# The cross-link between demographic and clinical characteristics and level of Lipoprotein-associated Phospholipase $A_2$ in diabetic type 2 and non-diabetic with and without coronary artery disease

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**Keywords:**

Diabetes Mellitus Type 2 (DM2) - Healthy controls (HC) - Non-diabetes Mellitus (NDM) - Coronary Artery Disease (CAD) - Body Mass Index (BMI) - C-reactive protein (CRP) - Glycated Haemoglobin ($HbA_{1c}$) - Lipoprotein-associated Phospholipase $A_2$ (Lp-$PLA_2$) - Platelet-Activating Factor - Acetylhydrolase (PAF-AH) - CT angiocardiography (CTA) - Percutaneous Coronary Intervention - (PCI) Triglyceride glucose index (TyG) - lysophosphatidylcholine (LysoPC).

## ABSTRACT

Coronary artery disease (CAD) is quite common among diabetics in all age groups and its risk is independently affiliated with Lipoprotein-associated phospholipase $A_2$ (LP-$PLA_2$). There is strong evidence that the elevated plasma level of Lp-$PLA_2$ indicates vascular inflammation associated with plaque formation within the arteries. Therefore, the relationship between the plasma Lp-$PLA_2$ level and coronary artery disease risk factors between non-diabetic and diabetic patients will be studied.

**Materials and Methods:**

A total of 181 individuals were recruited from the National Heart Centre Tajoura and divided into 4 groups (40 healthy controls (HC), 43 non-diabetics (NDM) who had CAD, 54 DM2 without CAD, and 44 DM2 who had CAD). The relationship between Lp-$PLA_2$ and the potential biochemical risk markers for CAD will be studied between the groups.

**Results:**

All the groups that participated in this study have similar body mass index. There is statistical significance in the level of plasma level Lp-$PLA_2$ between DM2 with CAD, DM2 without CAD and HC with ($^{***}P<0.0001$, $^{**}P<0.006$), respectively; and likewise between NDM with CAD and HC with $^{*}P<0.05$. Although there is a significant difference between the plasma levels of Lp-$PLA_2$ between the groups below the age of 60 years, there is no statistical significance between the groups above the age of 60 years. Correspondingly, there is a statistical difference in the plasma level of Lp-$PLA_2$ between healthy controls and both DM2 without CAD and DM2 with CAD with (**P<0.006, and ***P<0.0001), respectively. Once more, there is statistical significance in the plasma level of Lp-$PLA_2$ between NDM with CAD and healthy controls with *P<0.05.

Furthermore, there is a statistical difference in the serum level of CRP between DM2 with CAD and HC. In addition, there is a positive relationship exists between the duration of diabetes mellitus without CAD and the level of glycated haemoglobin ($HbA_{1c}$) with **P <0.003 in both genders.

**Conclusion:**

In this study, the high level of Lp-$PLA_2$ and CRP strongly indicate the presence of vascular inflammation associated with the formation of arterial atherosclerosis with thrombus formation. These findings are also correlated with diabetic duration and they could be used in the diagnosis and prognosis of coronary artery disease.

## INTRODUCTION

Coronary artery disease (CAD) is a pathological condition that has several mechanisms, including systematic inflammation that causes structural changes on the vascular wall, endothelial dysfunction, arterial atherosclerosis, and discrete plaque formation within the coronary arteries [(1)]. Lp-$PLA_2$ is also known as platelet-activating factor acetylhydrolase

(PAF-AH) [2,3], is an enzyme encoded by the $PLA_2G7$ gene located at chromosome 6p12-21 and is included in different signal transduction pathways, and is primarily secreted de novo from inflammatory cells (macrophages) or bound to circulating low-density lipoprotein (LDL) and high-density lipoprotein (HDL) [4,5,6].

Lp-$PLA_2$ may play a role in the development of atherosclerosis, and this enzyme degrades phospholipids in oxidized low-density lipoproteins (ox-LDL) to produce two pro-inflammatory and pro-atherogenic products, lysophosphatidylcholine and oxidized free fatty acids. These inflammatory factors promote atherosclerosis [7,8].

Based on the local and systemic inflammatory responses observed throughout the spectrum of atherosclerotic disease from an initial lesion formation to plaque destabilization and rupture, inflammation plays a primary role in the progression of human atheroma [9].

Several serum markers have been identified as independent predictors of adverse clinical outcomes in CAD, such as C-reactive protein (CRP), interleukin-6, matrix metalloproteinases, myeloperoxidase, and neutrophil-to-lymphocyte ratio. CRP is an acute-phase reactant that has been linked to an increased risk of CAD, though the causality of the link has been debated. The question of whether adding CRP to modern risk prediction models provides incremental predictive utility remains unanswered. Large observational studies have found a strong link between CRP levels and the morbidity and mortality associated with CAD [10,11,12].

Cardiovascular disease is one of the most common deaths worldwide, with a mortality rate of approximately 30%. CAD is an important threat to sustainable development in the 21$^{st}$ century. An increasing number of individuals with non-fatal CAD live with chronic disabilities and poor quality of life [13,].

The primary pathological process that leads to CAD is atherosclerosis, an inflammatory disease of the arteries associated with lipid deposition and metabolic alterations due to multiple risk factors. More than 70% of at-risk individuals have multiple risk factors for IHD, and only 2% to 7% of the general population has no risk factors. The increasing incidence of CAD is expected to continue, due not only to the increased prevalence of

obesity, diabetes mellitus, and metabolic syndrome but also to population ageing [14,15]. The past two decades have witnessed a steep rise in global population ageing.

Major guidelines for grading Coronary Artery Disease–Reporting and Data System (CAD-RADS) for assessing patients presented with low to intermediate chest pain by using a coronary CT angiography (CTA). The key aspect of using CTA is to minimize errors in clinical and to ultimately improve patient outcomes. Therefore, the main goal of the CAD-RADS classification system is to propose a reporting structure that provides consistent categories for final assessment, along with suggestions for further management as described in Table (1).

