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**RESEARCH ARTICLE**

# COMPARATIVE EFFECT OF COMBINATION SITAGLIPTIN AND METFORMIN VERSUS METFORMIN ALONE ON BLOOD GLUCOSE AND OXIDATIVE STRESS IN TYPE 2 DIABETES MELLITUS PATIENTS

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**ABSTRACT**

*Objective; of the this study was to investigate the effect of sitagliptin on blood glucose ,oxidative stress and total antioxidant status (TAS) in people with type 2 diabetes mellitus.*

*Design: Case-control study*

*Selection; The patients were selected from main diabetic clinics in al-Wafaa' Diabetic Clinic and from private clinics in Mosul city during the period from 1st of November 2020 to the 30 t h April 2021.*

*Methods; It was conducted randomly 60 patients (32 males , 28 females ) of Non-insulin diabetes mellitus , who were already on routine anti-diabetic therapy ,those separated into 2groups. First Group : included 30 patients( 17 males,13 females) of T2DM treated with oral metformin alone Second group : included 30 patients( 15 males ,15 females) of T2DM treated with combination of Sitagliptin + metformin for at least 3 months , measured fasting plasma glucose, glycosylated hemoglobin another 30 healthy subjects , age ,sex matched with the patients, were considered as a control groups . Malondialdehyde (MDA) and Total antioxidant status (TAS) were measured in all groups*

*Results; of this study, serum MDA levels were found high and TAS concentrations was lower in diabetic patients of both groups than the healthy control, the combination of sitagliptin + metformin caused significant reduction( P-value > 0.05) in the level of glycosylated hemoglobin ( HBA1c ) and malondialdehyde (MDA) in comparison with group that received metformin monotherapy ,as well as ,non-significant change in fasting blood group( FBG) and total antioxidant status (TAS )in both groups As a conclusion of the present study sitagliptin can attenuated MDA in patient with NIDDM and greater reduction in serum level of HBA1c than the use of metformin monotherapy.*

*Keywords: diabetes mellitus ,sitagliptin , oxidative stress, antioxidants*



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## INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by dysregulation of carbohydrate, lipid and protein metabolism caused by reduced secretion of insulin, resistance of insulin, or a combination of both ( ADA ,2009 , Eizirik et al ., 2020).

Increased blood glucose level in diabetes produces free radicals (Yaribeygi et al ., 2018) These free radicals cause oxidative stress and in turn weakens the endogenous body antioxidant defense mechanism (Furukawa et al ., 2017).

Oxidative stress plays a fundamental role in the progression , development and the pathogenesis of diabetic complications ( Zhang et al ., 2020) Oxidative stress is a phenomenon caused by the imbalance between the formation and removal of free radicals (Poprac et al.,2017).It will lead to impaired glucose uptake in muscle and fat cells and decreases insulin secretion and function from beta cells resulting in a variety of microvascular and macrovascular complications (Aypola et al ., 2014 ; Maddux et al ., 2014). Despite this, the specific mechanisms are not fully understood. T2DM is linked to increased oxidative stress leading to several abnormalities , as while as the antioxidant defence system decreased in type 2diabetes mellitus ( Laight et al ., 2000).

Metformin(Biguanide class) is the most popular first-line oral therapy for the treatment of type 2 diabetes ( Apostolava et al ., 2020) is works by suppressing hepatic glucose synthesis , that lead to reducing in the level of fasting plasma glucose and HbA1c . Metformin has no effect on the function of ß-cell ( Rena et al ., 2017) .

On the other hand ,Sitagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances the effect of incretin hormone , which are required for reducing blood glucose, regulation of endogenous insulin secretion, and regulation of fatty acid metabolism ( Blauschmit et al ., 2017 ; Hung et al ., 2020) Their primary effect to improve glycemic control is mediated by inhibition of glucagon secretion and reduction in basal hepatic glucose production ( Saha et al ., 2020 ).It also protect against oxidative stress and possess antioxidant that boost antioxidant capacity (Bigagli et al ., 2020 ).The current study aimed to assess the effects of sitagliptin on blood glucose , oxidative stress and total antioxidant status.

## METHOD

The present study had approval from Mosul College of medicine. Sixty patients ( female 28 and male 32) whose ages range between ( 36 ± 75) years . The patients were divided into two groups ; First group included thirty patients taking metformin alone (Piophage® tablet provided by Pioneer Co. Iraqi) of different doses ranged from (1000 to 1700 mg daily) for at least 3 months duration .

