



N^ε-Carboxymethyllysine Correlates with the Extent of Macrovascular Complications in Type 2 Diabetic Patients.

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ABSTRACT:

Background: The effect of diabetes on health resides majorly in its characteristic complications. It is linked to a higher frequency of macrovascular complications (MVC), such as atherosclerotic vascular disorders, which are the foremost cause of death for people with diabetes. Several studies have shown inconsistent methods by which diabetes accelerates atherosclerosis. **Aim:** The study explored the circulating N^ε-carboxymethyllysine (CML) role as one of the advanced glycation end-products (AGEs) in MVC development in type 2 diabetic (T2D) patients. Also, the possible relationship between it and the other predictors of MVC. **Methods:** The study included 3 groups: Group I (Control): 15 healthy individuals. Group II: 20 T2D patients without MVC. Group III: 20 T2D patients with stable MVC. **Results:** This study indicated that Oxidized LDL appears to be atherogenic and its plasma level can be used as a marker/predictor for macrovascular MVC, also the ratio of OxLDL and HDL serves as a valuable new atherogenic marker instead of conventional atherogenic markers. The circulating level of CML is increased in diabetic patients with MVC compared with diabetics without MVC. CML correlates significantly with carotid Intima-media thickness, denoting the role of AGEs in the development of MVC in these diabetic patients. **Conclusion:** CML plasma level may be used as a diagnostic and prognostic marker for MVC development in diabetic patients.

Keywords: Diabetes mellitus, macrovascular complications, advanced glycation end-product, N^ε-carboxymethyllysine

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease marked by persistently high levels of blood sugar and disturbances in the carbohydrates, fats, and proteins metabolism due to deficiencies in insulin secretion, its action, or both. According to the International Diabetes Federation (IDF), about 537 million adults worldwide (10.5%) are diagnosed with DM. In early 2020, Egypt had the

ninth-highest incidence of diabetes worldwide; the condition affected 8,850,400 adult patients or 15.2% of the total. The IDF estimated that there will be 108 million diabetic patients in the Middle East and North Africa (MENA) region by 2045, doubling the current population ([Farag, Elrewany, Abdel-Aziz, & Sultan, 2023](#)).

Micro- and macroangiopathy are

triggered by DM. Patients with diabetes die mainly due to forms of macroangiopathy, such as cardiovascular disease ([Vetrono et al., 2019](#)). Multiple variables, such as hyperglycemia, hyperlipidemia, and oxidative stress, have been implicated in atherosclerosis, the main root cause of cardiovascular disease ([Abouzeid, Ali, Elkhawas, & Elshafei, 2022](#); [Yoo, Choo, & Lee, 2020](#)).

One of the major atherogenic variables associated with diabetes is advanced glycation end product (AGE). Hyperglycemia hastens the Maillard process, which is the nonenzymatic glycation of proteins. This is followed by a convoluted sequence of oxidative reactions and rearrangements that result in the creation of AGEs (such as N^ε-carboxymethyllysine (CML)). There are two general processes through which AGE formation (e.g., CML) may speed up the process of atherosclerosis: non-receptor-mediated effects and receptor-mediated effects ([Singh, Bali, Singh, & Jaggi, 2014](#)).

Non-receptor-mediated effects depend on direct alteration of the functional characteristics of various crucial matrix molecules with a slow turnover rate. AGEs accumulate on proteins of the extracellular matrix (ECM), altering the characteristics of the large matrix proteins and altering the stiffness of the vessel wall which eventually impairs arterial elasticity ([Jadhav & Kadam, 2005](#); [Reddy, 2004](#)). Besides the AGEs formation on proteins, AGEs can also

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form on lipids. Additionally, it has been demonstrated that AGEs make low-density lipoprotein (LDL) more prone to oxidation. This oxidized LDL (OxLDL) reduces the production of nitric oxide (NO) by inhibiting the activity of NO synthase, which leads to impaired vasodilatation and endothelial dysfunction in diabetes ([Vekic et al., 2023](#))

Additionally, AGEs bind to LDL apolipoprotein-B (Apo-B), which increases its oxidative modification susceptibility and hinders its hepatic receptor-mediated absorption and elimination. On the other hand, glycated Apo-B causes the aorta wall to retain LDL longer and increases macrophage identification. As a result, the scavenger receptor on macrophages is activated upon uptake of AGE-modified low-density lipoprotein (AGE-LDL), which in turn stimulates the production of "foam" cells, which are indicative of an early atherosclerotic lesion. Therefore, atheroma development is spread more by glycated LDL than by "naked" LDL ([Aronson & Rayfield, 2002](#))

The receptor-mediated effects of AGEs depend on their interaction with its receptor (RAGE). AGE and RAGE play a critical role in the pathogenesis of the chronic complications of DM. Upon AGE and RAGE interaction, several signaling mechanisms are activated leading to increased oxidative stress, inflammation and atherosclerosis ([Basta, Schmidt, & De Caterina, 2004](#); [Schiekofer et al., 2003](#)).

Oxidative stress has a prominent role in the pathogenesis of late diabetic complications. It leads to oxidation and modification of critical molecules such as lipoproteins, especially LDL. According to the oxidation theory, atherogenesis requires altered lipoproteins in the artery wall. These lipoproteins cause endothelial activation and damage, recruiting monocytes and supplying lipids that encourage the development of foam cells. Numerous physiologically active chemicals, including aldehydes, which are known to alter gene expression, are formed from oxidized lipoproteins. These molecules are immunogenic due to oxidative LDL modifications, which predict the advancement of coronary and carotid atherosclerosis ([Williams & Fisher, 2005](#)).

The high-density lipoprotein (HDL) possesses cardioprotective, anti-inflammatory, and antiatherogenic qualities. Beyond their capacity to encourage the release of cholesterol from cells, HDL demonstrates a variety of signaling functions that counteract the harmful effects of OxLDL and other proatherogenic substances, thereby lessening the atherogenic potential of oxidized lipoproteins. In addition to its anti-inflammatory qualities, HDL also regulates cell migration and proliferation and provides robust cytoprotection against apoptosis driven by proatherogenic substances such as OxLDL ([Negre-Salvayre et al., 2006](#); [Nofer et al., 2002](#)).

Although the atherosclerotic process develops sooner and can be severe, it is identical to the process affecting people without diabetes ([Unachukwu & Ofori, 2012](#)). The mechanisms by which diabetes hastens atherosclerosis are not consistent in various studies. The current study aimed to explore the possible role of the CML circulating levels as one of the AGEs in the progression of macrovascular angiopathy in type 2 diabetic (T2D) patients. Also, the possible relationship between it and the other predictors of MVC was assessed.

2. Subjects and methods

2.1. Subjects:

The study was included 3 groups:

- **Group I (Control):** 15 healthy individuals (8 males and 7 females). All individuals were volunteers. Their ages ranged from 41 to 60 years.