**TABLE (1)** Coronary Artery Disease–Reporting and Data System (CAD-RADS): grading of coronary artery disease (CAD); Invasive Cardiac Angiography (ICA) Includes positive remodelling with no measurable stenosis adopted from (Cury RC et al, 2016) [37].

| Classification | Maximal Stenosis | Interpretation | Further Cardiac Investigation |
|---|---|---|---|
| CAD-RADS 0 | 0% | No CAD | None |
| CAD-RADS 1 | 1 – 24%* | Minimal non-obstructive | None |
| CAD-RADS 2 | 25 – 49% | Mild non-obstructive | None |
| CAD-RADS 3 | 50 – 69% | Moderate stenosis | Consider functional assessment |
| CAD-RADS 4 | A: 70 – 99% or<br>B: Left Main >50% or 3-vessel ≥ 70% | Severe stenosis | A: Consider ICA or functional assessment<br>B: ICA recommended |
| CAD-RADS 5 | 100% | Total coronary occlusion | Consider ICA and/or viability assessment |
| CAD-RADS N | Non-diagnostic | Obstructive CAD cannot be excluded | An additional or alternative evaluation may be needed |

The severity of coronary artery complications is related to the duration and severity of hyperglycaemia. The latter is also linked to other co-morbidities and disorders that aggravate the direct diabetic effect. These include hypertension, dyslipidaemia, and generalized glycosylation of cells and membranes aggravating atherosclerosis, and induced

formation of Lp-$PLA_2$ in both genders [16]. The main Pathological risk factor in macro-vascular diseases is the role of DM in developing atherosclerosis within the medium and large vessels and the formation of atheroma or plaque formation especially in obese adolescents with and without diabetes [17]. Also, there is an increased leukocyte adhesion to endothelial cells and hyperviscosity of blood in DM that contribute to macro-and microvascular complications [18].

This study aims to investigate the relationship between patients with and without coronary artery disease and the plasma level of Lp-$PLA_2$ and the impact of various demographic characteristics among diabetic type 2 with and without CAD, non-diabetic with CAD, and healthy controls.

# METHODS

## Study Design

All participants in this study gave informed written consent and Ethical approval was obtained from the Ethical Committee of Tajoura National Heart Centre, Tripoli-Libya. For this study, 181 subjects were recruited from Tajoura National Heart Centre (40 healthy controls, 43 non-diabetics (NDM) with the presence of CAD, 54 DM2 without CAD, and 44 DM2 with CAD). The relationship between Lp-$PLA_2$ and the potential markers of CAD, renal function, and diabetes control is estimated by $HbA_{1c}$ level.

The demographic characteristics regarding height, weight, age, gender, duration of diabetes, family history of diabetes, the strategy of medical and surgical treatment, and the cardiovascular risk factors were obtained from all the individuals as shown in Table (2). However, individuals with liver dysfunction, oncological disease, infectious disease, and autoimmune disease, were excluded from this study. The lifestyle risk factors of the participants were determined and recorded in the data sheet and saved as confidential.

**TABLE (2)** Baseline demographic, clinical and biochemical parameters of the participant groups in this work.

| **Clinical characteristics** | **HC** | **NDM+CAD** | **DM2 no CAD** | **DM2+CAD** |
|---|---|---|---|---|
| Number (n) | 40 | 43 | 16 | 82 |
| Age (years) | 49.3±2.1 | 60.62.7±0.8 | 60.1±2.8 | 65.1±1.0 |
| Duration of diabetes (years) | 0 | 0 | 11.6±1.6 | 10.4±0.8 |
| BMI ($Kg/m^2$) | 27.2±0.6 | 28.4±1.3 | 28±0.5 | 32.2±1.2 |
| Smokers (n) | 12 (30%) | 14 (32.5%) | 2 (12.5%) | 14 (17.1%) |
| Coronary heart disease (n) | 0 | 43 | 0 | 82 |
| $HbA_{1c}$ (%) | 4.9±0.1 | 5.1±0.1 | 8.1±0.4 | 9.0±0.2 |
| Fasting blood sugar (mg/dl) | 85.7±1.9 | 92.4±2.6 | 191.5±14.7 | 273.6±12.2 |
| Lp-$PLA_2$ (ng/ml) | 144.6±14.2 | 263.3±13.0 | 206.2±16.9 | 273.6± 12.2 |
| CRP | 2.5±0.3 | 4.5±0.4 | 5.4±0.8 | 5.7±0.4 |
| TyG index | 4.4±0.03 | 4.5±0.03 | 4.9±0.09 | 4.9±0.05 |
| Anti-angina therapy (n) | 0 | 22 | 0 | 38 |
| Percutaneous intervention (n) | 0 | 17 | 0 | 15 |
| Coronary artery bypass graft (n) (CABG) | 0 | 4 | 0 | 29 |

The severity of coronary artery diseases was determined according to the number of vessels affected, the depth and quality of download sloping of ST-depression detected by ECG, and Percutaneous Coronary Intervention (PCI).

**Collection of blood and preparation of samples**

Fasting blood samples and all the biochemical analyses were measured in the laboratories at the Tajoura National Heart Centre, Tripoli-Libya. In brief, the routine blood tests included fasting blood glucose, Triglyceride, $HbA_{1c}$, and CRP levels were measured by COBAS INTEGRA 400 plus analyser (Roche Diagnostics, Germany) and plasma level of Lp-$PLA_2$ were measured by sandwich-ELISA kit (Elabscience Biotechnology, Texas, USA) according to manufacturer instructions.

Blood samples for lipid profiles {total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglyceride (TG)}, Plasma Lp-$PLA_2$, C reactive protein (CRP), fasting blood sugar (FBS), and $HbA_{1c}$ were analysed for this study

## Statistical analysis:

All variables of data collected registered in the questionnaire and biochemical blood tests were analysed using SPSS software version 16 (Statistical Package for Social Sciences) and GraphPad Prism version 6. The t-test and Fisher's test were used for correlation between Lp**-**$PLA_2$ and all relevant risk variables in both diabetic and non-diabetic individuals with and without coronary artery disease in both genders. P-value < 0.05 will be recognized as statistical significance.

