



PRE-ANALYTICAL INSURANCE OF HYPERLIPIDEMIC SAMPLE FOR D-DIMER ASSAY IN DIABETIC PATIENTS

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ABSTRACT

Background; Lipid abnormalities are commonly associated with diabetes, particularly in those with type2 diabetes. The most common lipid abnormalities in these patients include hypertriglyceridemia and high cholesterol (reduced high-density lipoprotein (HDL) and high (LDL)).^[4] In patients with diabetes, coronary artery disease is the most common cause of death due to strong relationship between all forms of vascular disease in patients with type 2 diabetes and hyperlipidemia.^[5] Lipemia besides pathophysiological conditions, preanalytical laboratory errors account for a large proportion of lipemic samples. Several mechanisms causing lipemia interference in laboratory testing. Lipemia effect on coagulation test, and may falsely increase the D-dimer level.^[9] This study is aimed to compare the reality of D-dimer results after performed the pre-analytical insurance of hyperlipidemic sample in Diabetic patients. **Materials and Methods;** The study was recruited 50 patients with Diabetes mellitus type2 and diagnosed by hyperlipidemia A 2.5 ml citrated venous blood sample was collected from each patient for classic centrifugation (protocol 1) and ultra-centrifugation (protocol 2). The D-dimer level was measured by quantitative immunoassay using I-CHROMATM kit and reader. **Results and conclusions;** Ultra centrifugation protocol for hyperlipidemic samples should be performed as pre-analytical insurance before D-dimer assay. There were significant associations between high level of D-dimer and increased level or abnormality of Lipid profile.

KEYWORDS: D-dimer, Diabetic, Hyperlipidemia, Ultracentrifugation.

INTRODUCTION

Hyperlipidemia includes several conditions, but it usually means that you have high cholesterol and high triglyceride level.^[1] The causes of hyperlipidemia are genetic (familial or primary hyperlipidemia), acute hepatitis, Systemic lupus erythematosus, hypothyroidism, nephrotic syndrome, steroid uses, alcoholism, obstructive liver disease, obesity, diabetes mellitus and pregnancy, obstructive liver.^[2] The lipid profile typically includes low-density lipoprotein (LDL), high-density lipoprotein (HDL), Triglycerides and total cholesterol.^[3] The results of these tests can identify certain genetic diseases and can determine approximate risks for cardiovascular disease, certain forms of pancreatitis, and other diseases. Lipid abnormalities are commonly associated with diabetes, particularly in those with type2 diabetes. The most common lipid abnormalities in these patients include hypertriglyceridemia and high cholesterol (reduced high-density lipoprotein (HDL) and high (LDL)).^[4] In patients with diabetes, coronary artery disease is the most common cause of death due to strong relationship between all forms of vascular disease in

patients with type 2 diabetes and hyperlipidemia.^[5] D-dimer is a fibrin degradation product (or FDP), a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis.^[6] Values of estimation D-dimer is estimation of quantitative D-dimer level has many diagnostic and prognostic values, like; (a) *diagnosis of venous thromboembolism (VTE)* (b) *identification of individuals at increased risk of first thrombotic event (both arterial and venous)* (c) *identification of individuals at increased risk of recurrent VTE* (d) *establishment of the optimal duration of secondary prophylaxis after a first episode of VTE* (e) *pregnancy monitoring* (f) *diagnosis monitoring of disseminated intravascular coagulation (DIC)*.^[7] Lipemia besides pathophysiological conditions, preanalytical laboratory errors account for a large proportion of lipemic samples. Several mechanisms causing lipemia interference in laboratory testing. Lipemia interferes with nearly all photometric measurements by light scattering and absorption. The extent of interference is related to lipoprotein sample size and number of particles.^[8] Lipemia effect on

coagulation test, and may falsely increase the D-dimer level.^[9]

Methods

An observational comparative study was performed at the Faculty of Medical Laboratory sciences, Alneelain University, Khartoum, Sudan from Nov to May 2016. The study was recruited 50 patients with Diabetes mellitus type 2 and diagnosed by hyperlipidemia after informed consent and the structured questionnaire was filled. Non lipemic diabetic patients, bilirubinemia, liver disease, RA and hemolytic anemia were excluded from the study. A 2.5 ml citrated venous blood sample was collected from each patient for classic centrifugation (protocol 1) and ultra-centrifugation (protocol 2).

