THE EFFECT OF LONG TERM ADMINISTRATION OF METFORMIN ON PROLACTIN LEVEL AND C-REACTIVE PROTEIN IN NEWLY DIAGNOSED WOMEN WITH TYPE II DIABETES MELLITUS

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ABSTRACT

Introduction: The role of hypothalamus and neurohormonal circuitry in glycemic control that incorporates the cross talk between blood born factors and neurons is considered an important mechanism involved in diabetes pathophysiology. The theory emphasizes that the circadian neuroendocrine rhythm (Dopamine, 5-HT and norepinephrine) in the hypothalamus is altered in diabetes mellitus. Many experimental studies indicate an influence of prolactin on type 2 diabetes mellitus. This study aims to investigate the effect of different doses of metformin on prolactin and C-reactive protein in newly diagnosed female with diabetes mellitus. Material and methods: 60 newly diagnosed type 2 diabetic patients were divided into three groups according to the dose of metformin in addition to 20 diabetic free (control group). All patients were treated with metformin for 6 months. Result: Treatment with different doses of metformin result in a significant reduction in prolactin and C-reactive protein levels. Our data indicate that metformin significantly decreases prolactin level possibility via increasing the dopamine tone and attenuating the pro-inflammatory response via reduction in CRP. This can have an important role in improving the insulin resistance and other outcome of diabetes. Further investigations required to determine the exact role of prolactin in diabetes.

KEY WORDS: Type II diabetes mellitus, prolactin, C-reactive protein, metformin.
INTRODUCTION

Diabetes mellitus is a group of common metabolic disorders that is characterized by a phenotype of hyperglycemia. Diabetes was once regarded as a single disease entity; however due to the diversity of etiologies that results in a state of chronic hyperglycemia, it is now seen as a heterogeneous group of disease.\(^1\) There are two types of diabetes mellitus: Type I diabetes; which is an autoimmune disease that causes the death of pancreatic beta cell and a subsequent lack of Insulin production. The other type is type II diabetes, a scientifically more challenging disease, which is characterized by elevated insulin resistance and glucose intolerance.\(^2\)

Type II diabetes occur as a result of a defect in glucose, lipid and energy homeostasis in organs/systems such as liver, muscle, adipose tissue and gastrointestinal tract. The imbalances in lipid and carbohydrate metabolism causes high levels of serum glucose and free fatty acids, which in turn lead to endoplasmic reticular stress in pancreatic beta cells, and consequent death.\(^3\) Although there is a great understanding of the underlying pathophysiology and diligent efforts towards management of diabetes, the disease and is associated complications are still increasing due to multiple defects.

The role of hypothalamus and neurohormonal cirquitry in glycemic control that incorporates the cross talk between blood born factors and neurons is considered an important mechanism involved in diabetes pathophysiology. The theory emphasizes that the circadian neuroendocrine rhythm (Dopamine, 5-HT and norepinephrine) in the hypothalamus is altered in diabetes mellitus.\(^4,5\) Prolactin (PRL) is a pituitary hormone important for various physiological functions in the human body.\(^6\) In addition to its role for the initiation and maintenance of lactation, it is also involved in reproduction, growth and development, immune regulation, osmoregulation, brain function, behavior, and metabolism.\(^7\) The wide expression of prolactin receptors in different tissues and cells such as lymphoid cells, prostate, endometrium, and adipocytes\(^8\), further suggests the different functions of prolactin.

Despite the rare potential effects of prolactin in type 2 diabetes mellitus and its complications shown in previous investigations, existing experimental studies indicate an influence of prolactin on type 2 diabetes mellitus via its metabolic effects on adipose tissue\(^7,8\), development and growth of pancreatic β-cells\(^9,10\), insulin resistance\(^8,11\), and lipid metabolism.\(^3,12\) The ability of prolactin to stimulate insulin\(^9\) and suppress adiponectin in addition to interleukin-6 release further suggests an important role in the manifestation of
insulin resistance. In addition, high prolactin levels may increase proinflammatory response indicating an involvement in human immune dysfunction.

A recent shift towards novel markers of cardiovascular risk has emerged, particularly towards C reactive protein (CRP) which has been found to be a good predictor of vascular events. In addition to being an inflammation marker, there are several data that suggest a direct role of CRP in atherogenesis via complement activation, expression of adhesion molecule, and mediation of the uptake of low density lipoprotein (LDL) by macrophages. Elevated CRP accompanied by hyperinsulinemia is a significant risk factor for cardiovascular diseases.

The aim of diabetes management is to maintain normal glucose levels and to prevent macro and micro-vascular complications of diabetes mellitus.