Second group included thirty patients taking sitagliptin + metformin( Sitavia plus®, manufactured by pioneer ,Iraq, 50/500 – 50/ 1000 mg twice daily ) for at least 3 months duration ,

Third control groups included apparently healthy subjects (15 males ,15 females ), Patient with concomitant disease, pregnant and lactating women, smoking ,alcoholic intake or receive other antihyperglycemic therapy was excluded from this study .The present study was approved by the Ethics Committee of Mosul medical college, For MDA and TAS using Colorimetric Assay Kit Elabscience® . (USA) (TBA Method) ) following the instructions included in the leaflet of the kit. HbA1c was analyzed on Roche/ Hitachi immunologically Cobas c501 using Tina-quant® Hemoglobin A1c Gen.3 (Roche Diagnostics, Mannheim, Germany).

### Statistical analysis

Computer feeding was conducted by prepared computer Standard statistical methods were used for analysis the data of the study using Minitab (version 18) software statistical program . Multiple comparisons were carried out between healthy and diabetic groups by using one way analysis of variance (ANOVA) with Tukey's Pair wise comparisons was applied ,also to compare the results between diabetic groups ,Independent sample ( unpaired t-test ) was used for quantitative variables and chi-square test used for catigoral data. for all statistical analysis, data represent by the mean ∓ stander deviation p-value > 0.05 was considered statisticallysignificant.

## RESULTS

In comparison the clinical feature such as age , gender , body mass index in addition to oxidative stress( MDA) and total antioxidant status between healthy and diabetic groups seem not statistically significant differences (p > 0.05) in age and gender while statistically significant in BMI , MDA and T-AOC ( p = 0.005 , 0.001 ,0.002 respectively ) as shown in table 1. table ( 2) although , the ( Mean∓ SD ) of group (sitagliptin + metformin) is fewer than metformin group but not reach statistically significant (p – value > 0.05) in both FBG and TAS parameter . While the amount of reduction of HbA1c and MDA in group combination of (sitagliptin + metformin) is significantly than metformin monotherapy ( p= 0.024 ,0.038) respectively as shown in table 2 .

Table 1. Self-Administered Questionnaire Sheet Related to Demographic Characteristics of students. No. = 218.

Table 1. Comparison in personal parameters between healthy and the two sampled groups of NIDDM.

| Parameters | Mean ± SD | | | P-value* |
|---|---|---|---|---|
| | Sitagliptin + metformin Cases [n = 30] | metformin Control [n = 30] | Healthy control [n = 30] | |
| **Age (years)** | 57.03 ± 7.37 A | 53.20 ± 9.21 A | 54.00 ± 7.52 A | 0.159 |
| **BMI (kg/m$^2$)** | 30.43 ± 3.81 A | 28.55 ± 4.23 AB | 27.17 ± 1.00 B | **0.005** |
| **Gender** | **No. (%)** | **No. (%)** | **No. (%)** | --- |
| **Male** | 15 (50.0) | 17 (56.7) | 15 (50.0) | 0.804 |
| **Female** | 15 (50.0) | 13 (43.3) | 15 (50.0) | |
| **MDA (nmol/ml)** | 3.85 ± 0.73 A | 4.470 ± 1.42 A | 2.92 ± 0.70 **B** | **0.001** |
| **T-AOC (U/ml)** | 7.62 ± 4.47 B | 7.59 ± 2.75 B | 10.33 ± 2.56 A | **0.002** |

* One-way ANOVA-test with Tukey's Pair wise comparisons was applied, means that do not share a letter are significantly different and chi-square test for catigoral data.

Table 2. Comparison in the blood sugar and oxidative stress parameters between the two sampled groups of Type 2 diabetes patients.

| Parameters | Mean∓ SD | | P-value* |
|---|---|---|---|
| | Sitagliptin+metformin n=30 | Metformin n=30 | |
| **FBS (mg/dl)** | 144.30 ± 27.17 | 149.25 ± 24.80 | 0.482 |
| **HbA1c %** | 6.64 ± 0.70 | 7.32 ± 1.45 | **0.024** |
| **MDA (nmol/ml)** | 3.85 ± 0.73 | 4.470 ± 1.42 | **0.038** |
| **T-AOC (U/ml)** | 7.62 ± 4.47 | 7.59 ± 2.75 | 0.972 |

* Independent T-test of two means was applied for quantitative variables and chi-square test for catigoral data

## DISCUSSION

The goal of this study was to compare the effect of sitagliptin plus metformin versus metformin monotherapy on blood glucose , oxidative stress and total antioxidant status in groups of NIDDM patients in Mosul. The two study groups were picking to be match as closely as possible in order to rule out any substantial difference in feature between them .

In the present study the total number involved 90 subjects divided into three groups of 30 subjects ,the groups were matched concerning number of males, females, their ages and statistically confirmed by lack of significant variation between the groups. This matching of the number of individual groups, gender, age may eliminate any influence of this parameters on the results .

