Original Article

Evaluation Of The Relation Between Fetuin-A Protein And Adipocytokine Levels, Insulin Resistance In Iraqi Over-Weight Women With Polycystic Ovary Syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder and its etiology appears to be complex and multifactorial. It is one of the most frequent endocrine diseases, affecting about 5-12% of reproductive women. This study was designed to determine serum Fetuin-A levels and establish whether serum Fetuin-A level is related with insulin resistance, ovarian hyperandrogenism and dyslipidemia in women with polycystic ovary syndrome (PCOS). In addition to the selected one hundred PCOS patients with mean age of (29.01 ± 0.63years), and the mean body mass index (BMI) (28.29±0.99 Kg/m^2). A group of seventy-five healthy women without PCOS matching in the mean age and BMI to the PCOS women (28.57±0.81 years, 29.68±1.06 Kg/m^2) respectively were also recruited in the study as a control group.

Serum Fetuin-A, lipid fractions, glucose, insulin, and other hormone (gonadotropins, androgens) levels measurement conducted, Then estimate of insulin resistance calculated by homeostasis model assessment (HOMA-IR). The women with PCOS has significantly higher Insulin Serum, Luteinizing hormone (LH), Fetuin-A levels, and LH/FSH (Follicle-Stimulating hormone) ratio, Free Androgen Index (FAI), HOMA-IR, Interleukin 6, Interleukin 10, TNF-α, Leptin and hs-CRP, than healthy women($p<0.05$). However, Sex Hormone-Binding Globulin (SHBG), Adiponectin and HDL-ch levels were significantly lower in patients with PCOS compared with controls. Fetuin-A was positively correlated with insulin, HOMA-IR and FAI ($p< 0.05$).

Serum Fetuin-A level related with insulin resistance and ovarian hyperandrogenism in women with PCOS. These results suggest that Fetuin-A may have a role in triggering the processes leading to insulin resistance and androgen excess in PCOS.

Keywords: Fetuin-A protein, Adipocytokine, Polycystic Ovary.

1. INTRODUCTION

Polycystic ovary syndrome: a very complex condition that affects women health during their lifespan. It has psychologic, reproductive, and metabolic effects, one in each five women diagnosed with PCOS, which reveals its importance clinically and publicly. PCOS has multiple and different clinical relations like infertility, hirsutism, hyperandrogenism, insulin resistance, type two diabetes mellitus, also it has a multiple psychological effect like increased anxiety, and depression. Due to its heterogenous nature and the specialty of the condition, researchers and clinical disciples try to understand its uniqueness.

A wide range of signs and symptoms shown on PCOS patients, which makes it difficult to diagnose precisely. In 2003, ESRHE/ASRM diagnosis based on at least two out of three symptoms Oligoanovulation, hyperandrogenism and polycystic ovary on ultra sound. From 2006 Androgen Excess Society diagnosis required clinical and/or biochemical hyperandrogenism simultaneously with Oligoanovulation and polycystic ovary on ultra sound evidence.

Fetuin-A also known as alpha 2-HS-glycoprotein mostly secreted by the liver to circulation, affect insulin receptors and can inhibit tyrosine kinase activity in vetro. It also related to insulin resistance, obesity, and metabolic syndrome.

2. AIM OF STUDY

This study designed as an open labeled clinical trial. It was conducted on one-hundred patients, the age of patients enrolled were 18-39 years and the BMI were 29-33 Kg/m^2, and all were free of D2M and CVD, in addition a group of seventy-five healthy women free of PCOS matching the same age and BMI were recruited as a control group.

The clinical and biochemical parameters measured including: BMI, waist to hip ratio (WHR), scoring of hirsutisms, acne, menstrual irregularity, serum levels of total testosterone. Also, sex hormone binding globulin (SHBG), free androgen index (FAI), androstenedione, dehydroepiandrosterone sulfate (DHEAS), luteinizing hormone (LH), follicle stimulating hormone (FSH), LH/FSH ratio, glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR). highly sensitive C-reactive protein (hs-CRP), and lipid profile [total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C), in addition to leptin, adiponectin, Fetuin-A (α2-Heremans Schmid glycoprotein) and some cytokine like interleukin 6(IL-6),IL-10 and tumor necrotic-α(TNF- α) evaluated in patient and control group.

The relation between fetuin-A levels and the clinical features, metabolic features, lipid profile and hormonal profile respectively in both control women and PCOS women studied to assess its correlation.

3. Materials And Sample Collection

Table (1) the general laboratory equipment and instruments that used in this study, while table (2) contains the used Chemicals, biological materials and kits.

Blood samples (10 mL) collected from both control and all PCOS patients and divided into two tubes. The first whole blood samples to obtain serum, which needed to deliver into sterile tubes with caps or evacuated collection tubes, left to clot undisturbed for approximately half an hour at room temperature (18-25°C). The tubes then centrifuged for about 10 min under approximately 3000 rpm. The supernatant serum then pipetted into another tube and then was stored at -20°C in the freezer until biochemical and immunological tests performed. The second, blood samples delivered into tubes with anticoagulant in order to use in a special assay.

