THE EFFECT OF METFORMIN ON CYTOKINES IN IRAQI PATIENTS WITH TYPE 2 DIABETES

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes and is characterized by disorders of insulin action and insulin secretion, and associated with increase problem of insulin resistant. High plasma levels of insulin and glucose due to insulin resistance often lead to metabolic syndrome. Chronic inflammation associated with metabolic and immune system involves a network of cellular and systemic responses that integrates many complex signaling pathways. Inflammation cells in adipose tissue, abnormal pro-inflammatory cytokines reduction effect on these three groups, had significant reduction in HbA1c and also significant reduction in cytokines.

Introduction

Type 2 diabetes mellitus (T2DM) is characterized by disorders of insulin action and insulin secretion. Metformin activates AMPK in macrophages and this results in the reduction in cytokines, which is associated with both a localised and systemic chronic inflammation characterised by infiltration of inflammatory cells in adipose tissue, abnormal pro-inflammatory cytokine production. This phenomenon, referred to as meta-inflammation (metabolic inflammation).[7] Recently, it has been reported that metformin activates AMPK in macrophages and this results in the inhibition of biosynthesis of phospholipids as well as neutral lipids and down-regulates the expression of LPS-induced pro-inflammatory cytokines.[8] Metformin has suppressed IL-8 release from human adipose tissue in vitro. [9] Thus, metformin seems to exert its anti-inflammatory role by reducing pro-inflammatory cytokine secretion in specific cell types.[9]

Materials, Subjects and Methods

The study was conducted between October 2012 up to March 2013; during this period thirty Iraqi patients of newly diagnosed patient with Type 2 diabetes were attended of the (The National Diabetes Center, University of Al-Mustansiriya) Their age ranging (35-77) years, mean±SD (51.5±10.248) years. They had no history of smoking or alcohol drinking. The diagnosis of T2DM was made on the basis of the recommended criteria by WHO.[1] The selected patient diabetic patients were treated with different doses of oral hypoglycemic agents (Glucophage® (Metformin 500mg) tablets-Merck, France-500, 1000, 1500mg) according to the patient condition and the physician opinion and randomized into three groups:

1- First group: includes (10) patient diabetic patients were treated with oral hypoglycemic agent metformin in a dose (500mg/day) for (3) months.
2- Second group: includes (10) patient diabetic patients were treated with metformin in a dose (1000 mg/day) for (3) months.
3- Third group: included (10) patient diabetic patients were treated with metformin in a dose (1500mg/day) for (3) months.

Keywords: metformin, cytokines, diabetes
Ten ml of venous blood were drawn using a plastic disposable syringe of 10ml capacity. All patients were fasting (12-14) hr calories free diet. Two ml of blood collected in EDTA containing tubes for measurement of HbA1c. The remaining blood was allowed to clot and separated by centrifuge at speed of 3000 rpm for 20 minutes. Serum samples were stored at (-20 OC) until the time of examination for the other tests. Parameters used for analysis involved: Estimation of glycosylated hemoglobin (HbA1c). Glycated hemoglobin was measured by using the Variant Hemoglobin A1C program developed by Bio-Rad.[10]

Serum levels of cytokines (IL-8 & TNF-α) were quantitatively determined in patients by means of sandwich ELISA test using commercially available kit.

**Statistical Analysis**

Data were statistically evaluated using paired t-test to compare between pre- and post-treatment results. Another way analysis of variance (ANOVA) was utilized to compare between the results of studied parameters among different patients groups. Values with P<0.05 were considered significantly different.

**Results**

**HbA1c:**

Effect of treatment with different doses of metformin on HbA1c level in newly diagnosed patient with type 2 DM

Table – 1: showed that all T2DM patients treated with different doses of metformin (500, 1000 and 1500)mg showed significant decrease in HbA1c levels after 3 months (P<0.05) using Paired t-test compared with pre-treatment values. However, the percent reduction in HbA1c produced due to the use of different doses of metformin (500, 1000 and 1500)mg were (10.3%, 18.3% and 27.9%) respectively compared with baseline values; while the patient groups treated which was statically not Significant (P>0.05) according to ANOVA test as in (Table -1) and (Figure -1).

