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Original Article

Prolonged hyperglycemia in diabetic patients, its effect on inducing dyslipidemia and increasing the risk of cardiovascular disease

Mohammed E A Mohammed^{a,b}, Elsadig Mohamed Ahmed Fadlallah^c, Abdelrahim O Mohamed^d

^aDepartment of chemistry, Faculty of Science, King Khalid University, Abha, Saudi Arabia,

^bDepartment of biochemistry, faculty of medicine, International University of Africa, Sudan

^cFaculty of laboratory sciences, Elimam Elmahdi University, Kosti, Sudan

^dDepartment of biochemistry, Faculty of medicine, University of Khartoum, Khartoum, Sudan

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ABSTRACT

Background: Diabetes mellitus (DM) is the most common endocrine-metabolic disorder in children and adults. Cardio Vascular Disease (CVD) is a common complication of diabetes mellitus. The cause of the increased risk of CVD in diabetes is multi-factorial, including poor glycaemic control and dyslipidaemia. Aim of Study: This study was conducted to investigate the effect of prolonged hyperglycemia on the lipid profile (Lp(a), LDL, HDL, ApoB, ApoA, Cholesterol and Triglycerides) of Sudanese diabetic patients. HbA1C percentage was used as an indicator of prolonged hyperglycemia. Material and methods: 201 type 2 diabetic patients were included in this study after verbal informed consent. Ten milliliter of venous blood samples were obtained after 12 hours fasting for measurement of HbA1C and lipid profile. 84 patients were suffering from prolonged hyperglycemia (HbA1C mean percentage was $14.17\% \pm 3.71$) and 125 patients were found with normal HbA1C percentage (7.36 ± 1.32). The difference between the two means of the two groups was significant (p-value was less than 0.000). Results: This study showed that prolonged hyperglycemia significantly increased the concentration of cholesterol, LDL and Lp(a) since the p-values were 0.023, 0.001, 0.009 respectively. The triglycerides were insignificantly increased (p-value= 0.197), while Apo A, Apo B and HDL were insignificantly decreased (p-values were 0.23, 0.34 and 0.54 respectively). However, there was variation between the males and females. Conclusion: High percentage of HbA1C was associated with abnormal lipid profile and dyslipidemia. The results showed that males are more susceptible to dyslipidemia and Cardio Vascular Disease (CVD) than females.

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1. Introduction

Diabetes mellitus (DM) is a significant worldwide health burden with a growing prevalence globally [1, 2]. The prevalence of DM is increasing at alarming rate [3, 4, 5]. It is expected that more than one billion people will suffer from DM by the end of 21st century [6, 7]. Mortality wise, diabetes Mellitus was the seventh cause of death in USA in 2007 [8]. However, the WHO stated that high blood glucose is the third leading risk for mortality in the world (6%) [9].

DM is a risk factor for cardiovascular disease (CVD). Cardiovascular disease includes coronary heart disease, stroke and peripheral vascular disease. CVD is one of the factors that increase the death rate from diabetes mellitus [10, 11, 12]. Nearly 80% of

people with diabetes mellitus die as a result of CVD [12]. The cause of the increased risk of CVD in diabetes mellitus is multi-factorial; important factors include dyslipidaemia, hypertension, hyper-coagulability, poor glycaemic control, smoking, obesity, and lack of physical activity [13].

Dyslipidemia is a character of insulin resistance syndrome and it is associated with lipid abnormalities including high level of blood triglycerides and low HDL concentration. However, dyslipidemia has a greater negative impact on risk for cardiovascular disease in woman compared to men [14, 15, 16].

The aim of this study was to investigate the effect of prolonged hyperglycemia (high blood percentage of HbA1C) on the lipid profile and on inducing dyslipidemia in diabetic patients. The lipid profile parameters analyzed were Lp(a), LDL, HDL, ApoB, ApoA, cholesterol and triglycerides.

* Corresponding Author : **Dr. Mohammed Elimam Ahamed**

Assistant professor of biochemistry
Faculty of Science- department of chemistry
King Khalid University- Abha- Saudi Arabia
Mobile in Saudi Arabia: 00966556568650
mobile in Sudan: +249 912695221
E mail: meahmad@kku.edu.sa or mohammedelimam@yahoo.com

MATERIAL AND METHODS

Study design:

This research was classified as non experimental, quantitative, descriptive and case control hospital based study.

