Effect of Metformin on Adenosine Deaminase Activity in Polycystic Ovarian Syndrome patients

Faisal Gh. Al-Rubaye*, Taha Shawi Morad **, Maysoon A. Ahmed **

*Department of Chemistry & Biochemistry, College of Medicine, Al-Nahrain University

**Department of Medical Biology, College of Medicine, Al-Nahrain University.

E-mail: faisal3ghazi@yahoo.com

Mobile: +9647702640792

Abstract

This study was done to assess the role of adenosine deaminase (ADA) in women with polycystic ovarian syndrome (PCOS) on metformin therapy. The present study is a cross-sectional study (2011/2012) at Al-yarmouk teaching hospital. A total of 80 patients with PCOS were involved in this study, they were classified as newly diagnosed women with PCOS G1: (n=40);women with PCOS on metformin therapy 500 mg (bid) for 90 days G2: (n=40). A matching group of forty apparently healthy women who were included as controls(n=40). Serum ADA was measured in all women and it was significantly reduced in women with PCOS receiving metformin therapy when compared with newly diagnosed patients with PC ($p < 0.001$) also a significant reduction was found when controls compared with the newly diagnosed women ($p < 0.001$); however, the reduction was insignificant when controls compared with treated group ($p < 0.05$). In conclusion, the results of this study suggest that the metformin currently used by PCOS patients has indirect effect on ADA activity through improving insulin sensitivity.

Keywords: adenosine diaminase, PCOS, metformin.

تأثير عقار المتفورمين على فعالية أنزيم الأدينوسين دي أمنيدز لدى مريضات متلازمة تكيس المبيض المتعدد

د. فيصل غازي الربيعي*، طه شاوي مراد**، ميسون عبد الامير احمد**

*فرع الكيمياء و الكيمياء الحياتية، كلية الطب، جامعة النهرين، بغداد، عراق

**فرع البايولوجي الطبي، كلية الطب، جامعة النهرين، بغداد، عراق

الخلاصة

الهدف من هذه الدراسة هو لتَقييم منزلةِ انزيم الأدينوسين دي امينيز في متلازمة تكيس المبيض المتعدد.

إنّ الدراسةَ الحاليةَ هي دراسة مقطعية–عرضية للفترة (2011–2012) في مستشفى اليرموك التعليمي، اشترك في هذة الدراسة 80 مريضة بمتلازمة تكيس المبيض المتعدد، تم تقسيمهم كالآتي:

- مريضات مصابات بمتلازمة تكيس المبيض المتعدد المشخصين حديثا وبدون علاج ج1 (العدد =40)
- مريضات مصابات بمتلازمة تكيس المبيض المتعدد وعلى علاج بالمتفورمين بجرعة 500 ملغم مرتين في اليوم لمدة 90 يوم ج2 (العدد =40)

اضافة الى مجموعة مقارنة من نساء اصحاء ظاهريا تم اعتمادهم كمجموعة سيطرة ج 3(العدد = 40).

تم قياس تركيز انزيم الادينوسين دي امينيز لكل النساء واظهرت النتائج انخفاض معنوي في تركيز انزيم الادينوسين دي امينيز في مصل الدم في المريضات مصابات بمتلازمة تكيس المبيض المتعدد وعلى علاج بالمتفورمين بجرعة 500 ملغم مرتين باليوم لمدة 90 يوم ج2 كما هو مُقارن بالمريضات المصابات بمتلازمة تكيس المبيض المتعدد المشخصات حديثا وبدون علاج ج1 ولم يسجل اي فرق معنوي بالمقارنة مع مجموعة السيطرة ($P<0.05$)على الرغم من وجود ارتفاع معنوي في ج 1 بالمقارنة مع السيطرةِ ($p <0.001$). أظهرت النتائج ان عقار المتفورمين المستعمل كعلاج لمريضات تكيس المبيض المتعدد له تاثير مثبط غير مباشر على انزيم الادينوسين دي امنيزمن خلال تخفيض مقاومة الانسولين بزيادة حساسية الانسجة للانسولين.

الكلمات المفتاحية: الادينوسين دي أمينيز، المتفورمين، متلازمة تكيس المبيض المتعدد.