- **Group II:** 20 T2D patients without cardiovascular complications (9 males and 11 females). Their ages were from 32 to 64 years. At the time of the study, the individuals in this group were being treated by diet plus oral hypoglycemic agents. They were among those seen regularly by the medical staff in the diabetes outpatient clinic of the Medical Research Institute hospital.

- **Group III:** 20 T2D patients with stable cardiovascular complications (8 males and 12 females). They were within the patients diagnosed, treated and followed up in the Cardiology Unit of the Medical Research Institute Hospital. Their ages were from 53 to 72 years. At the time of the study, the individuals in this group were being treated by diet plus oral hypoglycemic agents.

Exclusion criteria from the study comprised a prior record of renal or hepatic impairment, tobacco use, consumption of vitamin or antioxidant supplements, utilization of cholesterol-lowering therapies, or other medications that have an impact on blood lipid levels.

2.2. Clinical Investigation

All subjects were interviewed for a full clinical examination. The duration of diabetes and the medications they received were recorded. Coronary heart disease was reported by history, clinical examination, ECG changes and/or previous coronary angiography.

The intima-media thickness (IMT) of the extracranial carotid arteries was measured by a carotid ultrasound scanner for all individuals ([Handa et al., 1990](#)).

2.3. Blood Sampling

A fasting blood sample was obtained from each subject under medical supervision and was divided into 2 aliquots. In one aliquot EDTA was used to prevent coagulation. In the second aliquot, no anticoagulant was added and it was used to prepare serum.

Blood samples with EDTA anticoagulant were immediately analyzed for glycated hemoglobin (HbA1c). EDTA plasma was stored at -70°C for determination of OxLDL, CML and TBARS. The serum was stored at 4°C for determination of lipid parameters and fasting blood glucose (FBG) which had been performed within 3 days.

2.4. Biochemical investigations

2.4.1. Serum parameters

Serum levels of FBG, triglycerides (TG), total cholesterol (TC), and HDL-cholesterol (HDL-C) were assessed using kits commercially accessible (Bio-Med Diagnostic INC, USA). LDL-C was calculated following Friedewald's equation, $LDL-C (mg/dl) = TC - (HDL-C) - (TG/5)$.

2.4.2. Determination of glycated hemoglobin (HbA1c)

The glycated hemoglobin (HbA1c) was assayed using a commercially available kit from TECO DIAGNOSTIC, USA following the manufacturer's protocol.

2.4.3. ELISA parameters

N^ε-carboxymethyllysine (CML) and OxLDL were analyzed using ELISA kits following the manufacturer's protocols; CML (CycLex co, Japan) and ox-LDL (Mercodia, Sweden).

2.4.4. Determination of thiobarbituric acid reactive substances (TBARS)

The method of Draper and Hadley was used to assess TBARS. The plasma samples and thiobarbituric acid (TBA) were heated at acidic pH. The resulted pink color was measured at absorbance of 532 nm (Draper & Hadley, 1990).

2.5. Statistical analysis

Data analysis was done using SPSS software package version 18.0 (SPSS, Chicago, IL, USA). The data were described as mean \pm standard deviation (SD) and analyzed using the ANOVA test. The Pearson correlation coefficient was used

for the correlation analysis between different studied parameters; the significance limit for all comparisons was considered as $P < 0.05$ (Hagen, 2002).

3. Results

3.1. Glycemic Control:

As expected, diabetic patients showed higher levels of FBG amounting (65.3%) and (74.9%) in diabetic patients without macrovascular complications [MVC (-)] and diabetic with macrovascular complications [MVC (+)] compared to non-diabetic controls (Table 1). In addition, HbA1c showed higher levels amounting (95.2%) and (105.3%) in diabetics without and with MVC, respectively, compared to controls. Even though MVC (+) diabetics have slightly increased levels of FBG and HbA1c than MVC (-) patients, these increases are not significant.

Table (1): Glycemic parameters in the studied groups.

	Reference range	Control	T2D patients	
			MVC (-)	MVC (+)
FBG (mmol/L)	3.9 - 7.0	4.45 \pm 0.541	7.35* \pm 2.21 (+ 65.3 %)	7.77* \pm 2.07 (+ 74.9 %)
HbA1c (%)	< 7.0	3.89 \pm 0.65	7.59* \pm 0.87 (+ 95.2%)	7.98* \pm 0.57 (+ 105.3 %)

Data represented as mean \pm standard deviation.

*Significantly different from control group by ANOVA test ($p < 0.05$).

#Significantly different from MVC (-) by ANOVA test ($p < 0.05$).

Numbers between parentheses represent the percentage change from controls.

+ Increase

MVC (-): without macrovascular complications, MVC (+): with macrovascular complications

3.2. Lipid Pattern and atherogenic indexes:

The results of the lipid pattern and atherogenic indexes are summarized in Table (2). The lipid pattern of T2D deviated from healthy controls in all assessed lipid fractions. The concentrations of TG in diabetic patients were, in general, significantly higher than the non-diabetic controls. In diabetic patients without MVC, the TG level showed a (30.3 %) increase over the controls, but this change is not significant, and the level is still within the normal reference values of TG (Up to 1.82 mmol/L). While the diabetic group with MVC showed a significant increase of about (61.4%) over the control. There is no significant difference observed between the diabetic groups with and without MVC.

The levels of TC in diabetic patients were, in general, significantly higher than in non-diabetic controls. The TC level in diabetic patients without MVC showed an increase of about (22.74%) over the controls. In contrast, the diabetic group with MVC showed a more prominent increase of about (28.2%) over the control. There was no significant difference between the two diabetic groups. In addition, LDL-cholesterol levels showed a significant increase in patients without MVC amounting to a (37.8%) increase over controls,

while patients suffering from MVC showed higher levels amounting to a (46.4%) increase over controls with no significant difference from diabetics without complications.

In contrast, the concentrations of HDL-Cholesterol in diabetic patients were, in general, significantly lower than in non-diabetic controls. The HDL-cholesterol level in diabetic patients with MVC showed (28.4%) decrease than the controls. While in diabetics without MVC the HDL-cholesterol level is still in the clinically accepted range with no significant change from controls. There was a detectable significant difference between the diabetic groups with and without MVC.

The atherogenic indexes (TC/HDL and LDL/HDL) were markedly elevated in diabetic patients, especially in patients with MVC, which showed 79.4 and 103.4 increases in these indexes respectively compared to controls while in diabetic patients without MVC these increases were mild amounting 42.7% and 60.5% respectively over the controls. In addition, both indexes showed significant differences between the diabetic groups with MVC and the diabetic group without MVC.

Table (2): Lipid pattern and atherogenic indexes in the studied groups.

