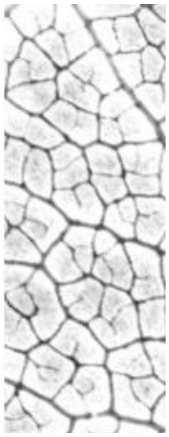

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 4493-4501 (2020)

Metformin effects on vaspin levels and other parameters in Iraqi polycystic ovarian women

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Abstract

Patients with polycystic ovarian syndrome showed hormonal imbalance and hyperandrogenemia because of higher secretion of androgen from theca cells of ovaries and adrenal gland due to excess LH or hyperinsulinemia. Obesity and increase fat mass aid the pathophysiology of PCOS, therefore, Different studies try to find the relationship of PCOS with Vaspin protein, which expressed in visceral adipose tissue resulting in increased insulin sensitivity by different mechanisms. This study is designed to show the effect of metformin treatment on Vaspin levels in newly diagnosed PCOS patients treated by this drug and the association of PCOS hormonal changes with Vaspin level

Forty three patients of newly diagnosed PCOS patients are included in the study with no abnormal thyroid or adrenal function, they do not use oral contraceptive or other treatment to treat their PCOS before the study, they agree to start metformin 850 mg twice daily for three months and provide fasting blood sample with measuring BMI, at the second day of menstruation before starting treatment and after completing three months of treatment. FSH, LH, SHBG, total testosterone, insulin, glucose, estradiol are determined by cobas technique, while Vaspin and free testosterone are determined by ELISA technique. Homeostatic model assessment is used to measure insulin resistance (HOMA-IR) is determined according to the following formula: HOMA-IR=(Fasting Insulin multiplied by Fasting glucose)divided by 22.5

It was found that there were no significant changes in BMI, FSH,LH, SHBG, total testosterone, estradiol and vaspin after end of treatment for all patients, but free testosterone and HOMA-IR showed significant decrease at p-value 0.02 and 0.03 respectively.

The study reveals that metformin can induce decrease in free testosterone and insulin resistance but cannot produce sever weight loss or changes in hormones regulating menstrual cycle, SHBG or Vaspin. The study suggested that there are different factors can affect Vaspin level in PCOS patients other than insulin resistance as its level failed to decrease after intake of insulin sensitizing drug, metformin.

Keywords: PCOS, polycystic ovarian syndrome, FSH, follicle stimulating hormone, LH, luteinizing hormone, SHBG, sex hormone binding globulin, HOMA-IR, homeostatic model assessment of insulin resistance, ELISA, enzyme-linked immunosorbent assay

Ibarhim MA, Ahmeid MS (2020) Metformin effects on vaspin levels and other parameters in Iraqi polycystic ovarian women. Eurasia J Biosci 14: 4493-4501.

INTRODUCTION

Polycystic ovarian syndrome (PCOS), found in women, is one of the most common endocrine disorder, characterized by excess androgen, hirsutism and infertility (Okoroh et al.2012). Human theca cells of PCOS ovaries secret higher androgen level than normal theca cell ovaries which may play a role in production of PCOS ovarian features (Nelson et al. 1999). In PCOS patients, gonadotrophin releasing hormone produced in rapid pulses, which favors LH production and decrease FSH production, net result is increment of androgen production from theca cells and fall of granulosa aromatase, which is important to change androgen to estradiol (Conway et al. 2014). In PCOS, the increased of ovarian volume with follicle numbers (Rackow et al. 2018), menstrual dysfunction and infertility are highly correlated with LH level (Kumar and Sait 2011). Hyperinsulinemia and insulin resistance are common features in PCOS women (Dunaif 1997),this is more obvious in amenorrheic PCOS women or those with anovulatory menses than those with regular cycle. Decrease in peripheral sensitivity to insulin in PCOS patients, may be attributed to obesity, distribution of fat, and muscle mass (Dunaif 1997). However, increase insulin level directs ovarian steroidogenic enzymes

