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Renoprotective Potency of Sitagliptin versus Pioglitazone in Type 2 Diabetic Patients: Impact on LncMIAT

Mohamed A. Kandeil, Mohamed A. Shaarawy, Hamdy A. Mourad, and Mohamed O. Mahmoud*

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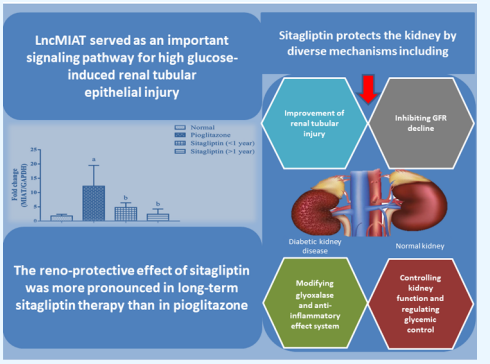
ABSTRACT: *Background:* Diabetes mellitus (DM) represents one of the most important reasons for chronic kidney diseases due to the high level of blood glucose that destructs blood vessels. *Objective:* The present study focused on investigating the protective impact of sitagliptin on kidney complication in type 2 diabetes mellitus (T2DM) patients in comparison to pioglitazone to examine which has the superior effect against the nephritic complication of DM. *Methods:* Eighty adult subjects were classified into four groups: control group, pioglitazone-treated T2DM patients (P group), sitagliptin-treated T2DM patients for less than one year (SL group), and sitagliptin-treated T2DM patients for more than one year (SM group). Blood samples were withdrawn from all subjects for analysis of neutrophil gelatinase-associated lipocalin (NGAL), vanin-1, kidney injury molecule-1 (KIM-1), glyoxalase-1 (Glo-1), methylglyoxal (MG), cystatin-C, and interleukin-18 (IL-18) using competitive ELISA kits. Also, long noncoding myocardial infarction associated transcript (lncMIAT) was measured in whole blood using qRT-PCR. *Results:* The present study revealed that the lncMIAT expression level was significantly higher in the P group as compared to the SL group, SM group, or healthy control group. Additionally, serum NGAL, vanin-1, KIM-1, Glo-1, MG, and cystatin-C were significantly higher in the P group and SL group as compared to the SM group and healthy control group. *Conclusion:* Sitagliptin protected the kidney through downregulation of lncMIAT besides amelioration of kidney injury marker levels, which was more preferable than in pioglitazone therapy.



1. INTRODUCTION

Diabetes mellitus (DM) is one of the most rapidly growing health concerns that impacts health and healthcare resources worldwide. Currently, the spread of type 2 diabetes mellitus (T2DM) in Egypt is approximately 15.6% of all adults, with ages ranging from 20 to 79 years. The International Diabetes Federation (IDF) has classified Egypt as the ninth main country for the number of patients with T2DM around the world.[1] In 2018, nearly 8.2% of the United States' population, 26.9 million persons of all ages, were diagnosed with DM, while about 210,000 children and adolescents less than 20 years of age, or 25 per 10,000 teenagers, have been diagnosed with DM.[2]

Patients suffering from DM for several years are prone to diabetic renal complications, including diabetic nephropathy (DN), which is one of the most dangerous and epidemic complications in diabetic patients leading to end-stage kidney disease with an increased probability of mortality and cardiovascular morbidity.[3] Generally, diabetic complications include microvascular complications such as nephropathy, neuropathy, and retinopathy, while macrovascular complications encompass peripheral arterial disease, stroke, and coronary artery disease, and their pathologies contribute to disease morbidity and mortality.[4]

Recent antidiabetic medications with renoprotective effects involving dipeptidyl-peptidase-4 (DPP-4) inhibitors, which elicit their protective effects independent of blood pressure and glucose-lowering actions, have been paid great attention.[5] They prevent decomposition of glucagon-like peptides 1 and 2 (GLP-1 and -2), which mimic glucose-dependent insulinotropic peptide (GIP) and its role in increasing insulin production by pancreatic β-cells results in lower blood glucose levels.[6]

Sitagliptin, a DPP-4 inhibitor, is involved in T2DM management via controlling glucose supply or enhancing incretin activity, which plays a role in treatment of DN. Besides, it has the ability to protect β-cells from damage and hence prevents the reduction in insulin secretion.[7] Other mechanisms of sitagliptin as the DPP-4 inhibitor involve GLP-1-dependent and GLP-1-independent pathways associated with preservation of mesangial cells and keeping of sodium,

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Table 1. Clinical Characteristics and Biochemical Analyses of All Studied Groups