# RESULTS

## Participants

All the participants in this study are age-matched, there is no statistical difference between all the groups as shown in Figure (1A). Besides, there is no statistical significance in body mass index ($Kg/m^2$) between DM2 without CAD between females and males ($27.4 \pm 0.9$ (n=33) *vs* $25.3 \pm 2.2$ (n=23); P>0.3), but there is statistical significance in DM2 with CAD

treated medically in both genders (31.76 ± 2.224 (n=13) vs 27.61 ± 0.6264 (n=45); *P<0.02), and also in patients treated surgically in females and males (32.26 ± 1.627 (n=9) vs 28.07 ± 0.5272 (n=58); **P 0.007), respectively, as shown in figure (1B).

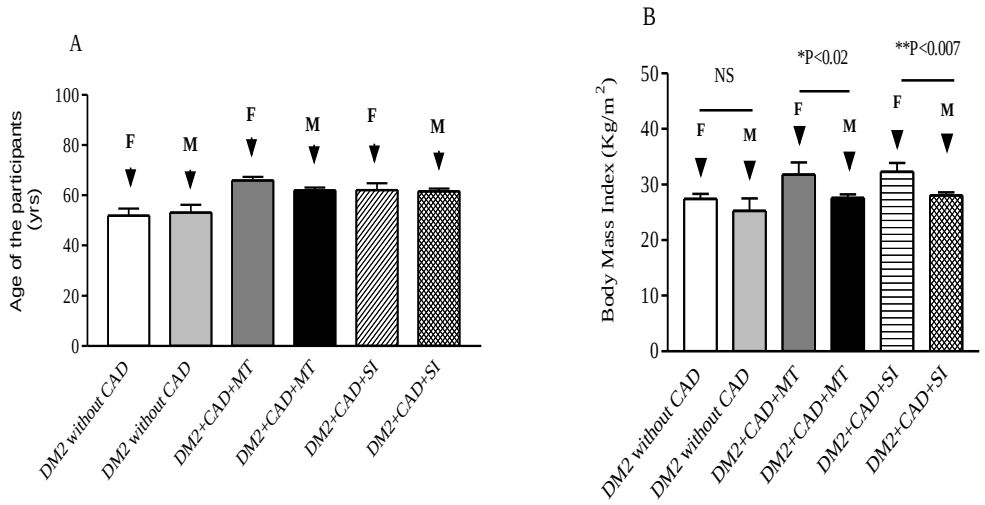


*Figure (1A) There is no statistical difference between the ages of participants in all the related groups in both genders (age-matched). Although there is no statistical significance in the Body Mass Index (kg/m$^2$) in DM2 without CAD. However, there is statistical significance in DM2 with CAD between both genders who are treated medically (*P< 0.002 and treated surgically (**P<0.007), respectively, as shown in Figure (1B). All data are expressed as mean ± SEM.*

In addition, there is no statistical significance in the duration of diabetes between all groups in both females and males. Moreover, the duration of DM2 without CAD between females and males (8.3 ± 2.9) *vs* (10.0 ± 3.9), and the duration of DM2 with CAD either treated medically (8.9 ± 2.2) *vs* (11.6 ± 1.2) or treated invasively (13.3 ± 2.9 *vs* 9.829±1.0) by (PCI and CABG) are insignificant between females and males, respectively as shown in figure (2).

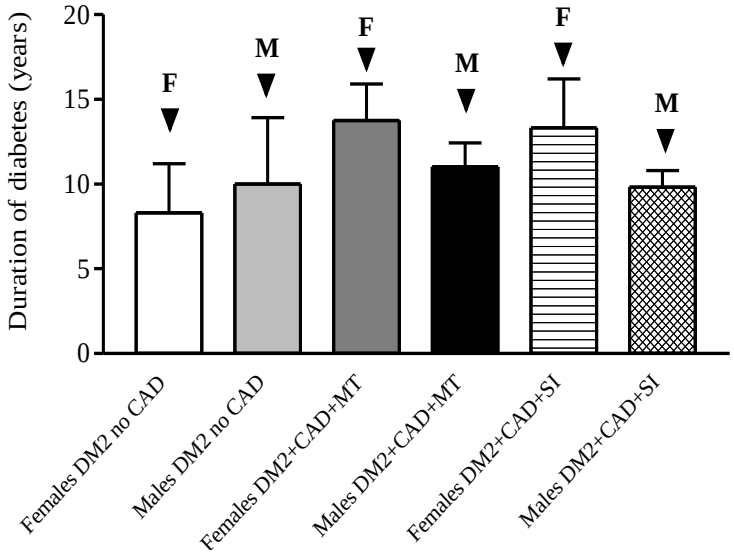


*Figure (2) The duration of diabetes type 2 (DM2) expressed in years in all the groups in both genders involved in this study, did not show any difference in duration of diabetes between all the groups in both genders. All data are expressed as mean ± SEM.*

## Fasting blood sugar and the level of glycated haemoglobin ($HbA_{1c}$) in all groups

There is statistical significance in the fasting blood sugar between healthy controls (HC) and NDM with CAD (85.7 ± 1.9 (n=40) vs 92.4 ± 2.6 (n=43); *P < 0.05), DM2 without CAD (85.7 ± 1.9 (n=40) vs 191.5 ± 14.7 (n=16); ***P < 0.0001), and DM2 with CAD (85.7 ± 1.9 (n=40) vs 197.3 ± 10.0 (n=82); *** P < 0.0001). In addition, there is a statistical difference in the level of fasting blood sugar between NDM with CAD and DM2 without CAD {92.4 ± 2.6 (n=43) vs 191.5 ± 14.7 (n=16); *** P <0.0001} and with CAD {92.4 ± 2.6 (n=43) vs 197.3 ± 10.0 (n=82); ***P<0.0001}. However, there is no statistical difference in fasting blood sugar between DM2wit and without coronary artery disease as shown in Figure (3A).

