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EFFECTS OF METFORMIN AND DAPAGLIFLOZIN ON GLYCEMIC INDICES AND HOMA-IR IN TYPE 2 DIABETES MELLITUS PATIENTS

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ABSTRACT

Type 2 diabetes mellitus is a prevalent and progressive disease with a need for innovative therapeutic agents to continue advancing disease management. Dapagliflozin is the second agent in a new class of oral antihyperglycemic drugs; sodium-glucose cotransporter 2 (SGLT2) inhibitors. Sodium-glucose cotransporter 2 inhibitor is responsible for the majority of renal glucose reuptake. Inhibition of the cotransporter allows for increased renal glucose excretion that consequently leads to reduced plasma glucose levels. Because this mechanism does not require the action of insulin, dapagliflozin rarely causes hypoglycemia and is effective in patients both early and late in the course of their disease. Studies of dapagliflozin have demonstrated efficacy both as monotherapy and in combination with oral antihyperglycemic agents and insulin. The most common adverse reactions observed with dapagliflozin in clinical trials were female genital mycotic infections, urinary tract infections, and nasopharyngitis. Dapagliflozin approved by FDA at 2014, is a new oral agent for type 2 diabetes with short-term efficacy similar to dipeptidyl peptidase 4 inhibitors. Metformin is the most recommended euglycemic monotherapy of T2DM belonging to Biguanides group. 1,5 anhydro glucitol (1,5-AG) is 1-deoxy form of glucose, is a validated marker of short-term glycemic control. Homeostasis model assessment (HOMA) is a mathematical model which can estimate an individual's degree of insulin sensitivity and level of beta cell function. ***Objectives:*** *The aim of this study was to investigate the effects of metformin alone, metformin with dapagliflozin on fasting serum 1,5AG, fasting plasma glucose (FPG), glycated hemolglobin A1c (HbA1c) and HOMA of insulin resistance (HOMA-IR). Methods: The study included 63 male and female patients with T2DM whose ages ranged between 38 and 72 years. The enrolled patients were divided into two groups, group 1: consisted of 30 patients, treated by oral metformim alone over a period of 24 weeks, which is the period of the study. Group 2: consisted of 33 patients, treated by oral metformim with dapagliflozin. Thereafter each patient was submitted to have fasting serum 1,5AG, FPG, HbA1c and fasting serum insulin. All parameters were measured initially before any intervention and later on at two steps, the 12th and the 24th week of the study time. Standard kits were used to measure biochemical profiles suggested in this study. Tests were performed and interpreted following instruction outlined in each kit.* ***Results:*** *After 24 weeks of treatment with metformin alone, metformin with dapagliflozin there was significant improvement in both 1,5AG (p 0.013, 0.030) respectively and HbA1c (p 0.012 ,0.020) respectively. For HOMA-IR, p value with metformin monotherapy and metformin plus dapagliflozin was (p 0.110, 0.126) respectively, both results showed an apparent improvement only which was statistically insignificant.* ***Conclusion:*** *Metformin alone and metformin plus dapagliflozin are associated with statistically significant improvement in 1,5AG serum level and HbA1c. HOMA-IR in both treatment groups showed only insignificant apparent improvement.*

KEY WORDS

apagliflozin, 1,5Anhydroglucitol (1,5AG), sodium-glucose transporter 2, type 2 diabetes mellitus, Homeostasis model assessment for insulin resistance (HOMA-IR), glycated haemoglobin A1c (HbA1c), fasting plasma glucose (FPG).

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a worldwide problem that is growing in prevalence. An estimated 347 million people worldwide are diagnosed with diabetes, and 90–95% of those have T2DM [1]. Chronic hyperglycemia due to diabetes is associated with both microvascular and macrovascular complications and can ultimately result in death [2] Despite these known consequences, many patients are unable to optimally regulate their blood glucose control [3]. Current guidelines advocate for initiating lifestyle modifications and metformin as first-line therapy, but beyond that, antihyperglycemic management becomes very patient specific. Even when patients are able to achieve a target glycated hemoglobin A1c (HbA1c) goal of less than (<7%), it is difficult to maintain this for a long term as their disease progresses [1].

