Research Article

Effects of Metformin Alone and in Combination with Sitagliptin on Oxidative Stress and Proinflammatory Markers in Patients with Diabetes Mellitus Type-2

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ABSTRACT

Diabetes mellitus describes a group of chronic metabolic disorders of multiple etiologies. It is characterized by chronic hyperglycaemia together with disturbances of carbohydrate, fat and protein metabolism resulting from defects of insulin secretion, insulin action or both. Several findings further support the hypothesis that chronic inflammation is a predictor of type 2 diabetes development. More recent data suggest that interleukin-6 (IL-6) is associated with type 2 diabetes. Regulation of hepatictumor necrosis factor alpha (TNF-α) levels and oxidative stress in the diabetic state could be of therapeutic relevance for the improvement or delay of the hepatic complications linked to chronic hyperglycaemia Epidemiological studies have demonstrated an increase in plasma levels of inflammatory markers such as IL-6 and TNF-α in patients with metabolic syndrome and also in those with clinically overt T2DM. Peroxidation of lipids produces highly reactive aldehydes, including malondialdehyde (MDA). MDA has been documented as a primary biomarker of free radical mediated lipid damage and oxidative stress. Increased level of MDA in diabetics suggests that peroxidative injury may be involved in the development of diabetic complications. The aims of this study were to investigate the effects of Metformin alone and in combination with sitagliptin on oxidative stress and pro inflammatory markers in patients with diabetes mellitus type-2 and to compare the effect of Metformin alone versus Metformin in combination with sitagliptin on clinical outcome in patients with diabetes mellitus type 2. Standard kits were used to measure biochemical profiles suggested in this study using double-sandwich ELISA technique. Tests were performed and interpreted following instruction outlined in each kit. The study included newly or recently diagnosed male and female patients with T2DM whose ages ranged between 25 and 65 years. The enrolled patients were divided into two groups, Group 1: consisted of 30 patients, treated with oral Metformin alone over a period of 12 weeks, which is the period of the study. Group 2: consisted of 33 patients treated with Metformin plus sitagliptin. TNF-α, IL-6 and MDA were measured as baseline and after treatment for 12 weeks. After 12 weeks of treatment with Metformin alone, Metformin with sitagliptin there were significant reductions in serum levels of TNF-α, IL-6 and MDA. Metformin with sitagliptin group had more significantly reduction in levels of (TNF- α and MDA) than Metformin alone group. As a conclusion Metformin alone and in combination with sitagliptin can attenuated (MDA) and (TNF-α, IL-6) in patients with T2DM. Metformin in combination with sitagliptin had greater effect than Metformin alone on (MDA) and (TNF-α).

Keywords: Metformin, Sitagliptin, oxidative stress, pro inflammatory markers.

INTRODUCTION

Diabetes mellitus describes a group of chronic metabolic disorders of multiple etiologies. It is characterized by chronic hyperglycaemia together with disturbances of carbohydrate, fat and protein metabolism resulting from defects of insulin secretion, insulin action or both.[1] These are associated with the development of micro vascular complications (retinopathy, nephropathy and neuropathy) and macro vascular complications (cerebral vascular disease, coronary heart disease and end-stage renal disease)[2]. Pathogenesis of T2DM ranging from predominantly insulin resistance with relative insulin deficiency to predominantly an insulin secretory defect with insulin resistance, which accounts for ~90–95% of those with diabetes[3].

Metforminis one of the most effective oral medications used in the treatment of type2 diabetes as a biguanide. It is the preferred initial treatment at the time of diagnosis and can be used virtually throughout the lifetime of a person with type 2, Metformin consider as a first-line agent to treat type 2 diabetes[4]. Metformin improves glycemic control mainly through inhibition of hepatic gluconeogenesis, and there also are improvements in peripheral glucose uptake and lipid metabolism, the proposed mechanism of action through Metformin inhibits mitochondrial complex I resulting in altered AMP/ATP ratios in the cell. One consequence is activation of AMP-activated protein kinase which has pleiotropic effects on lipid and glucose metabolism[5]. Although Metformin has the capacity to decrease the level of prolactin, it decreases the number and activity of sperms[6].