	Reference range	Control	Type 2 diabetic patients	
			MVC (-)	MVC (+)
Triglycerides (TG) (mmol/L)	0.57- 1.71	1.19 ± 0.3	1.55 ± 0.58 (+ 30.3 %)	1.92* ± 0.62 (+ 61.4%)
Total cholesterol (TC) (mmol/L)	< 5.2	4.23 ± 0.44	5.2* ± 0.95 (+ 22.7%)	5.43* ± 1.00 (+ 28.17 %)
HDL-cholesterol (mmol/L)	0.91–1.56	1.24 ± 0.23	1.11 ± 0.26 (-10.6 %)	0.89*# ± 0.14 (-28.4 %)
LDL- cholesterol (mmol/L)	< 3.37	2.46 ± 0.31	3.39* ± 0.85 (+ 37.8 %)	3.61* ± 0.84 (+ 46.4 %)
TC/HDL		3.49 ± 0.47	4.97* ± 1.64 (+42.7%)	6.27* ± 1.44 (+79.4%)
LDL/HDL		2.05 ± 0.44	3.296* ± 1.31 (+60.5)	4.18*# ± 1.19 (+103.4)

Data represented as mean ± standard deviation.

* Significantly different from control group by ANOVA test (p<0.05).

Significantly different from MVC (-) by ANOVA test (p<0.05).

Numbers between parentheses represent the percentage change from controls.

+ Increase - Decrease

MVC (-): without macrovascular complications, MVC (+): with macrovascular complications

3.3. Oxidative stress parameters:

3.3.1. N^ε-Carboxymethyllysine (CML)

N^ε-Carboxymethyllysine (CML) is an important consequence of oxidative stress on proteins. The CML level of T2D patients showed a significant increase from non-diabetic controls. Diabetic patients without MVC had a significant increase amounting (77.9%) over controls, while patients suffering from MVC showed a further increase to be (277.5 %) over controls and a significant increase of about (199.6%) over diabetics without MVC (Figure 1)

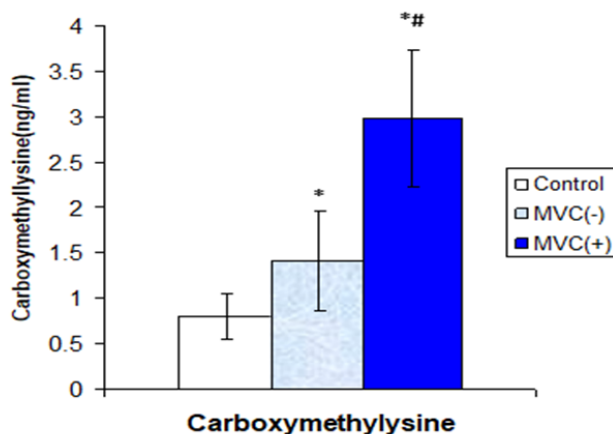


Figure (1): Mean level (± SD) of N^ε-Carboxymethyllysine (CML) (ng/ml), presented in different study groups.

Data represented as mean ± standard deviation.

*Significantly different from control group by ANOVA test (p<0.05).

#Significantly different from MVC (-) by ANOVA test (p<0.05).

MVC (-): without macrovascular complications, MVC (+): with macrovascular complications

3.3.2. Lipid peroxidation product measured as TBARS

The TBARS level of T2D without MVC showed a non-significant increase by about (32.71%) over non-diabetic controls. While patients suffering from MVC showed significantly higher levels amounting to (130.75 %) over controls. There was, also, a detectable significant difference between the diabetic groups with and without MVC. Where diabetic group with MVC showed a (98.04 %) increase over diabetic individuals without MVC (Figure 2)

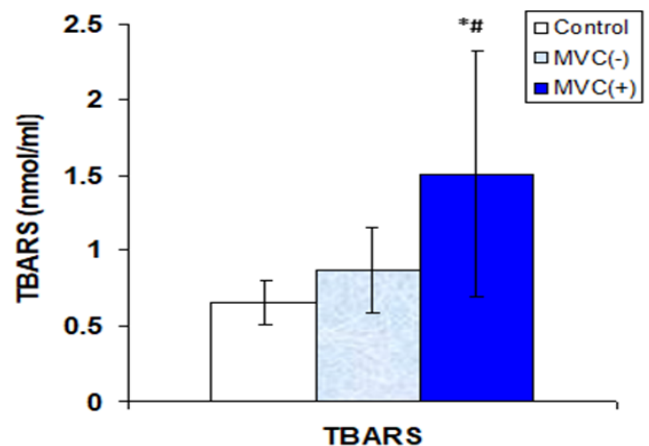


Figure (2): Mean level (± SD) of thiobarbituric acid reactive substances (TBARS) (nmol/ml), presented in different study groups.

Data represented as mean ± standard deviation.

*Significantly different from control group by ANOVA test (p<0.05).

#Significantly different from MVC (-) by ANOVA test (p<0.05).

MVC (-): without macrovascular complications, MVC (+): with macrovascular complications

3.3.3. Oxidized LDL (OxLDL) and related ratios (OxLDL/HDL and OxLDL/LDL)

Oxidized LDL levels in T2D were, in general, higher than in non-diabetic controls. Patients without MVC had a significant increase by about (47.7 %) over controls, while patients suffering from MVC showed higher levels amounting (81.4%) over controls. The most important finding is that diabetic patients with MVC showed significantly higher levels than diabetic individuals without MVC by about (33.69 %) (Figure 3).

The ratios of OxLDL to HDL-Cholesterol and LDL-Cholesterol were recently considered a promising predictor for cardiovascular complications. OxLDL/LDL ratio is an

estimate of the percentage of OxLDL particles, this ratio showed no significant change in T2D without MVC, while in diabetics with MVC, this ratio is significantly increased (Figure 3).

The ratio of OxLDL to HDL showed the same pattern of change as OxLDL/LDL but the increase in the ratio was more apparent and significant in the two diabetic groups. It increases by (66.5%) in diabetics without MVC and by (149.5%) in diabetics with MVC instead of (12.4%) and (29.7%) respectively of OxLDL/LDL which makes the OxLDL/HDL ratio a good biomarker for MVC in type2 diabetic patients (Figure 3)