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are a new therapeutic class of oral agents for the treatment of T2DM. This therapeutic class currently includes three agents: canagliflozin, dapagliflozin, and empagliflozin [5]. Dapagliflozin was approved in the US on 8 January 2014 and has previously been approved and is used in 38 other countries, including Europe, under the trade name Forxiga (Bristol-Myers Squibb Company, Middlesex, UK) [AstraZeneca, 2012; Bristol-Myers Squibb Company, 2014]. A fixed-dose combination of dapagliflozin and metformin (Xigduo) [Bristol-Myers Squibb Company, Middlesex, UK] was also recently approved in Europe [AstraZeneca, 2014].

Dapagliflozin competitively, reversibly, and highly selectively inhibits SGLT2. Type 2 SGLT2s are expressed in the kidney and on the epithelial lining of the S1 segment of the proximal convoluted tubule. Physiologically, these transporters are responsible for approximately 90% of renal glucose absorption [4]. By blocking SGLT2 with dapagliflozin, reabsorption of glucose into the blood stream is diminished. Dapagliflozin promotes glucose filtration through the kidneys and into the urine to be eliminated from the body. Dapagliflozin doses of 20–100 mg have resulted in urinary glucose excretion of approximately 60 g over 24 h in healthy volunteers [35]. In subjects with T2DM who received dapagliflozin doses between 2.5 and 20 mg, the 24 h glucose excretion after 1 day ranged between 38 and 77 g and after 14 days ranged between 42 and 73 g [36].

Studies have demonstrated that the 24 h urine glucose excretion with dapagliflozin represents only about 40–50% of the human-filtered glucose load. One potential reason for this ceiling effect is that when SGLT2 is inhibited, SGLT1 may compensate by increasing reabsorption of glucose [37].

Dapagliflozin is dosed starting at 5 mg orally in the morning and can be titrated up to 10 mg orally in the morning if clinically indicated. Dapagliflozin is not known to have any meaningful drug–drug interactions. Dapagliflozin has been evaluated in combination with glimepiride, metformin, pioglitazone, and sitagliptin; it neither affects the metabolism of these antihyperglycemic agents nor is its metabolism affected by them and there are no known pharmacokinetic (PK) alterations [Bristol-Myers Squibb Company, 2014] [36]. Because dapagliflozin can cause decreases in systolic blood pressure (BP) *via* its osmotic diuretic effect, patients receiving antihypertensive agents, especially loop diuretics, or those known to experience hypotension should be closely monitored when initiating or titrating dapagliflozin [Bristol-Myers Squibb Company, 2014].

Metformin has been the most recommended monotherapy of T2DM. The UK Prospective Diabetes Study (UKPDS) found metformin more effective than chlorpropamide, glibenclamide and insulin [31] The American Diabetes Association (ADA) recommended metformin as the first drug of choice for treating T2DM patients, especially those who are overweight [2] These agents are termed "euglycemic" agents. The proposed mechanisms of action include reduced hepatic and renal gluconeogenesis, slowing of glucose absorption from the gastrointestinal tract, increased glucose to lactate conversion by enterocytes, direct stimulation of glycolysis in tissues, with increased glucose removal from blood and, lastly, reduction of plasma glucagon levels [26].

Maida *et al.*, 2011 [10] have recently reported that metformin acutely increases plasma levels of glucagon-like peptide 1 (GLP-1) and induces islet incretin receptor gene expression through a mechanism that is dependent on peroxisome proliferator-activated receptor (PPAR)-α.

