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AStudy on APO B100/APO A1 Ratio as A Predictive Parameter for Assessmentof CAD Risk in Uncontrolled Type 2 Egyptian Diabetic Patients

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ABSTRACT

Background: this study was undertaken to study the biochemical effects of lipoproteins and apolipoproteins on uncontrolled Egyptian patients with type 2 diabetes mellitus, and to study if Apo B100/Apo A1 ratio can be used as a predictive factor for assessment of CAD risk in this patients.

Patients & Methods: this study was carried out on a group of 60 patients; 20 were non diabetic controls and 40 diabetic patients, who were subdivided into : controlled type 2 diabetic patients (Group I) and uncontrolled type 2 diabetic patients (Group II), based on their glycated hemoglobin level. Levels of triacylglycerols or triglycerides (TG), total cholesterol (TC), HDL-C, Apolipoprotein A1 and Apolipoprotein B100, in addition to fasting blood glucose (FBG) and glycated hemoglobin (HbA_{1c}) were estimated,and also, levels of LDL-C were calculated by Friedewald's formula in all studied cases. Blood samples were collected after an overnight fasting of 10-12 hours, and divided into two parts, the 1st for separation of serum and the 2nd collected in an EDTA-containing tube for HbA_{1c} determination. **Results:** the obtained results revealed that, levels of total cholesterol, LDL-C and Apo B100 were comparable with no statistical significance between uncontrolled type 2 diadetic patients (group II) with controlled type 2 diabetic patients (group I) and control group.While, a highly significant increase in triacylglycerols levels in group II compared to group I and control group. In contrast, a highly significant decrease in HDL-C and Apo A1 levels in group II compared to group I and control group. Highly significant increase in Apo B100/Apo A1 ratio in group II compared to group I, and a significant increase compared to control group, while it was comparable with no statistical significance in group I compared to control group.

Conclusion: this study showed that there was an adverse effect of prolonged hyperglycemia on the apolipoproteins in diabetic patients which is associated with dyslipidemia, good glycemic control is able to make an improvement on this dyslipidemia. Apo B100/Apo A1 ratio can be used as a predictive parameter for assessment of CAD risk in type 2 diabetes mellitus.

Key words: Type 2 diabetes mellitus, Apo B100/Apo A1 ratio, CAD.

INTRODUCTION

Diabetes mellitus is a heterogeneous chronic metabolic disorder, which is due to combination of insulin resistance and relative insulin deficiency due to pancreatic β cell failure, and characterized by hyperglycemia which has a lot of various life threatening complications like atherosclerosis, retinopathy and nephropathy. Type 2 DM often have both quantitative and qualitative abnormalities of lipoproteins that are responsible for increased incidence of coronary artery disease (CAD), which is three to four folds higher in patients with type 2 DM compared to non diabetics.

The most characteristic lipid abnormalities in non-insulin-dependent DM are hypertriglyceridaemia, increased VLDL cholesterol concentration and decreased HDL levels, which frequently remain unchanged even upon the proper treatment of glucose metabolism. The alterations are related to increased free fatty acid levels and decreased glucose uptake resulting from elevated insulin levels [(1)]. Also, lipoprotein oxidation (especially LDL-C) are more common in diabetes and are associated with a poor glycaemic control.

Apo A1 which is the protein component of HDL-C, and ApoB 100 which is the protein component ofLDL-C, have been reported that they are better predictors of atherosclerotic diseases more than the routine lipid and lipoprotein analysis[(2)]. And, the Apo B100/Apo A1 ratio has been described to be a better parameter for the prediction of CAD better than any other lipids, lipoproteins and its ratios [(3)].

Little studies worked on this Apolipoproteins and Apo B100/Apo A1 ratio in diabetic patients. So, this study was undertaken to study the biochemical effects of lipoproteins and apolipoproteins on uncontrolled Egyptian patients with type 2 diabetes mellitus, and to study if Apo B100/Apo A1 ratio can be used as a predictive

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factor for assessment of CAD risk in these patients.

PATIENTS AND METHODS

A group of 40 type 2 diabetic patients who were attending the biochemistry lab. at Alarish hospital, North Sinai, Egypt, included in this study, in addition to 20 healthy non-diabetic controls (Fasting blood glucose less than 107 mg/dl), while the diabetic cases has FBG more than 126 mg/dl, who were further sub-divided into two groups; group I had 20 patients with HbA_{1c} level up to 8% of totalhaemoglobin (Hb), group II had 20 uncontrolled diabetic patients with HbA_{1c} levels of more than 8% of total Hb. In order to study the biochemical effects of apolipoproteins on uncontrolled type 2 diabetic patients. Exclusion criteria included diabetic patients with overt complications like neuropathy, nephropathy, retinopathy and ischemic heart disease, patients with acute complications like diabetic ketoacidosis, non ketosis hyperosmolar coma and hypoglycemia, patients with any concurrent illness like chronic liver disease, patients on drugs like diuretics, steroids and lipid lowering agents, and finally smokers and hypertensive patients.