Metformin is indicated as an oral hypoglycemic main therapy in diabetes management worldwide. Due to its favorable effect on metabolic indices of glucose, lipid, and weight control, the guidelines of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) recommend the use of metformin in patients regardless of age, body weight, and degree of baseline hyperglycemia.

The aim of this study is to demonstrate the long term effect of different doses of metformin on the levels of prolactin and C-reactive protein in diabetic female patients.

MATERIALS AND METHODS

The present study was a single center, open label, randomized parallel group study conducted at National Diabetes Center in Baghdad. 60 newly diagnosed type II diabetes mellitus female patients were enrolled in the study in addition to 20 healthy females matched with patients for age were included. The local clinical research ethics committee in accordance with Helsinki declaration 1998, approved the study protocol and all subjects gave written informed consent to participate in the study.

The patients evaluated for inclusion/exclusion criteria. The inclusion criteria include newly diagnosed female with type II diabetes according to ADA diagnostic criteria. The exclusion criteria include drugs and medical conditions that affect serum prolactin such as a medical history of prolactinemia, hypothyroidism or drugs that increase prolactin level, pregnancy and breast feeding. All patients were asked to monitor their blood glucose level
(fasting), prolactin and C-reactive protein levels at the initial visit to the center and after 3 and 6 months to measure the possible changes in studied parameters.

The patients were randomized into three groups according to the dose of metformin they received. First group includes 20 patients treated with 1000 mg metformin daily for 6 months; second group includes 20 patients treated with 1500 mg metformin daily for 6 months. The third group includes 20 patients treated with 2000 mg metformin daily for 6 months. Blood samples were obtained by venipuncture from a peripheral vein after 12 hour fasting and prior to any treatment as baseline then after 3 and 6 months the blood was allowed to clot and serum was separated and stored at −20 °C. Fasting serum glucose (FSG) was measured by enzymatic colorimetric test using kit provided commercially. The C-reactive protein (CRP) was measured using commercially available kit based on high sensitivity monoclonal antibody assay and the serum level of prolactin was measured by a commercial ELISA kit. Body mass index (BMI) was defined by national institution of health as body weight in kilogram divided by the height in squared meter (kg/m²). Normal weight was defined as BMI 25 kg/m². Over weight was defined as BMI ≥ 26 kg/m² and obesity was defined as BMI ≥ 30 kg/m²

Statistical Analysis
All data were statistically evaluated using paired t test to compare between pre- and post-treatment result. Two way analysis of variance (ANOVA) followed by Benferroni’s post hoc test was used to compare between the results of studied parameters among different patients groups. Values with p < 0.05 were considered significantly different.

RESULTS
As shown in table 1 treatment of newly diagnosed diabetic females with metformin in different doses (1000 mg, 1500 mg and 2000 mg) for 6 months significantly decrease fasting glucose levels in all groups compared with pretreatment baseline values.

Metformin significantly decreased BMI values in all groups after 6 months compared to baseline values (p < 0.05) as shown in table 1
Table (1): Effect Of Different Doses Of Metformin On Glucose Level And Body Mass Index In Newly Diagnosed Females With Type II Diabetes Mellitus.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Period</th>
<th>Control (n=20)(Baseline)</th>
<th>T2DM Treated with 1000mg/day (n=20)</th>
<th>T2DM Treated with 1500mg/day (n=20)</th>
<th>T2DM Treated with 2000mg/day (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting serum glucose (mmol/L)</td>
<td>Baseline Pre-treatment</td>
<td>4.6 ± 0.07</td>
<td>13.06 ± 0.16*</td>
<td>13 ± 0.16*</td>
<td>13.09 ± 0.15*</td>
</tr>
<tr>
<td></td>
<td>After 3 months</td>
<td>-</td>
<td>10.6 ± 0.19* †</td>
<td>10.10 ± 0.25* †</td>
<td>9.91 ± 0.24* †</td>
</tr>
<tr>
<td></td>
<td>After 6 months</td>
<td>-</td>
<td>7.10 ± 0.09* †</td>
<td>7.6 ± 0.23* †</td>
<td>7.14 ± 0.16* †</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Baseline Pre-treatment</td>
<td>28.34 ± 0.13</td>
<td>28.26 ± 0.16</td>
<td>28.37 ± 0.16</td>
<td>28.34 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>After 3 months</td>
<td>-</td>
<td>26.56 ± 0.28* †</td>
<td>26.51 ± 0.30* †</td>
<td>26.14 ± 0.28* †</td>
</tr>
<tr>
<td></td>
<td>After 6 months</td>
<td>-</td>
<td>26.03 ± 0.27* †</td>
<td>25.79 ± 0.28* †</td>
<td>25.45 ± 0.27* †</td>
</tr>
</tbody>
</table>

Data were expressed as mean ±SEM; n=number of patients.
*P<0.05 with respect to control group; †P<0.05 with respect to pre-treatment value.