Zhou *et al.*, 2001 [31] reported that the activation of AMP-activated protein kinase (AMPK) by metformin in the liver, and probably in other tissue provides a unified explanation for the pleiotropic beneficial effects of this

drug. The most common toxic effects of metformin are gastrointestinal that occur in up to 20% of patients. Absorption of vitamin B_{12} appears to be reduced during long-term metformin therapy. Metformin is contraindicated in patients with renal disease, alcoholism, hepatic disease, or conditions predisposing to tissue hypoxia e.g. chronic cardiopulmonary dysfunction, because of an increased risk of lactic acidosis induced by biguanide drugs in the presence of these diseases [14]. Maida et al [10] and Zhou et al [31] reported that the activation of AMP-activated protein kinase (AMPK) by metformin in the liver, and probably in other tissue, provides a unified explanation for the pleiotropic beneficial effects of this drug.

1,5 Anhydroglucitol (1,5AG) was first discovered in the plant family *Polygala senega*in 1888. The presence of the compound in human blood [6] and cerebrospinal fluid was established in 1972 and 1973, respectively. 1,5AG, a 1-deoxy form of glucose, is a major metabolically inert circulating polyol arising primarily from ingestion and excreted competitively with glucose [7]. Research studies have shown that 1,5AG originates mostly from foods with a mean daily intake of ~4.4 mg/day. The rate of intake is matched by the rate of daily excretion. A bodily pool of about 500–1000 mg of 1,5AG is constantly maintained [8].

1,5AG is a validated marker of short-term glycemic control and its levels in blood respond within 24 h as a result of glucose's competitive inhibition of 1,5AG reabsorption in the kidney tubule, since reabsorption of filtered 1,5AG in the proximal tubule is competitively inhibited by glucose, so it is an indicator to identify rapid changes in hyperglycemia [9].

Type 2 diabetes mellitus (T2DM) is caused by a combination of progressive β-cell dysfunction, relative insulin deficiency, and variable degrees of insulin resistance that lead to dysregulation of glucose homeostasis [11]. Homeostasis model assessment of insulin resistance (HOMA-IR) is a mathematical model which can estimate an individual's degree of insulin sensitivity (HOMA%S) and level of beta cell function (HOMA%B) from simultaneous measurements of fasting plasma glucose and insulin or C-peptide concentrations. Insulin resistance is a condition in which normal amounts of insulin are inadequate to produce normal responses from fat, muscle (promote glucose uptake) and liver (inhibit glucose output) cells [32]. It is a major hallmark in the development of T2DM and a number of associated metabolic disorders, and is also implicated in obesity, hypertension, cancer or autoimmune diseases [33]. In non-diabetic individuals, the best HOMA-IR cut-off levels ranged from 1.85 in men to 2.07 in women. A lower cut-off values for diabetic than non-diabetic individuals ranged from 1.60 in men to 2.05 in women probably because in the diabetic population there is an increased prevalence of hypertension, obesity, and dyslipidemia [34].

Patients and Methods

This study is a randomized, single blinded, interventional, dose escalation study of 24 weeks treatment duration, comparative and prospective study. The study was conducted on adult patients with T2DM attending a Diabetic and Endocrine Diseases Clinic over the period from March 3, 2017 through September 15, 2017.

The study included 63 male and female patients with T2DM whose ages ranged between 38 and 72 years.

The study excluded patients who were known to have hepato-biliary disease, hypothyroidism, chronic kidney disease and nephrotic syndrome. Cigarette smoking, the use of any glucose altering medications, such as oral contraceptive, diuretics, steroids and neuroleptics during the last month were also considered in exclusion in addition to pregnant or lactating women and patients with hematological abnormalities.

To have an idea about the normal values of study and in order to assess how much the drugs used in the study were able to normalize the abnormal parameters, A 35, apparently healthy volunteers whose age matched the enrolled patients were involved. Their data were obtained, manipulated, tabulated and analyzed in the same way as the study patients.

All the enrolled participants (n. 63) were informed about the aim of the study and an oral consent was obtained from each of them. Thereafter, the patients were divided into two groups as follow:

Group 1: consisted of 30 patients, treated by oral metformim alone (Dialon-Julphar), 500 mg b.d initially and the dose was adjusted according to the HbA1c and FPG readings over a period of 24 weeks, which is the period of the study.