		Control group	P group	SL group	SM group
Age (years)		47.50 ± 8.90	47.90 ± 7.20	49.10 ± 7.20	51.20 ± 6.80
Sex	Male	15 (75%)	14 (70%)	14 (70%)	17 (85%)
	Female	5 (25%)	6 (30%)	6 (30%)	3 (15%)
BMI (kg/m^2)		27.20 ± 4.30	26.80 ± 1.70	27.30 ± 1.80	27.60 ± 1.80
Duration of DM (years)		—	9.30 ± 2.90	9.60 ± 3.50	9.50 ± 4.10
Systolic BP (mmHg)		123.60 ± 9.20	125.0 0 ± 9.00	122.20 ± 5.80	122.60 ± 8.50
Diastolic BP (mmHg)		78.00 ± 4.40	80.10 ± 5.40	78.70 ± 3.60	79.80 ± 5.10
HbA_{1C} (%)		4.09 ± 0.49	7.74 ± 0.67[a]	7.35 ± 0.49[a]	7.19 ± 0.57[a,b]
Glucose (mg/dL)		102.10 ± 10.90	181.30 ± 23.40[a]	180.90 ± 20.20[a]	175.60 ± 16.70[a,b]
Creatinine (mg/dL)		1.10 ± 0.13	3.20 ± 0.47[a]	1.90 ± 0.41[a,b]	1.10 ± 0.14[b,c]
Creatinine clearance (mL/min)		91.30 ± 11.20	72.50 ± 5.80[a]	80.50 ± 8.00[a,b]	92.80 ± 7.80[b,c]
Urea (mg/dL)		23.10 ± 6.90	79.40 ± 21.40[a]	53.50 ± 10.80[a,b]	23.90 ± 8.10[b,c]
Sodium (mmol/L)		143.10 ± 5.00	123.40 ± 9.40[a]	139.20 ± 5.80[b]	142.40 ± 4.10[b]
Potassium (mmol/L)		4.08 ± 0.49	3.39 ± 0.59[a]	3.74 ± 0.32[b]	3.98 ± 0.27[b]

P group, pioglitazone-treated T2DM patients; SL group, sitagliptin-treated T2DM patients for less than one year; SM group, sitagliptin-treated T2DM patients for more than one year; HbA_{1C}, glycated hemoglobin. Results are presented as mean ± SD (n = 20 for each group). [a]Significant difference from the control group at P-value <0.05. [b]Significant difference from the pioglitazone group at P-value <0.05. [c]Significant difference from the sitagliptin more than one year group at P-value <0.05.

acid−base, and fluid homeostasis, which decrease blood pressure.[8] The renoprotective aptitude of sitagliptin also includes some mechanisms reported such as lowering albuminuria[9] and preventing the deterioration of the glomerular filtration rate (GFR).[10] Moreover, sitagliptin could decrease diabetic renal complications such as acute kidney injury (AKI), inflammation, abnormality in the glyoxalase system, and elevated renal function by reducing inflammation and oxidative stress due to its antiapoptotic and antiproliferative properties.[11] Low-dose sitagliptin was proposed to prevent inflammatory response in diabetic rat kidney[12] through prevention of increment in expression of proinflammatory cytokines IL-1β and tumor necrosis factor-α (TNF-α) mRNA in diabetic rat kidney.[13]

Glitazone is another antidiabetic class from which pioglitazone is the currently available member for clinical use. It exerts its clinical effect via activation of peroxisome-proliferator-activator-receptor-γ (PPARγ). It prevented the progress of diabetic kidney disease in animal models[14] and could be used in patients with CKD but with caution due to side effects such as water retention.[15]

There are contradictory results about the harmful or beneficial effects of antidiabetic medications on kidney function, especially with a long therapeutic duration. In our study, we compared two antidiabetic drugs belonging to different classes to assess the superiority regarding the renoprotective effect in T2DM patients.

AKI is a complex and heterogeneous process that leads to high morbidity and mortality between critical cases. Thus, earlier diagnosis of patients with diabetic renal disease may be very important to prevent further renal injury. Several biomarkers have been correlated with kidney function involving vanin-1, neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury molecule-1 (KIM-1), which are used as early markers of tubular injury instead of traditional markers, which lack sensitivity in fast measurement of kidney damage.[16] The level of vanin-1 increased in diabetic rats with DN.[17]

NGAL is a protein that results from tubular damage caused by DM where stimulated tubular cells trigger an increased NGAL level.[18] NGAL may be used as an early diagnostic marker of tubular injury, particularly in patients with normal blood creatinine levels.[19] In patients with DN, KIM-1 and NGAL biomarkers were interrelated with the degree of proteinuria.[20]

Another biomarker used in kidney disease is cystatin-C, which functions as an early marker for GFR and as an inflammatory marker as it suppresses cysteine proteases and can be utilized as an earlier biomarker in the disclosure of DN better than serum creatinine[21] because of its high specificity and sensitivity.

Methylglyoxal (MG) and glyoxalase-1 (Glo-1) as glyoxalase system markers are elevated in diabetic patients and have been involved in the etiology of kidney complications. Regarding MG, it is a glycating agent that reacts with protein residues resulting in the formation of a number of products involving advanced glycation end products, which play important roles in some pathologies, like inflammation, diabetes, and Alzheimer's disease.[22] Glo-1 is a highly active enzyme that is responsible for regulating the kidney sensitivity to hyperglycemic-induced renal pathology.[23] Moreover, it was reported that MG and Glo-1 can be used as early markers of the glyoxalase system that were reported to be elevated in DN.[24]

Inflammatory cytokines, mainly interlukin-18 (IL-18), are involved in the development and progression of diabetic kidney disease. Generally, macrophages generate IL-18, which is part of the inflammatory process. Tubular epithelial cells are the primary source of kidney IL-18.[25] A study by Navarro-González et al.[26] indicated that the levels of IL-18 in the diabetic group were higher than in the normal group, which had a significant impact on the development of DN. Also, IL-18 has been proposed as a biomarker for kidney injury by Yong et al.[27]