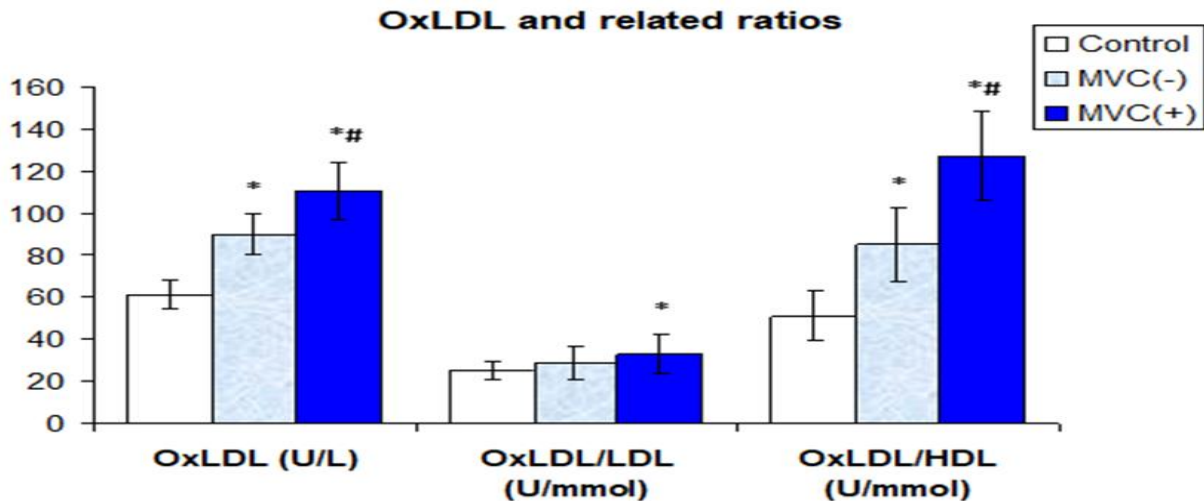


Figure (3): Mean level (± SD) of oxidized LDL (U/L), oxidized LDL / LDL ratio (U/mmol) and oxidized LDL /HDL ratio (U/mmol), presented in different study groups.

Data represented as mean ± standard deviation.

*Significantly different from control group by ANOVA test ($p < 0.05$).

#Significantly different from MVC (-) by ANOVA test ($p < 0.05$).

MVC (-): without macrovascular complications, MVC (+): with macrovascular complications

3.4. Intima-media thickness

Intima media thickness (IMT) of the carotid artery gives a reflection of the degree of atherosclerosis. The IMT of T2D showed a significant increase from non-diabetic controls. Patients without MVC had a (65.2 %) increase over controls, while patients suffering from MVC showed even higher levels by about (150.5 %) increase over controls. Diabetic patients with MVC showed significant elevation amounting (85.43%) over the diabetics without complications (Figure 4). Also, the IMT and OxLDL level in diabetic patients showed an interesting relationship; when the OxLDL level was beneath 100 – 105 U/L the IMT appeared to be constant or among the clinically accepted range (0.8 – 0.85 mm) and showed a sharp increase as the level of OxLDL increases above this level as shown in Figure (5A). In addition, IMT showed a significant positive correlation with OxLDL/HDL ratio in diabetics with MVC ($r = 0.606$, $p < 0.002$) showing a significant pattern in diabetics with MVC as the IMT measurements were in the clinically accepted range (0.8 - 0.85 mm) as the OxLDL/HDL level below 130 then showed a sharp increase as OxLDL/HDL exceeds 130 (Figure 5B). Also, IMT is significantly negatively correlated with HDL-

cholesterol level in diabetics with MVC ($r = -0.452$, $p < 0.02$), and this relationship between IMT and HDL gives a wonderful pattern in which the IMT measurements remains in the accepted level as the HDL levels above 0.78 – 0.91 mmol/L (30-35 mg/dl) and the IMT sharply increases as HDL was lower than this value (Figure 5C)

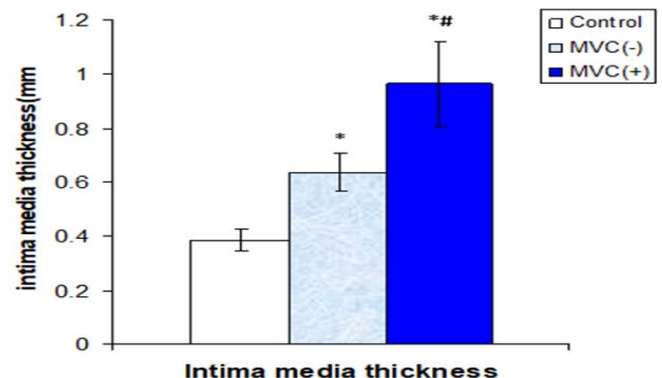


Figure (4): Mean level (\pm SD) of Intima-media thickness (IMT) (mm), presented in different study groups.
 Data represented as mean \pm standard deviation.
 *Significantly different from control group by ANOVA test ($p < 0.05$).

#Significantly different from MVC (-) by ANOVA test ($p < 0.05$).
 MVC (-): without macrovascular complications, MVC (+): with macrovascular complications

