DISCUSSION

The incidence and prevalence of type 2 diabetes are increasing; [91] and it is considered as one of the most serious health problems in the world. [92] It is projected that the total number of people with diabetes will rise from 171 million in 2000 to 439 million by 2030. A major complication of diabetes is angiopathy, which is characterized by abnormal angiogenesis in many organs causing diabetic nephropathy, retinopathy and neuropathy. [92] Moreover, diabetes increases the risk of cardiovascular disease which is considered as a primary cause of death. [91]

In the present study, significant elevation in blood glucose levels, glycated hemoglobin and lipid abnormalities characterized by elevation in TC, LDL-C, VLDL, TG and apo-E as well as significant reduction in HDL-C and apo-A1 were observed in both diabetic patients' groups who not receiving statin therapy as compared to controls.

Previously it has been reported that T2DM consists of an array of dysfunctions characterized by hyperglycemia and resulting from the combination of resistance to insulin action, inadequate insulin secretion and excessive or inappropriate glucagon secretion. [19]

Donath et al., **[29]** demonstrated that insulin resistance persists the entire period of T2DM from early stage of pre-diabetes to later stage of overt T2DM. In order to compensate insulin resistance, pancreatic islets increase their cell mass and insulin secretion but when these pancreatic islets become unable to compensate insulin resistance, insulin deficiency occurs in peripheral tissues, which may lead to the development of T2DM. Once T2DM occurs, it imparts long-term consequences which may include the development of microvascular and macrovascular complications.

In terms of pathogenesis, glucolipotoxicity can be stated as one of the essential determinants of T2DM. [93] Glucotoxicity has damaging effect on normal function of β-cells and finally decreases insulin secretion. Similarly, lipotoxicity also lead to β-cell dysfunction. This glucolipotoxicity in turn may cause endoplasmic reticulum (ER) stress within pancreatic islets ending up with cell apoptosis. [94]

Moreover, hyperglycemia may also induce oxidative stress in β-cells resulting in excessive production of pro-inflammatory mediators (cytokines and chemokines), which have been known for their involvement in causing β-cell dysfunction leading to insulin resistance and inflammation attributing to T2DM. [95]

The present study showed significant reduction in fasting and post prandial blood glucose and glycated hemoglobin levels in statin-treated type 2 diabetic patients compared to corresponding values of those treated with conventional treatment of diabetes alone.

Previously it has been reported that statin therapy reduce the risk of developing diabetes by as much as 30%, and it confer improved sensitivity to insulin as measured by an increase in the insulin sensitivity index. [96] The underlying molecular mechanisms for the increased sensitivity to insulin in diabetic patients treated with statins are largely unknown. The first step in insulin-stimulated glucose signaling is the transport of glucose into the cells through insulin-sensitive facilitative glucose transporters (Glut-4). Glut-4 is stored in intracellular vesicles. Insulin by binding to its receptors in the plasma membrane

results in phosphorylation of the receptor with the subsequent activation of PI3/AKT pathway, which in turn mediates the translocation of insulin-responsive Glut-4 containing vesicles to the membrane. Statins have been found to activate Akt and PI3-kinase, thereby stimulating the expression of Glut-4 and providing a mechanism by which cellular glucose uptake may be enhanced by this unique class of drugs. [97, 98]

Increased VLDL production is a major feature of diabetic dyslipidemia with consequences on the metabolism of other lipoproteins such as LDL-C and HDL-C. More precisely, an increased production of VLDL particles that is potentially detrimental by generating atherogenic remnants, small dense LDL particles and triglyceride-rich HDL particles has been previously observed in type 2 diabetes. Several pathophysiological factors are responsible for increased VLDL production, in type 2 diabetes. Among those, insulin resistance plays an important role. Indeed, defective activation of PI3-kinase, secondary to insulin resistance, is associated with a reduction of apo-B degradation in the hepatocytes, a rise in microsomal TG transfer protein (MTP) expression and an increased activity of phospholipase D1 and ADP-ribosylation factor-1 (ARF-1), which are involved in VLDL formation. [99]