Biochemical Determinations

Blood samples were collected after an overnight fasting of 10-12 hours, about 4 -5 ml of whole blood was collected via venus puncture with the help of a disposable syringe and then divided into two parts; The 1st part collected and centrifuged at 3000 rpm for 10 min. to separate serum, The 2nd part of venous blood collected in an EDTA-containing tubefor HbA_{1c} determination.

Levels of total cholesterol (TC) (CHOD-PAP method)[6], triglyceride (TG) (GPO-PAP method)[7], HDL-C (enzymatic method)[8], apo A1 and apo B100 (Radial Immunodiffusion (RID) plates), in addition to fasting blood glucose levels (FBG) (Glucose oxidase-peroxidase method)[4] and glycated hemoglobin (HbA_{1c})[5]were determinated in all cases of this study, also, LDL-C levels were calculated using Friedwald's formula[9].

Statistical analysis

Statistical differences were calculated with t-test using the SPSS software (version 15.0). Values are expressed as mean± st. dev., and a *P*-value < 0.01 was considered significant.

RESULTS

Lipid profile alterations in type 2 diabetic patients

As depicted in figures (1) and (2), levels of total cholesterol were comparable with no statistical significance between uncontrolled type 2 diadetic patients with controlled type 2 diabetic patients and control group, and also, between controlled type 2 diabetic patients with control group. There was a highly significant increase in triacylglycerols levels in uncontrolled type 2 diadetic patients compared to controlled type 2 diadetic patients and control group, also a highly significant increase in triacylglycerols levels in controlled type 2 diabetic patients compared to control group.In contrast, HDL-C levels were significantly lower in uncontrolled type 2 diabetic patients compared to controlled type 2diabetic patients and control group, also a highly significant decrease in HDL-C levels in controlled type 2 diabetic patients compared to control group. But the LDL-C levels between the three groups were comparable with no statistical significance.

Apolipoprotein B100 and Apolipoprotein A1

As depicted in figures (3) and (4), there was a highly significant decrease in Apo A1 levels in uncontrolled type 2 diabetic patients compared to controlled type 2 diabetic patients and control group, also a highly significant decrease in Apo A1 levels in controlled type 2 diabetic patients compared to control group. But, the mean Apo B100 levels between the three groups were comparable with no statistical significance. So, there was a highly significant increase in Apo B100/Apo A1 ratio in uncontrolled type 2 diabetic patients compared to controlled type 2 diabetic patients, and a significant increase compared to control group, while it was comparable with no statistical significance in controlled type 2 diabetic patients compared to control group.

DISCUSSION

Lipid profile alterations in type 2 diabetic patients

The obtained results were in agreement with what [10] reported that dyslipdemia in DM is affected by the type of DM and glycemic conditions, the characteristics of dyslipidemia in DM, especially in NIDDM are the increase in triacylglycerols accompanied by the decrease in HDL-C levels.

Also, [11] observed that In females with type 2 DM, the levels of FBG and triacylglycerols were significantly elevated, while HDL-C levels were significantly decreased as compared with those in non-diabetic females.

Similarly, LDL-C : HDL-C ratio increased in the diabetic patients compared to non-diabetic patients as [12] reported.

Regarding triacylglycerols only, [13] informed that poor glycemic control in patients with type 2 DM was related to higlytriacylglycerols levels. Also, [14] reported that severe hypertriglyceridaemia is associated with hyperglycaemia in heterogeneous group of patients with Type 2 DM.

Furthermore, the results of this study was completely in agreement with what [15] reported that Poorly controlled diabetic cases had significantly lower levels of HDL-C and significantly higher levels of triacylglycerols compared to highly controlled diabetic patients. But, total cholesterol and LDL-C levels were comparable in both groups. And also, what [16] reported, that there was highly significant increase in triacylglycerols and decrease in HDL-C in type 2 DM when compared to control group, and there was no significant change in total cholesterol levels between the two groups, but there was a difference that LDL-C increases significantly whereas, it was comparable with no statistical significance in this study.

[17] examined the impact of glycemic control on the lipid profile of diabetic patients and informed that there was a highly significant correlation between HbA_{1c} and FBG. Both HbA_{1c} and FBG exhibited direct correlations with total cholesterol, triacylglycerols and LDL-C. But, inverse correlation with HDL-C. There was a linear relationship between HbA_{1c} and dyslipidemia. The levels of serum total cholesterol and triacylglycerols were significantly higher and of HDL-C significantly lower in patients with worse glycaemic control as compared to patients with good glycaemic control.