Table (1): Laboratory equipment and instruments that used in this study

page	Equipment	Origin	Company
1.	Centrifuge	Germany	Universal 16A
2.	Spectrophotometer uv-visible	Japan	Shimadizo
3.	Incubator	England	Gallenkamp
4.	Water bath	Germany	GLF
5.	pH- Meter	USA	Criston
6.	ELISA Systems	Germany	Human Reader
7.	Distiller	Italy	Citron
8.	Deep freeze	Japan	Sanyo
9.	Oven	Germany	Memmert

4. Statistical Analysis

All values expressed as mean ± standard error of the mean. Data were analyzed using computer program Excel 2010 for all mathematical and statistical analysis. The paired Student's t test used to determine the difference between the means of parameters tested. The difference between results were considered statistically significant if $P< 0.05$.

Table (2): Chemicals and kits that used in this study

	Chemicals and biological materials	Origin	Company
1.	Kit for HDL-cholesterol determination	Spain	Spinreackt
2.	Kit for LH hormone	USA	Abcam
3.	Kit for glycoslated hemoglobin determination	USA	Abosystem
4.	ELISA kit for insulin determination	USA	Abcam
5.	ELISA kit for testosterone determination	USA	Abcam
6.	ELISA kit for adiponectin	USA	Abcam
7.	ELISA kit for leptin	USA	Abcam
8.	ELISA kit for DHEAS determination	USA	Abcam
9.	ELISA kit for SHBG determination	USA	Abcam
10.	Kit for glucose determination	Spain	Spinreackt
11.	Kit for cholesterol determination	Spain	Spinreackt
12.	Kit for triglyceride determination	Spain	Spinreackt
13.	ELISA kit for IL-6	USA	Abcam
14.	ELISA kit for IL-10	USA	Abcam
15.	ELISA kit for TNF-α	USA	Abcam
16.	ELISA kit for fetuin A	China	Usuf
17.	Ichroma kit for C-reactive protein	South Korea	Ichroma

5. The Demographic, Clinical Characteristics Of The Study Population

The analysis of the clinical and biochemical characteristics of the healthy control subjects and obese patients with PCOS shown in Table (3). Concerning the diagnostic criteria of PCOS, we followed the 2003 ASRM/ESHRE Rotterdam consensus. According to the criteria, patients diagnosed with PCOS when they have two of the following three features: oligo- , amenorrhea, clinical or biochemical hyperandrogenism and ultrasonography polycystic ovarian morphology. The demographic and basic laboratory data of the groups presented in Table (3). No significant differences identified between female ages. The mean age ± standard error (SE) of all females with PCOS participate in this study was (29.01±0.63) years, which is ranging between 17 and 40 years, and for control (the mean age ± SE was (28.57±0.81) years which ranging between 19 and 39 years.

The women with PCOS in all groups demonstrating comparable clinical, metabolic and biochemical characteristics at base line and about 88% of PCOS women were less than 36 years of age. This finding is in agreement with finding reported by Koivunen R, et al (1999) in which the prevalence of PCOS was more in younger women (<35 years of age) than in older women (> 35years of age). These age-related changes related to a physiological decline of the follicular cohort after the age of 35 (Scott R & Hofmann G. et al. 1995).

The mean ± SE of BMI of PCOS patients in the present study was (29.68±1.06) Kg/m^2, and in control group, the mean ± SE of BMI was (28.29±0.99) Kg/m^2. The statistical analysis showed no significant difference ($p>0.05$) in the BMI between PCOS and control groups and each of two group under study considered as overweight (BMI 25.0–29.9 kg/m^2). This finding is approximate with finding described by another study in Iraqi patients conducted by Abdulrazak H., and Nada K. (2007) in which they found that, about 63% of 107 women with PCOS were overweight or obese. However, this finding is higher than that reported by Bulent and his workers who found that about 50 % of

PCOS women are overweight or obese (Bulent, et al 2008). On the contrary, Herriot et al found that 66% of women with PCOS were below 24.9 Kg/m^2 BMI (Herriot, et al 2008). Indeed, there is widespread variability in the prevalence of obesity and overweight in PCOS women across different countries. The highest prevalence of obesity demonstrated in studies conducted in the United States and Australia, with 61% to 76% of women with PCOS considered obese (Elting, et al 1990). However, the increasing global prevalence of obesity may play a significant role in promoting the development of PCOS in susceptible individuals (Pasquali, et al 2006).

Table 3: Clinical, biochemical, hormonal characteristics and lipid profile for PCOS group and control group.