Another tubular injury marker reported in many studies is long noncoding myocardial infarction associated transcript (lncMIAT) belonging to long noncoding RNAs (lncRNAs) that consisted of up to 200 nucleotides; they originate from the transcription of genome, and their biological effects in DN pathogenesis were established well.[28] Generally, the role of lncRNAs in many human pathogenesis was proved.[29] They

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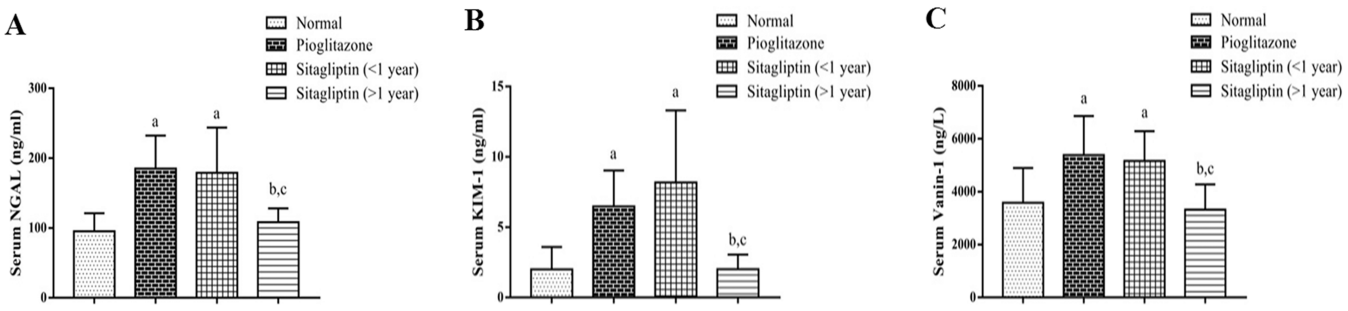

Figure 1. Serum levels of NGAL (A), KIM-1 (B), and vanin-1 (C) in control, pioglitazone-treated T2DM patients, sitagliptin-treated T2DM patients for less than one year, and sitagliptin-treated T2DM patients for more than one year groups. NGAL, neutrophil gelatinase-associated lipocalin; KIM-1, kidney injury molecule-1. Data are presented as mean ± SD (n = 20). Statistical significance was considered acceptable at P-value <0.05. [a]Significant difference from the control group, [b]significant difference from the pioglitazone group, [c]significant difference from the sitagliptin more than one year group.

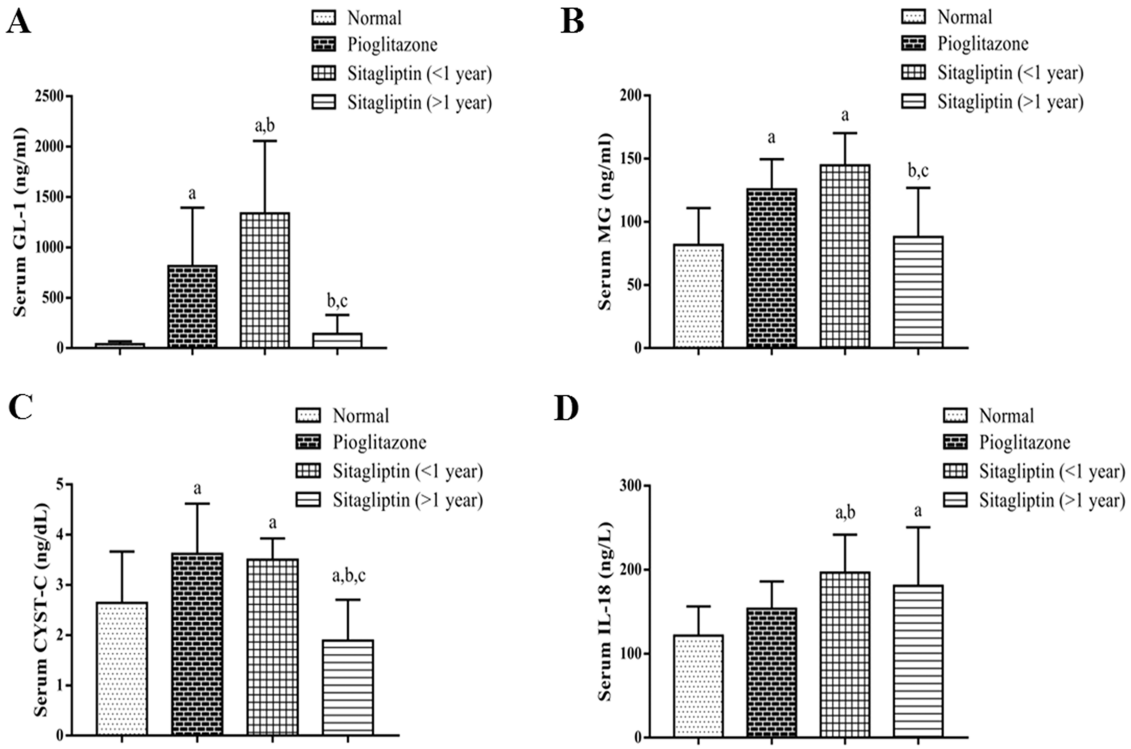

Figure 2. Serum levels of Glo-1 (A), MG (B), CYST-C (C), and IL-18 (D) in control, pioglitazone-treated T2DM patients, sitagliptin-treated T2DM patients for less than one year, and sitagliptin-treated T2DM patients for more than one year groups. Glo-1, glyoxalase-1; MG, methylglyoxal; CYST-C, cystatin-C. Data are presented as mean ± SD (n = 20). Statistical significance was considered accepted at P-value <0.05. [a]Significant difference from the control group, [b]significant difference from the pioglitazone group, [c]significant difference from the sitagliptin more than one year group.