The trend of $HbA_{1c}$ level is similar finding as the relation to the plasma level of fasting blood sugar between the groups as stated therefore, the P value between (i) HC and

NDM+CAD P> 0.08, DM2 without CAD ***P<0.0001, and DM2 with CAD ***P<0.0001. Once more there is statistical significance between NDM+CAD and DM2 with and without CAD ***P<0.0001, respectively. There is no difference between DM2 with and without CAD P > 0.0764 as seen in Figure (3B)

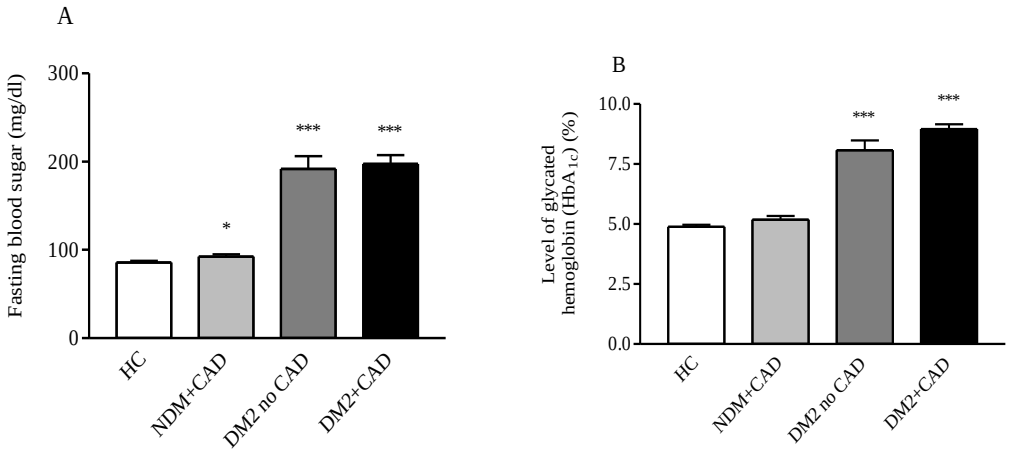


*Figure (3) The plasma level of fasting blood sugar and plasma level of $HbA_{1c}$ between the participants groups. The fasting blood sugar shows statistical significance between healthy controls and NDM+CAD, DM2 without and with CAD with *P<0.05, ***P<0.0001, and ***P<0.0001, respectively. However, there is a significant difference between healthy controls, and DM2 without and with CAD ***P<0.0001 as shown in figure ((3A and 3B). All data are expressed as mean ± SEM.*

## Relationship between diabetes duration and the level of glycated haemoglobin ($HbA_{1c}$) in all groups

Although there is a strong positive correlation between the duration of diabetes mellitus and the level of glycated haemoglobin ($HbA_{1c}$) in diabetic type 2 without the presence of coronary artery disease {r=0.734, with **P<0.002} in both genders. Once more, the data show no correlation between the duration of diabetes and the level of $HbA_{1c}$ in the diabetic patient with CAD receiving medical therapy {r=0.116, P>0.4} or who had surgical intervention {r=0.174, P>0.2} in females and males as shown in figure (4).

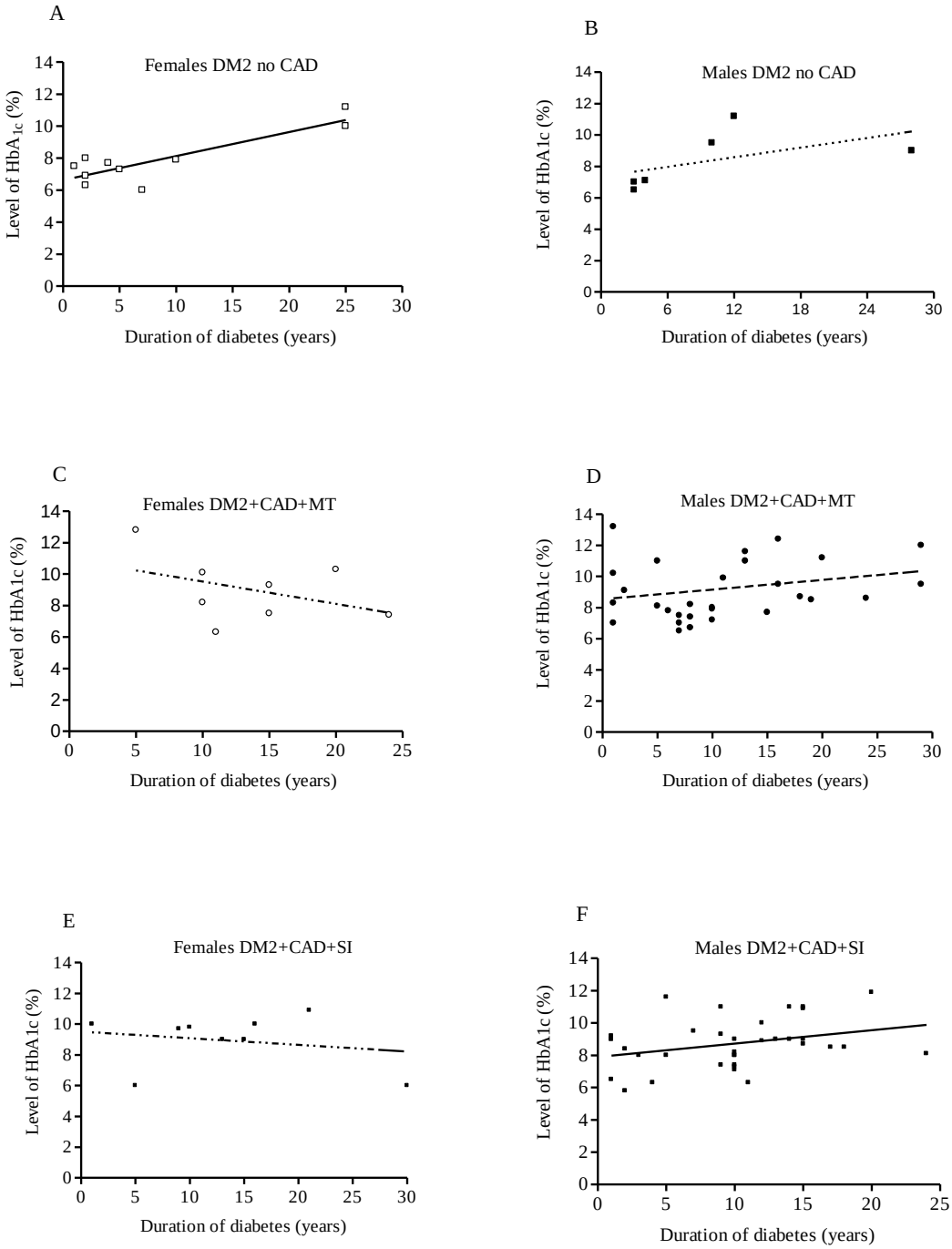


*Figure (4) There is a strong relationship between the duration of diabetes and glycated haemoglobin (%) in females DM2 without CAD (r =0.734; **P<0.002), but there is no correlation between the duration of diabetes and HbA1c level in diabetics with coronary artery disease on medical therapy P>0.4, and the group who had surgical intervention (PCI and CABG) P>0.2.*