Characteristics	Control Group No:75	PCOS group No:100	P Value
Age (year)	28.57±0.81	29.01±0.63	>0.05
BMI(Kg/m2)	28.29±0.99	29.68±1.06	>0.05
WHR	0.76±0.01	0.86+ 0.01	>0.05
Menstrual irregularity	6%	88%	
Hirsutism score	4.12+0.27	8.81+0.34	>0.05
Acne	7%	18%	
FSH (mIU/mL)	5.8±0.4	6.1±0.5	>0.05
LH (mIU/mL)	8.1±0.5	11.5±0.7	>0.05
LH/FSH ratio	1.39±0.2	1.88±0.2	>0.05
Estradiol (pg/ml)	50.6±0.7	59.5±1.7	>0.05
DHEAS (µg/dl)	237±22	240±24	>0.05
T. Test (ng/dl)	0.12±0.05	0.26±0.03	>0.05
SHBG (nmol/l)	33.8±1.9	15.6±2.1	<0.001
FAI	0.7±0.9	1.3±0.4	>0.05
B. sugar (mg/dl)	89±1.9	115±2.4	<0.05
insulin (µIU/ml)	7.2±1.03	16.8±1.4	<0.05
HOMA-IR	2.1±0.2	3.5±0.3	>0.05
T. Chol (mg/dl)	185.1±5.3	212.7±6.5	>0.05
HDL (mg/dl)	46.5±2.3	39.3±2.5	>0.05
LDL (mg/dl)	112.2±1.9	136.1±2.3	<0.05
VLDL (mg/dl)	16.0±1.1	24.1±1.2	>0.05
TG (mg/dl)	135.3±6.1	180.2±7.5	<0.001
Fetuin-A (µg/ml)	156.2±6.9	212.7±10.7	<0.001
TNF-α (µg/mL)	10.5±1.9	31.5±2.3	<0.001
IL-6 (µg/mL)	15.2±3.5	66.8±4.0	<0.001
IL-10 (µg/mL)	350±43	315±22	<0.05
leptin (ng/mL)	5.92±1.1	10.72±1.2	>0.05
adiponectin (mg/ml)	18.7±0.2	12.5±0.3	>0.05
CRP (mg/dl)	2.3±0.9	6.4±1.1	>0.05

Obesity particularly central visceral obesity as indicated by an increased waist to hip ratio is a risk factor for the development of diabetes and heart disease when present in a woman with PCOS worsens the clinical manifestation (anovulation, hyperandrogenism and insulin resistance) of the syndrome compared with healthy women. PCOS women in the present study had significantly higher values of WHR (0.86± 0.08; versus 0.72 ± 0.06) (Hoeger, et al

2007).This finding is in agreement with other studies which demonstrated that women with PCOS have abdominal obesity as determined by having a WHR of 0.85 or greater (Coviello, et al 2006; Zhao, et al 2010).

Menstrual cycle disorder is frequently early and dominant symptoms of the anovulatory component of PCOS. The incidence of cycle disorder in women with PCOS seems to be quite variable (Najem, et al 2008). In the current study, about 88% of women with PCOS were with oligomenorrhea compared to 6% in healthy women. Ansam A. Al-Bayatti (2006) found that high percentage of women with PCOS had oligomenorrhea and/ or amenorrhea; this is in contrast to the results reported by Balen et al. (1995) in which 59.9% of 1741 PCOS patients were with oligomenorrhea.

This variation in incidence of menstrual irregularity among women with PCOS may be attribute to ethnic difference among the patients that participate in the study and variability in definition and criteria used for diagnosis of PCOS among different studies. In a large series of patients diagnosed by varying criteria, 75–85% of PCOS women demonstrated clinically evident menstrual irregularity (Conway, et al 1984).

The cardinal feature of PCOS is hyperandrogenism which can be suggested by clinical signs including hirsutism and acne, and biochemically by measuring the serum concentrations of androgens, including testosterone, androstenedione and androgen precursor DHEAS (Howard AZ, 2003). In the present study 63% patients with PCOS, had hirsutism and 10 % had acne. This is similar to the figure reported by Abdulrazak H., and Nada K., (2007) in which about 64.49% of patients were suffering from hirsutism. In a meta-analysis by Azziz et al. (2000), the cumulative incidence of hirsutism was approximately 60% in PCOS patients of all racial groups. In women with PCOS, hirsutism may result from the combined influence of increased androgen production or greater androgen activity within the pilosebaceous unit (Bart, et al 2012). Androgens participate in the development of acne by stimulating sebum production, thereby providing optimal conditions for bacterial colonization with organisms such as Propionibacterium acnes (Burkhart, et al 2004).

6. FETUIN A ASSESSMENT IN PCOS

Results from table 3 and figure 1 observed that Fetuin-A concentration was increased significantly ($P<0.01$) in PCOS women to 212.7±10.7 µg/ml in comparison with control women which were 156.2±6.9 µg/ml. In the current study, we evaluated the association of serum Fetuin-A level and overweight PCOS women. We found a difference in Fetuin-A levels between PCOS and control group.

A previous observation noted that liver-derived fetuin-A in overweight PCOS women may play a significant role in mechanisms that regulate postprandial glucose disposal, insulin sensitivity, weight gain and fat accumulation. Because, as in animal and human studies the inhibitory function of fetuin-A on insulin receptor tyrosine kinase in the muscle and in the liver proved (Srinivas et al 1993; (Mori et al 2006)). Fetuin A may considered as an important link between obesity and insulin resistance.

Our finding, that fetuin-A levels in obese women with polycystic ovary syndrome increased in comparison with healthy subjects. A few other human studies, that did not involve women with PCOS, found higher levels of fetuin-A in obese subjects with metabolic disturbances compared to lean individuals. In obese children, initially elevated fetuin-A levels decreased during exercise- and diet-induced weight loss. In patients with morbid obesity, fetuin-A was markedly increased and significantly declined after weight loss resulting from bariatric surgery. This fall related to changes in insulin resistance but not directly to BMI (Brix et al. 2010).