The incretin-based therapies, incretin-based medications make use of the glucose-lowering activity of the gut incretin hormone glucagon-like peptide-1 (GLP-1), which stimulates insulin secretion glucose-dependent manner, suppresses postprandial glucagon secretion, decelerates gastric emptying, reduces appetite and food intake, and may have other actions in the nervous and cardiovascular

systems[7]. They are including GLP-1 receptor agonists and DPP-4 inhibitors represent advances in treating patients with type2 diabetes. Both classes of drugs stimulate insulin secretion in a glucose-dependent manner and reduce postprandial glucagon.[8] (DPP)-4 inhibitors are a new class of anti-hyperglycaemic agents which were developed for the treatment of type 2 diabetes they differ in terms of their chemistry; they are all small molecules which are orally available. There are some differences between them in terms of their absorption, distribution, metabolism and elimination, as well as in their potency and duration of action; they improve glycaemic control, reducing both fasting and postprandial glucose levels to lower HbA1c levels, without weight gain[9].Sitagliptin is a low–molecular weight pyrazine phosphate that selectively and competitively inhibits the enzyme DPP-4, which increases endogenous GLP-1, it has a long half-life allowing for once daily[7]. Sitagliptin is indicated for use in patients with type 2 diabetes, either as mono therapy or in combination with Metformin, pioglitazone, or glimepiride, with or without Metformin. The recommended dosage of sitagliptin (supplied as 25, 50, and 100mg tablets)[10] .

Several findings have supported the hypothesis that chronic inflammation is a predictor of type 2 diabetes development. More recent data suggest that interleukin-6 (IL-6) is associated with type 2 diabetes[11]. Regulation of hepatic TNF-α levels and oxidative stress in the diabetic state could be of therapeutic relevance for the improvement or delay of the hepatic complications linked to chronic hyperglycemia[12]. Epidemiological studies have demonstrated an increase in plasma levels of inflammatory markers such as IL-6 and TNF-α in patients with metabolic syndrome and also in those with clinically overt T2D[13]. Oxidative stress plays a pivotal role in cellular injury from hyperglycemia. High glucose level can stimulate free radical production. Weak defence system of the body becomes unable to counteract the enhanced ROS generation[14]. Oxidative stress acts as mediator of insulin resistance and its progression to glucose intolerance and installation of diabetes mellitus, subsequently favouring the appearance of atherosclerotic complications, and contributes to rise in many micro- and macrovascular complications[15]. MDA has been documented as a primary biomarker of free radical mediated lipid damage and oxidative stress[16]. Increased level of MDA in diabetics suggests that peroxidative injury may be involved in the development of diabetic complications[17].

Patients and Methods

The present study is prospective randomized control clinical trial. The study is conducted from December /2015 to July/2016 and carried out mainly in Thi-Qar diabetes centre and in coordination with specialist clinic and some of the patients recruited from Al Hussain teaching hospital in Nasiriya. The treatment and follow up period for the patients is 12 weeks. Fasting plasma glucose, glycosylated hemoglobin, tumor necrosis factor-α, interleukin 6 and malondialdehyde are measured at baseline and after 12 weeks. This study is approved by The Ethics Committee of Al Nahrain medical college. The participants included in this study were Seventy five patients with newly diagnosis T2DM are attended to the Dhiqar diabetes center. Out of the total enrolled patients, 12 patients dose not complete the study due to many reasons such as poor compliance, converted to the other drugs and/or discontinuing their visits.

The remaining patients included in this study are 63 patients (27 females and 36 males) all patients were informed about the study and their written agreements were obtained before they are included in the study. 63 enrolled patients were divided into two groups as follow:

Group A

Comprise of 30 patients, treated with oral Metformin alone (Merck Serono. France), 1000 mg once daily initially plus dietary control & life style modification over a period of 12 weeks, which is the duration of the study.

Group B

Comprise of 33 patients treated with Metformin 1000mg/sitagliptin 50 mg MSD, USA pharmaceutical company (Janumet) oral tablets once daily plus dietary control and life style modifications for 12 weeks.

To have an idea about the normal values of study parameters and in order to assess how much the drugs used in the study were able to normalize the abnormal parameters, other 20 in addition to 63 patients, apparently healthy volunteers were enrolled in this study. Their data were obtained, tabulated and analyzed in the same way as the study patients, but they don't receive treatment.