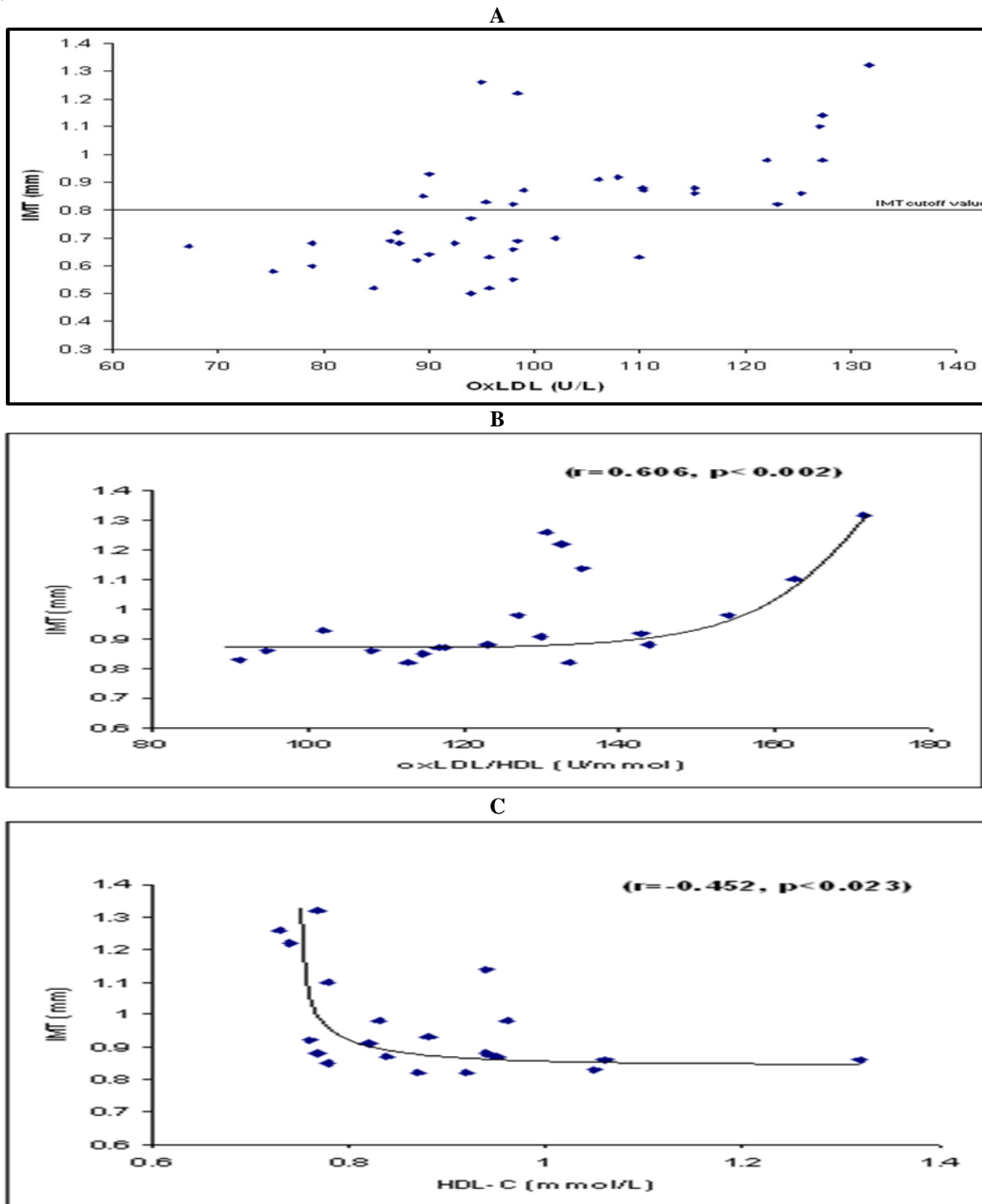


Figure (5): Linear Relationship between (A) IMT and OxLDL in all diabetic patients with and without MVC, (B) Curvilinear relationship between IMT and OxLDL/HDL in T2D patients with MVC, and (C) IMT and HDL-C in diabetic patients with MVC

3.5. Correlation studies

The correlation studies of T2D patients without MVC indicate that; CML levels showed a significant positive

correlation with TG level ($r=0.510, p < 0.01$; Figure 6A) and OxLDL/HDL ratio ($r=0.381, p < 0.05$; Figure 6B). The OxLDL/HDL ratio is positively correlated with TG level

($r=0.654$, $p<0.001$; Figure 6C) and total cholesterol ($r=0.434$, $p<0.03$; Figure 6D). In addition, correlation studies of IMT indicate that it is positively correlated with CML levels ($r=0.379$, $p<0.05$; Figure 6E). In addition, IMT showed a significant positive correlation with OxLDL/HDL ratio ($r=0.388$, $p<0.05$; Figure 6F).

The correlation studies of T2D patients with MVC indicate that; OxLDL levels showed a significant positive correlation

with TG level ($r=0.412$, $p<0.04$; Figure 7A), and a strong significant positive correlation with TBARS ($r=0.562$, $p<0.01$; Figure 7B). The OxLDL/HDL ratio is positively correlated with TBARS level ($r=0.448$, $p<0.02$; Figure 7C). In addition, correlation studies of IMT indicate that it is positively correlated with CML level ($r=0.606$, $p<0.002$; Figure 7D).

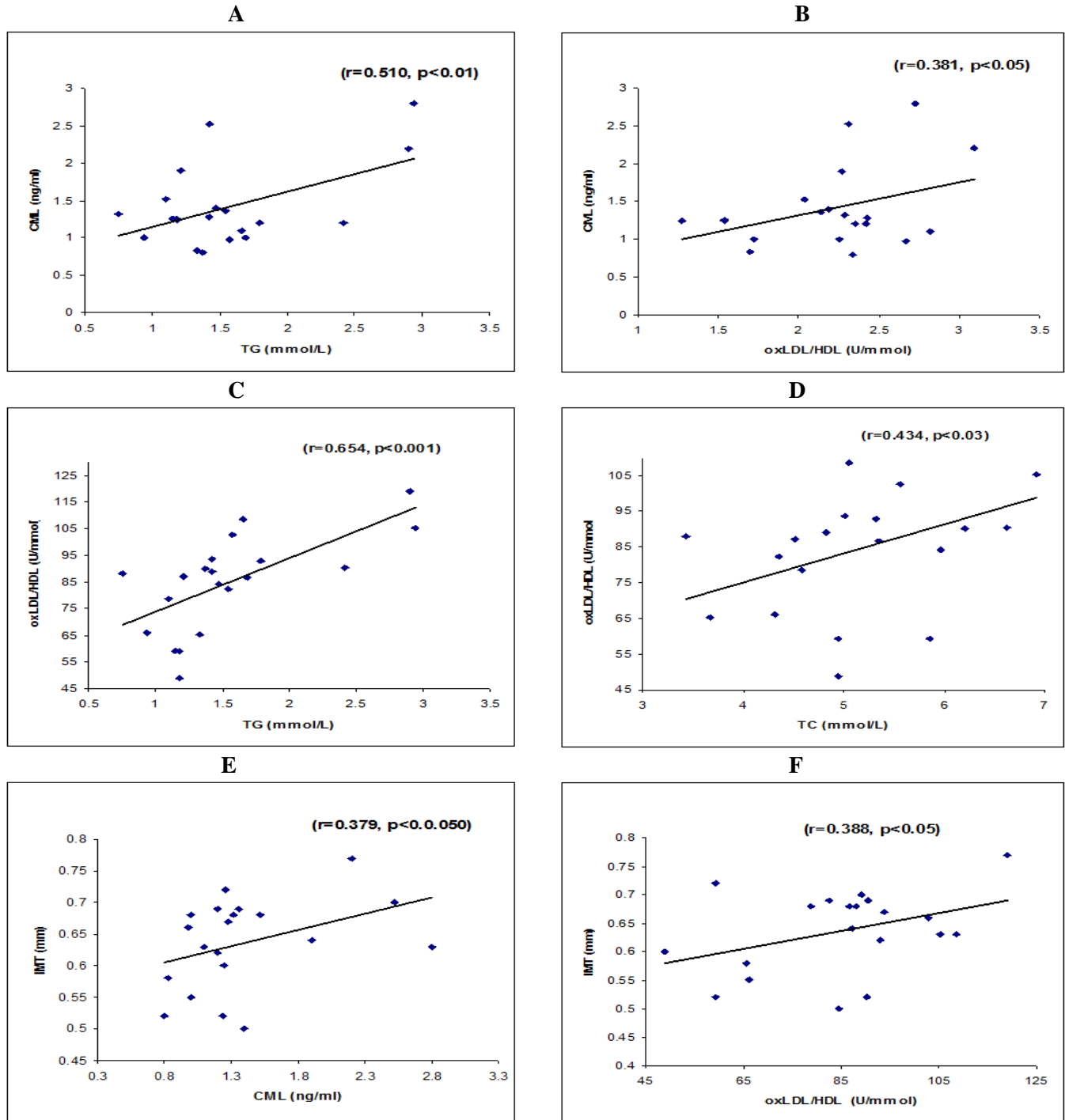


Figure 6: Correlation studies in T2D patients without macrovascular complications. **A:** Correlation between CML and TG, **B:** Correlation between CML and OxLDL/HDL, **C:** Correlation between OxLDL/HDL ratio and TG, **D:** Correlation between OxLDL/HDL ratio and TC, **E:** Correlation between IMT and CML, **F:** Correlation between IMT and OxLDL/HDL.

