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Research Article

INFLUENCE OF LARGED AILY DOSES OF METFORMIN ON THE EXPRESSION OF INTERLEUKIN-1B AND C-REACTIVE PROTEIN IN TYPE 2 DIABETES PATIENTS

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ABSTRACT

Objective: This prospective study aim to compare the inhibitory effect of different doses of metformin on the plasma levels of interleukin-1β(IL-1β) and C-reactive protein (CRP) in Iraqi patients with type 2 DM.

Methods: The study includes 30 newly diagnosed type 2 diabetic patients. All patients were treated with Metformin in different doses (group1=500 mg/day, group2=1000 mg/day, group3=1500 mg/day) for 3 months. FBG, BMI, CRP, and IL-1βwere measured pre- and post-treatment.

Results: After 3 months of treatment, the percentage of reduction in BMI was (3.1%, 4.9%, and 6.9%) in groups 1, 2, and 3 respectively. FBG was significantly decreased in all patients within the study groups(P< 0.05). There was no significant decrease in CRP level in all patients compared to baseline values (P>0.05), but still the higher percentage of reduction ingroup3 patients (22.5%). IL-1β was significantly decreased in groups2 and 3(P<0.05), with high percentage of reduction with 1500mg/day metformin (77.38%).

Conclusion: Our data indicate that metformin significantly attenuate the pro-inflammatory response via reduction in CRP and IL-1β levels in high doses, giving a promise to reducing risk of heart disease and stroke in T2DM patients.

Keywords: Metformin; T2DM; Interleukin-1β; CRP; Inflammation.

INTRODUCTION

The primary goal treatment in type 2 diabetes mellitus (T2DM),is to achieve and maintain good glycemic control, and to reduce the mortality and risk of microvascular and macrovascular complications [1].Chronic hyperglycemia greatly influences the positive feedback loop that drives mediators of metabolic inflammation and oxidative stress such as tumor necrosis factor alpha(TNF-α), IL-1β,and IL-6[2], and predispose toa disease state referred to as oxidative-inflammation cascade (OIC). Monocytes express predominantly IL-1β, a potent immunomodulator, which mediates a wide range of immune and inflammatory responses including the activation of B and T cells[3].Modulation of OIC mechanism involved in metabolic and immune process can improve glucose metabolism, insulin resistance, improve vascular function[4].

In addition to diabetes and cardiovascular disease, inflammation is also likened to obesity. Understanding the mechanisms that correlate obesity to inflammation is important for designing novel therapies to reduce the morbidity and mortality of obesity, probably through the prevention of its associated chronic inflammatory disorders[5];this idea suggests that anti-inflammatory and anti-atherosclerotic properties of anti-hyperglycemic agents could provide a theoretical advantage through such selective use[6]. Beside its effect on the cardiometabolic risk factors (hyperglycemia, insulin resistance, obesity, dyslipidemia, and high blood pressure) observed in T2DM patients, metformin, the well-known anti-diabetic drug, has been shown to possess antithrombotic and anti-inflammatory activities with a wide safety profile[7].Recently, AMP-activated protein kinase (AMPK) proved to plays an important role in inflammation[8-10], and therapies designed to block one single cytokine, such as TNF-α or IL-1β have shown limited efficacy probably due to the early and transient kinetics of these inflammatory cytokines[11]. Metformin, dose-dependently, inhibits release of many pro-inflammatory cytokines such asIL-1β, IL-6 and IL-8 in endothelial cells, smooth muscle cells, and macrophages via targeting AMPK[8,12]. It also reduces circulating CRP levels [13].Moreover, metformin decreases oxidative stress and inhibits oxidation of LDL and HDL[14],and experimental evidences suggest that metformin may inhibit differentiation of monocytes to macrophages and their transformation into foam cells[15].There are limited data high lighted the comparable inhibitory effects of different doses of metformin on IL-1β and CRP plasma levels in patients with T2 DM. Accordingly, the current study was designed to compare the inhibitory effect of different doses of metformin on the plasma levels of IL-1β and CRP in Iraqi patients with type 2 DM.

MATERIALS AND METHODS

The present study was a single center, open-label, randomized parallel group study conducted at the National Diabetes Center, Baghdad during the period from January to May 2013. Out of 100 selected patients, only 30 newly diagnosed 2TDM patients (12 female and 18 male) were enrolled in the study. 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 should not be previously treated with oral hypoglycemic agents before the time of enrollment. Patients with current insulin therapy or received insulin for more than six weeks in last 3 months; patients who had known hypersensitivity to metformin and are on chronic medication known to affect glucose metabolism were excluded from the study. All patients instructed to follow restricted diet and asked to monitor their blood glucose level, both fasting and postprandial, glycosylated hemoglobin and lipid profile at the initial visit to the center. The patient's records were maintained for the next three month after their initial visit to hospital; they were observed for weight, height and blood pressure measurement. The patients were randomized into three groups according to the treatment they received. First group includes 10 patients treated with 500 mg metformin tablet daily for 3 months; second group includes 10 patients treated with 1000 mg metformin tablet three times daily for 3 months; third group includes 10 patients treated with 15000mg metformin daily for three months. Another group of 20 healthy females matched with patients for age were included and served as control. Five milliliters of venous blood were drawn from fasting patients (12-14 hr). The blood was allowed to clot and the serum was separated and stored at −20°C. Fasting serum glucose (FSG) was measured by enzymatic colorimetric test using diagnostic kit provided commercially. The CRP was measured using commercially available kit based on a high sensitivity monoclonal antibody assay (hsCRP). Serum level of IL-1β was quantitatively measured by means of sandwich ELISA test using