Group 2: consisted of 33 patients treated by metformin plus dapagliflozin (forxiga- AstraZeneca) starting at a dose of 5 mg orally in the morning and titrating up to 10 mg orally in the morning if clinically indicated.

Thereafter each patient was submitted to have fasting serum 1,5AG, FPG, HbA1c and fasting plasma insulin (FPI) were measured. Standard kits were used to measure biochemical profiles suggested in this study using double-sandwich ELISA technique. Both fasting plasma glucose and fasting plasma insulin that required in HOMA-IR equation (HOMA-IR=FPG (mg/dl) x FPI(µIU/L) /405) was measured by an enzymatic immunoassay technique. Tests were performed and interpreted following instruction outlined in each kit.

An initial physical examination was conducted, and all the above parameters were assessed initially, before any intervention, and later on at two steps, the 12th and the 24th week of the study time. The data were recorded in specially preformed case record.

Statistical Analysis: After data gathering, the data were tabulated, organized and introduced into a computer file. The statistical analysis was carried out using Statistical Package for the Social Science (SPSS); version 21. The descriptive statistic; mean ±standard deviation (±SD), was used to describe the value of the numerical data [12]. The differences between the means were considered significant at the 5% confidence level and the *p* values of $p<0.05$, $p<0.01$ and $p<0.001$ were considered as significant, highly significant and very highly significant respectively.

The inferential statistics; one-way analysis of variance (ANOVA) was used to determine the differences between the parameters in the same treatment group. The *one* sample *t*-test was used to compare between parameters within treatment groups. Independent *t*-test was used to compare between the results of studied parameters obtained at the 24th week from treatment for each group with their corresponding at the controls to identify which parameter approaches more the normal value.

RESULTS

The patients were 63; 35 males and 28 females. Their ages ranged between 38 and 72 years with a mean age ±SD (41.5±7.00) for females and (49.5±11.37) for males. After 24 weeks of treatment with metformin alone, there was significant improvement in glycemic indices with a mean ± SD for (HbA1c, FPG, and 1,5AG) at base line level was 9.7±1.6, 258.1±21.2, 6.9±1.5 respectively. At week 24, mean±SD was (6.1±1.00, 130.9±17.4, 10.4±0.8) respectively.

HOMA-IR mean ± SD improvement was 20.2 +10.2→5.1 +3.2, this alteration was statistically insignificant (Table 1).

On comparing the results of metformin at the 24th week with the control group, the parameters didn't approach the control group values (Table 2)

Using metformin with dapagliflozin for 24 weeks showed a significant improvement in 1,5AG with a Mean±SD before and after treatment was (6.7 +1.2→12.9 +1.6). A significant increase in HbA1c, FPG with a Mean±SD before and after treatment was (9.8±1.2→6.00±1.1) for HbA1c and (235.5 ±48.3→120.2 ±19.8) for FPG.

HOMA-IR, apparently improved after 24-week treatment with metformin and dapagliflozin with mean ± SD was (16.3+ 5.6→3.2+ 1.9), this alteration was statistically insignificant (Table 3)

On comparing effect of therapy by metformin with dapagliflozin after 24 weeks of treatment with the control group values, it was shown that the parameters didn't approach the control group values (Table 4)

Table.1 Effect of treatment by metformin on HbA1c, FPG, HOMA-IR, 1-5 AG from baseline and at the12th and 24th week of treatment

Drug	Duration	HbA1c	FPG	HOMA-IR	1,5AG
Metformin	Base line	9.7 + 1.6	258.1±21.2	20.2 + 10.2	6.9 + 1.5
	Week 12	7.3 ±1.4	I66+ 31.7	8.1 + 4.3	9.2 + 1.5
	Week 24	6.1±1.00	130.9 + 17.4	5.1 + 3.2	10.4 + 0.8
p value (t-test)		0.012	0.026	0.110	0.013

Table.2 Comparison between metformin group and control group in regard to HbA1c, FPG, HOMA-IR, HbA1c, FPG HOMA-IR and 1,5AG.