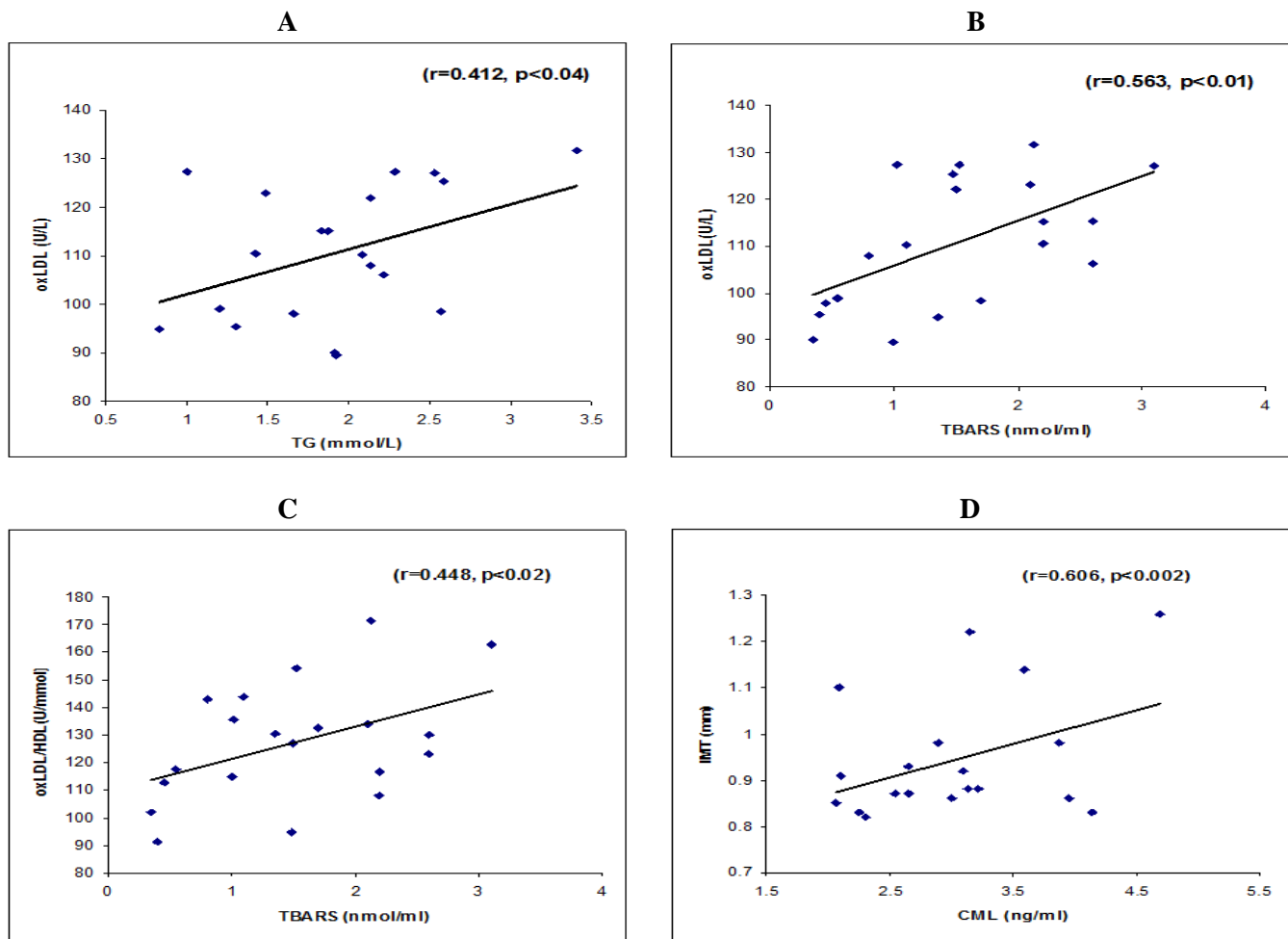


Figure 7: Correlation studies in T2D patients with macrovascular complications. **A:** Correlation between OxLDL and TG, **B:** Correlation between OxLDL and TBARS, **C:** Correlation between OxLDL/HDL ratio and TBARS, **D:** Correlation between IMT and CML

4. Discussion

The data on glycemic control in the current study was uneventful. As hyperglycemia is the hallmark of DM, the FBG levels were higher in the diabetic groups (without and with macrovascular complication) compared to the control non-diabetics. However, no significant difference was observed in the FBG level between the two groups of diabetic patients. In addition, the present study has shown that glycosylated hemoglobin level, which represents patient compliance to proper hypoglycemic treatment and efficacy of the treatment in the 3 months before the tested sample, was significantly elevated in diabetic groups by comparison with the control group. However, no significant difference was observed between the two diabetic groups, and the level of glycosylated hemoglobin was equal to or below the 8% recommended cut-off value to ensure proper glycemic control and avoid the development of complications. The relationship between glycemia and macrovascular disease in T2D is less pronounced, despite the fact that the degree of glycemia in diabetic patients is closely connected to the risk of microvascular complications (nephropathy and retinopathy). (Lazarte & Hegele, 2020).

Diabetes is a known risk factor for atherosclerosis, and people with diabetes are two to five times more likely to

progress coronary heart disease than people without the illness. (Neil, 2003). The precise role of hyperglycemia in the pathogenesis of long-term complications of diabetes like atherosclerosis is still not consistent (Lee, Yun, & Ko, 2022). However, an attractive hypothesis and one that has received considerable interest is the elevation in lipids and proteins subjected to non-enzymatic glycation, resulting in the irreversible production and accumulation of AGEs (such as CML) (Wang et al., 2012).

The results of the present study indicate that the circulating level of CML in T2D is significantly elevated compared with non-diabetic control subjects. Diabetic patients with MVC showed a significantly higher level of CML, about double, compared with diabetics without MVC. Our results are in accordance with other studies that confirm the association between diabetes and increased AGEs (Huebschmann, Regensteiner, Vlassara, & Reusch, 2006). A study showed a 20 – 30% increase in AGE levels in uncomplicated diabetic subjects and 40 – 100% higher levels in T2D patients complicated with coronary artery disease or microalbuminuria (Rigalleau et al., 2015; Tan, Chow, Tam, Bucala, & Betteridge, 2004).

The level of CML showed a low significant positive correlation with the IMT in diabetic patients without MVC,

while they are strongly positively correlated in diabetics with MVC. This pattern of change and relationships of CML in diabetic patients suggests a degree of causality for the progression of atherosclerosis in those patients. Also, the CML level was shown to be significantly positively correlated with convenient lipid fractions, TG and a promising atherogenic index, OxLDL/HDL.