Ethical license:

This study was implemented after approval from the health authorities of White Nile state- Sudan and from Kosti teaching hospital. Study subjects were verbally informed and they agreed to participate in the study. However, they have been informed by the benefits that they will gain from this study (risk of cardiovascular disease assessment).

Study population:

Two hundreds and one patients with diabetes mellitus type 2 were involved in this study. 84 patients were found with high percentage of HbA1C (> 9%) and 117 patients had normal percentage (\leq 9%). Unlike the previous studies, this study considered that 9% HbA1C reflects controlled blood glucose level while prolonged hyperglycemia is characterized by more than 9% HbA1C to be 100% sure that the patients are suffering of prolonged hyperglycemia (uncontrolled DM).

The females were one hundred and twelve, 48 were with high HbA1C percentage and 64 were normal. Ninety eight of the patients were males (36 of them had high HbA_{1c} and 53 were with normal percentage of HbA_{1c}).

Sample collection and storage:

10 mls of venous blood samples were collected from each patient in an EDTA container, centrifuged and the plasma was collected in two Eppendorffs' tubes; one for immediate determination of HbA1C percentage and the other was kept at -20°C for the analysis of lipid parameters.

Methodology:

Determination of HbA1C percentage: HbA1C was extracted using chromatographic spectrophotometric ion-exchange method (Cypress Diagnostic, Belgium) and the concentration was determined by colorimeter (Lab Tech, India). Regarding the extraction step, haemolysate was prepared and the labile fraction is eliminated; the haemoglobins are retained by cationic exchange resin. HbA1C is specifically eluted after washing away the HbA1A+B fraction. The HbA1C percentage was determined through five steps. Step one: The haemolysate was prepared by addition of 200 μ l of reagent1 (Potassium biphatalte detergent, pH 5.0 (50mmol/L) to 50 μ l of EDTA blood sample, the mixture was shaken thoroughly and then left at room temperature for 15minutes ready for use. Preparation of the column was the second step; the column was prepared by removal of the upper cap first then the lower cap. Using the rounded end of a pipette the upper disc was pushed down to the resin surface carefully. The third step was the separation of HbA1C: 50 μ l of the haemolysate were pipette carefully to the upper filter of the column. 200 μ l of reagent 2 (Phosphate buffer, pH6.5

(48mmol/L), Sodium azide (0.95g/L) were added in order to drain any sample residue left above the upper disc in the step before. Then 2ml of reagent 2 were added and the column was left to drain. The fourth step included addition of 4ml of reagent 3 (buffer, pH6.4 (72mmol/L), Sodium azide (0.95g/L) were added after the column was placed over a test tube (16 \times 100mm), then elute was collected, shaken thoroughly and the absorbance of the elute was determined at 415 nm against distilled water. Step five was the reading of total Haemoglobin; 12ml of reagent 3 were pipetted into a test tube (16 \times 160ml) then 50 μ l of haemolysate were added. The mixture was shaken thoroughly and the absorbance was read against distilled water at 415nm. The percentage of HbA1C was determined as follows; % of HbA1C = Absorbance of HbA1C / (3 \times Absorbance of total haemoglobin) \times 100

Lp (a) measurement

Measurement of Lp (a) was performed using Latex Enhanced Turbidimetric Test for Quantitative Measurement Technique (antigen antibody reaction) according to manufacturer instructions (Gesellschaft for Biochemica and diagnostica mbH, Germany). All reagents were brought to 37°C and well shaken before analysis. Then the instrument was adjusted to zero using distilled water. 800 μ l of reagent 1 (pH7.0); Sodium chloride (0.9%), Sodium azide (0.095%) were added to 30 μ l of diluted sample or STD (Liquid, stable human serum basis, Sodium azide (0.095%). After mixing and incubation for 10 seconds absorbance A1 for test or STD was measured at 570nm. 60 μ l of reagent 2 (Latex reagent, Latex particles coated with anti-Lp(a) antibodies (goat) (0.5%), Glycine buffer (pH 8.3), Bovine serum albumin (1%)), Sodium azide (0.095%).were added to the mixture, and incubated for 10min at 37°C, and then the absorbance A2 was read at 570nm ((Hitachi photometer 4020) from Boehringer Mannheim, Japan). The absorbance difference of sample or STD was calculated as (A2-A1). The concentration of the Lpa was calculated as follows: Concentration of test = (Absorbance difference of test / absorbance difference of STD)* concentration of STD.