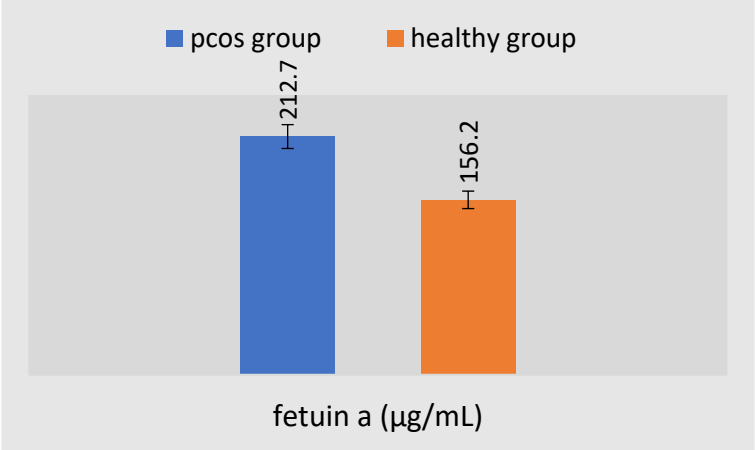

Figure 1: Mean fetuin-A levels in PCOS and healthy group

In two previously published independent trials, there was divergent data on serum fetuin-A levels in women with PCOS. Abali et al. (2013) was found that mean serum fetuin-A concentrations were considerably elevated in PCOS women compared to healthy controls, whereas Gulhan et al. (2012), observed no difference between women with PCOS and healthy subjects with regard to fetuin-A levels. Probably, all these discrepancies related to differences in age, BMI, liver fatness, level of insulin resistance and other yet undefined metabolic factors.

7. CORRELATION TO FETUIN-A

The relation between fetuin-A levels and the clinical features, metabolic features lipid profile and hormonal profile respectively in both control women and PCOS women studied to assess its correlation.

From figure 2 and 3, the correlation diagram of fetuin-A and BMI, in control group was observed and that there is no significant correlation where r= +0.13, while in PCOS group there is a positive correlation where r= +0.37 which indicate to the rising of fetuin-A with the increasing in BMI which corresponds the increasing in weight.

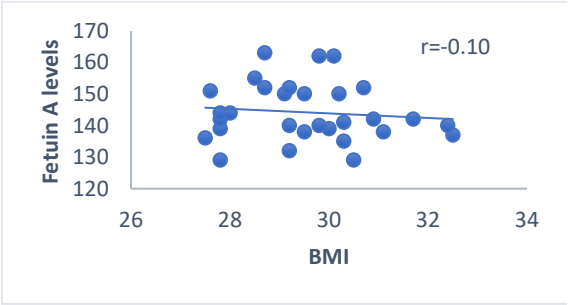

Figure 2: Correlation between fetuin-A and BMI in control women

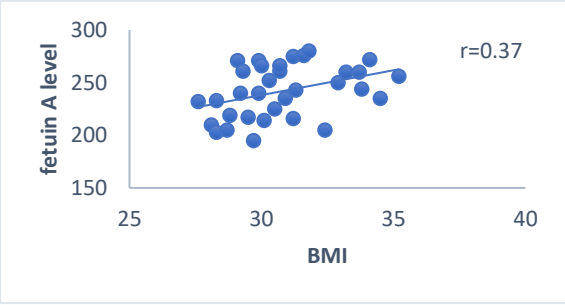

Figure 3: Correlation between fetuin-A and BMI in PCOS women

From figure 4 and 5, the correlation diagram of fetuin-A and adiponectin, in control group was done and there is slightly significant correlation where r= +0.20, while in PCOS group there is no significant relation between fetuin-A and adiponectin.

From figure 6 and 7, we have the correlation diagram of fetuin-A and leptin, in control group we see a direct positive correlation where r= +0.40, while in PCOS group there is no significate relation between fetuin-A and leptin.

From figure 8 and 9, we have the correlation diagram of fetuin-A and TNF-α, in control group we see that there is slightly positive correlation where r= +0.23, while in PCOS group there is a slightly negative correlation where

r= -0.17, which indicates to the different in correlation which increases in healthy control group and decreases in PCOS group.

From figure 10 and 11, we have the correlation diagram of fetuin-A and IL-6, in control group we noticed that there is slightly positive correlation where r= +0.17, while in PCOS group there is a slightly negative correlation where r= -0.17, it can refer to the different in correlation which increases in healthy control group and decreases in PCOS group.