Drug	HbA1c	FPG	HOMA-IR	1,5AG
Metformin	6.1±1.00	130.9 + 17.4	5.1 + 3.2	10.4 + 0.8
Control	4.7+ 0.8	99.4 + 8.2	3.00+ 1.5	25.9 + 8.6
p value (independent Samples t-test)	0.006	0.000	0.036	0.000

Table 3. Effect of treatment by metformin with dapagliflozinon HbA1c, FPG, HOMA, 1-5 AG from baseline and at the 12th and 24th week of treatment.

Drug	Duration	HbA1c	FPG	HOMA-IR	1,5AG
Met. plus dapagliflozin	Base line	9.8±1.2	235.5 + 48.3	16.3 + 5.6	6.7±1.2
	Week 12	7.4 + 1.4	160.4 + 33.1	8.2 + 3.5	9.0 + 1.4
	Week 24	6.00±1.1	120.2 + 19.8	3.2+ 1.9	12.9±1.6
p value (t-test)		0.020	0.015	0.126	0.030

Table.4 Comparison between metformin with dapagliflozin group and control group in regard to HbA1c, FPG, HOMA, 1-5 AG at the 24th week of treatment.

Drug	HbA1c	FPG	HOMA-IR	1,5AG
Met. Plus dapagliflozin	6.00±1.1	120.2 + 19.8	3.2 + 1.9	12.9±1.6
Control	4.7+ 0.8	99.4 + 8.2	3.00+ 1.5	25.9 + 8.6
p value (independent Samples t-test)	0.008	0.087	0.885	0.000

DISCUSSION

No studies about the effects of metformin on 1,5AG to be compared with the current results, this registers originality of this research in this regard. In this study, there was a significant reduction in HbA1c level after treatment with metformin for 24 weeks (*p*0.012).

A study by Ponssen *et al* [16] reported that treatment with metformin 1000 mg daily for 6 months produced significant reductions in HbA1c from baseline level (*P* = 0.005) which was in agreement with this study. Arai Keiko *et al* [13] study showed that the baseline Hb Alc level became lower after treatment with metformin with an HbA1c <7.0% (p<0.05).

Manuel González *et al* [15] claimed that HbA1c concentrations decreased significantly with metformin glycinate administration for 2 months (*P*=0.008) while HOMA-IR was not modified by the intervention.

Leyco T *et al* [17] reported that there was no significant difference in the HbA1c at 6-month follow-up compared to baseline after metformin discontinuation in patients with impaired renal function.

Zhang J P*et al* [19] reported that there was a significant reduction in HOMA-IR in which after 24 weeks treatment, metformin decreased HOMA-IR significantly with *P*<0.001. Baptista T.*et al* [25] also showed that HOMA-IR improved with 24 months of metformin therapy.

No available data regarding the relation between dapagliflozin and fasting serum 1,5AG, so this article is original in this regard.

The complimentary mechanisms of action of a combination of dapagliflozin and metformin may have clinically beneficial effects in the treatment of patients with T2DM.