[18] compared the lipid profile of diabetic patients and healthy controls, and the result was that the mean total cholesterol, triacylglycerols, LDL-C and the fasting blood sugar levels were highly significant in the diabetics as compared to those in the controls. The correlation studies showed a non significant negative correlation of FBG with HDL-C and a positive correlation of FBG with total cholesterol, triacylglycerols and LDL-C.

Other studies carried out on patients with coronary artery diseases (CAD) as [19] study, which informed that type 2 DM is an important factor in predicting factors for CAD development, and confirmed that the patients had higher cholesterol, triacylglycerols, LDL-C and decreased HDL-C.

From studies carried out by [17] and [20], which clarified that there was a highly significant correlation between HbA_{1c} and FBG, and recommended that HbA_{1c} is not only a useful biomarker of long-term glycemic control, but also a good predictor of lipid profile. Thus, monitoring of glycemic control using HbA_{1c} could have additional benefits of identifying diabetic patients who are at a greater risk of cardiovascular complications. So, HbA_{1c} had been choosen as a good factor for classification of the studied cases in accordance with fasting blood glucose level into control group, controlled type 2 diabetic patients and uncontrolled type 2 diabetic patients, as done in this study.

All studies confirmed that levels of triacylglycerols increases significantly in uncontrolled type 2 diabetic patients or poorly glycemic controlled compared to controlled type 2 diabetic patients or highly glycemic controlled and control group, and this is in consistent with the results of this study, and this is due to that the most characteristic feature of diabetic dyslipidemia is hypertriglyceridemia which is characterized by increased chylomicron and VLDL in serum depending upon the glycemic control [21],[22]. And this proves that with glycemic control and treatment, levels o triacylglycerols decreases gradually and approaches the normal value.

Also, HDL-C levels decreases in all studies that carried out in diabetic patients compared to non-diabetic and also, from poorly glycemic controlled to highly glycemic controlled, and this is in agreement with this study, which may be because of animpairment of VLDL lipolysis, the activity of hepatic lipase increased which a direct influence on HDL-C clearance and alterations inHDL-C composition which includes non enzymatic glycosylation, which may be operative particularly in patients with hypertriglyceridemia [22], [23].

The observed differences in total cholesterol and LDL-C, as in this study, they were comparable with no statistical significance, but in other studies, they were increased significantly in uncontrolled type 2 diabetic patients compared to controlled type 2 diabetic patients and control group, could be attributed to the different genetic backgrounds, different study population and environmental differences. As an example, levels of total cholesterol were comparable with no statistical significance between all study groups in [16] and [15] studies which were carried out on an Indian population, while it was increased significantly in poor glycemic control compared to good glycemic control in [17] and [20] studies which were carried out on Saudi Arabian population.

Apolipoprotein B100 and Apolipoprotein A1

The obtained results were in consistent with [15] study, which reported that Poorly controlled diabetic cases had significantly lower levels of Apo A1, but Apo B100 levels were comparable in both the groups. Apo B100/Apo A1 ratio was significantly elevated in poorly controlled diabetics.

Also, these results were in agreement with [11] study, which investigated the changes of the apolipoproteins (apo A I, AII, B100, C II, C III and E) in patients with type 2 DM regarding Apo A1, and reported that in male type 2 diabetic patients, the levels of Apo A1 were significantly lower than those in male non-diabetic subjects.

On the contrary regarding Apo B100 levels, as it were compared in patients with coronary heart diseases (CHD) with patients without CHD, and these levels were significantly higher.

The main aim of this study is to estimate the Apo A1 and Apo B100 levels, and study their effect with other lipids and lipoproteins in the diabetic patients according to their glycemic control, this two apolipoproteinswhich are the major protein components of HDL and LDL respectively have showed highly significant correlations with them as depicted in figures (5) and (6).

A significant decrease in Apo A1 levels were observed in uncontrolled diabetic patients compared to good controlled diabetic patients. This decrease in Apo A1 ratio is due to the same reasons for HDL-C decrease which described before,as there were highly significant relation between Apo A1 and HDL-C as shown in this study and depicted in figure (5) with correlation coefficient **r = 0.917**.

This suggests that though the apo A1 and HDL-C levels were significantly reduced in diabetics, improvement of glycemic control raises the Apo A1 and the HDL-C levels. However it was also observed that decrease in apo A1 levels was more significant than the decrease in HDL level.

Diabetes mellitus has two opposing effects on LDL-C metabolism, Itmay decreases the LDL-C clearance in order to increase LDL-C levels, and also it may directly removes the VLDL Apo B in order to decrease its levels. The resultant concentration of LDL-C depends upon the relative magnitude of these two opposite processes [22]. In this study, both the LDL-C and the Apo B100 levels in uncontrolled diabetics were comparable to those of controlled diabetics and controls.