## Plasma level of Lp-PLA$_2$ between healthy controls and DM2 with and without CAD

There is statistical significance in the plasma level of Lp-PLA$_2$ between (i) healthy controls (HC) and NDM with CAD (144.6 ± 14.2 (n=40) *vs* 263.3 ± 13 (n=43); ***P< 0.0001). (ii) DM2 without CAD and HC (144.6 ± 14.2 (n=40) *vs* 206.2 ± 16.9 (n=16); *P < 0.02). (iii) DM2 with CAD and HC (144.6 ± 14.2 (n=40) *vs* 273.6 ± 12.3 (n=82); ***P< 0.0001). (iv) DM2 with and without CAD (206.2 ± 16.9 (n=16) *vs* 273.6 ± 12.2 (n=82); *P< 0.03). However, there is no statistical difference between DM2 and NDM with CAD (263.3 ± 13 (n=43) *vs* 273.6 ± 12.3 (n=82); P > 0.6) as shown in Figure (5), respectively. All the experiments are measured in triplicate.

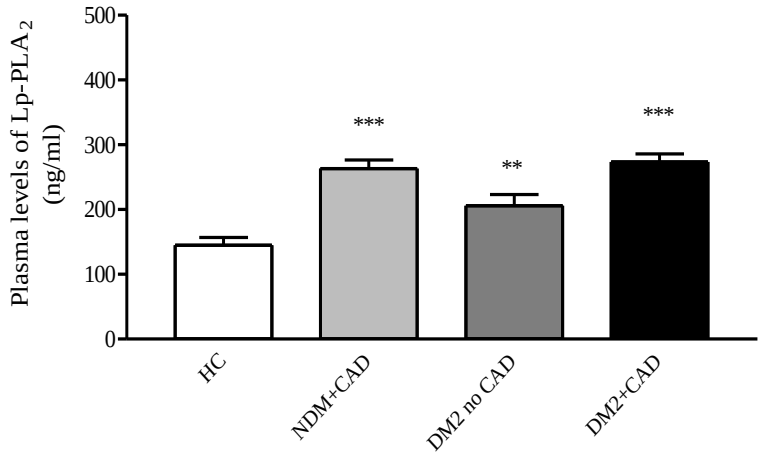


*Figure (5) There is a statistical significance for plasma level of Lp-PLA$_2$ between healthy controls and NDM with CAD ***P<0.0001, and also between healthy control and both DM2 without and with CAD **p<0.008 and ***P<0.0001, respectively. However, there is no statistical difference between DM2 and NDM with CAD P>0.6. The normal value of plasma Lp-PLA2 is <200 ng/ml. The data is represented as Mean ± SEM.*

## Relationship between Plasma levels of Lp-PLA$_2$ concerning the age of participant groups

There is a strong relation between the plasma levels of Lp-$PLA_2$ in age groups < 60 years old. The plasma level of Lp-$PLA_2$ is statistically significant between healthy controls and non-diabetic with coronary artery disease (120.3 ± 14.7 (n=30) *vs* 230.0 ± 25.9 (n=14); ***P < 0.0001), and also between healthy controls and both DM2 without CAD (120.3 ± 14.7 (n=30) *vs* 263.5 ± 39.4 (n=8); **P < 0.008) and DM2 with CAD (120.3 ± 14.7 (n=30) *vs* 269.7 ± 20.4 (n=32); ***P<0.0001) among age groups < 60 years old, respectively. However, there is no statistical significance in plasma levels of Lp-$PLA_2$ between healthy controls, NDM with CAD, and DM2 without and with CAD in the age groups > 60 years old as illustrated in Figure (6).

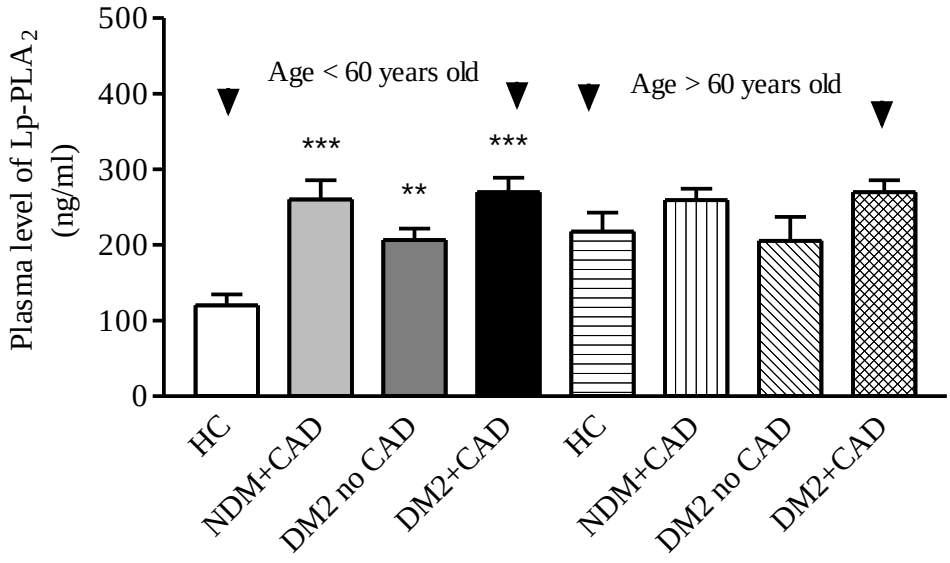


*Figure (6) Relation between age of participants and plasma level of Lp-$PLA_2$. The groups were divided into two groups according to their ages. The individuals are subdivided into both < 60 years and > 60 years old. The plasma levels of Lp-$PLA_2$ show statistical significance between healthy controls and NDM with CAD with ***P <0.0001. In addition, there is a strong statistical difference between HC and both DM2 without and with CAD **P <0.008, and ***P <0.0001 in the age group < 60 years old, respectively. However, there is no statistical significance in plasma Lp-$PLA_2$ levels in the age groups of > 60 years old.*

## Relationship between plasma level of Lp-PLA$_2$ and duration of diabetes mellitus

There is no relationship between the duration of diabetes and Plasma level of Lp-PLA$_2$ in DM2 without coronary artery disease (r = 0.21, P > 0.4) and DM2 with CAD (r = 0.054, P > 0.6), respectively as shown in figure (7A and 7B).