play a role as essential biomarkers in the diagnosis, treatment, and progression of DN through regulating inflammatory response, oxidative stress, and immune response via altering their expression level in renal tissues, blood, and urine.[30] LncRNAs are involved in renal fibrosis, and the lncRNA-based treatment will be of great importance in treatment of DN in the future.[31]

LncMIAT is a highly preserved lncRNA.[32] It was reported that lncMIAT is involved in many diseases like microvascular dysfunction, myocardial infarction, and cancers[33] besides its involvement in DM, where it is the main regulator in DN.[34] Moreover, lncMIAT is involved in diabetic complication associated with the injury of renal tubules by management of the Nrf2 gene expression.[35] Downregulation of the lncMIAT expression level results in the reduction of both cells' proliferation and the secretion of proteins responsible for cell fibrosis.[36]

From the above considerations, we hypothesized that sitagliptin may have superior protective effects on the kidneys of T2DM patients through several mechanisms including improvement of renal tubular injury, preventing decline of GFR, modifying the glyoxalase system, and anti-inflammatory effect, besides regulating glycemic control. To our knowledge, this is the first article to compare the renoprotective effects of short-term and long-term therapy with sitagliptin versus pioglitazone in T2DM patients.

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2. RESULTS

The clinical characteristics and biochemical analyses of all subjects included in this study are summarized in Table 1. As depicted in Table 1, there was no significant difference between the studied groups as compared to each other or to the healthy control group in age, sex, BMI, duration of diabetes, and diastolic and systolic blood pressures.

2.1. Effect of Hypoglycemic Therapies on Glycemic Control. The current results showed a significant increase in the HbA_{1C} levels in P, SL, and SM groups as compared to the control group. For serum glucose, the results showed a significant increase in P, SL, and SM groups as compared to the control group. Additionally, treatment with sitagliptin for more than one year (SM group) led to significant decreases in both glucose and HbA_{1C} levels compared to those seen in the pioglitazone-treated group, which highlights the superiority of sitagliptin in monitoring the glycemic state; Table 1.

2.2. Effect of Hypoglycemic Therapies on Regulating Kidney Function and the Electrolyte System. Serum creatinine significantly increased in P and SL groups compared to either the normal control or SM groups. Interestingly, creatinine levels in the SM group were comparable to those of the normal control. Similarly, serum urea significantly increased in P and SL groups as compared to either normal control or SM groups. This indicates that treatment with sitagliptin for more than one year successfully suppressed the deterioration of kidney function compared to pioglitazone treatment, Table 1.

Regarding sodium and potassium levels, they were within the normal range in all diabetic groups and the control group despite a significant decrease in the P group as compared to both sitagliptin-treated groups and the control. This indicates the role of sitagliptin in the improvement of kidney function; Table 1.

2.3. Effect of Antidiabetic Therapies on Tubular Injury Markers (NGAL, KIM-1, and Vanin-1). For serum NGAL, KIM-1, and vanin-1 levels, our results showed significant increases in P and SL groups as compared to those of SM and the control groups. Interestingly, the levels of these markers in the SM group were comparable to those of the control group. This confirms the beneficial impact of long-term treatment with sitagliptin on renal tubular injury markers; Figure 1.

2.4. Effect of Antidiabetic Therapies on Enhancing the Glyoxalase System. As shown in Figure 2, serum MG and Glo-1 levels were significantly increased in P and SL groups compared with both SM and the control groups. The increments in Glo-1 levels were 21-fold and 35-fold in P and SL groups, respectively, compared to the control group, while the increase reached only 3-fold in the SM group when compared to the control group. On the other hand, the increases in MG levels were 1.5-fold and 1.8-fold in P and SL groups, respectively, compared to the control group, while the MG level in the SM group was close to that of the control group. These results highlight the significant role of long-term treatment with sitagliptin in amelioration of the glyoxalase system.

2.5. Effect of Antidiabetic Therapies on Glomerular Filtration Marker (Cystatin-C and Creatinine Clearance). For serum cystatin-C, the present results showed a significant increase in P and SL groups as compared to the control and SM groups (Figure 2). On the other hand, creatinine clearance was significantly decreased in P and SL groups as compared to the control and SM groups, while its level was restored to the normal level in the SM group. This demonstrates the impact of long-term treatment with sitagliptin on keeping intact renal glomerular filtration; Table 1.

2.6. Effect of Antidiabetic Therapies on the Inflammatory Marker (IL-18). For serum IL-18, our results showed unexpected significant increases in SL and SM groups as compared to P and the control group, while the level in the P group was analogous to that in the control group; Figure 2.