Received: June 2019
Accepted: March 2020
Printed: October 2020

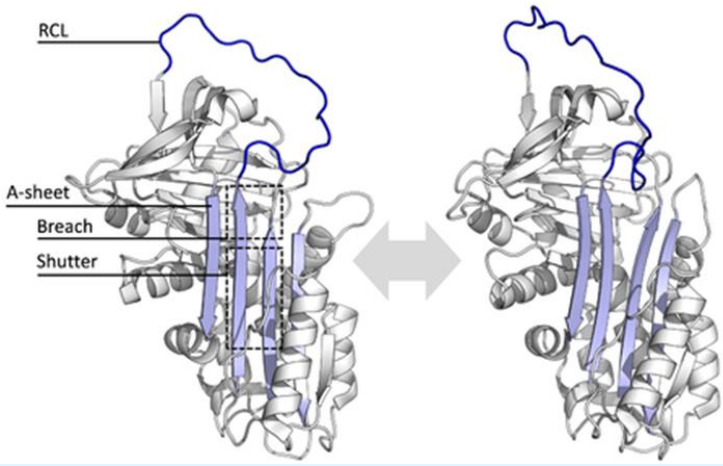

Fig. 1. The structure of Vaspin protein

(Bloomgarden 2010), leading to increase androgen, decrease SHBG and abolish ovulation (Legro et al. 2005). Insulin may also exert an effect on the hypothalamus and /or pituitary resulting in an abnormal gonadotrophins level augmenting LH action on ovary (Maqbool et al. 2019).

Adipose tissue is not only for energy store in form of fat, but also act as a multifunctional endocrine tissue, that express and secret bioactive peptide called 'adipokines", which can act on local, or systemic level (Kershaw and Flier 2004). Hormones such as insulin, adrenaline, noradrenaline and cortisone act on adipocytes regulating their function (Lehr et al. 2012; Saidi et al., 2018). Disruption of secretion of adipokine results in metabolic disorder (Kershaw and Flier 2004).Vaspin or (Visceral Adipose Tissue Derived Serpin) is a novel Adipocytokine that regulate glucose and lipid metabolism (Li et al. 2008), it is a protein consists of 415 amino acids in human, expressed in visceral adipose tissue (Hida et al. 2005). Vaspin is belonging to Serine protease inhibitors and consists of three β-sheets (A,B,C), nine α-helix and one active site loop, as **Fig. 1** (Hida et al.2000). Proteases are trapped in the structure of Vaspin between helices and sheets, therefore, vaspin is considered as a "suicide " enzyme inhibitor (Silverman et al.2001). Vaspin inhibit proteases that play a role in the degradation of substances resulting in direct or indirect glucose lowering effect (Hida et al. 2005) and increase glucose transporter type 4(GLUT4) expression and translocation in obese old myotubes (Nicholson et al. 2019), pancreatic cells (Liu et al.2017),pre-adipocytes (Liu et al. 2015), and endothelial progenitor cells (Sun et al. 2015). Vaspin also regulates feeding behavior by triggering anorectic pathway in hypothalamus mediating food inhibition(Brunetti et al. 2011) and stimulates receptor of FSH, LH, progesterone and estradiol, and resulted in increase secretion of progesterone and Estradiol (Kurowska et al. 2020)and increases expression of factors that suppress cytokine induced inflammation in adipocyte, cause attenuation of inflammatory response (Liu et al. 2015).

Increase androgen in females cause increase in fat mass, intra-abdominal mass, decrease adiponectin level, and disturbing adipose tissue function (Caldwell et al. 2017, Kauffman et al. 2015, Dumesic et al. 2016). While, Testosterone increase release of non- esterified fatty acid from visceral adipocyte Xu et al. 1990), increase lipolysis, impair adipocyte insulin signaling and generation of adipokine (O'Reilly et al. 2014). Moreover, enzymes expressed in adipose tissues that activate or inactivate androgen precursors are disturbed in PCOS adipose tissues, these enzymes in normal women regulate adipocytes proliferation, differentiation, insulin sensitivity, adipokine signaling and lipoid metabolism (O'Reilly et al. 2014). Therefore, visceral fat has great contribution to insulin resistance via adipokine and fatty acid release (Zhuang et al. 2009; Khodaie et al., 2019).