Introduction

Polycystic Ovarian Syndrome [PCOS] is a relatively common endocrine disorder in women of reproductive age group. It is found in around 70% of women who have ovulation difficulties leading to sub-fertility [1].

PCOS is a condition that has cysts on the ovaries that prevent the ovaries from performing normally. Symptoms of PCOS include Amenorrhea or infrequent menstruation, irregular bleeding, infrequent or no ovulation, multiple immature follicles, increased levels of male hormones, male pattern baldness or thinning hair, excess facial and body hair growth, acne, oily skin or dandruff, dark colored patches of skin specially on neck, groin, underarms, chronic pelvic pain, increased weight or obesity, diabetes, lipid abnormalities and high blood pressure [1].

Fertility problems experienced by women with PCOS may be related to the elevated hormone, insulin or glucose levels, all of which can interfere with implantation as well as development of the embryo [1]. Increased Leutenizing hormone reduces the chance of conception and increase miscarriage. Additionally abnormal insulin levels may also contribute to poor egg quality, making conception more difficult [1].

Insulin resistance (IR) is known to play a critical role in the pathophysiology of PCOS [2]. The administration of insulin sensitizer metformin (MET) is recognized as a successful treatment for many metabolic and reproductive dysregulations characteristic of women with PCOS [3].

Adenosine DeAminase[ADA] is an enzyme that converts adenosine into inosine through an irreversible deamination reaction [4]. Previous studies have reported that the highest ADA activity was observed in the lymphoid and fatty tissues, liver, skeletal muscle, and heart, although the activity was widely distributed in most organs [5,6]. An increase in ADA activity in type 2 diabetic (T2DM) patients has been reported [7-9]. While the mechanism that increases serum and tissue ADA activity is not well known, with higher ADA activity in insulin-sensitive tissues, the level of adenosine, which increases glucose uptake into cells, will be reduced [8]. Thus, if ADA activity is suppressed, insulin sensitivity may be improved, and cellular proliferation, inflammation, and T-cell activity, all of which are associated with the pathophysiology of insulin resistance, can also be affected. Therefore, insulin resistance may have an important relationship with ADA activity. However, it is difficult to conclude whether changes in ADA activity are the cause or result of actual insulin resistance [9, 10]. In

addition to its association with diabetes, serum ADA activity is also increased in patients with liver cirrhosis as well as in patient with PCOS and infectious diseases such as hepatitis, tuberculosis, brucellosis, and typhoid fever [11, 12].

Since ADA activity is associated with insulin resistance, in the present study, we measured serum ADA activity in PCOS patients with or without metformin therapy to check if metformin as, insulin sensitizer, affect ADA activity in PCOS patients.

Materials & Methods:

Subjects:the study was a cross-sectional study carried out at Obstetric Department at Al-Yarmouk Teaching Hospital, during the period from October, 2011 till the end of September, 2012. The diagnosis of PCOS was made from the history of chronic oligomenorrhoea (cycle length>35 days, or less than 9 cycles per year), amenorrhoea (cycle length> 12wks), infertility with hirsutism or acne, and with an ultrasonographic findings of polycystic ovaries [13]

Exclusion conditions included the following systemic and endocrine disorders: late-onset congenital adrenal hyperplasia, Cushing's syndrome, thyroid dysfunction, hyperprolactinemia, diabetes mellitus, coronary artery disease, and spontaneous abortion. Furthermore, subjects accepting treatment with medications known to alter insulin hemodynamics, ovulation induction, anti-obesity, or oral contraceptives (OCs) within 3 months were excluded from the study. All subjects were nonsmokers, and none reported chronic alcohol consumption. The protocol for the study was approved by the Ethical committee of Al-Nahrain Medical College, and informed signed consent was given by each subject.

A total of 80 patients with PCOS were enrolled in this study: forty of them were newly diagnosed to have PCOS who receives no therapy for PCOS (G1); the remaining 40 patients were women with PCOS who receive Metformin 500 mg (bid) for 90 days as a therapy (G3) as in Table 1.

The study included another 40 apparently healthy subjects, they were neither alcoholic nor smoker with no family history of any type of DM who serve as healthy controls; they were matched with patients` groups for age as in *table 1*.