Moreover, peripheral insulin resistance is responsible for increased lipolysis of adipose tissue leading to augmented portal flux of free fatty acids (FFA) to the liver and, as a consequence, activation of VLDL production. In addition, increased de novo lipogenesis has been observed in type 2 diabetes. This is secondary to increased activation of sterol regulatory element-binding protein-1c (SREBP-1c), mainly by endoplasmic reticulum stress, and of carbohydrate responsive element-binding protein (ChREBP), mainly by hyperglycemia. Furthermore, decreased plasma adiponectin observed previously in type 2 diabetes, may also play a role in increased VLDL production by decreasing liver 5' adenosine monophosphate kinase (AMP-kinase) activation and by increasing plasma FFA levels as a consequence of reduced muscle FFA oxidation. [100]

In the present study, after simvastatin therapy serum levels of TC, LDL-C, VLDL, TG and apo-E were significantly decreased, while, those of HDL-C and apo-A1 were significantly increased. These results are in agreement with previous studies concerning the effect of statin in management of diabetes mellitus. [101-103]

Several large clinical trials have provided ample evidence supporting the use of statins in dyslipidemia for primary and secondary prevention of cardiovascular disease in type 2 diabetes. Nonetheless, there is a widely adopted view that statins exert pleiotropic effects independent of lowering cholesterol, and a significant proportion of these effects may be considered to be anti-inflammatory as well as immunomodulatory properties.[104,105]

Statins inhibit hydroxy methylglutaryl coenzyme-A (HMG-CoA) reductase enzyme resulting in decreased hepatic cholesterol synthesis and lower LDL-C level through upregulation of LDL receptors on hepatocytes in diabetic patients. [106,107] Van Tits et al.,[108] confirmed that simvastatin has been shown to decrease all LDL subfractions in diabetic patients. The finding of the present study concerning the reduction of LDL-C in simvastatin-treated patients is in agreement with this observation as shown in **(table 17)**.

The significant increase in HDL-C in diabetic patients could be attributed to the upregulatory effect of statin on hepatic ATP-binding cassette transporter-A1 (ABC-A1) gene expression, [109] reduction of hepatic lipase activity which in turn inhibits the

triglyceridation of HDL-C [110] and finally, inhibition of cholesteryl ester transfer protein (CETP) leading to a decrease in CETP-mediated transfer of cholesteryl ester (CE) from HDL-C to TG-rich lipoproteins. **[111]** It is perhaps not surprising that the baseline level of plasma TG is a predictor of the HDL-C response to statins or that the statin-induced reduction in plasma TG correlated significantly with the increase in HDL-C. [112]

Simvastatin has been reported to improve reverse cholesterol transport (RCT), which is crucial for preventing atherosclerosis in type 2 diabetic patients with dyslipidemia. This anti-atherogenic mechanism involves export of cholesterol from lipid-laden macrophages in the artery wall back to the liver for excretion. [113]

Among the underlying mechanisms explaining the increase in HDL-C after using simvastatin include the increase in hepatic apo-A1 and plasma apo-A1 concentrations as well as hepatic expression of lecithin cholesterol acyltransferase (LCAT) providing a crucial insight into the anti-atherosclerotic effect of simvastatin beside lipid lowering effect in type 2 diabetes as shown in **(table 17)**. [114]

The reduction in TG level in simvastatin-treated diabetic patients, conducted to the present study, is in alignment with previous findings and could be attributed to the improvement of TG clearance and stimulation of intravascular triglyceride rich lipoproteins-TG (TRL-TG) lipolysis, decrease in production of VLDL particles resulting from decreased availability of cholesterol or TG for particle assembly, or increased catabolism of VLDL in simvastatin-treated diabetic patients. [115,116]

Previous finding of an increase in intravascular lipase activity in the diabetic subjects treated with simvastatin provides confirmatory evidence that the effects of the drug on TRL-TG are mediated by increased intravascular lipase-mediated TG clearance. [115] This finding is consistent with a report by Schneider et al., [117] that statin treatment increased lipoprotein lipase (LPL) activity in diabetic subjects. Increased post-heparin LPL activity has been also recently seen in subjects with familial combined hyperlipidemia treated with statin. [118] The results obtained in the present study regarding the decrease in TG level in simvastatin-treated diabetic patients could partly confirm the previously reported findings as shown in **(table 18)**.