2.7. Effect of Antidiabetic Therapies on LncMIAT in T2DM. As illustrated in Figure 3, LncMIAT showed a significant increase in the P group as compared to the SL, SM, and the control groups. The LncMIAT level dramatically increased to 6.8-fold in the P group and declined to a 3-fold increase in the SL group compared to the control group, while it returned to a nearly normal level in the SM group. This demonstrates the renoprotective effect of long-term treatment with sitagliptin through downregulation of LncMIAT.

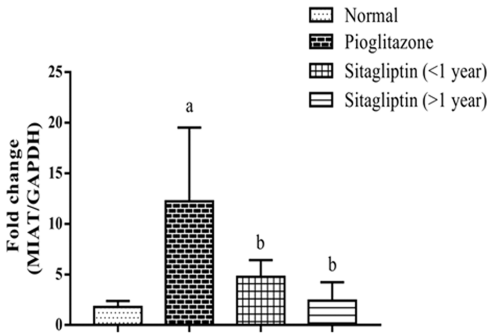

Figure 3. Expression level of long noncoding myocardial infarction associated transcript (LncMIAT) in the control, pioglitazone-treated T2DM patients, sitagliptin-treated T2DM patients for less than one year, and sitagliptin-treated T2DM patients for more than one year groups. Data were presented as mean ± SD (n = 20). Statistical significance was considered accepted at P-value <0.05. [a]Significant difference from the control group, [b]significant difference from the pioglitazone group.

2.8. Correlation between Different Variables. Univariate analysis revealed that the lncMIAT level was positively correlated with cystatin-C (r = 0.51, P = 0.001) and vanin-1 (r = 0.48, P = 0.001) in patients with T2DM. Additionally, there was a significant inverse correlation between lncMIAT and creatinine clearance in patients with T2DM (r = −0.50, P = 0.001).

Moreover, there was a significant inverse correlation between creatinine clearance and KIM-1 in patients with T2DM (r = −0.41, P = 0.001).

There was a significant positive correlation between vanin-1 and KIM-1 in patients with T2DM (r = 0.37, P = 0.004).

A multivariate linear regression analysis was used to evaluate the relationship between lncMIAT and clinical and biochemical parameters, in which lncMIAT was involved as the dependent variable. All variables graphed in Figures 1 and 2are used as independent variables. The significant predictors of lncMIAT were vanin-1 (β = 0.30, P = 0.023) and cystatin-C (β = 0.25, P = 0.04).

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3. DISCUSSION

In DM, the high glucose level is responsible for the progression of long-term diabetic complications.[37] In T2DM, an incretin defect resulting from rapid inactivation by DPP-4 appears through the reduction in incretin bioavailability. Hyperglycemia and hyperlipidemia are associated with oxidative stress, which is the main mediator of disturbance in the β-cell function and is involved in exacerbation of the diabetic case.[38]

The current work revealed that chronic T2DM patients suffered from diabetic kidney disease (DKD) that resulted from diabetic-induced renal tubular epithelial injury, decline in glomerular filtration rate, glyoxalase system disturbance, and inflammation. These findings are in harmony with those reported by Nielsen et al.,[39] who disclosed that diabetic patients are exposed to two types of stress: metabolic and hemodynamic. Metabolic stress is accompanied by hyperlipidemia and hyperglycemia, while hemodynamic stress includes hypertension. These two types of stress cause inflammation and atherosclerosis, which lead to endothelial dysfunction and tubular damage, that cause an increase in tubular biomarker levels.

In the present study, our results are partially consistent with those of a previous study regarding the renoprotective effect of sitagliptin in T2DM when compared with pioglitazone treatment. Generally, sitagliptin protects the kidney through different mechanisms, including anti-inflammatory and antioxidant effects. Sitagliptin has two actions: direct action through DPP-4 inhibition and indirect action through improving insulin secretion.[7] Actually, the DPP-4 level increases in the kidneys of diabetic mammals as narrated by Hasan and Hocher.[5] Hence, inhibition of DPP-4 by sitagliptin is required for the management of DN. Also, we consider the findings of Ren et al.,[12] who found that in the diabetic rat kidney, the inflammatory profile and the proapoptotic state were prevented by low-dose sitagliptin, which resulted in improvement of renal function and tissue lesions. Our results are in line with those of Ye et al.,[40] who showed that sitagliptin treatment played a role in ameliorating fasting glucose, HbA_{1c}, systolic and diastolic blood pressures, or kidney function in T2DM patients suffering from increased blood pressure during a two-year study period, and with those of Yang et al.,[41] who established that in T2DM, a cytokine inflammatory profile acts by enhancing the interaction between GLP-1 with its receptor, which is responsible for the progression of glomerular sclerosis and interstitial fibrosis, and sitagliptin delayed this progression.

In the current investigation, kidney function assessed by creatinine and urea levels was significantly increased in the P group and improved in sitagliptin-treated groups, and the improvement was obvious with long-term sitagliptin treatment, which elicits the role of long-term sitagliptin treatment in controlling kidney function. This is in agreement with the results of Esaki et al.,[42] who claimed that the renoprotective impact of sitagliptin took place via suppressing the decline in GFR in T2DM patients. Moreover, sitagliptin treatment improved the glycemic level, which was better in the SM group than in the P and SL groups, which was illustrated by Engel et al.,[43] who found that sitagliptin regulates hyperglycemia in both cases of postprandial or fasting blood glucose by various mechanisms superior to other antidiabetic therapies.