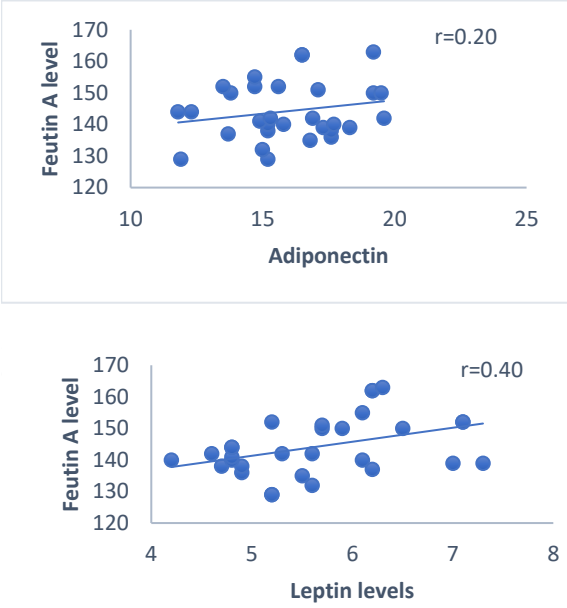

Figure 6: Correlation between fetuin-A and leptin in control women

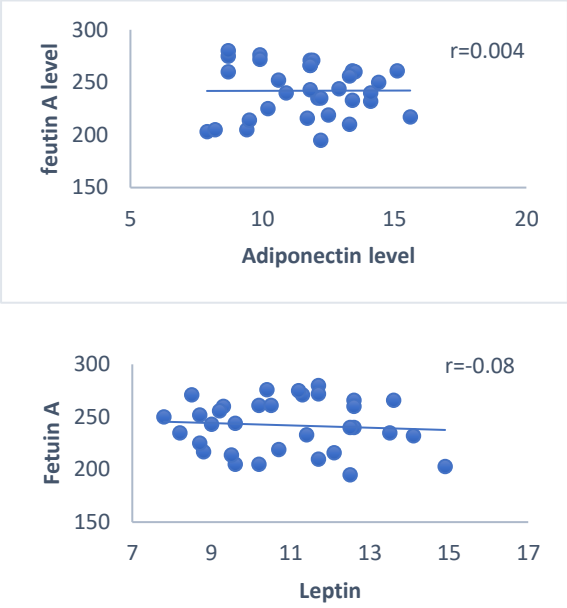

Figure 7: Correlation between fetuin-A and leptin in PCOS women

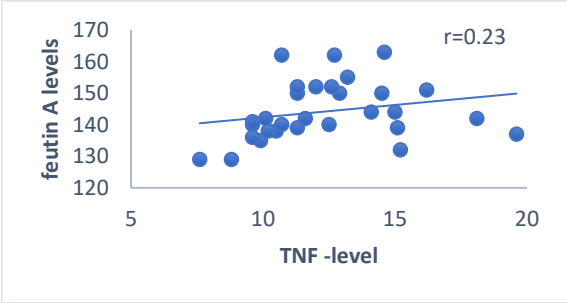

Figure 10: correlation between Fetuin-A and il-6 in control women

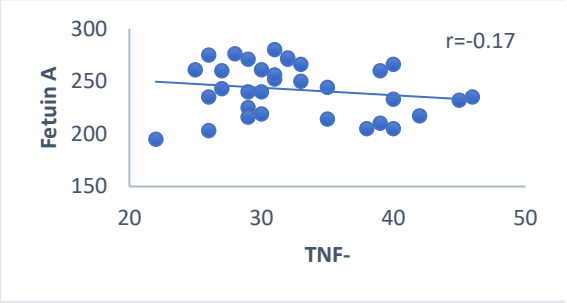

Figure 11: Correlation between Fetuin-A and IL-6 in PCOS women

From figure 12 and 13, the correlation diagram of fetuin-A and IL-10, in control group was showed a negative correlation where r = - 0.31, while in PCOS group there is a slightly positive correlation where r = + 0.16, which indicates to the different in correlation which decreases in healthy control group and increases in PCOS group.

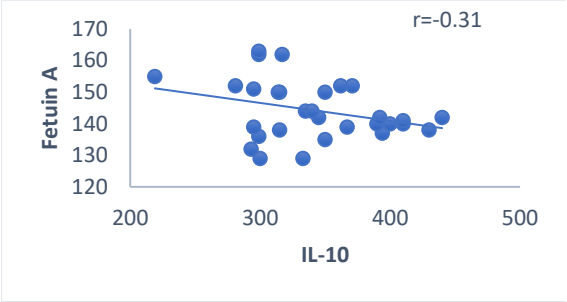

Figure 12: Correlation between fetuin-A and IL10 in control women

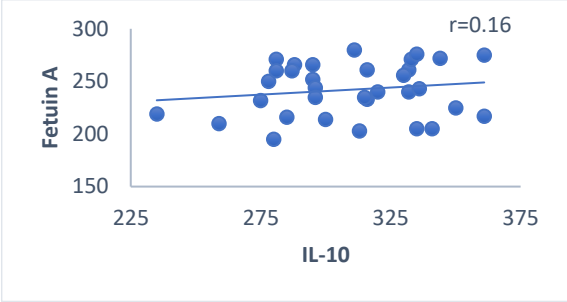

Figure 13: Correlation between fetuin-A and IL-10 levels in PCOS women

From figure 14 and 15, the correlation diagram between fetuin-A and insulin, was shown and that there is no significant correlation in both control and PCOS group where in control group r = -0.04 and in PCOS group r = +0.11.