Several studies suggested that enrichment of TG-rich lipoprotein with apo-E may have a role in the increased risk of coronary artery disease in subjects with diabetes. The mechanism by which apo-E might increase atherosclerosis relates to its ability to bind to retained proteoglycans as syndecan-1 in the artery wall. [119] The level of apo-E in diabetic patients showed significant decrease after using simvastatin indicating the anti-atherosclerotic effect of statin as shown in **(table 18)**. [120]

Recent investigation to identify serum biomarkers for the diagnosis of type 2 diabetic nephropathy found that apo-E was a potential biomarker and was associated with urinary albumin excretion rate (ACR) independently of other lipid parameters. [121] Moreover, several studies have shown that apo-E protein regulates mesangial cell proliferation and matrix production and it may act as an autocrine regulator of glomerular function. [122] Thus, internal dysfunction induced by an apo-E abnormality may contribute to the induction of diabetic nephropathy. [121,122] Accordingly, the positive correlation between apo-E and ACR observed in diabetic patients receiving conventional treatment of diabetes, conducted to the present study, could confirm the previously mentioned findings that

circulating apo-E is associated with urinary albumin excretion in type 2 diabetic patients indicating the susceptibility of these patients to diabetic nephropathy as shown in (**figure 11,12)**.

The present study showed significant elevation in two of inflammatory markers namely hs-CRP and IL-6 in all diabetic patients who did not receive statin therapy as compared to corresponding mean values of controls as shown in **(table 26)** .

Various pathogenic factors have been reported to participate in the pathogenesis of type 2 diabetes. Activation of pro-inflammatory mediators and macrophages infiltration are considered as important factors which may lead to β-cell dysfunction. [27,123,124] Our data are consistent with previous findings dealing with studying inflammatory markers in type 2 diabetes. [125] Recent findings reported that chronically elevated levels of IL-6 can promote insulin resistance in skeletal muscle and endothelial dysfunction, as well as liberation of CRP from the liver. Moreover, hyperglycemia also induces IL-6 production from endothelium and macrophages, which might worsen insulin liberation and signaling cascades. [28]

The present study revealed significant reduction in serum level of hs-CRP in statin-treated diabetic patients compared to those treated with conventional treatment of diabetes alone as shown in **(table 26)**. Previous findings reported that statin effect is primarily observed in patients with elevated levels of hs-CRP. Moreover, elevated levels of hs-CRP have been shown to be a strong predictor of future cardiovascular event, perhaps even stronger than LDL-C level. [71,126]

Considered a modifiable risk factor, hs-CRP is an inflammatory marker that can indicate the presence of active vascular inflammation and atherosclerosis and may be decreased with statin therapy. [127]

Simvastatin was significantly reported to decrease hs-CRP in healthy individuals by 30 %, similar to the decrease induced at high cardiovascular risk such as patients with diabetes mellitus or coronary heart disease. [128,129] The exact molecular mechanisms mediating the anti-inflammatory effect of statins have not been elucidated yet. Inhibitions of prenylation of small GTP-binding proteins, inhibition of nuclear factor kappa-B (NF-κB) as well as modulation of the peroxisome proliferator-acivated receptors (PPARs) have been proposed as possible mechanisms. **[129]**

Concerning reduction in IL-6 in statin-treated diabetic patients, conducted to the present study as shown in **(table 26)**, similar results were obtained previously which proposed that decreased cardiovascular events not only through lowering cholesterol levels, but also through pleiotropic properties, including anti-inflammatory actions. Moreover, statins suppress IL-6 induced monocyte chemoattractant protein-1 (MCP-1) gene expression and protein secretion as well as monocyte migration by inhibiting Janus activated kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway, supporting the proposition that statins have anti-inflammatory properties beyond cholesterol lowering effects. Accordingly, statins exert anti-inflammatory and anti-atherosclerotic properties in IL-6-mediated processes. **[130]**

Syndecans constitute a family of transmembrane proteoglycans. They play dual function as cell surface receptor by acting as both adhesion receptors and docking

receptors regulating both intracellular and extracelluar activities, potentially altering a variety of cell behaviors. [131]

The present study showed a significant reduction in serum syndecan-1 levels in both groups of diabetic patients receiving conventional treatment of diabetes (not receiving statin treatment) as compared to corresponding mean value of controls as shown in **(table 30)**.