commercially available kit processed according to manufacturer instruction. Body mass index (BMI) was defined by National Institution of Health (NIH) as body weight in kilograms divided by the height in squared meters (kg/m^2). Normal weight was defined as BMI < 25 kg/m^2. Overweight was defined as BMI ≥ 26 kg/m^2, and obesity was defined as BMI ≥ 30 kg/m^2.

Statistical analysis

The data were analyzed statistically using SPSS (version 19). Pearson Chi-square test was utilized to detect significant differences among demographic variables, while paired *t*-test was used to compare between pre- and post-treatment results. Analysis of Variance (ANOVA) was utilized to compare between the studied parameters among different patient groups. $P < 0.05$ was considered significant.

RESULTS

The percentage of the distribution of male to female among study groups was (60:40)in group 1, (70:30) in group 2, and (50:50) in group 3 patients(Table 1).

The mean age for patients in groups 1, 2, and 3 were55±11.4, 47.4±8.8 and 51.4±9.5 years, respectively. No significant differences were reported among study groups with respect to age and gender. In table 2, BMI in the study groups was illustrated; normal weight T2DM patients represent 30%, 20%, and 10%; overweight patients represent 30%, 60%, and 40%; while obese patients represent 40%, 20%, and 50% of the total sample. No significant differences were reported among groups before starting treatment.

Table 1: Demographic parameters distribution among newly diagnosed T2DM patients on different doses of metformin therapy

Parameter	Metformin 500mg/day	Metformin 1000mg/day	Metformin 1500mg/day	*P* value
Age (Years)	55.6±11.4	47.4±8.8	51.4±9.5	0.83
Sex				
Female	4 (40%)	3 (30%)	5 (50%)	0.66
Male	6 (60%)	7 (70%)	5 (50%)	
BMI (kg/m^2)				
Normal (18.5-24.9) (kg/m^2)	3 (30%)	2 (20%)	1 (10%)	0.50
Over weight (25-29.9) (kg/m^2)	3 (30%)	6 (60%)	4 (40%)	
Obese (=>30) (kg/m^2)	4 (40%)	2 (20%)	5 (50%)	

Values are presented as mean±SD and percentage; n= 10 patients in each group.

Table 2: Metabolic parameters in newly diagnosed T2DM patients on different doses of metformin therapy before and after 3 months

Treatment groups	BMI (kg/m^2)		Fasting Serum Glucose (mg/dL)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Metformin (500mg/day)	28.3±5.5	27.4±5.5*^{a}	190.3±22.7	152.2±21.2*^{a}
Metformin (1000mg/day)	28.4±4.7	27.0±4.2*^{a}	190.8±16.1	142.2±23.9*^{a}
Metformin (1500mg/day)	28.36±2.5	26.4±2.3*^{a}	191.7±24.6	134.6±16.6*^{b}

Values are expressed as mean±SD; n= 10patients in each group; * significantly different compared with pre-treatment values in each group ($P<0.05$); values with non-identical superscripts (a,b) are significantly different among different groups within the post-treatment data ($P<0.05$).

Table 3: CRP level in newly diagnosed T2DM patients on different doses of metformin therapy before and after 3 months

Treatment groups	Serum CRP (mg/L)	
	Pre-treatment	Post-treatment
Metformin (500mg/day)	5.01±3.18	4.82 ±3.22^a
Metformin (1000mg/day)	5.40±3.02	4.47±3.39^a
Metformin (1500mg/day)	5.82±3.37	4.51±2.84^a

Values are expressed as mean±SD; n= 10patients in each group; * significantly different compared with pre-treatment values in each group ($P<0.05$); values with non-identical superscripts (a,b) are significantly different among different groups within the post-treatment data ($P<0.05$).

Table 4: Interleukin-1β level in newly diagnosed T2DM patients on different doses of metformin therapy before and after 3 months

Treatment groups	Serum IL-1β (ng/ml)	
	Pre-treatment	Post-treatment
Metformin (500mg/day)	1.49±0.45	1.33±0.22^a
Metformin (1000mg/day)	1.65±0.56	0.88±0.57*^{b}
Metformin (1500mg/day)	1.68±0.63	0.38±0.38*^{c}

Values are expressed as mean±SD; n= 10patients in each group; * significantly different compared with pre-treatment values in each group ($P<0.05$); values with non-identical superscripts (a,b,c) are significantly different among different groups within the post-treatment data ($P<0.05$).