Blood samples: five milliliters of random venous blood were withdrawn from each patient, in supine position, without application of tourniquet. Samples were transferred into clean new plane tube, left at room temperature for 15 minutes for clotting, centrifuged at 1800 x g for 10 minutes at 4°C, and the separated serum was transferred into Eppendrof tube and was used for measurement of ADA. The tubes were stored at -20^{o} C until analysis, which was done within one month after collection. [14]

Methods: measurement of serum ADA was done by ELISA kit [14].

Statistical analysis: statistical analysis was done using Excel system version 2003 and includes descriptive statistics (mean and standard deviation) and inferential statistics (*t-test*) to test the significancy of mean

difference. When P-value was less than 0.05, the difference is considered statistically significant, and the difference is considered highly significant when P-value was less than 0.001.

Results & Discussion:

Serum Adenosine deaminase: Serum ADA was highly significantly reduced in PCOS group who receive treatment with metformin (G2) when compared with newly diagnosed PCOS group whom receive no therapy (G1) [$P < 0.001$]; however, no significant difference was found when compared with healthy controls (G3) [$P > 0.05$] despite the high significant elevation of ADA activity which was observed when newly diagnosed PCOS patients (G1) compared with healthy controls (G3) [$P < 0.001$] as in *Table 2*.

PCOS has been a subject of research and debate over past six decades. Insulin resistance accompanied by compensatory hyperinsulinemia is a common feature of PCOS [15].

Because ADA is closely related to T lymphocyte function [16] and insulin resistance, in the present study, we measured ADA activity in PCOS patients to evaluate this enzyme and to demonstrate whether ADA activity is affected by therapeutic drug (metformin). According to our results, ADA activity in PCOS patients was significantly higher than that in the control group, ADA activity comparisons showed that ADA activity was significantly lower in the metformin group than it was in the PCOS newly diagnosed group; however, no significant increase was found when metformin treated group compared with the control group. Metformin decreases insulin resistance, so ADA activity is expected to decrease in conjunction with metformin therapy. Reports from one study conducted on red blood cell lysates showed that metformin did not directly inhibit ADA activity [17]; however, the exact mechanism still remains unclear. Metformin's glucose-lowering effects and various other effects such as an anti-inflammatory actions, T-cell differentiation inhibition, TNF-α inhibition, and other immune regulatory effects [18] may be considered to have an effect on ADA activity.

In line with previous reports done by other researchers, ADA activity in PCOS patients in the present study are consistent with those reported by Hoshino et al. [19]. The limitation of this study was as follows: 1) ADA activity differences based on treatment and were compared in PCOS patients through a simple cross-sectional study, 2) comparisons before and after medical treatments were not performed, In conclusion, compared to the control group, ADA activity in newly diagnosed PCOS patients was higher. When metformin therapy was used for 90 days, ADA activity was nearly as controls.

Conclusion:

The results of this study suggest that the metformin currently used by PCOS patients do have an effect on ADA activity indirectly through improving insulin sensitivity. Additional studies are needed to evaluate the

activity of ADA in various insulin sensitive tissues.

Table (1): Clinical criteria of patients` groups with Polycystic Ovarian Syndrome & Control (presented as range and mean ± SD).

Group	***G1***	***G2***	***G3***
No	***40***	***40***	***40***
Age / year (Mean + SD)	***32±3.4***	***34±24***	***35 ±5***
Age range (years)	***28-41***	***31-40***	***33-41***
BMI -(kg/m²) (mean ± SD)	***21±4.8***	**27.2±4.5**	***22.1± 4.5***
BMI Range(kg/m²)	***16-23.9***	***22.4-34.7***	***15-26***

(G1): women with PCOS: newly diagnosed, on no treatment.

(G2): women with PCOS: on metformin therapy 500mg (bid) for 90 days.

(G3): HealthyControls.

Table (2): The mean serum Adenosine deaminase in different women with Polycystic Ovarian Syndrome and controls (presented as mean ± SD).

Variable	**G1**	**G2**	**G3**
serum ADA (IU/L)	26.4 ±4.9*§	17.9±3.5**	14 ±2.8

(G1): women with PCOS: newly diagnosed, on no treatment.

(G2): women with PCOS: on metformin therapy 500mg (bid) for 90 days.

(G3):HealthyControls.

* t-test: G1 versus G2, $p < 0.001$

** t-test: G2 versus G3, $p<0.05$

§t-test: G1 versus G3, $p<0.001$

The authors declare that they have no conflict of interest.