Classic centrifugation (Protocol 1)

A 2.5 ml was centrifuged at 3,000 rpm for 5 minutes. A 500 micro liter was stored in -80 for ultra-centrifugation and another 500 micro liter for D-dimer assay.^[10]

Ultracentrifugation (Protocol 2)

A 500 micro liter from the classic centrifuged sample was ultra-centrifuged at 18,000 rpm for 30 minutes to separate the chylomicrons, VLDL's and other fat particles from the serum (*Beckman coulter protocol*). Then the plasma sample was stored in -80 for D-dimer estimation.^[11]

Note: During ultracentrifugation, the large, low-density lipid particles are forced to the surface of the sample and the clear serum below is extracted and used for testing.

Body mass index (BMI)

Assessment of body mass index (BMI) was calculated as: Weight /Height² (Kg/m²) for each patient.

Clinical Records

Chemical assays includes fasting blood glucose (F.B.G), Haemoglobin A1c (HbA1c), lipid profile (cholesterol, LDL, HDL, T.G) were performed for each patients before D-dimer assay.

D-dimer performance

The D-dimer level was measured by quantitative immunoassay using I-CHROMATM kit and reader. A sandwich immune detection method, such that the detection antibody in buffer (*contains fluorescence-labeled anti D-Dimer antibody, fluorescence-biotin labeled BSA, BSA as a stabilizer, and sodium azide as a preservative in PBS*) was bound to D-Dimer in the plasma sample and antigen-antibody complexes were captured by antibodies that have been immobilized on the test strip as sample mixture migrates through nitro cellulose matrix.

The cut-off (reference value): 500 ng/ml

Statistical analysis

Data were analyzed by SPSS statistical package of social science (version 16.0; SPSS Inc.). The Paired t-test was employed to compare differences between the means of continuous variables and Pearson's correlation to correlate between study parameters and variables.

P values ≤ .05 were considered statistically significant.

RESULTS

This study involved 50 patients with diabetes mellitus type 2 and diagnosed by hyperlipidemia, their mean age was 56.100 ± 8.60 yrs. 23 (46%) of them were males and 27 (54%) were females. 78% of the patients were intake treatment for diabetes and 22% did not. A significant correlation was found between D-dimer level and age (P.value = 0.02).

Records and Clinical chemistry profile

The means of height, weight, body mass index (BMI), fasting blood glucose (FBG), Hb-A1C, Cholesterol, Triglyceride, LDL, HDL were 166 cm, 88 kg, 29,188 mg/dl, 9,212 mg/dl, 195 mg/dl, 135 and 38 respectively.

D-dimer

The D-dimer data were classified according to the quantitative level, some of showed normal level and the other showed high abnormal level.

Patients with normal D-dimer level

The mean of D-dimer levels in those patients after classical centrifugation and ultracentrifugation were 306 ng/ml and 263 ng/ml respectively. Significant correlations were found between D-dimer level, Cholesterol level, Triglyceride level, Hb A1C and Fasting Blood Glucose (P.value < 0.05). A significant difference of D-dimer level was found between the classic centrifugation and ultra-centrifugation (P.value = 0.02).

Patients with Abnormal D-dimer level

The mean of D-dimer levels in those patients after classical centrifugation and ultracentrifugation were 834 ng/ml and 773 ng/ml respectively. Significant correlations were found between D-dimer level, Cholesterol level, Triglyceride level, Hb A1C and Fasting Blood Glucose (P.value < 0.05). A significant difference of D-dimer level was found between the classic centrifugation and ultra-centrifugation (P.value = 0.02).

DISCUSSION AND CONCLUSION

Diabetic patients may progress to cardiovascular diseases and coagulopathy and D-dimer assay is one of the known biomarkers that manage and exclude these complications. Using of D-dimer assay for the prognosis management and follow up of the diabetic patients will preserve them in a safe manner. The effect of hyperlipidemia in the reality of D-dimer results will complicate the management approach. In addition to

incongruity of D-dimer results in hyperlipidemic sample by the photometric protocol, we also confirmed in this study the abnormality in D-dimer results by the immune assay protocol. The pre-analytical insurance of the hyperlipidemic blood sample by ultra-centrifugation will warranted the actuality of results. We concluded that, there was significant difference in D-dimer level in hyperlipidemic sample in diabetic patients. Ultra centrifugation for these samples should be performed as pre-analytical insurance before D-dimer assay. There were significant associations between high level of D-dimer and increased level or abnormality of Lipid profile.

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