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Research Article

PLEIOTROPIC EFFECTS OF ATORVASTATIN ON PRO-INFLAMMATORY CYTOKINES IN NEWLY DIAGNOSED IRAQI PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Objective: Atorvastatin therapy is now recommended for reduction of cardiovascular risk in type 2 diabetic patients (T2DM), based on convincing evidence of reductions in mortality and vascular events in major clinical outcome trials. The aim is to evaluate the effects of atorvastatin on pro-inflammatory markers (TNF-α, IL-6), HbA1c andleptin in obese patients with type 2 diabetes.

Methods: Sixty fivenewly diagnosed T2DM patients were randomly allocated into 2 groups; group I treated with metformin only; in group II atorvastatin was added with metformin. Twenty healthy subjects were enrolled as control group. While maintaining their usual eating habits, fasting blood samples were collected at baseline and after 12 weeks of treatment.

Results: HbA1c levelswere decreased significantlyonly in group I compared with baseline value; while serum levels of leptin, TNF-α and IL-6 were decreased significantly in both groups compared to baseline values.

Conclusion: Atorvastatin is effective in reducing leptin and pro-inflammatory cytokines levels but associated with an increased risk of worsening HbA1c in patients with T2DM.

Keywords: Atorvastatin, Inflammation, T2DM, Cytokines

INTRODUCTION

The role of inflammation in a wide range of diseases is not commonly regarded as immune- mediated disorders, and inflammatory mediators may not only be markers of metabolic aberrancies in T2DM, but may directly contribute to β-cell dysfunction and insulin resistance[1].Evidence has been accumulated in favor of the expected role of immuno-related mechanisms and factors in the pathogenesis of T2DM, both with regard to progressive β-cell failure and destruction and to the peripheral insulin resistance[2]. Many reports highlighted the correlation between obesity, insulin resistance, leptin, and pro-inflammatory cytokines including tumor necrosis factor (TNF- α) and interleukin IL-6 with the cardiovascular risks in T2DM[3].Meanwhile, low-grade inflammation has been considered as a risk factor of future development of T2DM, where lifestyle modifications and medical treatment to lower the inflammatory state are of significant value to decrease the risk of future development of T2DM[2].TNF-α is constitutively expressed in adipose tissue and its expression increased in adipose tissue of insulin resistant obese individuals[4], and correlated with the extent of obesity and the level of hyperinsulinemia[5]. Meanwhile, there is a positive correlation between elevated levels of IL-6 with obesity[6] and future risk of T2DM development, because it is considered as an insulin resistance–inducing agent in adipocytes[7]. Although leptin deficiency is rare in humans, obesity is associated with central and peripheral leptin resistance. Indeed, obesity impairs the leptin signaling cascade, potentiating an increase in the production of leptin[8], in obesity and T2DM; tissues are often exposed to high concentrations of both of leptin and insulin. The lipid lowering agent atorvastatin has beneficial pleiotropic actions comprise improvement of endothelial dysfunction, increased nitric oxide bioavailability, antioxidant properties, inhibition of inflammatory responses, and stabilization of atherosclerotic plaques[9], reduce oxidative stress, cytokines, even before a hypolipidemic effect becomes apparent[10].It has been reported that treatment with HMG-CoA reductase inhibitors might delay the development of diabetes, and statins might also improve insulin resistance[11]. The present study was designed to evaluate in impact of using atorvastatin in combination with metformin on the serum levels of leptin and the inflammatory markers, IL-6 and TNF-α, in Iraqi obese patients newly diagnosed with T2DM.

METHODS

In this prospective open label study, 65 patients (37 females and 28 males) with ages ranged between 32-60 years, who intend the Specialized Center for Diabetes and Endocrinology, were enrolled after signing informed consent; they arediagnosed for having type 2 diabetes mellitus(T2DM) according to the criteria of American Diabetes Association Expert Committee[12]. Additionally, another 20 healthy subjects (BMI=26.61±0.53kg/m^2), with ages matched with patients, were enrolled as control group. Patients with renal dysfunction (serum creatinine>2.0 mg/dl), severe hepatic or chronic or acute inflammation, uncompensated heart failure, myocardial infarction and uncontrolled hypertension were excluded. The study protocol was approved by the Medical Ethics Committee, Al-Kindy College of Medicine.

Patients' randomization and treatment

The patients were randomly allocated into two groups; group I, includes 32 patients with mean body mass index (BMI)=29.95±1.22 kg/m^2; they are treated with metformin (1500mg/day) administered orally in three divided doses. Group II, includes 33 patients with BMI=30.12±1.6 kg/m^2 and treated with metformin (1500mg/day) + atorvastatin (20mg/day); the outcome of treatment was followed after 12 weeks period.