In fact, metformin drug used in PCOS treatment, increase insulin mediated glucose uptake in liver, visceral fat and muscle (Diamanti-Kandarakis et al. 2010). Also affect on neurotransmitter expression which result in decrease Gonadotrophin releasing hormone secretion and hence decrease LH from pituitary(Oride et al. 2010), decrease infiltration of immune cells in liver and brown adipose tissue, possessing anti-inflammatory activity(Kim et al. 2018). Moreover, metformin downregulate level of obesity related factors like cholesterol, LDL and mitigate progression of obesity (Kim et al. 2016; Mirzaie, 2018).

Because of the complex interaction between adipose tissue, Vaspin, steroid hormone and insulin resistance in PCOS, this study is designed to show the effect of metformin treatment on Vaspin level and other parameters in PCOS patients.

SUBJECTS, MATERIALS AND METHODS

This study deals with forty three newly diagnosed PCOS women according to Rotterdam criteria, with no

Table 1. Comparison of different parameters before and after metformin intake for all patients participated in the study

Measured Parameters	Before treatment		After treatment		p-value
	Mean	se	Mean	se	
Body mass index	29.29	0.89	27.5	0.87	0.15
FSH (uIU/ml)	6.32	0.29	6.41	0.61	0.89
LH (uIU/ml)	10.66	0.9	8.68	1.04	0.15
TESTOSTE RONE(ng/ml)	0.55	0.06	0.42	0.05	0.12
FREE TESTOSTERONE(Pg/ml)	2.86	0.1	2.48	0.13	**0.02**
Estradiol (Pg/ml)	119.45	7.39	99.05	12.17	0.16
SHBG (nmol/L)	27.68	3.68	35.94	5.02	0.19
HOMA-IR	5.38	0.82	2.58	0.48	**0.03**
Vaspin (pg/ml)	662.24	75.93	710.9	116.06	0.73

thyroid and adrenal problem, and they do not take drugs to treat their condition before, their weight and height are measured. Their ages ranges 25-33 years. The study is conducted in city of Mosul/Iraq, all the patients are advised to take metformin drug 850 mg twice daily during the meal for three months and to measure fasting glucose, fasting insulin, FSH, LH, estradiol, total testosterone, free testosterone, SHBG and Vaspin in the second day of menstrual cycle before after three months of metformin treatment. FSH, LH, SHBG, insulin, estradiol and total testosterone are measured by cobas e 411 system, glucose is determined by cobas e 311 system, vaspin and free testosterone are determined by ELISA technique, serum Vaspin measurement is done by Elisa kit: Human VASPIN (Visceral Adipose Specific Serine Protease Inhibitor) ELISA Kit Catalog No: MBS2506005 96T from Mybiosource/USA origin. Homeostatic model assessment is used to measure insulin resistance (HOMA-IR) is determined according to the following formula: HOMA-IR=(Fasting Insulin multiplied by Fasting glucose)divided by 22.5(Wallace et al. 2004, Matthews et al. 1985). HOMA-IR is easily obtainable, safe, low cost, less invasive test than oral glucose tolerance test (Wongwananuruk et al. 2012).

RESULTS

It was found that there were no significant changes in BMI, FSH, LH Testosterone, Estradiol, SHBG, Vaspin after three months of metformin therapy 850 mg twice daily; but there were significant decreases in free testosterone p-value 0.02, HOMA-IR of P-value 0.03.This can be illustrated in **Table 1**, which show the comparison of different parameters before and after intake of metformin 850 mg twice daily for three months.