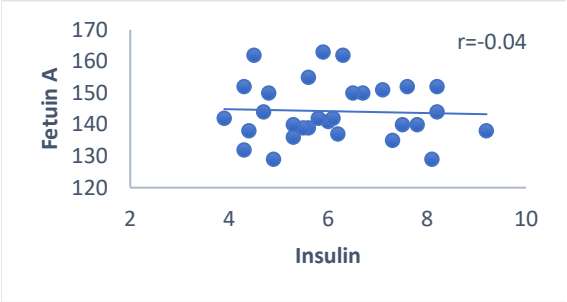

Figure 14: correlation between fetuin-A and insulin in control women

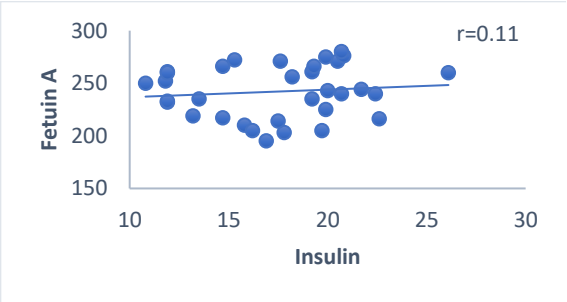

Figure 15: correlation between fetuin-A and insulin in PCOS women

From figure 16 and 17, we have the correlation diagram of fetuin-A and B. sugar, in control group we see that there is slightly negative correlation where r= -0.21, while in PCOS group there is no significant correlation.

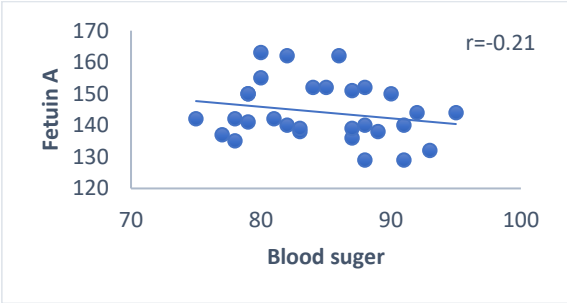

Figure 16: Correlation between fetuin-A and B. sugar in control women

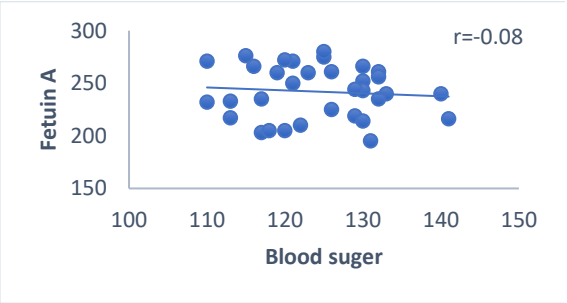

Figure 17: correlation between fetuin-A and B. sugar in PCOS women

From figure 18 and 19, we have the correlation diagram of fetuin-A and cholesterol, in control group we see that there is slightly negative correlation where r = -0.14, while in PCOS group there is a slightly positive correlation where r = +0.24, which indicates to the different in correlation which decreases in healthy control group and rise in PCOS group.

From figure 20 and 21, we have the correlation diagram of fetuin-A and Tg, in control group we see that there is slightly negative correlation where r = -0.26, while in PCOS group there is a slightly positive correlation where r = +0.17, which indicates to the different in correlation which descends in healthy control group and rise in PCOS group.

From figure 22 and 23, we have the correlation diagram of fetuin-A and HDL, we can sense no correlation in both control group and PCOS group, but it was somewhat negatively correlated in PCOS group where r = -0.14.

From figure 24 and 25, we have the correlation diagram of fetuin-A and LDL, in control group we see that there is no significant correlation in control group where r = -0.11, while in PCOS group there were a slight positive correlation where r = +0.20.