Table 2: shows that treatment with different doses of metformin significantly decreased the serum prolactin level in comparison with pre-treatment baseline values.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum prolactin (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>Control (n=20)</td>
<td>11.15 ± 0.70</td>
</tr>
<tr>
<td>Metformin 1000mg (n=20)</td>
<td>33.18 ± 1.60* (197.58%)</td>
</tr>
<tr>
<td>Metformin 1500mg (n=20)</td>
<td>32.85 ± 1.89* (194.62%)</td>
</tr>
<tr>
<td>Metformin 2000mg (n=20)</td>
<td>31.30 ± 1.45* (180.72%)</td>
</tr>
<tr>
<td>ANOVA (P value) Among metformin dosing groups</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SEM and percent elevation from control group n=number of patients.
*P<0.001 with respect to control group.
†P<0.01 with respect to pre-treatment value.
No significant differences were existed between groups pre- and post-treatment (P>0.05).
Figure 1 shows the percent decline in prolactin level after 3 and 6 months and the maximum dose of metformin used in the study (2000 mg/day) produce no significant differences in prolactin reduction compare to the other doses.

![Figure 1: Percent decline in Prolactin level in newly diagnosed female diabetic patients after 3 & 6 months treatment with different doses of metformin. * P < 0.05 with respect to base line value. Non –identical superscript (a, b) represent significant difference among groups regarding the same period.](image)

Table 3 shows that the different doses of metformin significantly decreased the CRP after 3 and 6 month of treatment and the (2000 mg/day) dose produce the maximum reduction after 6 month of treatment compared with other doses as shown in figure 2.

**Table (3): Effect of different doses of metformin on C-reactive protein level in newly diagnosed female with type II diabetes mellitus.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>C-reactive protein (mg/L)</th>
<th>Baseline</th>
<th>After 3 months</th>
<th>After 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=20)</td>
<td></td>
<td>2.46 ± 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin 1000 mg (n=20)</td>
<td>3.37 ± 0.12 * (37.00%)</td>
<td>3.09 ± 0.11 * † (25.61%)</td>
<td>2.67 ± 0.1 † (8.54%)</td>
<td></td>
</tr>
<tr>
<td>Metformin 1500 mg (n=20)</td>
<td>3.37 ± 0.10 * (37.00%)</td>
<td>3.07 ± 0.09 * † (24.8%)</td>
<td>2.53 ± 0.08 † (2.85%)</td>
<td></td>
</tr>
<tr>
<td>Metformin 2000 mg (n=20)</td>
<td>3.19 ± 0.12 * (29.67%)</td>
<td>2.94 ± 0.09 * † (19.51%)</td>
<td>2.31 ± 0.08 † (-6.8%)</td>
<td></td>
</tr>
<tr>
<td>ANOVA (P value) Among metformin dosing</td>
<td>0.45</td>
<td>0.49</td>
<td>0.67</td>
<td></td>
</tr>
</tbody>
</table>
Data were expressed as mean ± SEM and percent elevation from control group n=number of patients.
*P<0.001 with respect to control group.
†P<0.01 with respect to pre-treatment value.
No significant differences were existed between groups pre- and post-treatment (P>0.05).

Figure (2): Percent decline in CRP level in newly diagnosed female diabetic patients after 3 & 6 months treatment with different doses of metformin. * P < 0.05 with respect to base line value. Non–identical superscript (a,b) represent significant difference among groups regarding the same period.

DISCUSSION
The role and levels of prolactin in diabetes mellitus is still controversial. Despite being a common disorder, hyperprolactinemia exact metabolic consequences are unclear. Experiments in animals and humans showed that prolactin may exert a diabetogenic effect.[18, 19, 20] There is no previous study that was undertaken to address the effect of metformin on prolactin level in diabetic patients.