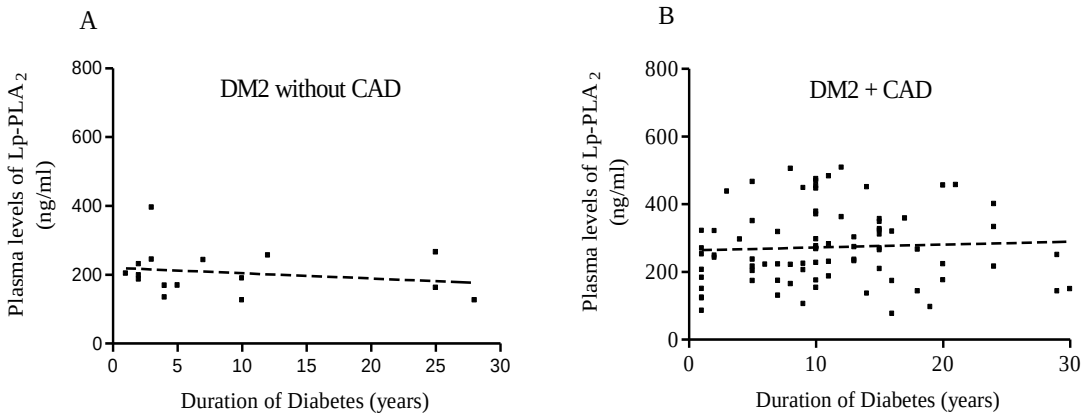


*Figure (7) The data show there is no relationship between the duration of diabetes and plasma levels of Lp-PLA$_2$ in diabetic type 2 without and with coronary artery disease as shown in Figures (7A and 7B).*

## Relationship between fasting blood sugar and plasma level of Lp-PLA$_2$

Although there is no correlation between the fasting blood sugar and Plasma level of Lp-PLA$_2$ in healthy controls (HC) {r = 0.082, (n=40); P > 0.6} and non-diabetic patients with coronary artery disease {r = 0.059, (n=43); P> 0.7} as shown in figure (7A and 7B). However, there is statistical significance in DM2 without coronary artery disease {r=0.954, (n=16); ***P < 0.0001} and DM2 with CAD with {r = 0.237, (n=82); *P < 0.04} respectively as shown in figure (7C and 7D).

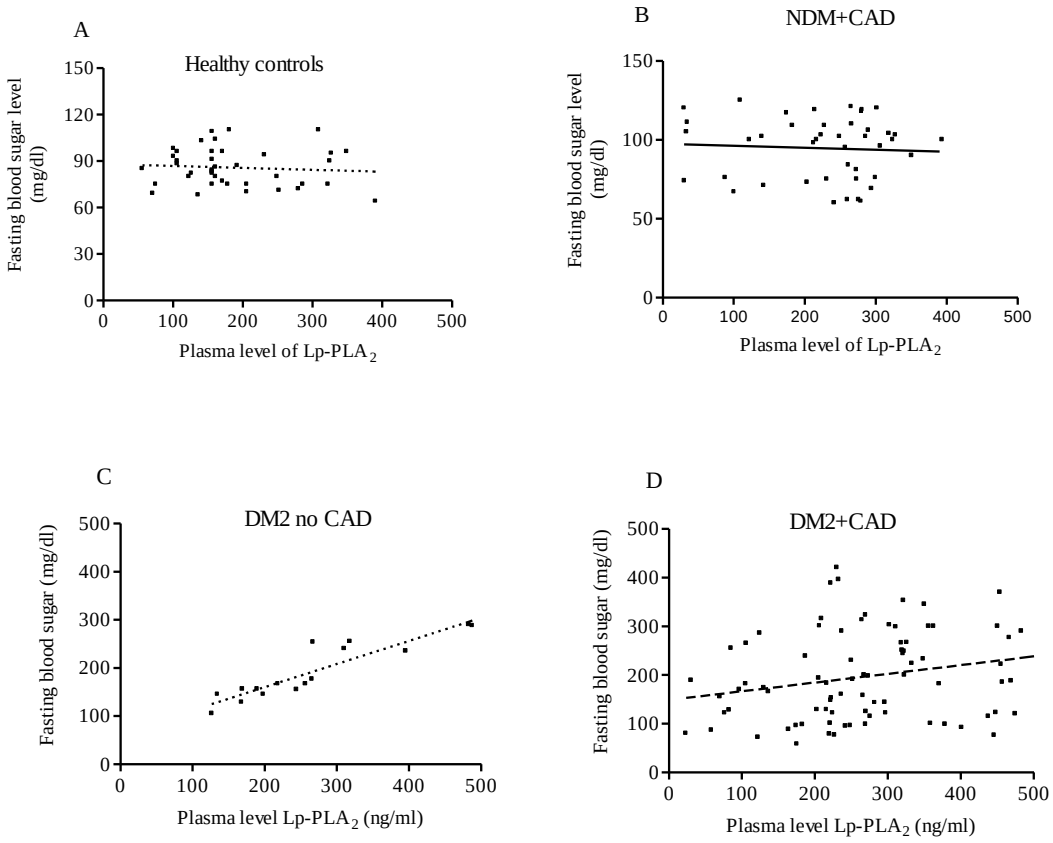


*Figure (8) Relationship between fasting blood sugar and plasma level of Lp-PLA2 between all the groups participating in this study. There is no statistical significance between fasting blood sugar and plasma level of Lp-PLA2 in healthy controls as shown in (Figure 8A) and non-diabetics with CAD as described in (Figure 8B). Besides there is a statistical difference seen in both DM2 without CAD demonstrated in figure (8C) and DM2 with CAD illustrated in figure (8D) with ***P <0.0001 and *P < 0.04, respectively.*

## The difference between level C-reactive protein in healthy controls, diabetic type 2 and patients with CAD

There is a statistical difference in the level of C-reactive protein (CRP) between healthy controls and NDM+CAD {2.5 ± 0.3 (n=40) *vs* 4.5 ± 0.4 (n=43), ***P<0.0001}, DM2 without {2.5 ± 0.3 (n=40) vs 5.4 ± 0.8 (n=16) ***P<0.0001}and with coronary artery disease (2.5 ± 0.7 (n=40) vs 5.7 ± 0.4 (n=82); ***P<0.0001), respectively as shown in

Figure (9). In addition, there is statistical significance between non-diabetic and type 2 diabetic patients with CAD {4.5 ± 0.4 (n=43) vs 5.7 ± 0.4 (n=82); *P <0.05}. However, there is statistical insignificance between NDM+CAD and DM2 without CAD, and also between DM2 with and without CAD with {P>0.2 and P>0.7}, respectively.