Statistical Analysis

Paired Student's *t* test was used to compare values obtained before and after treatment administration within each group while independent sample *t* tests were used for between all patients and healthy subjects. Multiple comparisons were also carried out by using Analysis of variance (ANOVA) with LSD post-hoc testing to compare changes in variables between groups before and after the 12 weeks treatment period. Data are presented as mean ± Standard deviation (SD). For all statistical analyses, $P<0.05$ was considered statistically significant using a two-tailed test. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 16.0 (SPSS, Chicago, IL)

RESULTS

In healthy group, the mean± SD for FPG, HbA1C, TNF-α, IL-6, MDA, were 84.55±10.23, 5.04±0.32, 18.42±6.50, 14.58±3.39, 1.40±0.32, 161.25±11.57, 137.50±25.67, 1.35±0.24, 2.48±0.57, 83.17±5.25, 122 ±7.67, 26.53±1.77, 12.15±3.55 respectively.

In patients group the mean± SD for FPG, HbA1C, TNF-α, IL-6, MDA, TC, TG, HDL, LDL, DYS., SYS., BMI,ESR were 177.98±20.35, 8.81±0.76, 64.06±14.33, 37.79±9.23, 3.88±0.92, 201.34±43.15, 206.41±58.51, 1.86±0.42, 2.68±0.59, 79.75±4.99, 128.09±8.90, 30.65±4.070, 12.39±3.43 respectively. as show in the table 1

Table 1: comparison parameters of patients T2DM at baseline with healthy subjects

Parameters	Patients (mean±SD)	Healthy (mean±SD)	P value
FPG	177.98±20.35	84.55±10.23	0.0012
HbA1C	8.81±0.76	5.04±0.32	0.0001
TNF-α	64.06±14.33	18.42±6.50	0.0014
IL-6	37.79±9.23	14.58±3.39	0.0001
MDA	3.88±0.92	1.40±0.32	0.0001

After 12 weeks of treatment with Metformin alone (1000mg), there were highly significant improvement in glycemic indexes with a mean±SD for (FPG, HbA1c) at base line level was (162.10±19.37, 8.35±0.91) respectively and at week 12, mean±SD were (123.30±22.10, 7.18±0.72) respectively (p< 0.001).there were highly significant reductions in TNF-α, IL-6 and MDA levels with a mean±SD at baseline level were (61.93±13.72, 36.58±10.08, 3.57±0.50) respectively, and after 12 weeks mean±SD were (56.87±14.41, 33.47±10.78, 3.12±0.55) respectively (p<0.05). As show in the table 2

Table 2: Effect of Metformin on glycemic indexes, inflammatory markers (TNF-α, IL-6) and oxidative stress (MDA)

Parameters	Baseline	After 12 weeks	P Value
FPG.	162.10±19.37	123.30±22.10	0.0001
HbA1C	8.35±0.91	7.18±0.72	0.0015
TNF-α	61.93±13.72	56.87±14.41	0.0011
IL-6	36.58±10.08	33.47±10.78	0.0016
MDA	3.57±0.50	3.12±0.55	0.0001

After 12 weeks of treatment with Sitagliptin plus Metformin, there were highly significant improvement in glycemic indexes with a mean±SD for (FPG, HbA1c) at base line level were (186.72±16.94, 8.97±0.65) respectively and at week 12, mean±SD was (146.13±26.07, 7.67±1.01) respectively (p<0.001).

Table 3: Effect of Metformin plus sitagliptin on glycemic indexes, inflammatory markers (TNF-α, IL-6) and oxidative stress (MDA)

Parameters	Baseline	After 12 weeks	P Value
FPG	186.72±16.94	146.13±26.07	0.0001
HbA1C	8.97±0.65	7.67±1.01	0.0002
TNF-α	64.96±15.20	50.23±7.75	0.0011
IL-6	38.32±9.97	32.90±8.56	0.0001
MDA	4.07±0.62	3.03±0.47	0.0001

There were highly significant reductions in TNF-α, IL-6 and MDA levels with a mean±SD at baseline level were (64.96±15.20, 38.32±9.97, 4.07±0.62) respectively and at week 12, mean±SD was (50.23±7.75, 32.90±8.56, 3.03±0.47) respectively (p<0.001). As show in the table 3.

