OGTT 120 min (mU/L)	107.6±79.4	126.3±82.0
HOMA index	4.9±2.1	2.9±1.2
ESR	18.2±19.2	7.0±5.2
MCP-1 pg/ml	413.7±83.6	438.9±101.9
Fetuin A mcg/ml	255.0±27.4	248.5±57.7

*- *<0,05*

between the change of MCP-1 levels (r=0.002, p=0.994) or fetuin A (r=0.283, p=0.287) and body weight after the treatment and there was no difference in MCP-1 and fetuin A levels change in patients who did or did not restore normal menstrual cycle after metformin use (p=0.85).

4. Discussion

MCP-1 is a likely contributor to obesity, hyperinsulinemia, and insulin resistance in POCS [31]. According to some research, overweight, rather than the PCOS diagnosis per se, appears to be the main explanatory variable for elevated adipose tissue inflammation in patients with PCOS [32]. On the other hand PCOS obese and non-obese patients show higher serum MCP-1 levels than controls [15]. In the present study there was no difference in MCP-1 levels between patients with obesity with and without PCOS, nor between patients with PCOS with and without obesity. This confirms the conclusion that both PCOS and obesity contribute to a proatherogenic inflammation. We did not find however any correlation between insulin resistance and fat tissue mass and MCP-levels. Further prospective studies should be performed to confirm the role of MCP-1 in cardiovascular risk in PCOS patients.

MCP-1 levels were found to be linked to androstendione levels [16] and in our study we found a correlation between MCP-1 levels and testosterone and significantly higher levels of MCP-1 in patients with hyperandrogenemia. It has been shown that testosterone can promote the expression of TNF-α and MCP-1 by activating both NF-κB and ERK1/2 signal pathways [33]. This is probably linked to the role of androgens in low-grade chronic inflammation and the pathogenesis of atherosclerosis. The positive correlation between testosterone levels and serum MCP-1 and the higher levels of MCP-1 in patients with hyperandrogenemia found in the present study could be a possible part of the link between PCOS and increased cardiovascular risk.

In one study pioglitazone treatment in PCOS patients significantly improved insulin sensitivity without affecting testosterone, body composition and MCP-1 [17]. In our study serum MCP-1 levels did not change significantly after several months of metformin treatment despite the beneficial changes in insulin sensitivity and the decrease of erythrocyte sedimentation rate. This could be a result of the small number of the patients and the relatively short duration of metormin treatment.

Studies show that fetuin A levels are an independent risk factor for insulin resistance [22,34]. Fetuin A levels are higher in patients with non-alcoholic fatty liver disease and are related to the features of the metabolic syndrome in both cross-sectional and longitudinal analyses. Therefore, fetuin A might be a new promising link between obesity and its comorbidities [35]. Higher human fetuin A concentrations are strongly associated with metabolic syndrome [27,36] and this has been confirmed for the first time in patients with PCOS in our study. There was no difference in fetuin A levels between patients with obesity with and without PCOS, nor between patients with PCOS with and without obesity. These results suggest that fetuin A is not independently linked to obesity and to PCOS status but rather depends on the presence of metabolic syndrome. Fetuin A levels correlate well with erythrocyte sedimentation rate probably due to chronic inflammation. In our study however we found no change in fetuin A levels after six months of metformin treatment despite the beneficial effect of the treatment on ESR.

One study shows that Fetuin A significantly decreased after 6 months of pioglitazone treatment in patients with type 2 diabetes while there were no changes in serum fetuin-A after intervention in either the metformin or aerobic exercise [37]. Authors hypothesize that pioglitazone could partially ameliorate insulin resistance

via modulating fetuin A levels. In our study fetuin A levels did not change after several months of metformin treatment despite the beneficial changes in insulin sensitivity. The reason for that is probably the fact that elevated fetuin A levels are a cause but not a consequence of insulin resistance.

5. Conclusions

1. MCP-1 and fetuin A levels do not differ between patients with obesity with and without PCOS, nor between patients with PCOS with and without obesity.
2. MCP-1 levels are higher in patients with hyperandrogenemia.
3. Fetiun A levels are higher in patients with metabolic syndrome.
4. MCP-1 and fetuin A levels do not change significantly after metformin treatment.

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Disclosure

The authors declare no conflict of interest.

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