DISCUSSION

This study revealed that there were no significant changes in BMI, total testosterone, while free testosterone and HOMA-IR exhibited significant decrease after treatment. Our study is in agreement with(Sharma et al.2019, Shahebrahimi et al. 2016),who show that metformin treatment for PCOS, had no significant effect on BMI although of lowering insulin level, as metformin can't encourage sever weight loss, but it favors redistributing of adiposity(Lashen 2010). Concerning total testosterone, Our results agree with (Singh et al. 2010, Baqer et al. 2018, Shahebrahimi et al. 2016) studies, as they found a decline in serum testosterone, but did not reach to significant value. While(Santana et al. 2004) study suggested that metformin improves insulin resistance and improve hyperandrogenemia through action on IGF-1(Insulin growth factor-1) and carrier protein, metformin also, can decrease androgen synthesis by direct effect of metformin on ovary(Kurzthaler et al. 2014), or by increase insulin sensitivity and decrease insulin level(Zahra et al. 2017) reversing insulin and LH synergism effect on ovary (Lakkakula et al. 2013, Baptiste et al. 2010), which explain the significant decrease in free testosterone in our study.

There was no significant change before and after metformin therapy in SHBG p-value 0.19, our result is in agree with (Eisenhardt et al. 2006, Harbone et al. 2003) while (Nestler and Jakubowicz 1997, Tang et al. 2012) showed an improvement in SHBG. Also,our study show that FSH,LH, Estradiol, values (p-values 0.89, 0.15, 0.16 respectively). A new meta-analysis study performed in 2017 (Patel and Shah 2017) included 532 women of 14 randomized studies, showed significant alteration in insulin ratio and testosterone, with no improvement in fasting blood glucose, SHBG, FSH, LH and Estradiol level, which is in agreement with our data. While, administration of metformin for six to twelve months by (Velija-Ašimi 2013) showed very high significant reduction in prolactin, total testosterone, Estradiol and (p-value 0.000), but no significant change in FSH level. Also, (Jindal et al. 2016) found that six months treatment decrease in BMI, LH at p-value 0.000 for both of them, while FSH remained unchanged. It is revealed that, longer duration of metformin intake can influence biochemical parameters more efficiently and balance an irregular cycle. FSH in study of (Deo Leo et al. 2006) do not increase after treatment, which also agree with our result.

This study show no significant differences in Vaspin level before and after intake of metformin 850 mg twice daily, which agrees with (Koiou et al.2011) study. However,(Koiou et al.2011) did not show significant differences in HOMA-IR after metformin intake in his patients, while, our study showed a significant decrease in HOMA-IR after metformin intake, indicating presence

of other factors other than insulin resistance may influence Vaspin concentration. This is obvious in study of (Kloting et al. 2010) who compared BMI,age, and gender matched insulin resistance and insulin sensitive healthy obese subjects, the Vaspin levels were indistinguishable between these two groups. Also, study of (Youn et al. 2008) failed to find any correlation between Vaspin, BMI, and insulin sensitivity in diabetic patients. Concerning PCOS, Vaspin is considered an important point of interaction between androgen, abdominal obesity and insulin resistance(Escobar-Morreale and San Millán 2007), but insulin resistance alone, cannot be considered the only marker or mechanism to change serum Vaspin, especially that circulating Vaspin levels were similar in women presenting with normal or abnormal glucose tolerance, irrespective of PCOS or obesity(Escobar-Morreale et al. 2009) and several studies failed to identify an association between Vaspin with insulin(von Loeffelholz et al. 2010, Cinar et al. 2011).