There was a highly significant increase in Apo B100/Apo A1 ratio in uncontrolled diabetics compared to controlled diabetics, and a significant increase compared to control group, while it was comparable with no statistical significance in controlled diabetics compared to control group, also, there were a strong positive correlation between this ratio and HbA_{1c} as depicted in figure (7). Therefore it can be assumed that diabetic patients who are under treatment and with well controlled plasma glucose levels, tends to show less chances of developing dyslipidemia.

CONCLUSION

Diabetic patients who were with well controlled plasma glucose levels, tends to show less chances of developing dyslipidemia. As in this study, in well controlled diabetic group, their Apo B100/Apo A1 ratio was almost same as those of controls, therefore we can conlude that, with the prolonged increasing of blood glucose concentration, the diabetics are at higher risks in development of CAD.

The Apo B100/Apo A1 ratio showed a significant correlation with HbA_{1c} indicating the adverse effect of prolonged hyperglycemia on the apolipoproteins, the use of these markers may be the next natural step in assessing the patient risk, and would represent an alternative to the routine lipid markers, So, Apo B100/Apo A1 ratio canbe used as a predictive parameter for assessment of CAD risk in type 2 diabetes mellitus.

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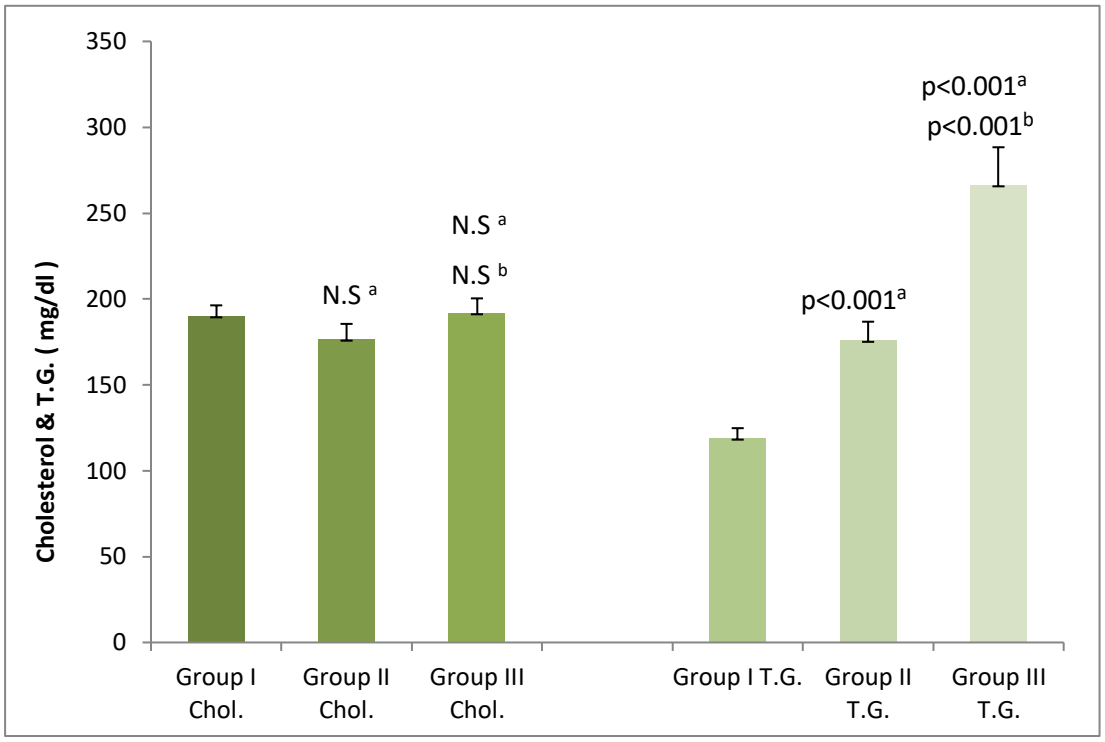

Figure 1 :T.cholesterol and T.G. levels in the different groups of the study (Mean ± St. Error)