Treatment follow up and measurements

While maintaining the usual dietary habits of participants, fasting blood samples(10 ml) were collected at baseline and after 12 weeks period of treatment, and transferred into plain tubes; left for 30 min for clotting, centrifuged and serum was prepared and stored at -20^{o}C until time of analysis. Serum levels of TNF-α, IL6, leptinand HbA1c were determined using ready made analytical kits according to standardized methods (enzyme linked immunosorbent assay).

Statistical analysis

The resultswere expressed as mean±SEM. The results were statistically analyzed using Student's t-test, statistical significance was set at P<0.05.

RESULTS

Figure 1 showed that baseline HbA1c levels weresignificantly increasedin both groups of patients (P<0.05) compared with healthy control group; HbA1c levels then significantly decreasedafter 12 weeks of treatment in group I only compared to baseline values; while in group II although HbA1c levels were decreased by 12.6%, the value was not significantly different compared with that reported before treatment. In figure 2, serum leptin levels were significantly increased in both groups of T2DM

patients compared to controls. Meanwhile, metformin or its combination with atorvastatin produced significant decrease (P<0.05) in serum leptin values in both groups after 12 weeks of treatment compared with baseline values; the effect of metformin alone is more prominent in this respect (27.6% *vs*. 19.8, respectively). Regarding the influence of atorvastatin on the inflammatory markers, figure 3 clearly showed that in patients with T2DM serum levels of TNF-α were significantly increased compared with that reported in healthy controls, and treatment with either metformin or its combination with atorvastatin results in significant decreases in serum TNF-α levels in both groups of patients, though the percent decrease was greater when combination is used (24.0% *vs*. 27.5%, respectively). Figure 4 shows that serum IL-6 levels were significantly increased in both groups of T2DM patients compared to healthy controls.Meanwhile, in both groups of patients, treatment with metformin aloneor its combination with atorvastatin produced significant decrease in serum levels of IL-6 after 12 weeks of treatment compared with baseline values; however, the combination protocol produces higher level of decrease in this pro-inflammatory marker compared with that produced due to treatment with metformin alone (21.3% *vs*. 36.4%, respectively) (Figure 4).

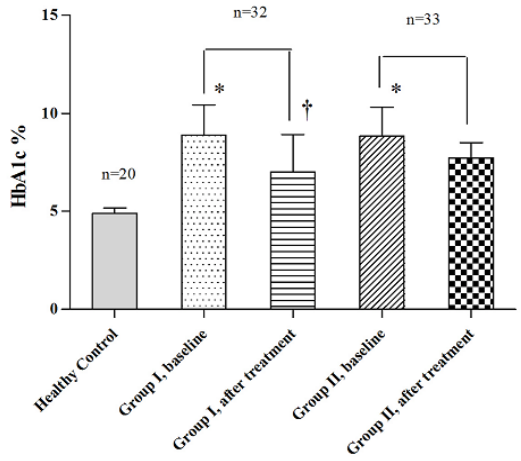

Fig. 1: Effect of metformin alone or its combination with atorvastatin on HbA1c level in T2DM patients; n= number of subjects; * significantly different compared with control (P<0.05); † significantly different compared with pre-treatment (P<0.05).

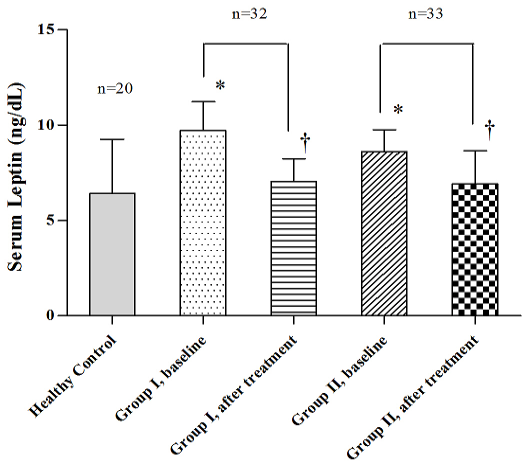

Fig. 2: Effect of metformin alone or its combination with atorvastatin on serum leptin level in T2DM patients; n= number of subjects; * significantly different compared with control (P<0.05); † significantly different compared with pre-treatment (P<0.05).

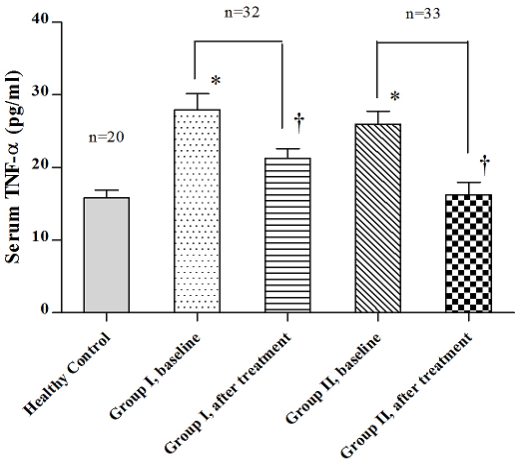

Fig. 3: Effect of metformin alone or its combination with atorvastatin on serum TNF-α level in T2DM patients; n= number of subjects; * significantly different compared with control (P<0.05); † significantly different compared with pre-treatment (P<0.05).