**Tab1: HbA1c distribution of newly diagnosed diabetic type 2 patient treated with different dose of metformin before and after 3 months.**

<table>
<thead>
<tr>
<th></th>
<th>Metformin 500 mg</th>
<th>Metformin 1000 mg</th>
<th>Metformin 1500 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HbA1C %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>9.54±1.95</td>
<td>9.16±1.55</td>
<td>9.80±1.94</td>
</tr>
<tr>
<td></td>
<td>(7.3-13.0)</td>
<td>(7.7-12.1)</td>
<td>(6.5-12.3)</td>
</tr>
<tr>
<td>After 3 months</td>
<td>8.55±1.89</td>
<td>7.47±1.08</td>
<td>7.06±1.23</td>
</tr>
<tr>
<td></td>
<td>(6.0-11.9)</td>
<td>(5.7-10.2)</td>
<td>(4.6-9.3)</td>
</tr>
<tr>
<td><strong>P value compare After to Before</strong></td>
<td>0.0001*</td>
<td>0.0001*</td>
<td>0.001*</td>
</tr>
<tr>
<td>Before P value compare to 500mg</td>
<td>-</td>
<td>0.635</td>
<td>0.768</td>
</tr>
<tr>
<td>P value compare to 1000mg</td>
<td>-</td>
<td>-</td>
<td>0.425</td>
</tr>
<tr>
<td>P value comparing All doses</td>
<td></td>
<td></td>
<td>0.734</td>
</tr>
<tr>
<td>After P value compare to 500mg</td>
<td>-</td>
<td>0.134</td>
<td>0.051</td>
</tr>
<tr>
<td>P value compare to 1000mg</td>
<td>-</td>
<td>-</td>
<td>0.411</td>
</tr>
<tr>
<td>P value comparing All doses</td>
<td></td>
<td></td>
<td>0.072</td>
</tr>
</tbody>
</table>

Data were presented as Mean±SD (Range)

*Significant using Paired t-test for difference between two dependent means (paired observations) at 0.05 level

#Significant using Student t-test for difference between two independent means at 0.05 level

@Significant using ANOVA test for difference among three independent means at 0.05 level
Interleukin 8

Effect of treatment with different doses of metformin on Interleukin 8 level in newly diagnosed patient with T2DM:

Tab -2: showed that there is a significant decrease in IL-8 in all groups of patients treated with different doses of metformin (500, 1000 and 1500 mg) after 3 months \((P<0.05)\) compared with baseline values according to Paired t-test. Moreover, (table -2) and (figure -2) showed that treatment with maximum dose of metformin (1500mg) produced a higher percent reduction in this parameter after 3 months (-44.20 %) compared with base line values, and metformin treated group (1000mg),(500mg) produced (-15.77% and -12.56% respectively). There is a significant difference among the three groups treated with metformin doses after 3 months \((P<0.05)\), and also a significant according to ANOVA test \((P<0.05)\).

Tab 2: IL-8 serum concentration of newly diagnosed diabetic type 2 patient treated with different dose of metformin before and after 3 months

<table>
<thead>
<tr>
<th></th>
<th>Metformin 500 mg</th>
<th>Metformin1000 mg</th>
<th>Metformin1500 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin 8 (pg/ml) Before</td>
<td>13.85±6.15</td>
<td>15.02±6.28</td>
<td>14.41±4.61</td>
</tr>
<tr>
<td>Interleukin 8 (pg/ml) After 3 months</td>
<td>12.11±4.82</td>
<td>12.65±5.24</td>
<td>8.04±1.02</td>
</tr>
<tr>
<td>P value compare After to Before</td>
<td>0.018*</td>
<td>0.005*</td>
<td>0.004*</td>
</tr>
<tr>
<td>P value compare to 500mg Before</td>
<td>-</td>
<td>0.899</td>
<td>0.820</td>
</tr>
<tr>
<td>P value compare to 1000mg</td>
<td>-</td>
<td>-</td>
<td>0.807</td>
</tr>
<tr>
<td>P value comparing All doses</td>
<td>-</td>
<td>0.048#</td>
<td>0.018#</td>
</tr>
<tr>
<td>P value comparing All doses to 500mg</td>
<td>-</td>
<td>-</td>
<td>0.014#</td>
</tr>
<tr>
<td>P value comparing All doses to 1000mg</td>
<td>-</td>
<td>-</td>
<td>0.039@</td>
</tr>
</tbody>
</table>