Increment of Vaspin can be found in females, not only in PCOS patients (Tan et al.2008), but also in girls after puberty (Körner et al. 2005) and after intake of oral contraceptive bills (von Loeffelholz et al.2010),giving rise to suggest that estrogen level may be an important factor in Vaspin concentration.. Recent in vitro animal study (Kurowska et al. 2019) found that gonadotrophins, insulin, estradiol and testosterone all can influence vaspin expression. Study of (Tan et al. 2008) suggested that the decreased Vaspin after metformin intake is due to effect of metformin treatment in decreasing gonadal steroid levels and estrogen. While (Akbarzadeh et al. 2012) suggested that changes of adipokines and Vaspin are due to consequences of fat accumulation. The suggestions of (Tan et al. 2008, Akbarzadeh et al. 2012) can be a good contributions to our result, especially that patients in our study showed no significant changes in BMI and estrogen level before and after metformin therapy.

According to study of (Klöting et al. 2006), Vaspin expression is a compensatory mechanism to obesity and Vaspin is correlated with BMI, as the increased fat mass decreases insulin sensitivity and induces Vaspin levels. The relationship between obesity and Vaspin is obvious, because Vaspin is secreted from visceral and subcutaneous adipose tissue of obese patients, although of that Vaspin can be expressed in non-fat cells (Fain et al. 2008), and ovary (Kurowska et al. 2019). Moreover, the relationship between Vaspin and BMI is strengthened when undetectable Vaspin levels has been shown in lean subjects and increased Vaspin level in overweight or obese individuals(Youn et al. 2008), and most of obese subjects showed significant decrease in Vaspin levels after bariatric surgery(Handisurya et al.2010)or after weight reduction program(Chang et al. 2010). On the other hand, Physical activity and changes of life style may be considered an another influencing factor on Vaspin concentration, that long term physical training can decrease Vaspin concentration(Oberbach et al 2010), although of that ten months of life style changes failed to affect Vaspin concentration in metabolic syndrome patients (Kim et al. 2011).).

Duration of metformin treatment is another factors that may affect Vaspin level, as the administration of metformin for six months in studies of (Kadoglou et al. 2011, Tan et al. 2008) showed significant decrease in Vaspin concentration. The study of (Kadoglou et al. 2011) involved Type 2 Diabetic patients who exhibited significant changes in BMI after six months treatment, the decrease of BMI in (Kadoglou et al. 2011) explains the differences in results of Vaspin changes between our study and his study. While, study of (Tan et al. 2008) involved regular menstrual cycle PCOS women, they exhibited significant decrease in glucose, estradiol, HOMA-IR and total testosterone after treatment. While in our study, the PCOS women were of sever PCOS with amenorrheic symptoms, Which explains the insignificant decrease of Vaspin in our study. In addition to that, other factors may influences Vaspin levels, these includes: nutritional status, age, gender, insulin sensitizer and changes of pituitary functions(González et al. 2009).

Another explanation of our result is that most of our patients started to take drug from summer and after they complete their course of metformin, the second time of sampling occurred during days of colder weather than from months of the first sampling. It was found that Vaspin is up regulated in brown adipose tissue, after cold exposure (Rosell et al. 2014),as cold exposure and high fat diet cause up regulation of Vaspin expression (García-Ruiz et al. 2015). In other word, The people who come at summer months : June, July, August, retuned back after three months : September, October and November, while those people come in September, October and November, return back in December, January,February months. In Iraq it is well known that, in the year of the study (2019), climate temperatures were very low reaching -6 degree Celsius or less in some days of January and February, while temperatures in summer were 35-40 degree Celsius or more in some days. The effect of climate temperature is very important factor that can affect on Vaspin levels, moreover, people at winter tend to consume high fat diet which may also tend to upregulate Vaspin expression, as free fatty acids activate target gene expression (Schoonjans et al.1996) also, Vaspin expression is increased upon feeding (González et al. 2009). The high differences in climate temperatures between date of two blood sampling may explain the insignificant decrease of serum Vaspin compared with (Tan et al. 2008)

CONCLUSION

Administration of metformin to treat PCOS patients for three months can influence decrease in free

testosterone and insulin resistance but has no significant effect on Vaspin level and BMI, Which suggest that other factors can influence Vaspin level a part from insulin resistance. Longer period of metformin treatment for six months and more may influence reduction in BMI, LH, and estradiol.

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