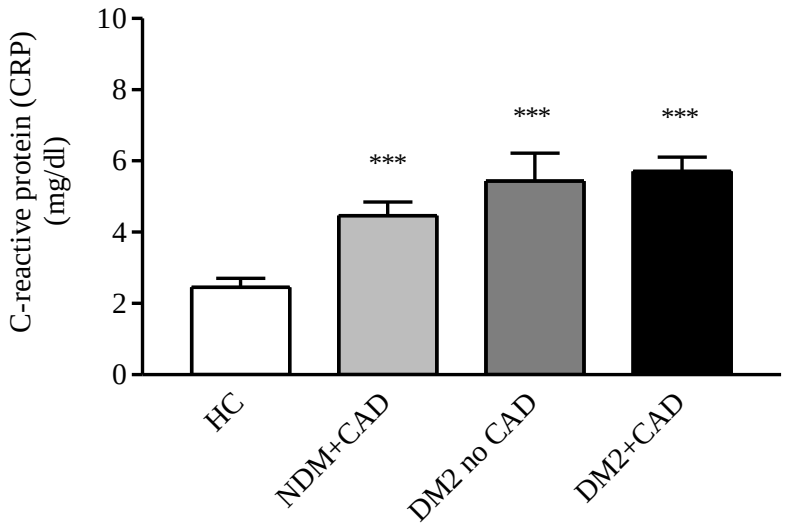


*Figure (9) There is a statistical difference in the level of C-reactive protein (CRP) between healthy controls and NDM+CAD, DM2 without and with coronary artery disease with ***P < 0.0001), and also between NDM+CAD and DM2+CAD with *P<0.05. However, there is no statistical significance in CRP level in the other groups as shown in the figure (8). Data expressed as mean ± SEM*

## The level of Triglyceride Glucose (TyG) index between the participant groups

Although there is no statistical significance in Triglycerides Glucose Index (TyG) between healthy controls and non-diabetic (NDM) with CAD {4.4 ± 0.03 (n=40) vs 4.5 ± 0.03 (n=43); P > 0.2} and also between DM type 2 without and with CAD {4.9 ± 0.09 (n=16) vs 4.9 ± 0.05 (n=82); P > 0.6}. However, there is a significant statistical difference in the TyG index between healthy controls and DM Type 2 without CAD {4.4 ± 0.03 (n=40) vs 4.9 ± 0.09 (n=16); ***P<0.0001} and DM type 2 with CAD {4.4 ± 0.03 (n=40) vs 4.9 ± 0.05 (n=82); ***P<0.0001} and also between NDM with CAD and DM Type 2 without CAD {4.5 ± 0.04 (n=43) vs 4.9 ± 0.09 (n=16); ***P<0.0001} and DM type 2 with CAD {4.5 ± 0.04 (n=43) vs 4.9 ± 0.05 (n=82); ***P<0.0001} as shown in Figure (10)

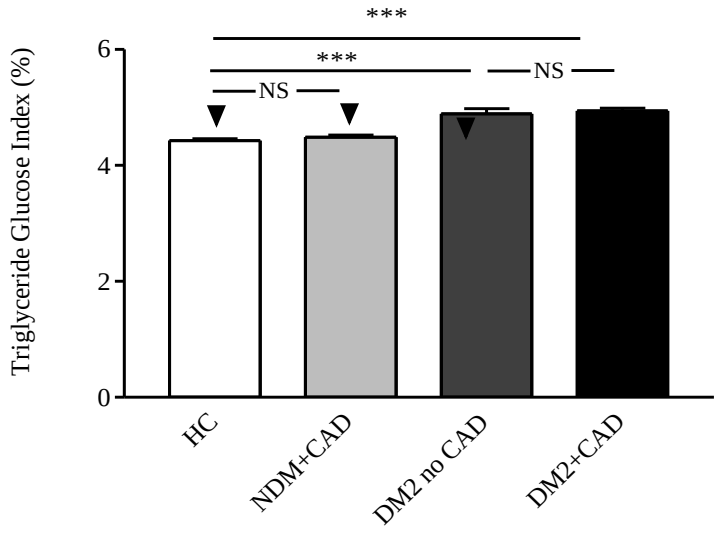


*Figure (10) This graph for the Triglyceride Glucose (TyG) index illustrates the statistical difference between all participating groups. The TyG index was calculated as "Ln(fasting triglyceride level (mg/dl) X fasting glucose level (mg/dl)/2)" and the normal value should be less than 4.49. however, the values > 4.49 indicate insulin resistance. The data represented as Mean ± SEM and ***P<0.001*

## DISCUSSION

The great majority of patients with coronary artery disease (CAD) can be diagnosed with a medical history, electrocardiography (standard or exercise), biochemical analysis, and non-invasive and/or invasive medical techniques. In this study, many risk factors have a significant role in the progression of CAD in the participants, which is why all the necessary measures need to be standardised some of these factors such as age, weight, body mass index, and strategic medical treatment are almost similar between these groups.

Therefore, increasing age is one of the risk factors of coronary artery disease (CAD), and the risk factor of physical sedentary is reflected by the presence of high BMI, and is an independent risk factor for the early incidence of CAD and is strongly associated with the presence of co-morbidities such as insulin resistance, diabetes type 2, hypertension, and hyperlipidemia [(20)].