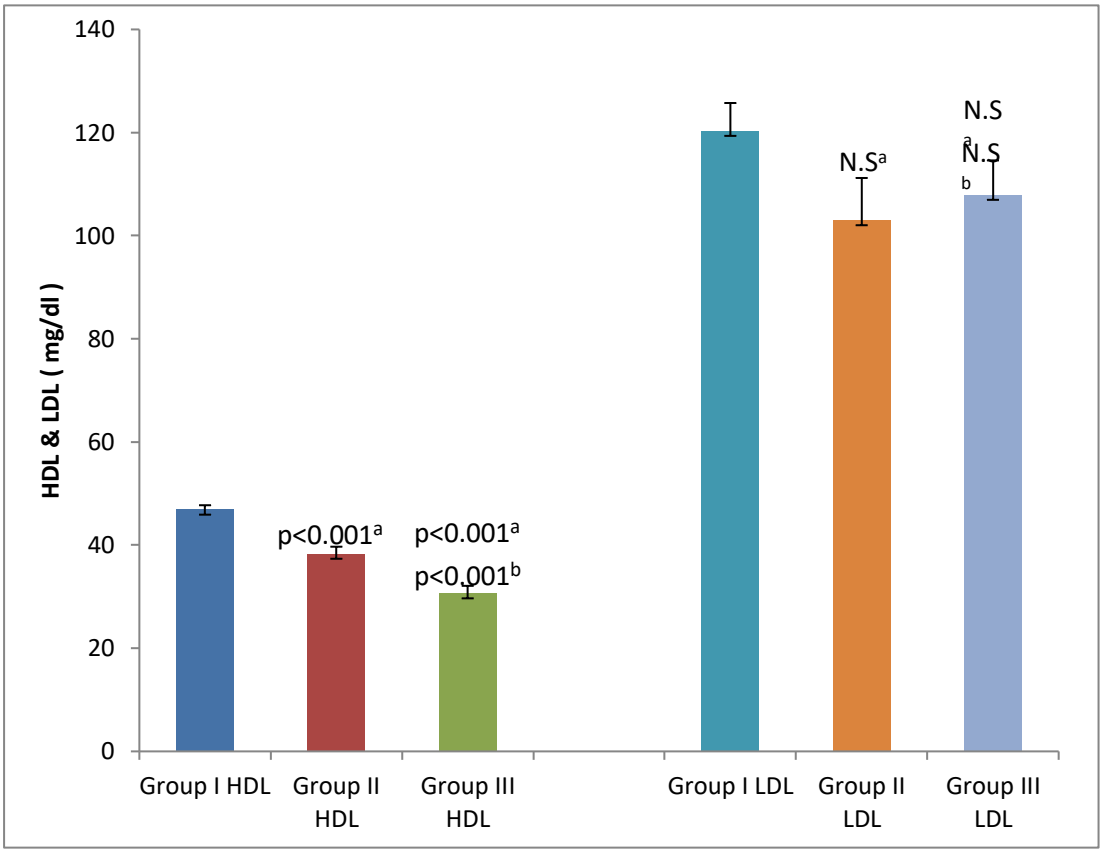

Figure 2 : HDL-C and LDL-C levels in the different groups of the study (Mean ± St. Error)