Estimation of Apolipoprotein B:

Turbidimetric immunoassay technique was applied to measure the apo lipoprotein B concentration using commercially available test kits obtained from Human Gesellschaft for Biochemica and Diagnostica mbH, Germany and the standard procedure was followed. Reagents were brought to 37°C prior to measurement. A 5 μ l of sample or STD were added to 1ml of assay reagent (ApoB mono-reagent; antihuman apoB serum (goat) stabilized in phosphate buffered saline (pH7.4), polymer enhance PEG 2%, sodium azide 0.095%. Test kits were from Human Gesellschaft for Biochemica and diagnostica mbH, Germany), then mixed well and immediately the cuvette was inserted into spectrophotometer. The absorbance A1 was read at 340nm (Hitachi photometer 4020 from Boehringer Mannheim, Japan) after 10scends, then and after incubation for 10mins at 37°C absorbance A2 was obtained. Absorbance difference was obtained (A2-A1). The apolipoprotein B was calculated following the equation; Concentration of test = (Absorbance difference of test / absorbance difference of STD) X concentration of STD.

Apolipoprotein A analysis:

Reagents are brought to 37°C prior to measurement by Turbidimetric immunoassay technique. 5µl of sample or STD were added to 1ml of assay reagent (ApoA mono-reagent; antihuman apoA serum (goat) stabilized in phosphate buffered saline (pH7.4), polymer enhance PEG 3%, sodium azide 0.095%. Test kits were from Human Gesellschaft for Biochemica and diagnostica mbH, Germany), then mixed well and immediately and the cuvette was inserted into spectrophotometer ((Hitachi photometer 4020 from Boehringer Mannheim, Japan). The absorbance A1 of sample or STD was read at 340nm after 10 seconds, then and after incubation for 10mins at 37°C absorbance A2 was obtained. Absorbance difference was obtained (A2-A1). The concentration of apolipoprotein A was determined following the equation; Concentration of test = (Absorbance difference of test / absorbance difference of STD) X concentration of STD.

Triglycerides, Cholesterol, LDL and HDL Estimation:

Enzymatic colorimetric test using kits from Human Gesellschaft for Biochemica and diagnostica mbH- Germany, was used to determine the concentration of triglycerides, cholesterol, LDL and HDL. The estimation was done according to the manufacturer instructions.

RESULTS AND DISCUSSION:

Description of study population

201 type 2 diabetes patients with age range between 40 and 80 years old were involved in this study. 84 patients were found with high HbA1C percentage (uncontrolled DM); 36 males and 48 females. 117 patients were found with normal HbA1C percentage (controlled DM); 53 males and 64 females.

Results of the all the patients

There was significant increase in HbA1C, Lpa, LDL and cholesterol (> 0.000, 0.009, 0.001 and 0.023 respectively) when patients with high HbA1C were compared to the patients with normal HbA1C. HDL, apolipoprotein A, and apolipoprotein B were insignificantly decreased while triglycerides were insignificantly increased in the patients with high HbA1C compared to those with normal HbA1C (Table.1).

Table.1: results of diabetic patients with high and normal HbA1C percentages

| Group | Diabetic patients with high HbA _{1c} percentage (n= 84) | | Diabetic patients with normal HbA _{1c} percentage (n= 117) | | Significance |
|--------------------------|--|--------------------|---|--------------------|--------------|
| | Mean | Standard Deviation | Mean | Standard Deviation | |
| %HbA1C | 14.18 | 3.71 | 7.36 | 1.32 | >0.000 |
| Lpa (mg/dl) | 92.13 | 36.01 | 76.50 | 31.96 | 0.009 |
| HDL (mg/dl) | 40.45 | 13.15 | 45.55 | 13.86 | 0.540 |
| LDL (mg/dl) | 1.44 | 92.86 | 1.09 | 41.83 | 0.001 |
| Apolipoprotein A (mg/dl) | 1.54 | 67.61 | 1.68 | 60.31 | 0.226 |
| Apolipoprotein B (mg/dl) | 1.48 | 64.68 | 1.51 | 58.98 | 0.342 |
| Cholesterol (mg/dl) | 1.97 | 68.20 | 1.81 | 50.54 | 0.023 |
| Triglycerides (mg/dl) | 2.06 | 61.04 | 1.83 | 57.21 | 0.197 |