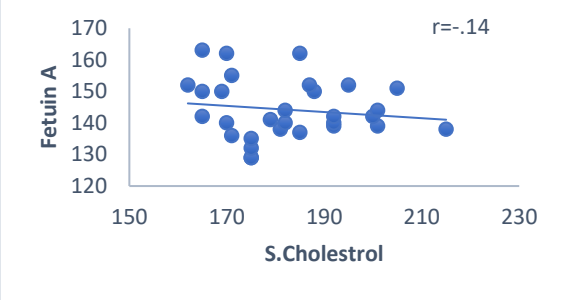

Figure 18: Correlation between fetuin-A and cholesterol in control women

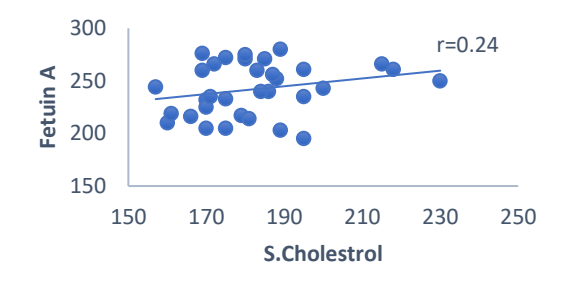

Figure19: Correlation between fetuin-A and cholesterol in PCOS women

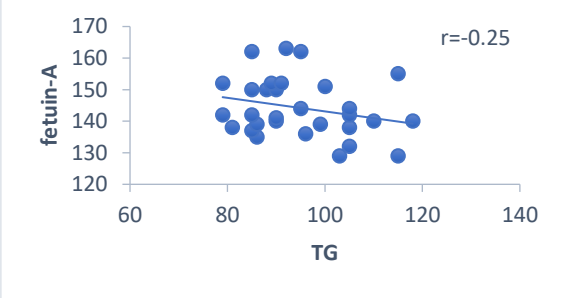

Figure 20: correlation between fetuin-A and TG in control women

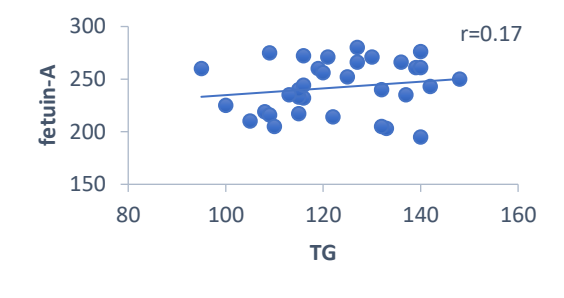

Figure 21: Correlation between fetuin-A and TG in PCOS women

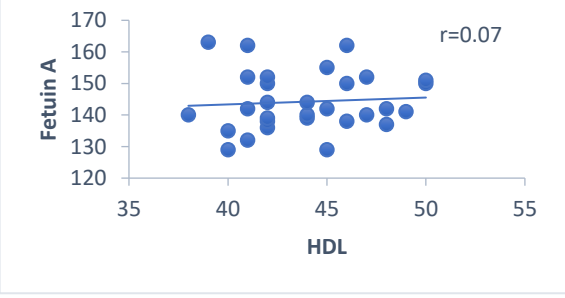

Figure 22: correlation between fetuin-A and HDL in control women

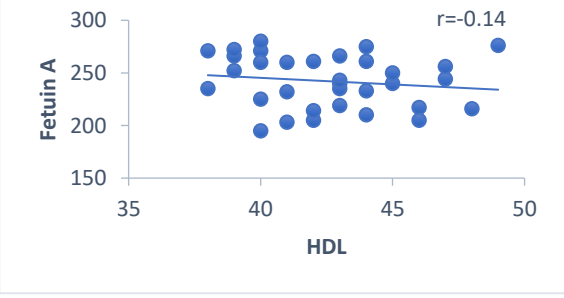

Figure 23: correlation between fetuin-A and HDL in PCOS women

From figure 26 and 27, we have the correlation diagram of fetuin-A and vLDL, both groups show a similar correlation where in control group r = -0.25 and in PCOS group r = - 0.23.

From figure 28 and 29, we have the correlation diagram of fetuin-A and HOMA, in control group we can sense no significant correlation where r= -0.15, while in PCOS group there is a direct correlation where r = +0.35.