Additionally, a high level of $HbA_{1c}$ is a critical and more accurate standard for determining the severity of DM than fasting blood glucose levels. $HbA_{1c}$ levels provide a greater advantage by providing a consistent indicator of long-term blood sugar status, which correlates between two to three months of the average plasma glucose concentration [(21)]. Therefore, the significantly high $HbA_{1c}$ levels correlate with acute coronary arteriosclerosis. In its recent position statement, the American Diabetes Association stated that $HbA_{1c}$ reduction may be associated with a decrease in microvascular and macrovascular complications for diabetic patients. Additionally, there is a potential use of $HbA_{1c}$ as a vital sign of hyperlipidemia as well as a potential indirect indication of CVD risk in patients with diabetes type 2 [(22,23)].

High fasting blood glucose and triglyceride levels are two components of metabolic syndrome, which is one of the most important risk factors for CAD [(24)]. Hypertriglyceridemia deteriorates diabetes by impairing the function of β-cell and causing peripheral insulin resistance (IR) [(25)]. The triglyceride-glucose (TyG) index combines both levels of triglycerides and fasting blood glucose, and it has been reported to be significantly correlated with insulin resistance and to be a reliable marker of insulin resistance [(26)]. Our data revealed that the TyG index is high in a few individuals in the NDM with CAD group, and those individuals may develop diabetes mellitus shortly.

This study determined the pivotal role in the measurement of CRP and Lp-$PLA_2$ in healthy controls and non-diabetic and diabetic type 2 with and without coronary artery disease were age-matched. In addition, the controllable and unchangeable risk factors such as male gender, the elderly, ethnicity, and family history of CAD were assessed, and the percentage of smokers in diabetic patients without CAD revealed (12.5%) and those with diabetes with CAD were (17.1%).

Studies are showing that lipoprotein metabolism, not only in type 2 diabetes but also in impaired glucose tolerance (IGT) tends to show changes such as decreased HDL and increased triglyceride levels, which may be associated with increased insulin and increased insulin resistance. High levels of triglycerides and fasting glucose are two components of metabolic syndrome, which is one of the most important risk factors for cardiovascular

disease [(27)]. The triglyceride glucose index (TyG) combines both triglyceride levels and fasting glucose and its strong association with insulin resistance and is a reliable mark of insulin resistance has been reported. These results were similar to the data shown in this study. Although obesity is a risk factor for CAD, the findings in this study showed a strong relationship between BMI and the TyG index, which is a major risk factor for CAD development [(28)].

However, it remains unclear whether there is a direct mechanistic link between Lp-$PLA_2$ function and atherosclerosis. The complexity of this issue is related to the fact that the catalytic products of the enzymatic reaction catalysed by Lp-$PLA_2$ namely lysophosphatidylcholine (lysoPC) and oxidized fatty acids, as well as Lp-$PLA_2$ substrates, including oxidized phospholipids and a platelet-activating factor that can be considered a proangiogenic factor. Although Lp-$PLA_2$ is secreted by macrophages, hepatocytes, and platelets, and mainly co-circulates with LDL, HDL, and Lp(a), and whether these native and oxidized forms of lipoproteins affect Lp-$PLA_2$ expression by macrophages and hepatocytes is largely unknown. Moreover, the specific role of Lp-$PLA_2$ in lipoprotein metabolism remains unclear.

Increased disease duration is associated with increased plasma $HbA_{1c}$ levels in diabetic without CAD and increases the risk of death with coronary heart disease supported by data from (*Sheng et al., 2019*) in two main outcomes: (i) patients with diabetic duration of $\geq 10$ years with higher $HbA_{1c}$ levels than those with a diabetes duration of < 10 years, and (ii) patients with DM who have been with STEMI, especially those with diabetic duration periods of $\geq$ 10 years, have a higher prevalence of lipid-rich plaques, a thin-covering fibroma (TCFA), and plaque rupture from those without diabetes. Furthermore, a study by (*Sacks et al., 2002*) suggested the effect of pravastatin and rosuvastatin in improving HDL and triglycerides in patients with coronary heart disease who have high LDL concentrations [(29,30,31)].

Some researchers are exploring whether treatment to lower Lp-$PLA_2$ levels will reduce a person's risk of CAD and ischemic stroke. However, several studies on selective inhibitors of Lp-$PLA_2$ (such as darapladib) found that inhibition leads to atherosclerotic protection

and reduces the risk of CAD [19]. Therefore, the Lp-$PLA_2$ test may be ordered more frequently and can be used to monitor a person's response to treatment. Further studies with larger sample sizes and more extended time frames for both medical or surgical intervention and follow-up are needed to validate the role of Lp-$PLA_2$ in the morbidity and mortality of CAD. Therefore, the Lp-$PLA_2$ activity test may be performed on individuals at intermediate or high risk for developing cardiovascular disease (CAD).

A study by (*Spiezia et al., 2018*) found that patients with type 2 diabetes showed increased platelet reactivity compared to patients without diabetes, despite combined treatment with clopidogrel and aspirin. An increase in clopidogrel dose was not sufficient to reduce increased platelet reactivity in patients with DM2, highlighting the need for further investigation of other anti-platelet drugs in this population [32-36].

## CONCLUSION

In conclusion, LP-PLA2 is a relatively new inflammatory marker, but more evidence indicates that it plays an important role in the occurrence and development of coronary atherosclerosis. The current study also shows that LP-PLA2 activity is positively correlated to the seriousness of CAD, and this can provide a strong basis for predicting the occurrence and prognosis of CAD, at the same time, it may provide a new way to treat CAD in the future

## 4.1 Limitations

We are aware that this study has some potential limitations. Some of the medication records of the patients have not been indicated in the present study. Since Lp-$PLA_2$ is considered a marker of vascular inflammation, which might be decreased by some medication (e.g., statin use), the effect of statin and other medications therapy on lipoprotein-associated phospholipase $A_2$ levels will be conducted in the Libyan population in the future. Furthermore, we did not determine Lp-$PLA_2$ activity; thus, the interaction between plasma Lp-$PLA_2$ activity and classical risk factors remains uncertain; However, previous studies have shown a strong correlation between Lp-PLA2 concentration and activity. Therefore,

we propose the novel hypothesis that the synergism of Lp-$PLA_2$ activity with classical risk factors in the risk of CAD may be significant.

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