Additionally, in the present study, it was demonstrated that tubular injury markers NGAL, KIM-1, and vanin-1 significantly increased in the P group and SL group as compared with both SM and control groups. These biomarkers are considered detectors of DKD in T2DM patients. It also reveals the role of sitagliptin treatment in improving renal tubular injury, especially in patients treated for more than one year.

For NGAL, results of the present study are in agreement with those of Siddiqi et al.,[44] who investigated the use of NGAL and cystatin-C as biomarkers in the early stage of DN in T2DM patients and disclosed that urinary biomarkers were significantly increased in normo-albuminuric T2DM patients in comparison with the control group and could be utilized as early markers of DN even before the progression of microalbuminuria. Moreover, Abbasi et al.[45] found increased excretion of NGAL ensued from DN followed by tubular damage; hence, it can be utilized as an early diagnosis marker of DN, especially with a normal serum creatinine level.

Regarding vanin-1, the findings of the present study are in line with those reported by Fugmann et al.,[17] who showed that rats with DN induced by streptozotocin had increased levels of renal vanin-1 besides increased urinary vanin-1 level in diabetic patients, so it could be validated as a marker of early detection of impaired kidney function noticed in DM patients.

With respect to KIM-1, the current result is in accordance with that of Gohda et al.,[46] who discussed the highest sensitivity of the ratio between urinary KIM-1 and creatinine as a noninvasive diagnostic marker for renal function in T2DM patients that can detect renal tubular injury before the occurrence of albuminuria. Also, Quang et al.[47] recognized that tubular injury markers KIM-1 and NGAL correlated with the degree of proteinuria in patients with DN.

Additionally, for glyoxalase system markers, MG and Glo-1 were significantly increased in the P group and SL group as compared with the SM and the control groups, which manifests the role of sitagliptin treatment in modifying the glyoxalase system in the SM group. These results in the present study are in agreement with those reported by Rabbani and Thornalley,[24] who found that experimental DN was accompanied by elevated levels of MG glycation and was also accompanied by an elevated renal expression of Glo-1, which can inhibit MG glycation. Also, these results are in agreement with those of Pácal et al.,[48] who found that MG production increased in DM. Glo-1 is responsible for detoxification of MG, where its activity significantly increased in diabetics with chronic renal disease compared with healthy control subjects. Thus, both DM and chronic renal disease influence the glyoxalase system. Also, Rabbani and Thornalley[24] found that MG is responsible for the progression of chronic renal disease. Cellular proteolysis of MG-modified proteins provides MG free adducts and glycated amino acids, which are excreted in the urine after washing in the kidney. MG free adducts are collected markedly in plasma and cause a decrease in GFR.

For glomerular filtration markers, cystatin-C and creatinine clearance, the current results found that cystatin-C significantly increased in the P group and SL group as compared with both SM and control groups, which illustrates the role of long-term sitagliptin treatment in preventing GFR decline in the SM group. The current results are in accordance with those of Al-Saedy et al.,[49] who showed the reverse relationship between cystatin-C and GFR and recommended the use of cystatin-C as an accurate marker of high sensitivity to detect changes in GFR. Moreover, serum cystatin-C level can be utilized as a predictable marker for kidney damage in T2DM with microalbuminuria. On the other hand, creatinine clearance significantly decreased in P and SL groups as compared with

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SM and control groups (Table 1). This is in line with the result of Kawasaki et al.,[10] who showed that sitagliptin slowed down the deterioration of GFR in DN.

Furthermore, the current work established that serum IL-18 levels were elevated in all diabetic patient groups as compared with the control group. These findings are in harmony with those of Al-Rubeaan et al.,[50] who estimated the predictive ability of this marker for the early diagnosis of DN before kidney injury occurs. The predictive ability of IL-18 and cystatin-C exceeds that of resistin, NGAL, TNF-α, and IL-6, especially for cases with macroalbuminuria. Also, as reported in many studies, our study showed a significant increase in IL-18 levels in both sitagliptin-treated groups as compared to pioglitazone and the control groups, which indicates the better anti-inflammatory effect of pioglitazone than sitagliptin. Our result is partially consistent with the previous studies regarding the impact of pioglitazone-induced reduction in leucocyte count and IL-6 level, which was not considered to be a result of glycemic control alone.[51] In contrast to our results, treatment with sitagliptin decreased plasma IL-6, IL-18, TNF-α, and nitro-tyrosine levels in T2DM compared with the control group.[52] The failure of sitagliptin to lower the IL-18 level better than pioglitazone in the current study may be explained by the finding of Tremblay et al.,[53] who claimed that treatment with sitagliptin was more effective in diabetic patients with raised levels of inflammatory markers than in those with decreased levels. In their study, the level of IL-18 in diabetic patients was about 2-fold that in the present work, where they recorded a 7.3% decrease in the parameter level.