In term of glycemic control ,the present study found no significant difference in baseline FBS between the two groups ,however (sitagliptin + metformin) had significantly lower in HbA1C level( p- value > 0.05) than metformin alone. The finding are compatible with previous research (Han et al. 2011; Reasner et al ., 2011) , Katzeff et al., 2015)shown 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 and ß-cell function improved over two years in diabetic patient .

DPP-4 inhibitors lower HbA1c via increasing plasma GLP-1/GIP levels, which lead to enhance glucose-stimulated insulin secretion and glucagon inhibition (Berger et al ., 2018 : Iannantuoni et al

., 2019). As a result a combination of Metformin (reduces hepatic glucose production) with DPP-4 inhibitor (insulin secretagogue) should have additive effect in lowering HbA1c ( Goldstein et al ., 2007) . Fasting glucose level did not changed significantly but HbA1c level did , Unfortunately we do not have data on glucose or insulin concentrations postprandially. Thus , the decreased in HbA1c via DPP-4inhibitor was attributed to an increase level of GLP-1/GIP which to enhanced secretion of glucose-stimulates insulin secretion and inhibits glucagon(Berger et al ., 2018 : Iannantuoni et al ., 2019). As a result the combining of Metformin (reduces hepatic glucose synthesis ) plus DPP-4 inhibitor (an insulin secretagogue) was expected to produce the additional effect to reduce HbA1c level ( Negre-Salvayre et al ., 2009).

Chronic hyperglycemia results in the production free radical which lead to increase in oxidative stress in a variety of tissues (Papachristoforou et al ., 2020) the serum level of malondialdehyde(MDA is an indicator of lipid peroxidation) is used to assessed Oxidative stress (Amin et al ., 2020) an increased in oxidative stress, which is likely to the abnormal metabolic mellitus contributes to rise many micro and macrovascular complication (Fernandez-Robredo et al ., 2020) In this study serum MDA levels were found high in diabetic patients of both groups than the healthy control (3.85 ± 0.73 , 4.470 ± 1.42 , 2.92 ± 0.70 nmol/ml , respectively ) (p-value = 0.001) .Increased MDA level which is the consequence of lipid peroxidation and a marker of oxidative stress is an evidence of exaggerated oxidative stress . in these patients found that serum MDA levels was statistically significant lowered (p< 0.05) in group treated with (sitagliptin + metformin ) than group treated with metformin monotherapy(3.85 ± 0.73; 4.470 ± 1.42 nmol/ml) , respectively (p- value = 0.038) ,this result is consistent with Ferreira et al study (2010) .Which found after treatment with sitagliptin for six weeks there was significant decrease in serum and tissue of the Zuker Diabetic Fatty(ZDF) rats.

Furthermore , Apaijai et al. study (2013) showed that (sitagliptin) have significant reduction cardiac MDA level in obese insulin-resistant rats after 12 weeks of treatment .

Ayaori et al (2013) on the other hand , found non-significant reduction in the level of malondialdehyde after (1-2months) of sitagliptin treatment . According to kelleni et al.,( 2015) study report that sitagliptin have antioxidant capabilities that decreased the accumulation of free radicals .

In this study TAS concentration was lower in T2DM (7.62 ± 4.47 , 7.59 ± 2.75 u/ml , respectively) , when compared with the healthy control group(10.33 ± 2.56 u/ml) , (P = 0.002) .Our results are in accordance with the results of some previous studies that compared the concentrations of TAS and individual antioxidant enzymes in T2DM patients. while ,NIDDM patient treated with( sitagliptin+ metformin) had minimum improvement in the mean of TAS than those treated with metformin only , although the difference was statistically non -significant these results was similar with those previously mentioned with (Hazman and Celik .,2014) on the contrary, Pujadas et al ( 2017) (31) study ,discovered that sitagliptin and other DDP4 inhibitors reduced yperglycemia-induced OS with a significant antioxidant effect by reversing of ROS and endoplasmic reticulum stress . As a result our finding suggest that sitagliptin has more antioxidant effect than metformin and lessen oxidative stress.

## CONCLUSIONS

Our data shown that sitagliptin combined with metformin is a well-tolerated and effective treatment for improving glycaemic control via significant reduction of HbA1c also, significant reduction of MDA that attenuate NIDDM induced oxidative stress , therefore a combination is recommended to diminished glucolipotoxicity and related OS damage suggesting that this combination is beneficial as thereby against the development and/or progression of diabetic complication.

## ETHICAL CONSIDERATIONS COMPLIANCE WITH ETHICAL GUIDELINES

This study was completed from the coolege of nursing, University of Mosul, Iraq. The participants were informed about the research's purpose and ensured anonymity and confidentiality of the information. A written informed, voluntary participation consent was obtained from each participant.

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## AUTHOR'S CONTRIBUTIONS

Study concept, Writing the original draft, Data collection, Data analysis, Reviewing the final edition, by All authors.

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