Chen et al., [132] provided a compelling pathophysiologic model; in which advanced glycation end-products (AGEs) and insulin resistance in T2DM could induce abnormal upregulation of sulfatase-2 (Sulf-2) in the liver and thereby impairs syndecan-1 function. Moreover, hepatic over-expression of Sulf-2 provided a novel molecular mechanism that contributes to post-prandial dyslipoproteinemia and hence arterial harm in T2DM and related disorder.

In humans, it has been recently reported that the primary defect in heparan sulfate proteoglycans (HSPGs) as syndecan-1 in T2DM appears to arise from accelerated de-sulfation, owing to Sulf-2 induction. [133-135] This finding could explain, in part, our observations of decreased syndecan-1 serum levels in diabetic patients as shown in **(table 30)**.

Abnormal persistence of post-prandial apo-B-containing lipoproteins in the circulation remains a significant and growing cause of cardiovascular morbidity and mortality. Typically, these particles have undergone substantial triglyceride hydrolysis and are, therefore, known as 'remnant' lipoproteins, but still carry significant amounts of both triglyceride and cholesterol. [136]

Concerning the role of membrane-bound syndecan-1 as a remnant receptor, Williams et al.,[135] and others[133 ,134] implicated syndecan-1 as a major hepatic receptor for remnant and other apo-B-containing lipoproteins, leading to hepatic catabolism of these particles. The membrane-bound syndecan-1 is a transmembrane molecule that is abundantly expressed on the sinusoidal surface of hepatic parenchymal cells, along microvilli facing the space of disse, where remnant lipoproteins are cleared. Moreover, it has been found that the membrane-bound syndecan-1 directly mediates endocytosis of LPL-enriched apo-B-containing lipoproteins.

The endocytic pathway mediated by the membrane-bound syndecan-1 differs considerably from LDL-receptor-mediated internalization. Efficient syndecan-mediated endocytosis is triggered by clustering as would occur upon binding a multivalent ligand such as a remnant lipoprotein and requires a tyrosine kinase activity. In contrast, endocytosis via members of the LDL receptor gene family proceeds through coated pits, and does not require clustering of tyrosine kinase. [137]

Recent investigations indicated that membrane-bound syndecan-1 and LDL receptor provide complementary pathway for remnant uptake that can not entirely replace each other. The syndecan pathway is high capacity ($>10^6$ lipoprotein-binding sites per hepatocyte), medium affinity, and leads to slower endocytosis. The LDL receptor is low capacity ($>5X10^4$ lipoprotein-binding sites per hepatocyte), high affinity, and leads to rapid endocytosis. [132-134]

Along similar lines, the clearance of LDL itself from the plasma of normal humans relies on significant contributors from both LDL receptor-independent and LDL receptor-dependent pathways. Much, if not most, of LDL receptor-independent clearance of LDL may also be through syndecan-1. [56,135] Accordingly, the significant decrease in serum soluble syndecan-1, observed in diabetic patients receiving conventional treatment of diabetes alone conducted to the present study, indicating reduction in syndecan-1 shedding, could at least in part explain the observed increase in LDL and VLDL concentrations which suspected to be retained within arterial wall increasing the risk of developing cardiovascular disease. Parallel with these findings, this decrease in syndecan-1 level could also explain the significant reduction in HDL-C concentration and apo-A1 levels in plasma of diabetic patients.

Recently, it has been reported that membrane-bound syndecan-1 on primary human hepatocytes can bind and take up native triglyceride-rich lipoproteins particles and considered the dominant proteoglycans clearance receptor in mice [138] as well as in humans. [139]

Membrane-bound syndecan-1 undergoes proteolytic processing resulting in shedding of ectodomains containing the attached glycosaminoglycan (GAG) chains.[139] The shedding process in hepatocytes is mediated by matrix metalloproteinases (MMPs). The inducibility of syndecan-1 shedding by insulin suggested that shed ectodomains might have functional significance. Shed syndecan-1 ectodomains might bind plasma lipoproteins in the space of disse and prevent their escape back into the plasma or facilitate their further processing prior to uptake. Because shedding of ectodomains increase plasma TG, shedding would appear to serve an alternative role, e.g., increasing the circulatory half-life of TRLs for more complete utilization of TGs in peripheral tissues.[139,140]

In alignment with these observations, the significant decrease in serum soluble syndecan-1, observed in the present study as shown in **(table 30)**, may refer to the impairment in the process of plasma TG binding and uptake. This impairment is obviously represented by the significant increase in TG, apo-E and VLDL in diabetic patients.