Finally, in the present study, it was found that LncMIAT was significantly elevated in the P group compared with both the control and sitagliptin-treated groups, which highlights the role of sitagliptin treatment in improving renal tubular epithelial injury especially in the SM group. In parallel, this result has been reported by Sani et al.[54] who stated that lncRNAs have a main role in management of kidney pathogenesis and are involved in kidney pathophysiological processes, and hence, they may be of great importance in the future as a therapeutic tool. Also, this result is in line with that of Tang et al.[30] who found that the disturbance between lncRNA expression levels is not the only effector in the progression of DN but it also has an important role in the management of DN, which makes it of therapeutic value in DN. Additionally, Leti and DiStefano[55] demonstrated the role of lncRNAs within the context of diabetic kidney disease and provided an accurate idea about the complex pathogenesis of T2DM and its related complications, resulting in the detection of therapeutic targets, and provide a new advanced method for the detection and diagnosis of T2DM. Furthermore, our finding is in accordance with that of Ji et al.[36] who found that the lncMIAT expression levelsignificantly increased in DN with an increased glucose level in mesangial cells. The mechanism of action of lncMIAT in the progression of DN depends on the induction of cell proliferation and extracellular matrix aggregation. This significance in the lncMIAT level provides a good tool for treatment of DN in the future. Although the number of patients in each group is relatively small, the effect size was 0.3, the α error probability was 0.05, and the actual power of sample size was 0.95, which indicates adequacy of sample sizes.

4. CONCLUSIONS

In summary, the present study found that chronic T2DM patients suffered from renal tubular epithelial injury, decrease in GFR, inflammation, abnormality in the glyoxalase system, elevated renal function, and changed glycemic control. Also, it was found that sitagliptin treatment ameliorated these deviations in T2DM as compared with pioglitazone treatment. Sitagliptin protected the kidney by diverse mechanisms including improvement of renal tubular injury, inhibiting GFR decline, modifying the glyoxalase system, anti-inflammatory effect, controlling kidney function, and regulating glycemic control. Moreover, serum NGAL, vanin-1, KIM-1, Glo-1, MG, and cystatin-C can be used as an accurate indicator in the early stage of DKD in patients with T2DM. Furthermore, lncMIAT was significantly higher in the pioglitazone group as compared to sitagliptin-treated groups and the healthy control group. Thus, it was suggested that lncMIAT served as an important signaling pathway for high-glucose-induced renal tubular epithelial injury. Overall, the renoprotective effect of sitagliptin was more pronounced in long-term sitagliptin therapy than in pioglitazone.

5. MATERIALS AND METHODS

The present study was performed on 80 adult subjects who were classified into four main groups: **Group 1** (healthy control group): this group contains 20 normal healthy adults with matched age and body mass index (BMI); **Group 2** (P group): this group contains 20 T2DM patients treated with pioglitazone (30 mg once daily) for at least five years; **Group 3** (SL group): this group contains 20 T2DM patients treated with sitagliptin (100 mg once daily) for less than one year; and **Group 4** (SM group): this group contains 20 T2DM patients treated with sitagliptin (100 mg once daily) for more than one year. Patients were selected from the Out-patient Clinic of El-Minia University Hospital. Diabetic patients were diagnosed according to blood HbA_{1C} and fasting serum glucose levels.[56] A complete medical history encompassing age, BMI, and duration of diabetes disease was executed for all patients enrolled in the study. Additionally, the measurement of blood pressure and screening for diabetic complications were performed. All diabetic patients had diabetic duration for more than five years and received antidiabetic therapy either pioglitazone or sitagliptin. The four groups are within the age range of 35−60 years.

This study was approved by the local ethical committee of Beni-Suef University (REC-H-PhBSU-21025). All patients in the study provided their informed consent. No subject included in the study suffered from respiratory disease, gastrointestinal infection disease, or malignancy to exclude any inflammation. Exclusion criteria also included treatment with antineoplastic agents, psychoactive agents, statins, antihypertensive, glucocorticoids, or vitamin supplements.

5.1. Sampling. About 6 mL of venous blood samples was withdrawn after overnight fast from each subject after 10 min rest in the sitting position and divided into two tubes. The first tube was allowed to clot for 15 min and then centrifuged at 4000 rpm for 10 min. The yielded serum was divided into two aliquots: the first aliquot was used for estimation of creatinine, urea, fasting blood glucose, sodium, and potassium levels, and the second aliquot was divided into seven small aliquots, stored at −20 °C until analysis of NGAL, vanin-1, KIM-1, Glo-1, MG, IL-18, and cystatin-C levels. The second tube contained EDTA in which blood was mixed gently and divided into two aliquots; the first aliquot was utilized for assessment of HbA_{1C}, while the second aliquot was kept at −80 °C for quantitative real-time polymerase chain reaction (qRT-PCR) estimation of

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lncMIAT. Additionally, urine samples were collected for 24 h and were used to measure creatinine in urine (diluted 1:50) with physiological saline. The volume of each 24 h urine sample was measured to calculate creatinine clearance.

5.1.1. Measurement of Fasting Blood Glucose and HbA_{1C}. Serum glucose was measured with a premade available kit obtained from Spinreact Co. and measured using an evo 1000 spectrophotometer (Thermo-scientific, England) at wavelength 546 nm.

HbA_{1C} was measured in EDTA blood samples and following the manufacturing protocol of the premade kit (Agappe Diagnostic, Switzerland) using an automated Mispa-*i2* analyzer (Agappe Diagnostics, Switzerland).