Table.2: results of diabetic female patients with high and normal HbA_{1c} percentages

| Group | Diabetic female patients with high HbA _{1c} percentage (n= 48) | | Diabetic female patients with normal HbA _{1c} percentage (n= 64) | | Significance |
|--------------------------|---|--------------------|---|--------------------|--------------|
| | Mean | Standard Deviation | Mean | Standard Deviation | |
| % HbA1C | 14.33 | 3.55 | 7.52 | 1.36 | >0.000 |
| Lpa (mg/dl) | 94 | 33.27 | 79.11 | 33.7 | 0.68 |
| HDL (mg/dl) | 39.25 | 12.64 | 44.03 | 13.00 | 0.998 |
| LDL (mg/dl) | 129.5 | 55.31 | 114.67 | 39.1 | 0.064 |
| Apolipoprotein A (mg/dl) | 156.67 | 73.25 | 165.89 | 60.67 | 0.19 |
| Apolipoprotein B (mg/dl) | 148.67 | 46.46 | 155.69 | 59.64 | 0.175 |
| Cholesterol (mg/dl) | 206.35 | 75.01 | 186.92 | 46.42 | 0.008 |
| Triglycerides (mg/dl) | 213.19 | 71.76 | 192.34 | 56.14 | 0.844 |

Table.3: results of diabetic male patients with high and normal HbA_{1c} percentages

| Group | Diabetic male patients with high HbA _{1c} percentage (n= 36) | | Diabetic male patients with normal HbA _{1c} percentage (n= 53) | | Significance |
|--------------------------|---|--------------------|---|--------------------|--------------|
| | Mean | Standard Deviation | Mean | Standard Deviation | |
| % HbA1C | 13.97 | 3.97 | 7.17 | 1.25 | >0.000 |
| Lpa (mg/dl) | 89.64 | 39.73 | 73.36 | 29.74 | >0.000 |
| HDL (mg/dl) | 42.06 | 13.82 | 47.38 | 14.76 | 0.465 |
| LDL (mg/dl) | 1.63 | 125.23 | 1.03 | 44.48 | 0.003 |
| Apolipoprotein A (mg/dl) | 1.51 | 60.13 | 1.7 | 60.37 | 0.800 |
| Apolipoprotein B (mg/dl) | 1.48 | 83.79 | 1.45 | 58.17 | 0.007 |
| Cholesterol (mg/dl) | 1.85 | 56.7 | 1.74 | 54.74 | 0.848 |
| Triglycerides (mg/dl) | 1.95 | 41.63 | 1.71 | 56.93 | 0.034 |

Discussion:

Our results showed that diabetic patients with high HbA1C percentage were characterized with abnormal lipid profile compared to diabetic patients with normal HbA1C percentage and there were variable changes in the lipid profile of the female patients compared to the male patients.

As previously mentioned, this study divided the study population to two groups; patients with prolonged hyperglycemia and patients with controlled blood glucose. This study ensured that the difference between the mean values of HbA1C percentages of the two groups was highly significant (p-value was less than 0.000). However, this study considered that prolonged hyperglycemia is characterized by more than 9% HbA1C and normal or controlled blood glucose is characterized by or less than 9% HbA1C to make

sure that the case is prolonged hyperglycemia. It is well known in the literature that the HbA1C percentage in normal subjects is in the range (4- 5.6%), in the patients with controlled DM is (7%) and in the patients with uncontrolled DM is (8% or more) [17]. Generally higher lipid profile is related to increased cardiovascular mortality in diabetic patients with long duration [18].

Regarding the Lpa results, its mean value in the patients with prolonged hyperglycemia was 92.13 mg/dl compared to 76.5 mg/dl for the patients with controlled blood glucose and the difference was significant (p= 0.009), i.e. the prolonged hyperglycemia significantly increased the concentration of Lpa. Lpa is well known to be an independent risk factor for atherosclerosis, Cardiovascular Disease (CVD) and thrombosis as stated by Saito T and his

colleagues [18] The conclusion of our finding is that prolonged hyperglycemia increased the concentration of Lpa which always increases the risk of CVD in diabetic patients.