In this study PRL level was significantly higher in all diabetic groups (33.18 ± 1.60, 32.85 ± 1.89, 31.30 ± 1.45) compared to control (11.15 ± 0.7). This finding is comparable with Mooradian et al and Shokoofeh et al.[21, 22]

High levels of prolactin exacerbate insulin resistance and impair insulin secretory capacity.[23] Prolactin may cause the insulin resistance via down-regulation of insulin receptors and/or due to post receptors defects.[24]
Many studies revealed that chronic elevation of prolactin was associated with increased food intake and high body weight.\(^{[25]}\)

The neuroendocrine control of prolactin secretion from the anterior pituitary gland involves several factors including prolactin releasing factors (PRFs) and prolactin-release inhibiting factors (PIFs), which exert a tonic inhibitory control.\(^{[26-29]}\) Dopamine (DA) secreted by the tuberfundibular dopaminergic neurons of hypothalamus is the main physiological PIFs.\(^{[29,30,31]}\) Dopamine at very low concentrations stimulates prolactin secretion from cloned GH4ZR7 cells and is inhibited at high concentrations.\(^{[32]}\) A hypothalamic deficiency of dopamine could explain the mild hyperprolactinemia frequently presents in women with polycystic ovary syndrome.\(^{[33]}\) This is further supported by the finding of a low DA hypothalamic tone with increased PRL bioactivity in obese, hyperinsulinemic women with polycystic ovary syndrome.\(^{[34]}\)

Several lines of evidence link the dopaminergic system to obesity, insulin resistance and type 2 diabetes in humans and animal models. There is an interaction between dopamine receptor D2 (DRD2) variants and energy homeostasis. The polymorphism Ser311Cys which impairs the DRD2 signal transduction pathways\(^{[35]}\) is associated with a high BMI and lower resting energy expenditure in Pima Indians.\(^{[36,37]}\)

The expression of DRD2 is reduced in specific brain areas of obese rats compared to lean control rats\(^{[38-40]}\), this decrease in DRD2 expression is also observed in the striatum of obese humans.\(^{[41]}\)

Dopamine levels are low during the insulin resistant state and increase to normal following the return to the insulin sensitive state.\(^{[42,43]}\) Bromocriptine (dopamine D2 receptors agonist) administration improved insulin sensitivity.\(^{[44]}\)

So in this study, there is a possibility for a decrease in dopaminergic activity and tone due to the increase in BMI, and may be due to insulin resistance in newly type 2 diabetic patients. This finding may explain the high prolactin levels in all diabetic groups.

The pituitary hormone and cytokine prolactin is one of the mediators of the bidirectional communication between neuroendocrine and immune system.\(^{[45]}\) Prolactin was shown to act as a stimulating factor for immune system and thus it might influence the development of autoimmune disease as in type 1 diabetes mellitus.\(^{[46]}\)
Hyperprolactinemia has been linked to dysfunction and low grade inflammation.\textsuperscript{[47]} In addition, it is associated with elevated high sensitive C-reactive protein\textsuperscript{[48]}, with reduction after treatment with DA agonist.\textsuperscript{[47, 48]}

High levels of CRP are strongly related to insulin concentrations\textsuperscript{[49]} and insulin resistance.\textsuperscript{[50]} Elevated levels of CRP were more closely related to insulin resistance than to obesity.\textsuperscript{[51]}

The data obtained from the present study revealed a significant elevation in CRP levels in all diabetic groups (3.37 ± 0.12, 3.37 ± 0.12 and 3.19 ± 0.12) compared to control (2.46 ± 0.11). Such findings confirm results obtained from previous studies.

Metformin administered in different doses for treatment of diabetic patients reduces blood glucose levels by inhibiting hepatic glucose production and reducing insulin resistance, predominantly in liver and skeletal muscle.\textsuperscript{[52]} Metformin decreases glucose intestinal absorption, and increase insulin sensitivity by enhancing glucose uptake and utilization in peripheral tissues.\textsuperscript{[53]}

Treatment of the diabetic patients in this study with different doses of metformin lead to a significant reduction in prolactin and C-reactive protein levels.

Six month treatment with (1000 mg/day or 1500 mg/day or 2000 mg/day) lead to significant reduction in prolactin level, but there is no significant difference between doses in prolactin reduction as shown in figure (1).

The reduction in prolactin may be due to the ability of metformin to increase the dopamine tone in hypothalamus in association with improvement in insulin resistance.\textsuperscript{[54]}

While six month treatment with 2000 mg/day of metformin produced the higher significant reduction in CRP level compared to other doses as shown in figure (2). This reduction in CRP may be due to the reduction in prolactin level in association with improvement in insulin resistance.

**CONCLUSION**

All diabetic female patients showed higher levels of prolactin and C-reactive protein compared to control group. Treatment with metformin for six months resulted in significant reduction of prolactin and C-reactive protein levels. The reduction of prolactin level is
possibly due to an increase in dopamine tone. Further investigations are required to explain the exact mechanisms of metformin to produce this effect.

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