References

1. Dutta D.C. (2005) Text book of Gynaecology. 4th edition. Culcutta: New central book Agency LTD: 421. (431, 523, 549, 558).
2. DeUgarte,CM.,Bartolucci, AA.,Azziz, R. (2005) Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. Fertility and Sterility. 83: 1454–1460.
3. Hoeger, K., Davidson, K.,Kochman, L., Cherry, T., Kopin, L., David, SG. (2008) The impact of metformin, oral contraceptives, and lifestyle modification on polycystic ovary syndrome in obese adolescent women in two randomized, placebo-controlled clinical trials. Journal of Clinical Endocrinology and Metabolism. 93. 4299–4306.
4. Brady, T. (1942) Adenosine deaminase. Biochem J. 36:478-84.
5. Ratech, H,Hirschhorn, R. (1981) Serum adenosine deaminase in normals and in a patient with adenosine deaminase deficient-severe combined immunodeficiency. ClinChimActa. 115: 341-7.
6. Niedzwicki, JG., Abernethy, DR. (1991) Structure-activity relationship of ligands of human plasma adenosine deaminase2.Biochem Pharmacol.41:1615-24.
7. Prakash, MS.,Chennaiah, S., Murthy,YS.,Anjaiah, E.,Rao, SA., Suresh, C. (2006) Altered adenosine deaminase activity in type 2 diabetes mellitus. JIACM: 7.114-7.
8. Mokhtari, M.,Hashemi, M.,Yaghmaei, M.,Molashahi, F.,Shikhzadeh, A.,Niazi, A.,Ghavami, S. (2010) Serum adenosine deaminase activity in gestational diabetes mellitus and normal pregnancyArchGynecolObste. 281.623-6.
9. Kurtul, N., Pence, S.,Akarsu, E.,Kocoglu,H.,Aksoy, Y.,Aksoy, H. (2004) Adenosine deaminase activity in the serum of type 2 diabetic patients. ActaMedica (Hradec Kralove). 47: 33-5.
10. Kather,H. (1990) Pathways of purine metabolism in human adipocytes. Further evidence against a role of adenosine as an endogenous regulator of human fat cell function. J Biol Chem. 265:96-102.
11. Koopmans,SJ., Sips, HC.,Bosman, J.,Radder, JK.,Krans, HM. (1989)Antilipolytic action of insulin in adipocytes from starved and diabetic rats during adenosine-controlled incubations. Endocrinology.125:3044-50.
12. Green, A.,Newsholme, EA. (1979) Sensitivity of glucose uptake and lipolysis of white adipocytes of the rat to insulin and effects of some metabolites. Biochem J. 180:365-70.

13. The Rotterdam ESHRE/ASRM—sponsored PCOS Consensus Workshop Group 2004 Revised 2003. Consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Fertile Steril 2004;81:19.
14. ELISA Kit for Adenosine Deaminase (ADA) E91390Hu; Instruction Manual.
15. Legro, RS., Finegood, D., Dunaif, A. (1998) A fasting glucose to insulin ratio is useful measure of insulin sensitivity in women with polycystic ovary syndrome. J ClinEndocrinolMetab. 83:2694–8.
16. Gorrel, MD.,Gysbers, V.,McCaughan, GW. (2001) CD26: a multifunctional integral membrane and secreted protein of activated lymphocytes. Scand J Immunol. 54:249-64.
17. Paiva, M.,Riksen, NP., Davidson, SM.,Hausenloy, DJ.,Monteiro, P.,Goncalves, L.,Providencia, L.,Rongen, GA., Smits, P.,Mocanu, MM., Yellon DM. (2009) Metformin prevents myocardial reperfusion injury by activating the adenosine receptor. J Cardiovasc Pharmacol.53:373-8.
18. Kwak, B.,Mulhaupt, F.,Myit, S., Mach, F. (2000) Statins as a newly recognized type of immunomodulator. Nat Med.6:1399-402.
19. Hoshino, T., Yamada, K.,Masuoka, K.,Tsuboi, I.,Itoh, K.,Nonaka, K., Oizumi, K. (1994) Elevated adenosine deaminase activity in the serum of patients with diabetes mellitus. Diabetes Res ClinPract. 25:97-102.