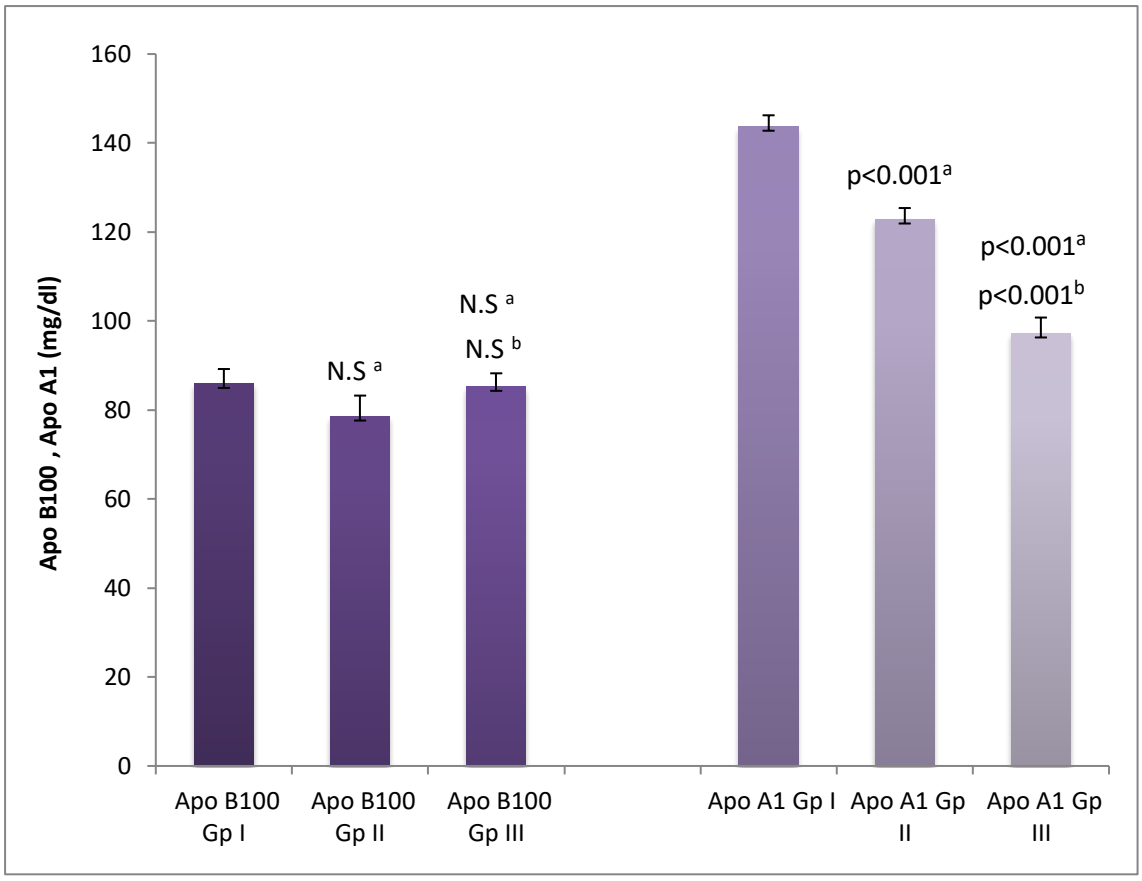

Figure 3 : Apo B100 and Apo A1 levels in the different groups of the study (Mean ± St. Error)

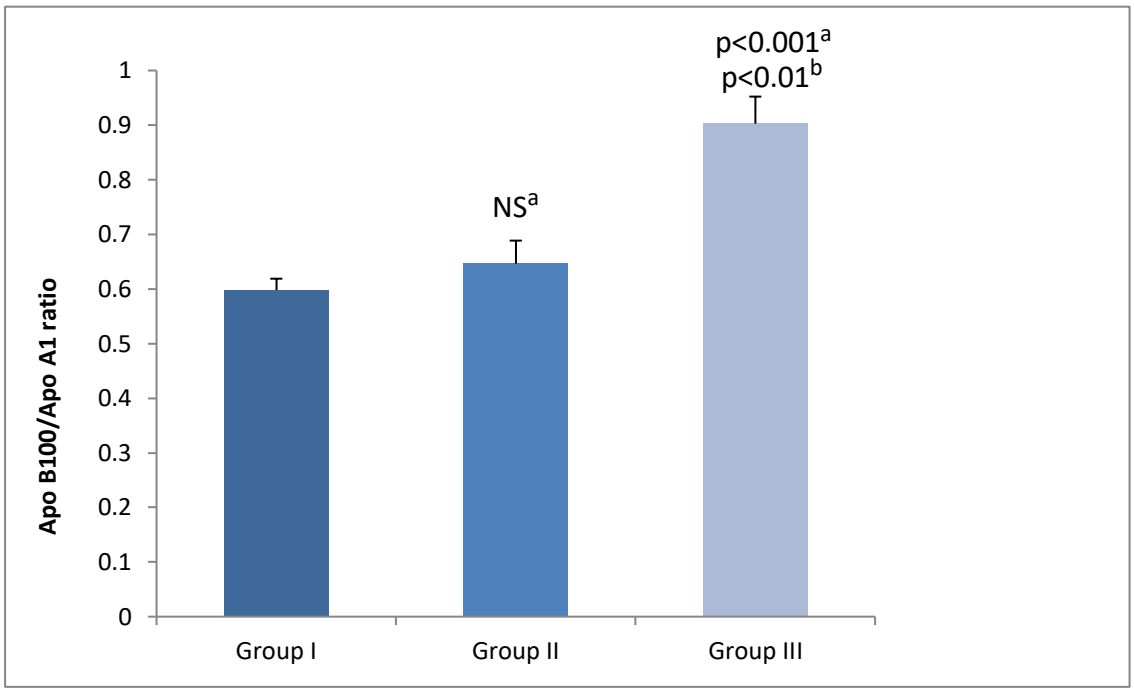

Figure 4 : Apo B100/Apo A1 ratio in the different groups of the study (Mean ± St. Error)

[a] Results are compared to control group (group I)

[b] Results are compared to controlled type 2 diabetic patients (group II)