Comparison the amount of reduction (mean±SD) of Metformin alone, Metformin plus sitagliptin, on study parameters by using ANOVA and post hoc test

The amount of reduction of TNF-α mean±SD (-14.73±7.45) in Metformin + sitagliptin group is significantly lower than Metformin alone (-5.05±7.34).

There were not significant differs in amount of reduction between groups (Metformin, Metformin plus sitagliptin) in regarding to IL-6 (-3.10±3.63, 5.41±5.61) respectively. In Metformin plus sitagliptin group the amount of reduction in MDA level was significant reduced mean±SD (-1.04±0.62) than Metformin alone (-0.45±0.30). HbA1c amount of reduction in Metformin plus sitagliptin (-1.29±1.06) was significantly lower than Metformin alone group mean±SD (-1.17±0.19). As show in the table 4:

Table 4: Comparison between the effects of Metformin alone, Metformin plus sitagliptin on different parameters

Parameters	Metformin	Metformin +sitagliptin	P Value
TNF-α	-5.05±7.34	-14.73±7.45	0.0001
IL-6	-3.10±3.63	-5.41±5.61	0.112
MDA	-0.45±0.30	-1.04±0.62	0.011
FPG	-38.80±26.26	-40.59±23.14	0.809
HbA1C	-1.17±0.19	-1.29±1.06	0.005

DISCUSSION

Effects of Metformin on study parameters

In the present study reveal that Metformin have potential action to decrease the inflammatory markers by significantly reduction in IL-6 and TNF-αafter 12 weeks treatment with Metformin alone. These findings agreed with Gómez-García et al (2007) which were proved the Metformin significantly reduced the IL-6 and TNF-α[18]. Metformin dose-dependently inhibited IL-1β induced release of the pro-inflammatory cytokines IL-6 and IL-8 in human vascular smooth muscle cells and endothelial cells[19]. Metformin can act as an inhibitor of pro-inflammatory responses through direct inhibition of nuclear translocation of nuclear factor-kappa B (NF-kB), this effect may partially explain the apparent clinical reduction of cardiovascular events not fully attributable to Metformin antihyperglycemic action[20]. In this study Metformin have potential effect on oxidative stress during significantly reduction in MDA level in T2DM patients after treatment for 12 weeks. This result is consistent with Pavlovic et al study (2000) that reported the highly significant reduction after 4 weeks treatment obese T2DM patient with Metformin monotherapy[21]. Metformin can reduce the MDA level but not significantly

after treatment for 12 weeks[22]. Interestingly, Metformin has antioxidant properties which are not fully characterized. It reduces reactive oxygen species (ROS) by inhibiting mitochondrial respiration and decreases advanced glycosylation end product (AGE) indirectly through reduction of hyperglycaemia and directly through an insulin-dependent mechanism[20]. The present study that reported there was statistically significant reduction in FBS and HbA1c in newly diagnosed T2DM patient after 12 weeks treatment with Metformin alone. Júnior*et al.*, 2006 showed that the use of Metformin causes a glycemic control to be significantly improved (p<0.001) within 4 weeks after treatment with Metformin[23]. HbA1C and FBS were significantly reduced after 12 weeks of Metformin administration for type 2 diabetic patients with inadequate glycaemic control[24]. After 12 weeks of treatment with Metformin alone, there was significant reduction in glycemic indexes (HbA1c and FPG)[3]. The anti-hyperglycemic properties of Metformin are mainly attributed to suppress hepatic glucose production, especially hepatic gluconeogenesis, and increased peripheral tissue insulin sensitivity[25].