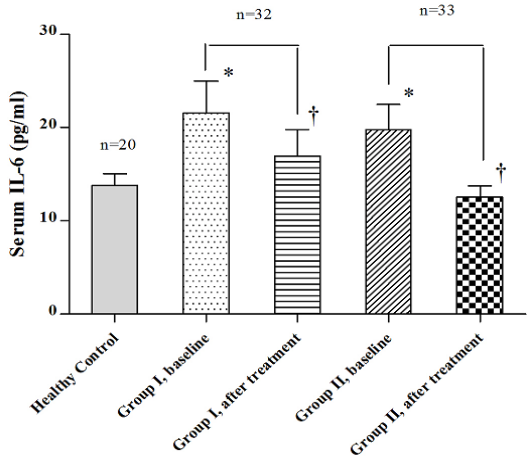

Fig. 4: Effect of metformin alone or its combination with atorvastatin on serum IL-6 level in T2DM patients; n= number of subjects; * significantly different compared with control (P<0.05); † significantly different compared with pre-treatment (P<0.05).

DISCUSSION

T2DM has been associated with chronic inflammation in adipose tissue. Increased expression in adipose tissue of key genes involved in inflammation pathways, such as those encoding cytokines and other macrophage-related factors, has been linked to obesity and insulin resistance[13].Many studies have provided clear evidence that circulating levels and adipose tissue expression of pro-inflammatory cytokines TNF-α and IL-6 are elevated in obese subjects and TIIDM[14,15]. In the present study, the significant increase in baseline serum leptin level reported in both groupswas consistent with previous studies, where obese individuals generally exhibit an unusually high circulating concentration of leptin[16], and those individuals are said to be resistant to the effects of leptin in similar way with insulin resistance in T2DM. After 12weeks of treatment, metformin produces greater decrease in serum leptin than that produced when atorvastatin added to the treatment protocol, but in both cases the results are significantly lower compared to baselinevalues. Patients with T2DM experienced a cardioprotective effect from low dose atorvastatin therapy[17] and the magnitude of protection may be greater than what reported in non-diabetic individuals[18]. This may be due to that statinsmay decrease elevated leptin levels in obese and T2DM patients, but such effect is not clear in normal weight, non diabetic individuals. Our

results are in tune with that reported by others,where 8 weeks of atorvastatin treatment (40 mg/d) in obese patients with T2DM reduced plasma leptin concentrations by 40%[18], and they did experience cardioprotective benefits from atorvastatin therapy, including reduced total and LDL cholesterol and triglycerides and reduced circulating concentrations of the inflammatory markers high-sensitivity C-reactive protein and TNF-α[19]. The reported significant increase in TNF-α and IL-6 at baseline in both groupscompared with controlwas consistent with other studies that indicate that inflammatory mediators may not only be markers of metabolic abnormalities in T2DM, but may directly contribute to β-cell dysfunction and insulin resistance[20]. In the present study, the addition of atorvastatin to the treatment protocol with metformin produces greater reduction in TNF-α and IL-6 level after 12 weeks. Statins have been consistently reported to affect inflammation index[2]; there is evidence that statins reduce oxidative stress, cytokines expression, and adhesion molecules in humans even before a hypolipidemic effect becomes apparent[21,22]. These anti-inflammatory effects may provide additional vascular protection and contribute to the clinical benefits reported in other studies[10]. As shown in the results,the significant increases in HbA1c baseline values in groups I and II was consistent with previous findings in T2DM. After 12 weeks of treatment significant decrease of HbA1c was observed only in group I,while non significant decrease in HbA1cwas observed in group II because the effect of metformin on glucose metabolism was neutralized by atorvastatin[23,24], which results in significant increases in glycated hemoglobin levels[25]; it was reported that lipophilic statins have pleiotropic actions that might cause unfavorable reduction of insulin secretion and exacerbation of insulin resistance[26].Moreover, the results of large-scale, randomized controlled clinical trials have raised the possibility that lipophilic statins might increase the rate of new onset diabetes[27]. The mechanisms by which statins may influence glucose metabolism suggested that statins may alter glycemic control by decreasing various metabolites, such as isoprenoid, farnesyl pyrophosphate, geranylgeranyl pyrophosphate, and ubiquinone, which enhance glucose uptake via glucose transporter type 4 in adipocytes and impair insulin release[28]. In conclusion, addition of atorvastatin to the treatment protocol with metformin is effective in reducing pro-inflammatory cytokines and leptin levels, but associated with an increased risk of worsening HbA1c in in obese T2DM patients.

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