P < 0.001 is considered highly significant

P < 0.01 is considered significant

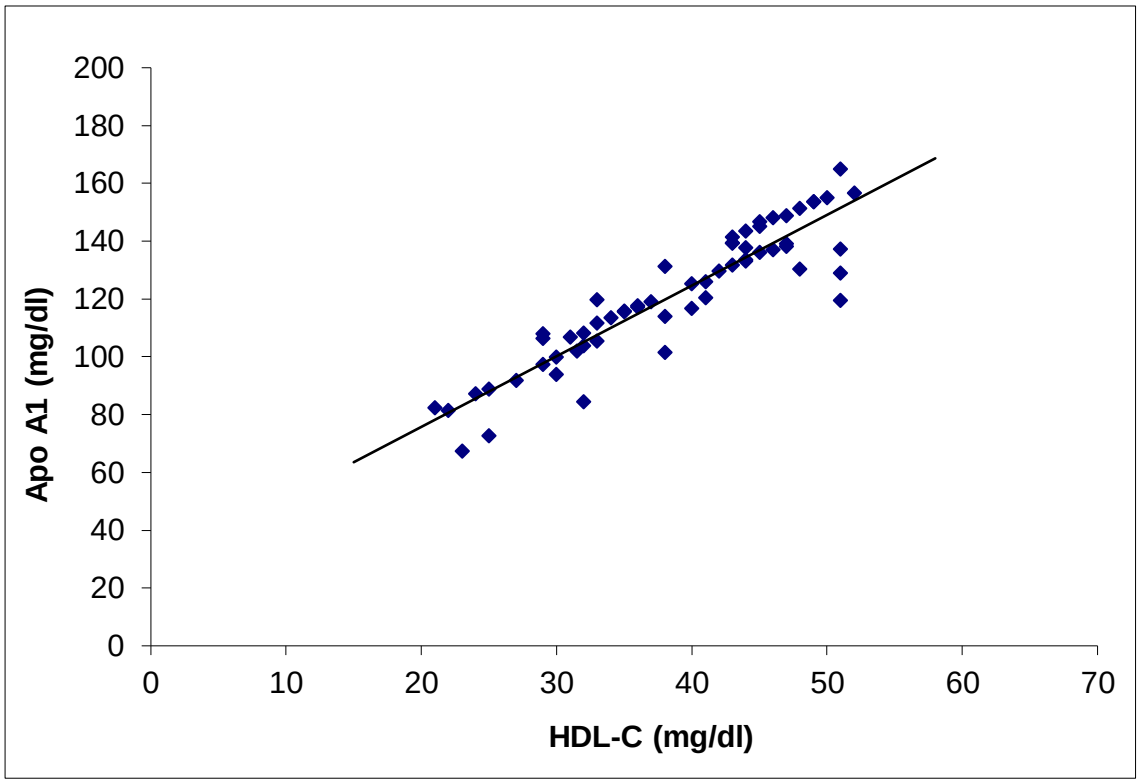

Figure 5 : Correlation between Apo A1 and HDL-C (r = 0.917 , p < 0.001)

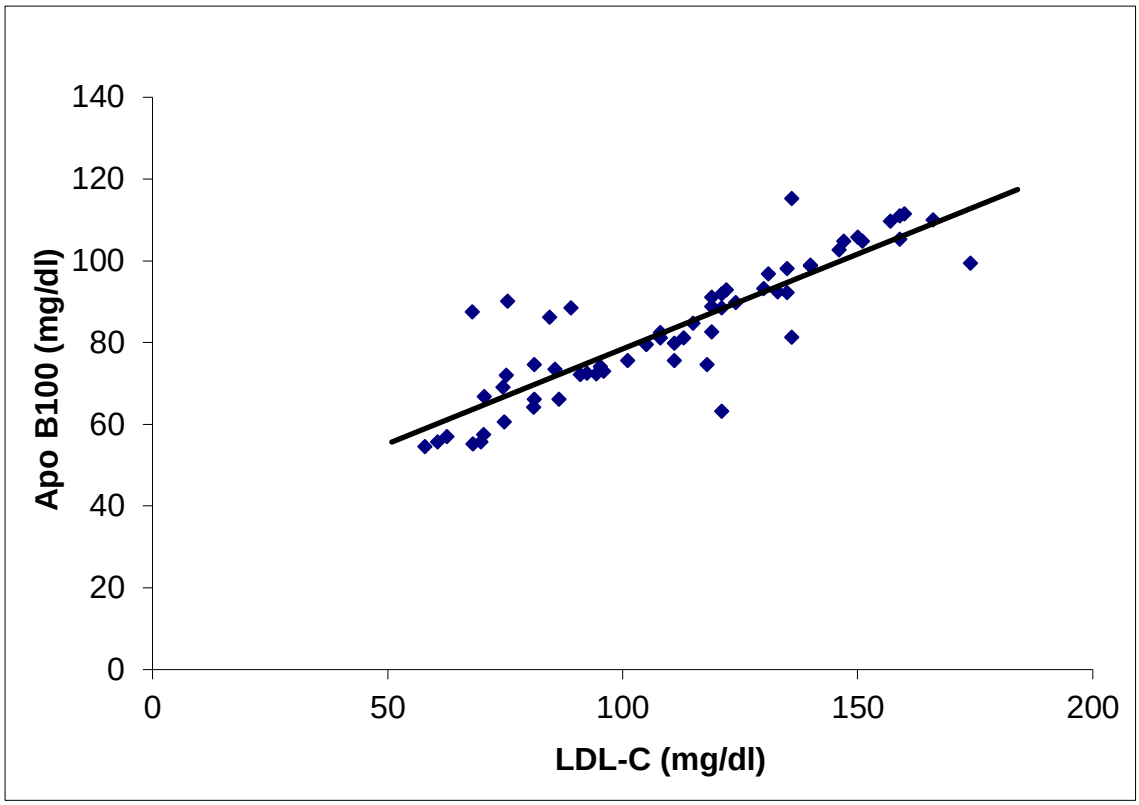

Figure 6 : Correlation between Apo B100 and LDL-C (r = 0.868 , p < 0.001)