Concerning the pleiotropic effect of statin, it has been reported that statin stimulated shed of syndecan-1. [72] Accordingly, the significant alteration in lipid fractions, including the significant decrease in LDL-C, VLDL, TG and apo-E and the significant increase in HDL-C and apo-A1 concentrations observed in statin-treated diabetic patients, conducted to the present study, could be explained on the basis of increased syndecan-1 shedding induced by statin treatment.

Retention of LDL-C in the artery intima is mediated by extracelluar matrix proteoglycans as syndecan-1, and plays an important role in the initiation of atherosclerosis. [141] Thus, current therapies targeting modification of proteoglycan synthesis and structure or shedding may represent a prime target to prevent LDL binding and entrapment in the vessel wall and thus prevent the development and progression of atherosclerosis in type 2 diabetes mellitus. [142]

Because statin inhibit vascular smooth muscle cells (VSMCs) proliferation, it has been hypothesized that statins would decrease the size of secreted syndecan-1 and decrease the binding affinity of this proteoglycan to LDL reducing the retention of LDL in the artery wall by a mechanism unrelated to serum LDL lowering. [141]

Syndecan-1 shedding is critical endogenous mechanism that facilitates the resolution of neutrophilic inflammation by aiding the clearance of pro-inflammatory chemokines in a heparan sulfate dependent manner. Recently, it has been suggested that syndecan-1 regulates leukocytes adhesion and migration from the blood circulation to an inflammation zone by inhibiting their binding to endothelial cell receptors: P- and E-selectins and cell adhesion molecules ICAM-1 and VCAM-1. At the same time, syndecan-1 binds with chemokines to form the proper gradient of chemotactic factors. [143,144] Accordingly, the reduction in inflammatory markers as IL-6 and hs-CRP after statin therapy, observed in the present study, and negative correlation between these markers and syndecan-1 could be attributed to the role of statin in stimulating syndecan-1 shedding. Till now, no data are available concerning the modulatory role of syndecan-1 in improving the inflammatory status of statin-treated diabetic patients.

The improvement in inflammatory status as indicated by the decrease in IL-6 and hs-CRP in statin-treated diabetic patients, enrolled in the present study, could be attributed to the role of statin in stimulating syndecan-1 shedding. [141] As inflammation plays an important role in the pathogenesis of type 2 diabetes mellitus and may predict it, syndecan-1 could therefore serve as a new target for the prevention of pathologic inflammatory events. Accordingly, the beneficial effects of statin therapy by inhibiting deposition of lipids and decreasing inflammation might be associated to changes in syndecan-1 level in patients with type 2 diabetes.

- **Safety considerations for simvastatin in diabetic patients:**

Regarding the changes in the liver function tests (ALT and AST) and the marker of muscle myopathy (CK). The results of the current study revealed clinically insignificant changes of all of these indices after 10 weeks of simvastatin treatment of diabetic patients.

The use of 40 mg doses of statins has been largely demonstrated to be safe and well tolerated [116]. It has been reported that the frequency of liver-related adverse effects during statin treatment is low (1.1%) in CVD patients and does not differ from rates reported in patients not treated with statins [145]. Another important side effect, although relatively uncommon, is myopathy. It happens especially when statins are used in high doses [146]. Recently, it has been reported that simvastatin appear to have much less intrinsic muscle toxicity. [116]

SUMMARY AND CONCLUSION

Diabetic dyslipidemia is one of the key risk factors for cardiovascular diseases in type 2 diabetes mellitus. Abundant evidence shows that patients with diabetes are at high risk for cardiovascular disorders which are considered as the leading causes of diabetes-related morbidity and mortality.