The mean value of the HDL of the patients with prolonged hyperglycemia was 40.45 mg/dl compared to 45.55 mg/dl of the patients with controlled blood glucose level. By comparing the two means it was clear that the prolonged hyperglycemia insignificantly decreased the blood concentration of HDL (p- value= 0.54). Our result was, to some extent, similar to the result of Bhaktha G and his research group and Lokhande Suryabhan L and his colleagues [19, 20] who concluded that there was significant negative correlation between HbA1C percentage and the HDL concentration in postprandial and fasting diabetic patients, however, our study showed that the difference between the HbA1C percentage and the HDL concentration was insignificant in fasting diabetic patients. Unlike our finding Ahmed N research [21] concluded that HDL was normal in type 2 diabetic patients with dyslipidemia. Low HDL concentration is an indicator for peripheral arterial disease and CVD [22], Coronary Artery Disease (CAD) [23] and macro and microvascular complications [24].

The LDL results showed that its mean value of the patients with prolonged hyperglycemia was 92.86 mg/dl compared to 41.83 mg/dl in the patients with controlled blood glucose. When the means were compared the p- value was 0.001 which means that prolonged hyperglycemia significantly increased the concentration of LDL. All of the contacted researches reached to the finding of our study e.g. Dukát A found that LDL was increased in type 2 diabetic patients [25], Chehade JM [26] stated that high concentration of LDL and triglycerides and low concentration of HDL are characters of dyslipidemia in diabetic patient type 2. It is registered that increased LDL concentration is associated with silent myocardial ischemia [27].

The apolipoprotein A results showed that the mean concentration in the patients with prolonged hyperglycemia was 1.54 mg/dl compared to 1.68 mg/dl in the patients with controlled blood glucose. The p- value of the compared means was 0.226 i.e., prolonged hyperglycemia insignificantly decreased the concentration of apolipoprotein A. Similar to our findings Taskinen [28] stated that low concentration of apolipoprotein A and high concentration of apolipoprotein B (high Apo B: Apo A-I ratio) are strong parameters in predicting CVD risk. Unlike our findings, Katulanda GW and his research team [29] concluded that type 2 diabetic patients in south east Asia had higher lipid profile including apo A-I and apo B suggesting that south eastern Asian patients are at increased risk of CVD.

Concerning the means of the apolipoprotein B, the mean value of it in the prolonged hyperglycemia group was 1.48 mg/dl and it was 1.51 mg/dl in the patients with controlled blood glucose. By comparing the means of the two groups it was clear that prolonged hyperglycemia insignificantly decreased the concentration of apolipoprotein B since the p- value was 0.342. however, changes in the concentration of either apo B or A are risk factors for initiation

and progression of atheromatous lesions in coronary, brain and peripheral arteries [30]. Bruck J and his research team [31] stated that determination of apolipoproteins concentration may be useful in the diagnosis of arteriosclerotic disease. In addition Jiang R research group [32] concluded that apoB and non HDL cholesterol are valuable predictors of CVD incidence among diabetic men.

The prolonged hyperglycemia significantly increased the cholesterol concentration (p- value= 0.023) and the two means of the patients with prolonged hyperglycemia and controlled blood glucose were 1.85 and 1.74 mg/dl respectively. All the contacted studies showed that cholesterol was significantly increased in type 2 diabetic patients postprandially and in fasting compared to normal subjects [20, 25, 26, 27].

The triglycerides were significantly increased in the patients with prolonged hyperglycemia (p- value= 0.034). However, the mean concentration of triglycerides in patients with prolonged hyperglycemia was 1.95 mg/dl compared to 1.71 mg/dl in the patients with controlled blood glucose level. The previous studies showed similar results to those of our study [26, 29].

When females patients with prolonged hyperglycemia were compared to the males results, this study stated that males were more susceptible to dyslipidemia than females since males lipid profile was more drawn to the picture of dyslipidemia (significant increase in Lpa and triglycerides and decrease in HDL). However, this study showed that male patients are characterized by significant increase in apolipoprotein B which put the males at high risk for CVD than females. However, Howard BV research group [33] and Kautzky-Willer and his colleagues [34] stated that diabetic women are at greater risk for CVD than diabetic men.

CONCLUSION:

Prolonged hyperglycemia induced dyslipidemia in type 2 diabetic patients with some differences between women and men. However, this study showed that men at higher risk for CVD than women.

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