Effect of Metformin with Sitagliptin on study parameters

In the present study showed the effect of combination of sitagliptin plus Metformin on pro inflammatory markers (IL-6 and TNF-α) and there were highly significant reduction after treatment the patients with T2DM for 12 weeks. this results are consistent with Rizzo et al (2012) study that reported there were significantly lowered plasma IL-6, TNF-α levels *P* (<0.001) after treatment patients T2DM with sitagliptin for 12 weeks[26]. Makdissi*et al* (2012) study reported there were significantly reduction in plasma CRP, IL-6 and TNF-α concentration after treatment with sitagliptin for 12 weeks[27]. These antiinflammatory actions of sitagliptin are consistent with the observations made by Dobrian*et al.* in experimental animals, there was significantly reduction in TNF-α after 6 weeks treatment with sitagliptin + Metformin group in a mice fed on a very high-fat diet[28]. According to the present study Metformin plus sitagliptin significantly reduced the MDA level after treatment T2DM patients for 12 weeks P= 0.000. This finding is agreed with Ferreira et al. study (2010) that showed a significant reduction in serum and tissue of the Zucker Diabetic Fatty (ZDF) rats after treatment with sitagliptin for 6 weeks, suggesting that Sitagliptin decrease in ROS and subsequent lipid peroxidation[29]. Apaijai et al. study (2013) reveal that DPPi (sitagliptin) have significant reduction on plasma and cardiac MDA level in obese insulin-resistant rats after treated for 12 weeks[30]. A potential mechanism of vascular protection by DPP-4 inhibitors may be by limiting oxidative stress,data with regards to the effect of DPP-4 inhibitors on oxidative stress are scant[31].

There were significantly reduction in FBS and HbA1C in Sitagliptin plus Metformin group (p<0.05) after 12 weeks from baseline in this present study. These results compatible with Williams et al (2009) study that reported significantly improvement in glycemin control after initial combination therapy with sitagliptin and Metformin in patients with type 2 diabetes[32]. Katzeff et al. (2015) showed that the Long-term efficacy of sitagliptin as either mono therapy or add-on therapy to Metformin can lead to significantly improvement in glycemic control (FPG and A1C) and β-cell function improved over 2 years in patients with type 2 diabetes[33]. Treatment of T2DM patients with Sitagliptin in combination with Metformin provides significantly reduction in HbA1C and FPG[34]. DPP-4 inhibitors lower HbA1c by increasing plasma GLP-1/GIP levels, leading to increased glucose-stimulated insulin secretion and inhibition of glucagon secretion[35]. Thus, the combination of Metformin (reduces hepatic glucose production) plus DPP-4 inhibitor (insulin secretagogue) would be expected to produce an additive effect to reduce HbA1c[36]. Combination of sitagliptin with Metformin produced additive decreases in FPG, 2h-PG, versus either therapy alone[37].

Comparison the effect between Metformin alone and Metformin + sitagliptin on study parameters

In the present study there was significant in change from baselin regarding to HbA1c level between Metformin alone and other groups (Metformin +sitagliptin).After 12 week with treatment. This results is agreed with Han et al. study (2011), there was significantly lowered the HbA1c level in combination group (Metformin +sitagliptin) than Metformin group alone[38].Jadzinsky et al study (2009) reported that the combination treatment (Metformin + saxagliptin) had statistically significant reductions in mean HbA1c than Metformin monotherapy[39]. In the present study Metformin +sitagliptin group had significant differences in the mean of reduction of TNF-α and MDA levels than Metformin alone group. These results reveal that sitagliptin have anti-inflammatory effect and attenuated the oxidative stress markers more than Metformin. these findings is similar to the results of Rizzo et al study (2012) that reported the vildagliptin had greater reduction in IL-6, TNF-α, and nitrotyrosine than sitagliptin, and this associated with reduction of oxidative stress and systemic inflammation markers in type 2 diabetic patients [26].sitagliptin has ameliorated the elevation of MDA and NO*x* levels in a model of endothelium dysfunction induced by atherogenic diet in rabbits[40]. Kelleni et al. (2015) study were reported sitagliptin possess antioxidant properties decreasing the accumulation of free radicals[41].

CONCLUSIONS

Metformin alone and in combination with sitagliptin can attenuated the oxidative stress (MDA) and pro inflammatory markers (TNF-α, IL-6) in patients with T2DM. Metformin in combination with sitagliptin had great effect than Metformin alone on oxidative stress (MDA) and pro inflammatory markers (TNF-α). Add Metformin to sitagliptin can lead to significant reduction in the level of HbA1c than Metformin alone.

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