The results of the present study may indicate that AGEs are not only associated to manifestations of cardiovascular diseases, but they can also provide prognostic information. It was documented that AGEs content predicts adverse cardiac events in patients after cardiac surgery ([de la Cruz-Ares et al., 2020](#); [Koyama et al., 2007](#); [Simm et al., 2007](#)).

Since CML is elevated in tissues of many diseases and conditions marked by oxidative stress, inflammation, and general tissue damage, it is widely regarded as a biomarker of oxidative stress. But without a corresponding rise in oxidative stress, an increase in oxidizable substrates like lipids or FBG may also lead to an increase in CML in tissue proteins. For instance, in both diabetes and hyperlipidemia, the elevation in plasma substrate concentrations (glucose and lipids) is adequate to explain the elevation in AGEs in collagen and atherosclerotic plaque without causing an increase in oxidative stress ([Rochette, Zeller, Cottin, & Vergely, 2014](#)). ([Shimoi et al., 2001](#)) have found that endothelial cells exposed to hyperglycemia suffer from increased DNA damage, but they also note that this damage happens without an increase in oxidative stress. Glyoxal had similar effects, indicating that processes other than oxidative stress may be responsible for AGEs, DNA damage, and glyoxal during hyperglycemia. Therefore, rather than viewing CML as a sign of increased oxidative stress in and of itself, it could be more reasonable to view it as a marker of continued oxidative damage in the absence of other oxidative stress indicators ([Choudhuri et al., 2013](#)).

[Qian & Eaton, 2000](#) have suggested that CML may work to enhance oxidative tissue damage in diabetes, uremia, atherosclerosis and other disorders by chelating and activating redox-active metal ions, such as iron and copper. While it is doubtful that the density of CML on proteins can cause multivalent chelation of metal ions, it is plausible that CML may capture and activate metal ions when it works in tandem with functional groups on other amino acids in proteins ([Piarulli, Sartore, & Lapolla, 2013](#)). Additionally, [Cameron & Cotter, 2001](#) have demonstrated that in diabetic rats, chelation treatment prevents peripheral vascular disease from developing.

The production of ROS coincides with the glucose autooxidation process that is metal-catalyzed, whether proteins are present or not. The production of CML and TBARS is also heightened when proteins, LDL, or phosphatidylcholine liposomes are incubated with glucose under oxidizing conditions while transition metals, such as copper, are present. Furthermore, CML is formed on proteins by reactive peroxynitrite ([Nagai et al., 2002](#)).

Hence, CML and other AGEs are formed as a result of both oxidative and glycation damage to macromolecules. Consequently, it has been postulated that the local oxidative environment, in addition to the relative glucose concentration, influences the generation of glycoxidation

products in vivo ([Shaw, Baynes, & Thorpe, 2002](#)). So, it can be suggested that oxidative/carbonyl stress, hyperlipidemia, hyperglycemia, and/or impaired renal clearance of AGE-precursors all contribute to the buildup of AGEs. Absorption of AGEs from food or smoking may exacerbate AGE accumulation, and decreased clearance of serum AGEs may enhance tissue AGE accumulation and de novo production ([Koska et al., 2018](#)).

AGEs may have a significant impact on the development of vascular problems through changes to the composition and functionality of ECM components ([Li et al., 2017](#); [R Meerwaldt et al., 2008](#)). AGEs formation (e.g., CML) may accelerate the atherosclerosis process in diabetic patients by affecting vascular function through alteration of matrix protein, artery wall stiffness and formation of AGE-LDL complexes with the consequential stimulation of “foam” cell establishment, characteristic of the early atherosclerotic lesion. In this way, glycated LDL is more atherogenic than “naked” LDL ([Aronson & Rayfield, 2002](#)).

Also, AGEs interaction with its receptor RAGE cause cellular activation resulting in the generation of oxidative stress and a variety of signaling pathways, including the generation of ROS inside cells. These ROS would in turn activate the NAD(P)H-oxidase system and the redox-sensitive transcription nuclear factor (NF- κ B). upregulation of NF- κ B in the context of oxidative stress and RAGE activation ultimately results in or modulates the transcriptional activation of several genes, many of which are extremely important for atherosclerosis, inflammation, and immunity ([Singh et al., 2014](#)).

In line with our results, it was documented that T2D patients with coronary heart disease have elevated serum levels of AGEs than patients without complications and AGEs are correlated with the severity of coronary heart disease ([Kiuchi, Nejima, Takano, Ohta, & Hashimoto, 2001](#)). Carotid IMT is positively associated with serum AGEs concentrations ([R. Meerwaldt et al., 2004](#)). AGE levels are elevated in T2D patients with peripheral artery occlusive disease (PAD) compared to those without ([Lapolla et al., 2007](#)). The serum level of CML may be used to differentiate between patients with and without MVC but this observation needs more work with a larger sample size to determine the critical cutoff value of CML.

Oxidative stress is vital to both the T2D progression and the cardiovascular consequences ([Molehin, Adefegha, & Adeyanju, 2020](#)). The current study's findings demonstrated that diabetic patients were overexposed to oxidative stress and had a discernible overproduction of free radicals. Compared to healthy individuals, both diabetic groups had significantly higher levels of the lipid peroxidation index, or TBARS. Moreover, by comparing the two diabetic groups together, there was a significant increase in TBARS in patients with MVC. These findings are compatible with various studies in the literature ([Atalay & Laaksonen, 2002](#); [Cederberg, Basu, & Eriksson, 2001](#)). The development of cellular oxidative stress appears to be a potential culmination of all cellular alterations brought on by hyperglycemia ([Giacco & Brownlee, 2010](#)).

According to certain theories, the development of AGEs, activation of protein kinase C (PKC), induction of the polyol

pathway, and elevation of hexosamine flow are specific pathologic consequences of hyperglycemia that are linked to mitochondrial dysfunction in DM. Oxidative stress has been linked to several of these mechanisms. The hyperglycemia-induced formation of AGEs and associated oxidative stress leads to the oxidation and modification of critical molecules, especially lipoproteins. Since modified lipoproteins are immunogenic and physiologically active, they promote inflammatory processes that hasten atherosclerosis ([Lorey, Öörni, & Kovanen, 2022](#)).

In our study, we found that the circulating level of OxLDL in T2D patients is higher than that of control subjects. Interestingly, the results indicate that diabetic patients with MVC have higher levels of OxLDL than those without these complications. These higher levels in diabetics with complications are positively correlated with their plasma level of TBARS which can indicate a causality relationship. Our results are in line with other published data ([Viigimaa et al., 2020](#); [Xu, Wang, Li, & Feng, 2016](#)).