Veronica Hackethal [28] and Liao X *et al* [30] reported that dapagliflozin may increase insulin sensitivity by increasing blood levels of zinc-A2-glycoprotein (ZAG). Zinc-A2-glycoprotein is an adipokine that may play an important role in metabolic regulation. Although ZAG's biological actions have yet to be fully described, ZAG is thought to be involved in promoting lipolysis, as well as modulating insulin sensitivity. Originally it was thought to be expressed only from tumors, but recent research suggests that ZAG may also be produced in white and brown adipose tissue, as well as in the liver, heart, and lungs. "These data suggest that some aspect of dapagliflozin positively regulates ZAG and adiponectin expression and their release into the circulation. These results further support that whole-body insulin sensitivity is increased after SGLT2 inhibitor treatment and the increase in ZAG and adiponectin may contribute to insulin sensitization [29]. Recent work found lower ZAG levels in individuals with T2DM. In these individuals, increasing ZAG levels were linked to increased levels of adiponectin, which is known to increase insulin sensitivity. In addition, increasing ZAG levels were linked to decreased BMI, waist to hip ratio, and HOMA-IR, suggesting a role for ZAG in insulin sensitivity [29]. Veronica Hackethal [28] and Liao X *et al* study showed that, compared to baseline, six months of dapagliflozin treatment with metformin was associated with significant increases in ZAG (*P*<0.01), significant decreases in HbA1c (*P*<0.01), fasting blood glucose (*P*<0.01), fasting insulin (*P*<0.01), and HOMA-IR (*P*<0.01) which is in agreement with this study.

Juan José *et al* [18] and Bailey C *et al* [27] found that when the patients become uncontrolled on submaximal doses of metformin, increasing the metformin dose or

adding a second agent are alternative management strategies for restoring glycemic control. They also found that treatment with metformin and dapagliflozin for 24 weeks in a dose of 2.5, 5 and 10 mg once daily significantly reduced HbA1c and FPG in patients with T2DM.

A study performed by Julio Rosenstock *et al* [20] showed that 24-week treatment with metformin and dapagliflozin, significantly improved the HbA_{1c} (P<0.02). Min SH *et al* [21] had mentioned that following oral antidiabetic drugs treatment period with dapagliflozin and metformin named commercially *(Xigduo XR*), there was a significant reduction in HOMA(P<0.05) improvement. both studies are in agreement with this study.

In Aki Okamoto *et al* [24] study, both fasting Blood glucose and HbA1c baseline level was significantly decreased with (p < 0.05) (p< 0.01) respectively.

The glucose-lowering effects of dapagliflozin have been confirmed in several clinical trials conducted in Japan and overseas. In a dose determination study of drug naive Japanese patients with T2DM, dapagliflozin 5 and 10 mg/day reduced HbA1c level by 0.41 and 0.45 %, respectively, from baseline at 24 weeks after treatment [22]. In another study in Japan that evaluated the add-on effect of dapagliflozin to an existing antidiabetic agent, dapagliflozin 5 mg/day for 12 weeks reduced HbA1c level by approximately 0.6 % [23]. the current study results showed that adding dapagliflozin to metformin improved glycemic control nearly at the same level of previous reports.

Sarah L. Anderson [5] found that dapagliflozin has been shown to decrease HbA1c values 6 mmol/mol (0.5%) to 8 mmol/mol (0.7%).

Ferrannini *et al.* [38] conducted a 24-week study. The patients were randomized to dapagliflozin 5 mg or 10 mg each morning. The dapagliflozin 5 mg and 10 mg doses produced an HbA1c reduction that was statistically significant (p< 0.001 for 5 mg and p<0.0001 for 10 mg). Fasting plasma glucose was also decreased significantly (p< 0.05 for 5 mg and p <0.01 for 10 mg). Similarly, Bailey *et al.* [27] showed that 71% from the original patient population was followed up for 102 weeks. At 102 weeks, patients in the dapagliflozin plus metformin groups continued to have more substantial reductions in HbA1c [−5 mmol/mol (−0.48%) to −9 mmol/mol (−0.78%)] and FPG (−19.3 to −24.5 mg/dl).

CONCLUSION

In conclusion dapagliflozin is a new addition to the antidiabetic drugs with similar glycaemic efficacy to other oral antihyperglycaemic agents and low risk for hypoglycaemia. Dapagliflozin offers additional clinical benefits including body weight and blood pressure reduction.

Metformin and metformin plus dapagliflozin were associated with statistically significant improvement in 1,5AG and HbA1c. HOMA-IR in both treatment groups showed insignificant apparent improvement

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