The hallmarks of type 2 diabetes are hyperglycemia, insulin resistance and insulin deficiency. Inflammation has been implicated as an important etiological factor in the development of both insulin resistance and type 2 diabetes mellitus. Moreover, insulin resistance contributes to the characteristic dyslipidemia associated with type 2 diabetes.

Syndecan-1, one of the heparan sulfate proteoglycans (HSPGs), is the major hepatic receptor for remnant and triglyceride-rich lipoproteins leading to hepatic catabolism of these particles. Moreover, it has been found that the membrane-bound syndecan-1 directly mediates endocytosis of LPL-enriched apo-B-containing lipoproteins. Syndecan-1 is also involved in inflammation and its shedding showed to be a critical mechanism that facilitates the resolution of neutrophilic inflammation by aiding the clearance of pro-inflammatory chemokines in type 2 diabetes.

Statins are the first-line agents used in treatment of hypercholesterolemia. Statins have other benefits apart from their lipid-lowering effects. Among these pleiotropic effects are the anti-atherosclerotic and the anti-inflammatory properties of statins.

The present study was designed to evaluate the alterations of serum levels of syndecan-1, lipids, interleukin-6 and C-reactive protein in type 2 diabetic patients and to assess the possible effect of statins on syndecan-1 level targeting the prevention of pathologic inflammatory events in these patients.

The present study conducted on 40 patients with type 2 diabetes mellitus and 10 healthy controls. The patients group was subdivided into 2 groups; the first included 20 type 2 diabetic patients treated with conventional therapy of diabetes, while, the other comprised 20 type 2 diabetic patients treated with conventional therapy of diabetes together with simvastatin with a dose of 40 mg per day for 10 weeks.

To all studied subjects, detailed history and full clinical examination were done. Some laboratory investigations were also done at the beginning of the study and repeated after 10 weeks of treatment for both groups of patients including: serum levels of glucose (fasting and post prandial), glycated hemoglobin (HbA1c), total cholesterol (TC), high and low density lipoprotein cholesterol (HDL-C and LDL-C), triglycerides (TG), apolipoprotein-A1 (apo-A1) and apolipoprotein-E (apo-E). In addition, urinary albumin to creatinine ratio (ACR) was estimated. Serum levels of inflammatory biomarkers were also estimated included high sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6). Moreover, serum level of syndecan-1 was also measured by using enzyme-linked immunosorbent assay (ELISA).

The present study revealed significant increase in FBG, PPG and HbA1c in both groups of diabetic patients at base line values compared to controls. Significant reduction in serum levels of TC, LDL-C, TG and apo-E and significant elevation in HDL-C and apo-

A1 were observed in diabetic patients receiving statin in combination with the conventional treatment of diabetes compared to the corresponding values before starting statin treatment. In contrast, there were no significant changes in these parameters after 10 weeks in diabetic patients receiving conventional treatment of diabetes alone compared to the corresponding base line values.

Regarding inflammatory biomarkers, serum levels of hs-CRP and IL-6 demonstrated significant elevation in both groups of diabetic patients at the beginning of the study compared to controls, while, they showed significant reduction in diabetic patients receiving statin in combination with the conventional treatment of diabetes compared to the corresponding values before starting statin treatment. After 10 weeks of starting the study, no significant changes in inflammatory markers were observed in patients receiving conventional treatment of diabetes solely compared to the corresponding values at the beginning of the study.

Serum levels of soluble syndecan-1 showed significant reduction in both groups of diabetic patients at base line values compared to controls, while, syndecan-1 level showed significant elevation in diabetic patients receiving statin in combination with the conventional treatment of diabetes compared to the corresponding mean value before starting simvastatin treatment. After 10 weeks of starting the study, no significant change in syndecan-1 was observed in patients receiving conventional treatment of diabetes solely compared to the corresponding mean value at the beginning of the study.

Conclusion:

From the results obtained in the present study, it could be concluded that statin-mediated increase of syndecan-1 shedding could serve as a new target for the prevention of atherosclerosis and associated-pathologic inflammatory events in type 2 diabetes. Although the present study could throw the light upon the importance of syndecan-1 as anti-atherosclerotic and anti-inflammatory modulator in type 2 diabetes mellitus, the exact nature, magnitude and role of syndecan-1 need further intense investigations.