The higher level of circulating OxLDL can serve as a marker and/or a predictor for MVC in diabetic patients as it is positively correlated with TG levels and plasma levels of TBARS in diabetics with MVC. Also, the relationship between OxLDL levels and IMT of the carotid artery showed a wonderful pattern; the IMT was among the clinically accepted range (equal to or less than 0.8 mm) ([Frauchiger, Schmid, Roedel, Moosmann, & Staub, 2001](#); [Okur et al., 2013](#)) if the OxLDL level was beneath 100-105 U/L. Once the OxLDL level exceeded this range, the IMT sharply elevated and showed proportional change with OxLDL level. This observation is in line with the results of a study that documented that the level of OxLDL should be kept below 100 U/L to avoid its serious atherogenic effect ([El-Bassiouni, Helmy, El-Zoghby, Kamel, & Hosny, 2007](#)).

We have shown in diabetic patients that OxLDL is a useful biomarker for distinguishing diabetic patients with and without MVC when combined with LDL and HDL cholesterol. It is unclear why ratios of plasma-oxidized lipoproteins are larger than their raw counts, but in the instance of OxLDL/HDL, it is most likely because it represents the balance between the primary target of lipoprotein oxidation (LDL) and its counterweight (HDL). We demonstrated that in diabetes patients, the OxLDL/HDL ratio is linked to vascular disease. Our findings concur with earlier research demonstrating that OxLDL plasma levels are a predictor of future occurrences in diabetic patients with coronary artery disease ([Shimada et al., 2004](#)) and that there is an association of OxLDL/HDL with vascular disease in diabetic patients ([Girona et al., 2008](#); [Srivastava, 2018](#)).

The OxLDL/HDL ratio showed a strong positive correlation with the traditional components of lipid fractions. Also, it showed a positive correlation with TBARS level and IMT in diabetic patients with MVC. The relationship between IMT and OxLDL/HDL ratio showed a very interesting finding. The thickness of the intima was among the clinically accepted range (0.8-0.9 mm) when the ratio was below 130 U/mmol. While the IMT sharply increased if the ratio exceeded 130 U/mmol. All of these findings may suggest that OxLDL/HDL ratio is a promising atherogenic index for the prediction and diagnosis of macrovascular events in T2D

patients. This is because the ratio combines two phenomena, the increase of OxLDL level which acts as a driving factor for the formation of foam cells and initiation of atherosclerosis, and the decrease in the antiatherogenic factor, HDL.

The HDL results in patients with MVC were in agreement with previous epidemiological studies, suggesting that high plasma levels of HDL are negatively associated with the incidence of various diseases, and HDL plays a significant role in preventing atherosclerosis development and other MVC ([Assmann & Nofer, 2003](#); [Sharrett et al., 2001](#)). HDL exerts cardioprotective, anti-inflammatory and antiatherogenic properties ([Libby, 2001](#); [Nofer et al., 2002](#)). As proposed by Nicholls et al. 2005, and due to their pleiotropic protective properties, HDL, apo-AI, and synthetic peptides that imitate their function constitute a novel therapeutic approach against atherothrombotic vascular disorders and, more broadly, proinflammatory pathologies. ([Nicholls, Rye, & Barter, 2005](#)).

Our results indicated a negative correlation between the plasma level of HDL-cholesterol and IMT in T2D with MVC and the relation showed a critical level of HDL (0.78 – 0.91 mmol/L, 30-35 mg/dl) below which the IMT showed a sharp increase. This is in agreement with the critical level of HDL in type 2 DM by the American Diabetes Association, > 40 mg/dl (1.04 mmol/L) for men and > 50 mg/dl (1.3 mmol/L) for women ([Chamberlain et al., 2018](#)).

The study also showed a shift in nearly all lipid fractions and traditional atherogenic indexes in diabetic patients, especially those with MVC. Both the diabetic groups had significant elevation in the levels of TG, LDL-cholesterol, moderately raised cholesterol levels, and declined HDL-cholesterol levels compared to the control group. The lipid disturbances found in the diabetic groups are in line with the findings reported by most workers in the fields ([Ballantyne et al., 2001](#)). These abnormalities (increased TG levels and decreased HDL-cholesterol levels) were reported to be highly associated with type 2 diabetes and to be definite risk factors in atherosclerotic cardiovascular disease ([Sprecher et al., 2003](#); [Sultani et al., 2020](#)).

Increased levels of small, dense LDL molecules, low HDL-cholesterol levels, and high TG levels make up the condition known as *diabetic dyslipidemia* (lipid triad). Dyslipidemia is a recognized risk factor for coronary atherosclerosis, and individuals with diabetes frequently have abnormal lipid levels ([Cefalu, 2008](#)). Diabetic people have a higher incidence of atherogenic lipid abnormalities, such as tiny, dense LDL particles, than non-diabetic patients, presumably due to a higher severity of coronary disease at all lipid levels. Furthermore, it has been observed that elevated LDL oxidation in individuals with diabetes has been linked to a higher risk of coronary artery disease, maybe through the promotion of endothelial dysfunction. Because it disrupts endothelial integrity and deactivates NO, OxLDL is cytotoxic to endothelium and reduces endothelium-dependent vasodilation ([Lee et al., 2022](#)).

5. Conclusion

From the above we can summarize the role of increased serum CML (as one of AGEs) in accelerated MVC in T2D

patients as follows: AGEs affect vascular function through alteration of matrix protein, artery wall stiffness and formation of AGE-LDL complexes. In addition, RAGE activation can propagate a surge of incidents, involving NADPH oxidase activation and various pro-inflammatory mediators which leads to excessive ROS production, and quenching of NO leading to endothelial dysfunction. Endothelial dysfunction and oxidative stress work together to mediate the development of diabetes-induced atherosclerosis, which is ultimately the leading cause of early death in individuals with diabetes. In light of this study and previous work in literature, it is possible to speculate on human atherosclerosis as a chronic inflammatory disorder with a history of problems with lipid metabolism. The formation of AGEs and OxLDL may play a role in relating inflammation with lipid accumulation in atheromatous plaque in vessel walls through its pro-inflammatory effect, resulting in oxidative stress and endothelial dysfunction. Finally, we must state that although many of the pathways in this field of study are still not fully understood, there are strong indications that AGEs are a major component of diabetes and that they are crucial in the initiation and spread of the oxidative stress and inflammation that drive endothelial dysfunction and atherosclerosis.

Declarations

Ethics approval

Approval is taken by Medical Research Institute Ethical Committee, University of Alexandria (approval number: E/C.S/N.R 29/2013). The research was performed for studies involving humans according to the World Medical Association's Code of Ethics (Declaration of Helsinki).

Informed Consent:

All participants in the study signed the consent before participating in the research.

Availability of data and materials

All materials used or analyzed are included in the manuscript.

Competing interests

The authors declare that they have no competing interests.

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No funding was received for conducting this study.

Authors' contributions

MB & HH: Performed practical work, acquisition of data, data analysis and interpretation, and writing the manuscript. AE & MK: Supervised the practical part and contributed to the analysis and manuscript critique. SE & MH: Supervised the practical part, data analysis and interpretation, and writing of the manuscript. All authors read and approved the final manuscript.

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