-Data were presented as Mean±SD (Range)

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#Significant using Student t-test for difference between two independent means at 0.05 level

@Significant using ANOVA test for difference among three independent means at 0.05 level

Fig 1: HbA1c distribution of newly diagnosed diabetic type 2 patient treated with different dose of metformin before and after 3 months.
TNF-alpha

Effect of treatment with different doses of metformin on TNF-α level in newly diagnosed patient with type 2 DM:

Table 3 showed that there is a significant decrease in TNF-α in all groups of patients treated with different doses of metformin (500, 1000 and 1500 mg) after 3 months (P<0.05) compared with baseline values according to Paired t-test. Moreover, table 3 and figure 3 showed that treatment with maximum dose of metformin (1500 mg) produced a higher percent reduction in this parameter after 3 months (-49.75 %) compared with base line values, and metformin treated group (1000 mg), (500 mg) produced (-34.42 % and -4.72 % respectively). There is a significant difference among the three groups treated with metformin doses after 3 months (P<0.05), and also a significant according to ANOVA test (P<0.05).

Table 3: Serum TNF-α concentration of newly diagnosed diabetic type 2 patient treated with different dose of metformin before and after 3 months.

<table>
<thead>
<tr>
<th></th>
<th>Metformin 500 mg</th>
<th>Metformin 1000 mg</th>
<th>Metformin 1500 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-alpha (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>260.1±123.7</td>
<td>230.86±49.26</td>
<td>207.42±40.45</td>
</tr>
<tr>
<td></td>
<td>(137.7-550)</td>
<td>(176.9-297.9)</td>
<td>(129.50-280.9)</td>
</tr>
<tr>
<td>After 3 months</td>
<td>247.8±127.0</td>
<td>151.74±52.26</td>
<td>104.22±29.55</td>
</tr>
<tr>
<td></td>
<td>(122.2-546.0)</td>
<td>(110.2-284.2)</td>
<td>(62.8-141.5)</td>
</tr>
<tr>
<td>P value compare After to Before</td>
<td>0.001*</td>
<td>0.002*</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Before P value compare to 500mg</td>
<td>-</td>
<td>0.496</td>
<td>0.217</td>
</tr>
<tr>
<td>P value compare to 1000mg</td>
<td>-</td>
<td>-</td>
<td>0.260</td>
</tr>
<tr>
<td>P value comparing All doses</td>
<td></td>
<td></td>
<td>0.354</td>
</tr>
<tr>
<td>After P value compare to 500mg</td>
<td>-</td>
<td>0.040#</td>
<td>0.003#</td>
</tr>
<tr>
<td>P value compare to 1000mg</td>
<td>-</td>
<td>-</td>
<td>0.025#</td>
</tr>
<tr>
<td>P value comparing All doses</td>
<td></td>
<td></td>
<td>0.002@</td>
</tr>
</tbody>
</table>

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DISCUSSION

Many specialist recommended the use of metformin as first drug of choice in newly diagnosed diabetic patients in The National Diabetes Center, University of Al-Mustansiriyyah Baghdad, strategy which was parallel to the strategy used in the treatment of diabetes mellitus in other countries.[11] Metformin lowers hyperglycemia by reducing hepatic gluconeogenesis in the present of insulin and increasing peripheral glucose uptake and utilization.[12,13,14] The results in the present study investigated the effects of different doses of metformin (500, 1000 and 1500)mg on HbA1c levels in newly diagnosed T2DM patients for periods of 3 months. The mean values of HbA1c intreated groups of diabetic patients are in agreement with other results.[15,16] However, the percent reduction in HbA1c produced following the use of different doses of metformin were (-10%),(-18.3%) and (-28.2%) respectively compared with baseline values; This finding agrees with other previous studies[17,18,19] and the possible explanation for these results could be attributed to the effect of the short duration of treatment and also, the baseline values of HbA1c at zero time indifferent treated groups were lower than the fasting glucose values, which could probably reflect that the HbA1c values were not in steady state at the start of treatment.[20] Moreover, (table -1) and (figure-1) showed that treatment with maximunmetformin (1500mg) produced the highest percent reduction in this parameter after 3 month rather than the other groups treated with 1000mg and 500mg doses compared with baseline values. These results are compatible with those of Garber et al (1997),[21] who demonstrated that the hypoglycemic activity of metformin on glycemic control (%HbA1c) in diabetic patients is exhibited generally, a dose-dependent manner.[21] High HbA1c regarded as predictor for the development of microangiopathy. This problem may be explained by the facts that HbA1c had high affinity for O2 resulting in marked difficulty in unloading O2 to peripheral tissues resulting in tissue hypoxia and microangiopathy.[22]