Table 2 shows that BMI values were significantly decreased in all groups of patients compared with pre-treatment levels, while no significant differences reported among groups after 3 months. Meanwhile, table 2 shows that FSG values were significantly decreased in all treated groups, and the level of reduction in FSG was highest in patients treated with 1500mg metformin/day and significantly different with the other two groups, which demonstrate comparable values ($P>0.05$). Table 3 shows that serum levels were not significantly changed after 3 months of treatment with different doses of metformin. In table 4, treatment with 500mg/day metformin for 3 months fails to produce significant changes in serum levels of IL-1β, while metformin, in daily doses of 1000 and

1500mg/day, significantly reduces serum levels of IL-1β, compared with pre-treatment levels (P<0.05).The level of decrease in IL-1β was found to be dose dependent, where metformin (1500mg/day) produced the greatest level of decrease in IL-1β, followed by 1000mg and 500mg/day, respectively.

DISCUSSION

Recently, many *in vivo* and *in vitro* studies suggested that metformin could be used in other pathological conditions beyond the improvement of glycemic control, such as oncological,

autoimmune, and cardiovascular disorders[10,16].It has been recognized that markers of vascular inflammation play an important role in the pathogenesis of T2DM, insulin resistance, and atherosclerosis[17],and the over-expression of pro-inflammatory cytokines clearly increases the incidence of insulin resistance[18].Several studies demonstrated that the infiltration of macrophages into adipose tissue and their subsequent release of pro-inflammatory cytokines into circulation occur at a greater rate in T2DM than in non-diabetics[18-20].In the present study, all of the patients are newly diagnosed with normal or overweightT2DM,in order to reduce the effect of obesity on the level of pro-inflammatory mediators. Metformin was prescribed as first-line mono-therapy for newly diagnosed middle-aged overweight/obeseT2DMpatients. The patients selected in this study were nearly matched these criteria. Investigation of potential signaling pathways demonstrated that metformin diminished IL-1β-induced activation and nuclear transformation of nuclear factor-kappa B (NF-κB) in smooth muscle cells[12]. Furthermore, metformin suppress IL-1β-induced activation of the pro-inflammatory phosphokinases in relevant plasma concentration of metformin of 20 μmol/L[21], thus it possess direct vascular anti-inflammatory effect. In experimental animals, metformin significantly improves survival rate in treated mice compared with untreated one [22]. When administered 12hr after lipopolysaccride (LPS) infusion, metformin improves the survival rate (75% *vs*. 17%), and decreases circulating cytokines, where the production of inflammatory cytokines TNF-α and IL-1β reaches maximum level within 4hr[23].This finding supports our claim that early treatment of T2DM patients with metformin may reduce the risk of early inflammatory response. Lavrenko et al used a short-term course of metformin (one month) to produce systemic anti-inflammatory activity; however, this approach was found not enough to implement a reliable effect on insulin resistance or improving the clinical features of coronary artery diseases (CAD)[24].Accordingly, in thepresent study, the duration of 3 months of treatment was expected to be enough to show the anti-inflammatory effect of metformin, mainly in terms of decrease in circulating hsCRP and IL-1β.In several studies, the combination of metformin with other hypolipidemic agents reduces expression of inflammatory markers like CRP[6,25,26]. Alaa et al reported that use of metformin in T2DM positively affects the oxidant/antioxidant balance with no significant effect on acute phase CRP [27]**.** Moreover, in two randomized placebo-controlled studies, high daily dose of metformin (3gm in divided dose),used for 90 days in patients with impaired glucose tolerance and on hypolipidemic therapy, produced marked decrease in the release of hsCRP, TNF-α, interferon-γ and IL-2 [28,29].In the present study, 3 months of treatment with metformin shows slight reduction in hsCRP level compared to pretreatment value with the three doses, but still with higher percentage of reduction associated with 1500mg/day of metformin compared to lower doses,suggesting that higher doses of metformin may produce marked effect. Additionally, many reported data in this regard revealed a well-recognized reduction in the oxidative stress markers, and the levels of many cytokines, including TNF-α, IL-8, IL-6,IL-10after treatment with metformin [30-33]. However, up to our knowledge, there is no study that investigates the dose-dependent effects of metformin on pro-inflammatory mediators, mainly IL-1β, as an initiator for other inflammatory mediators in T2DM patients. The use of metformin, during the first month of treatment in patients with CAD and T2DM,decreases insulin resistance and the circulating concentrations of IL-8 and TNF-α[34], and such effect of metformin on IL-8 and TNF-α in diabetic patients was found to a dose-dependent [35].Moreover, metformin in a dose-dependent pattern suppresses IL-1-induced IL-8 production in endometriotic stromal cell (ESCs) [36].In the present study, metformin in a daily of 1000mg and 1500mg produces significant reduction in IL-1β after 3 months of treatment, with a highly significant reduction achieved with the highest dose compared with the lowest doses. These results suggest that early treatment with high doses of metformin may have remarkable effect on inflammatory processes in newly diagnosed middle-aged overweight T2DM patients. In conclusion**,** early treatment of middle-aged and overweight T2DM patients with large daily doses of metformin may be beneficial in attenuating the inflammatory processes associated with poor glycemic control. Further studies are warranted to explore whether the effect of metformin on pro-inflammatory mediators is directly correlated to the reduction in the metabolic and obesity markers in T2DM patients.

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