5.1.2. Measurement of Kidney Function and Creatinine Clearance (Serum and Urine Creatinine, Urea, Sodium, and Potassium Levels). Serum and urine creatinine and urea levels were measured with premade available kits obtained from BioMed Co, Germany. All samples were measured using an evo 1000 spectrophotometer (Thermo-scientific, England) at wavelength 492 nm for creatinine and 578 nm for urea.

Serum sodium and potassium levels were measured using an automated Sensa Core electrolyte analyzer (Telangana, India).

Urine samples were collected during 24 h in clean containers, and the volumes were measured for each subject. Creatinine clearance was determined using serum and urine creatinine levels[57] applying the following equation

$$\text{creatinine clearance (mL/min)} = \frac{\text{urine creatinine (mg/dL)} \times \text{urine volume (mL) in 24 h}}{\text{serum creatinine (mg/dL)} \times 24\ \text{h} \times 60\ \text{min}}$$

5.1.3. Measurement of Tubular Injury Markers (Serum NGAL, KIM-1, and Vanin-1 Levels). Serum levels of NGAL (CAT. No E1719Hu), KIM-1 (CAT. No E1099Hu), and vanin-1 (CAT. No E5832Hu) were assayed using ELISA kits (Shanghai Korian Biotech Co, China) according to the manufacturer's instructions using a Statfax 2100 ELISA reader (Warness technology, USA).

5.1.4. Measurement of Glyoxalase System Markers (Serum Glo-1 and MG Levels). Serum levels of Glo-1 (CAT. No. E4016Hu) and MG (CAT. No. E4106Hu) were assayed using ELISA kits (Shanghai Korian Biotech Co, China) according to the manufacturer's instructions utilizing a Statfax 2100 ELISA reader (Warness technology, USA).

5.1.5. Measurement of the Glomerular Filtration Marker (Serum Cystatin-C). Serum levels of cystatin-C (CAT. No E1104Hu) were assayed using ELISA kits (Shanghai Korian Biotech Co, China) according to the manufacturer's instructions using a Statfax 2100 ELISA reader (Warness technology, USA).

5.1.6. Measurement of the Tubular Inflammatory Marker (Serum IL-18). Serum levels of IL-18 (CAT. No E0147Hu) were assayed using ELISA kits (Shanghai Korian Biotech Co, China) according to the manufacturer's instructions utilizing a Statfax 2100 ELISA reader (Warness technology, USA).

5.1.7. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). In the aseptic condition, quantitative determination of the mRNA lncMIAT expression was conducted according to the previously published method.[58] In brief, purified RNA was prepared from whole blood samples using a total RNA extraction kit (GF-1 Total RNA extraction kit, Vivantis, Malaysia) according to the manufacturer's instructions. The purity and quantity of extracted RNA were tested (SPECTROstar^{Nano} spectrometer, BMG Labtech, France). The produced RNA from each sample (1 μg) was further used for cDNA synthesis (two-step RT-PCR kit, Vivantis, Malaysia) according to the manufacturer's instructions. Then, the produced cDNA (1 μL) was used for qRT-PCR with the SYBR green master mix (Thermo Fisher, USA). Step one plus a Real-Time PCR system (Applied Biosystem, USA) was used. Primer sequences (Vivantis, Malaysia) are listed in Table 2. The relative expression of the lncMIAT gene was quantitatively calculated in relation to GAPDH following a previously described method.[59]

Table 2. Quantitative RT-PCR Primer Sequences

Target gene	Nucleotide sequence (5′−3′)
LncMIAT	F: AAGCAGGAAGCTCACACCTC
	R: CCACAGACCCCTGACCAATC
GAPDH	F: GCCACTAGGCGCTCACTGTT
	R: TCTAGACGGCAGGTCAGGTC

5.2. Statistical Analysis. Data of each patient was collected in a special file, and then, it was coded and fed to a computer on Windows7 worksheet version 5. Statistical analysis was performed by the SPSS program (version 16. California, USA). Categorical data was presented in the form of number (%), while continuous data was established as mean ± standard deviation (SD). The Kolmogorov−Smirnov test was used for testing the normality of data. Chi-square or Fischer's exact tests were used for categorical data, and the Mann−Whitney *U* test was used for continuous data in the comparison between groups. Analyses were done for parametric quantitative data among groups by a one-way ANOVA test followed by Tukey's test. $P < 0.05$ was considered statistically significant. The degree of relationship between the variables was calculated using the Pearson correlation analysis. Power calculations among the four groups were accomplished using PS Power and Sample Size Calculations Software, version 3.0.43 for MS Windows (William D. Dupont and Walton D., Vanderbilt).

AUTHOR INFORMATION

Corresponding Author

Mohamed O. Mahmoud − *Biochemistry Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt;* orcid.org/0000-0002-6737-8142;
Email: mohamed.omar@pharm.bsu.edu.eg

Authors

Mohamed A. Kandeil − *Biochemistry Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt*

Mohamed A. Shaarawy − *Internal Medicine and Nephrology Department, Faculty of Medicine, El-Minia University, El-Minia 61519, Egypt*

Hamdy A. Mourad − *Biochemistry Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c07008

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by [H.A.M.], [M.O.M.], and [M.A.S.]. The first

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draft of the manuscript was written by [H.M.A.], and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Notes

The authors declare no competing financial interest.

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