It is increasingly recognized that markers of vascular inflammation play a role in the pathogenesis of T2DM, insulin resistance, and atherosclerosis.[23] The infiltration of macrophages into fat tissue and their subsequent release of pro-inflammatory cytokines into circulation occur at a greater rate in type 2 diabetes than non-diabetics.[24,25,26] Pro-inflammatory cytokines clearly decrease insulin sensitivity.[27] The use of metformin during the first month of treatment of patient with coronary artery disease and T2DM led to the decrease of insulin resistance and reduced activity of systemic inflammation (significant decrease in the concentration of IL-8 and TNF-α).[28] The results in the present study investigated the effects of different doses of metformin (500, 1000 and 1500)mg on IL-8 & TNF-α levels in newly diagnosed T2DM patients for periods of 3 months. The mean values of IL-8 & TNF-α in treated groups of diabetic patients are in agreement with other results.[29,30,31] However, the percent of reduction in IL-8 & TNF-α produced following the use of different doses of metformin were (-12.56%,- 15.77% and -44.20%) for IL-8,(-4.72%, -34.42% and -49.75 %) for TNF-α respectively compared with baseline values; This finding agrees with other previous studies[22,23] and the possible explanation for these results could be attributed to the effect of the short duration of treatment and also, the baseline values of IL-8 & TNF-α in different treated groups were lower than that of newly diagnosed values, which could probably reflect that the IL-8 & TNF-α values were have significant decrease in the concentration compared with baseline values.[30] Metformin can exert a direct vascular anti-inflammatory effect by inhibiting NF-kappa B through blockade of the PI3K-Akt pathway. The novel anti-inflammatory actions of metformin may explain in part the apparent clinical reduction by metformin of cardiovascular events not fully attributable to its hypoglycemic action.[29]

Moreover, (table -2.3) and (figure -2) showed that treatment with maximumdose of metformin (1500mg) produced the highest percent reduction in this parameter after 3 month rather than the other groups treated with 1000mg and 500mg doses compared with baseline values. These results are compatible with those of Yoshiyuki Hattori et al (2006)[30] who demonstrated that the effect of metformin on cytokine (IL-8 & TNF-α) in diabetic patients is exhibited generally, a dose-dependent manner.[21] These results, therefore, should be discussed within this framework, and not necessarily to be comparable with other results. Furthermore, the exact mechanism action of metformin remains elusive.[35] On one hand, some experts consider metformin to be the drug of choice for newly diagnosed type 2 diabetes.[34] whereas the landmark Diabetes Prevention Program, on the other hand, concluded that metformin is efficacious in preventing the new onset of type 2 diabetes in middle-aged, obese persons with impaired glucose tolerance and fasting hyperglycemia but did not prevent diabetes in older, leaner prediabetics.[35] Finally, the present study did establish an improved efficacy of metforminuse at different doses (500-1500mg/day); most of the changes were observed as early as 12
weeks of treatment. Furthermore, this study demonstrated an improved insulin sensitivity of metformin & pro-inflammatory cytokines clearly decrease insulin sensitivity in the newly diagnosed type 2 diabetic patients. The current study was conducted on a small number of patients, and no power calculation was done, because of the scarcity of data, on dose calculation of metformin in newly diagnosed diabetic patient. Therefore, the number of patients was too small to draw a strong conclusion from it, and a larger number of patients are essential for a better understanding of the present results.

CONCLUSION

- The use of different doses of metformin as monotherapy in all treated groups significantly improved the biochemical markers (HbA1c) in newly diagnosed patient with type 2 diabetes.
- Serum levels of the inflammatory markers (IL-8 and TNF-α) are decreased in all treated groups with different doses of metformin after 3 months in different percent of reduction; metformin has an anti-inflammatory effect.
- We demonstrated that metformin inhibits the expression of pro-inflammatory resulting in suppression of cytokine-induced NF-κB activation. The present results suggest that metformin may serve for antithrombogenic drug for diabetic subjects.

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