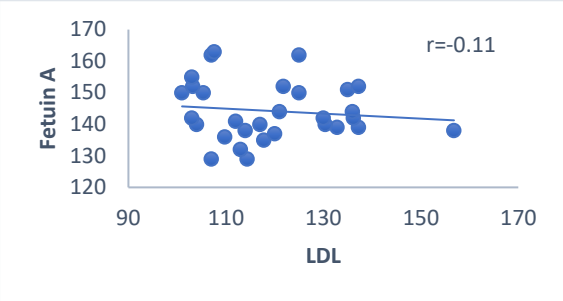

Figure 24: correlation between fetuin-A and LDL in control women

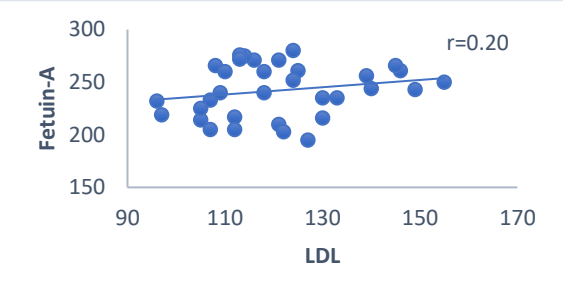

Figure 25: correlation between fetuin-A and LDL in PCOS women

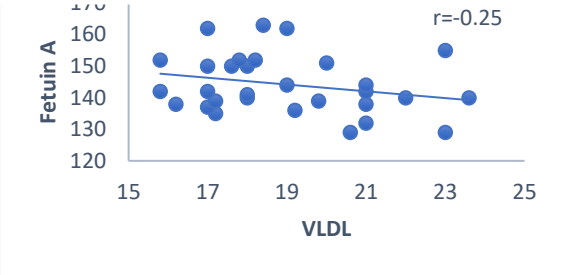

Figure 26: correlation between fetuin-A and vLDL in control women

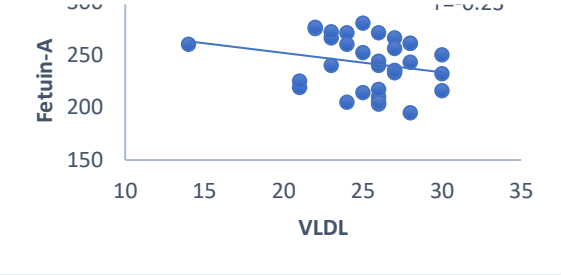

Figure 27: correlation between fetuin-A and vLDL in PCOS women

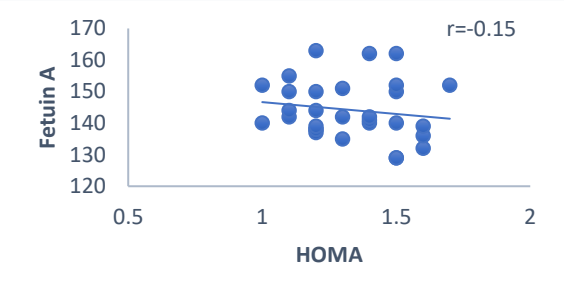

Figure 28: correlation between fetuin-A and HOMA in control women

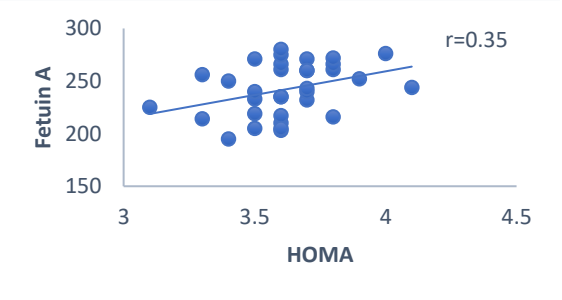

Figure 29: correlation between fetuin-A and HOMA in PCOS women

The women with PCOS, obesity and subsequent hyperinsulinaemia regarded as meaningful factors that contribute to androgen excess (Singh, et al 1990). It could be that fetuin-A is a factor that promotes insulin resistance, and in consequence hyperinsulinaemia may be associated with increased androgen production in women with PCOS, at least in adrenals. We could not demonstrate a similar correlation for testosterone and FAI. In the case of FAI, the impact of obesity on hepatic SHBG production and therefore on its serum levels should be taken into account additionally.

Serum fetuin-A levels reported to be negatively correlated with serum adiponectin in diabetes (Mustafa, et al 2014). A negative correlation between fetuin A and adiponectin also shown in general population studies (Ishibashi, et al 2010). There are some reports pointing out the association of the adiponectin and fetuin A independently of each other with some disease states.

On the other hand, Kozawkowski et al (2014) found that there were no differences between fetuin-A levels in lean and obese PCOS women, but they found a direct correlation between fetuin-a and DHEAS. Fetuin-A was positively correlated with insulin, HOMA-IR and FAI.

The present study showed that the level of Fetuin-A is associated with the PCOS. Moreover, Fetuin-A levels also related to insulin resistance and elevated testosterone levels frequently related to PCOS and other hormones in patients with PCOS. Fetuin-A, as a phosphorylation substrate, inhibits tyrosine kinase activity which results in impaired insulin action (Dunaif. et al 1995). It is possible that another factor may promote the Fetuin-A synthesis; for example, genetic factors and other cytokines secreted from adipose tissue could be involved in the regulation of hepatic synthesis of Fetuin-A. Further studies needed to elucidate the underlying mechanisms.

Giandalia et al. 2018 said, "PCOS women were heavier, had worse clinical hirsutism, higher biochemical androgen levels and worse metabolic profile (i.e. greater fasting insulin and HOMA-IR, greater triglycerides and lower HDL-cholesterol levels)." From their study data, it can summarized that all the characteristics, biological and hormonal tests that except from FSH, SHBG, HDL, and T. cholesterol that were found to be decreased in PCOS women, the rest were found to be increased in PCOS women.

(Ibrahim et al. 2018) also confirmed a decreasing in FSH concentrations in PCOS women compared to the control healthy women.

(Rashad et al 2018) said "In attempt to compare between control and PCOS groups we adjusted age and body composition parameters; body mass index (BMI), waist/hip ratio, fat mass index (FMI %) and fat free mass index (FFMI %). In PCOS group, we found significant higher levels of systolic blood pressure, TG, FSI, (percentage), HOMA-IR compared to control group. Regarding PCOS characteristic parameters, total testosterone, free testosterone, androstenedione, hirsutism score, ovarian volume, FSH, LH, LH/FSH, dehydroepiandrosterone sulphate (DHEA-S), and antral follicle count (AFC) values as we expected they were significantly higher. On the contrary, we detected significantly lower HDL cholesterol and SHBG levels in PCOS patients than in control group. Moreover, we observed significant higher levels of inflammatory markers; hs-CRP, WBC, neutrophil lymphocyte ratio (NLR) and neutrophil counts in PCOS cases compared to controls. ($P > 0.05$)", as it is shown it can be conducted that they found a lower FSH and SHBG concentrations compared to other parameters where they were higher in PCOS group more than the control group.

(Na Malini et al 2017) said that "PCOS patients were observed to have different ranges of LH: FSH ratios from < 1 range to 4.6-5.5 and subjects were classified into 7 PCO subgroups on the basis of their LH: FSH ratios. In whole PCO group, body weight, LH, FSH, LH: FSH ratio, insulin, HbA1c, estradiol, testosterone and TSH were significantly ($P < 0.05$) increased whereas progesterone and SHBG levels were significantly ($P < 0.05$) decreased in comparison to control. In various PCO subgroups as LH levels and LH: FSH ratios were increased, levels of insulin, testosterone and AMH were increased and SHBG levels were decreased accordingly."

8. CONCLUSIONS AND RECOMMENDATIONS

From the data collected and the study of the relation between fetuin-A and lipid profile, hormonal profile and the demographic features of the over weighted PCOS women, it is suggested that fetuin-A have a role in processes leading to insulin resistance and androgen excess. The collected result affected by the ethnicity, if there were any medication taken by the subjects, genetics, diet, and numerous effects can contradict with the data collected. Therefore, to gain a further knowledge including this topic it recommended doing as follow:

- A larger population scale is needed to identify the relation of fetuin-A to PCOS.
- Lean PCOS women could be included in the incoming research to gain a larger view.
- Enroll the subjects of the study to a diet plan to minimize the errors in lipid profile and hormones.

- Minimize the effect of medication in the later researches to minimize the errors in the tests.

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