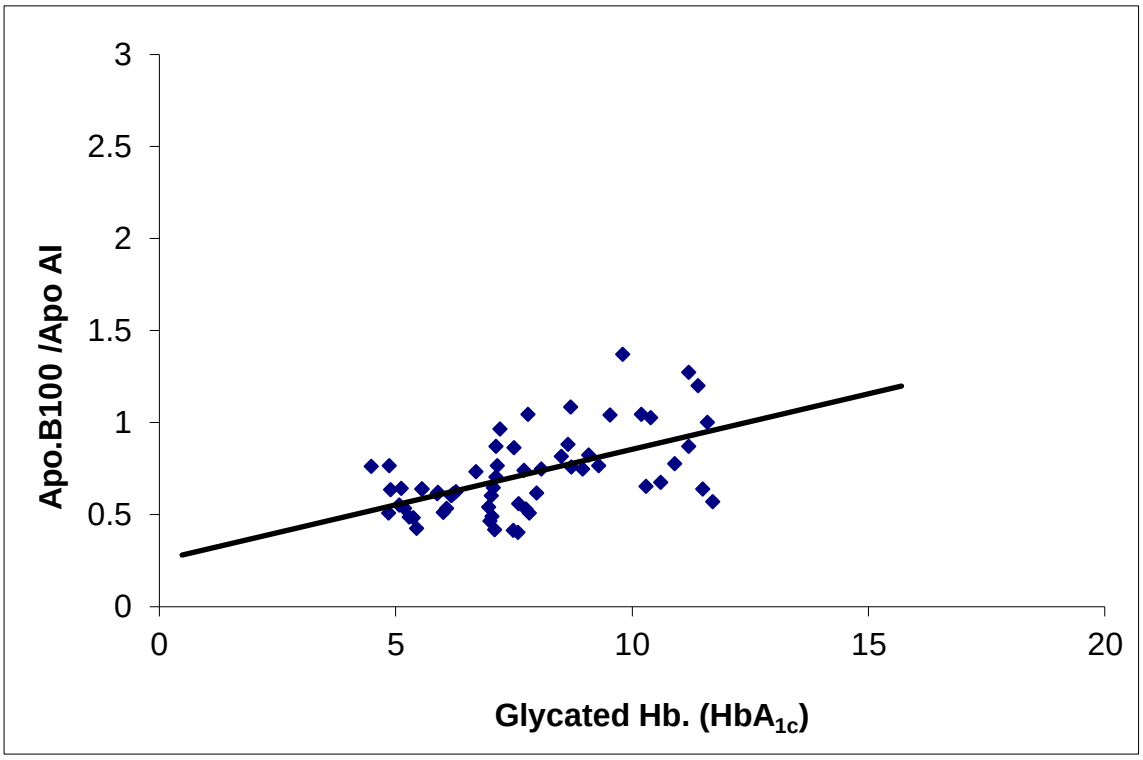

Figure 7 : Correlation between Apo B100/Apo A1 ratio and HbA_{1c} ($r = 0.559$, $p < 0.001$)

	Group I	Group II	Group III
FBG	96.32 ± 7.62	147.79 ± 13.798	282.2 ± 83.6
HbA_1c	5.602 ± 0.548	7.425 ± 0.366	10.18 ± 1.1

Table 1 : Levels of fasting blood glucose level (FBG) and glycated hemoglobin (HbA_1c) in the different groups of the study (Mean ± Standard deviation)

	Group I	Group II	Group III
Apo B100	85.955 ± 14.565	78.63 ± 20.603	85.305 ±13.152
Apo A1	143.765 ± 11.038	122.91±11.091	97.3 ± 15.573
Apo B100/Apo A1 ratio	0.598 ± 0.093	0.646 ± 0.188	0.903 ± 0.221

Table 2 : Levels of total cholesterol (T.cholesterol), triacylglycerols (T.G), HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C) in the different groups of the study (Mean ± Standard

	Group I	Group II	Group III
T.cholesterol	190.3 ± 26.788	176.75 ± 39.146	192.1 ± 36.986
T.G	119.1 ± 25.445	176.05 ± 47.949	266.6 ± 97.289
HDL-C	46.9 ± 3.726	38.35 ± 5.985	30.675 ± 6.37
LDL-C	120.36 ± 24.03	103.04 ± 36.485	107.96 ± 29.71

Table 3 : Levels of Apo B100, Apo A1 and Apo B100/A1 ratio in the different groups of the study (Mean ± Standard deviation)