Assessment of Lipoprotein (a) In Blood Samples of Sudanese Diabetic Patients Correlated With Glycosylated Hemoglobin
Elsadig Mohamed Ahmed Fadalla1, Abderahim Osman Mohamed2
1. Assistant professor of clinical chemistry, department of clinical chemistry, Faculty of Medicine and Health Science, University of El Imam El Mahdi, Kosti, Sudan. E-mail: alsadigm@yahoo.com
2. Professor of biochemistry, Department of Biochemistry, Faculty of Medicine, University of Khartoum, Sudan.
ABSTRACT:
The Sudanese diabetic patients may have high frequency of dyslipidaemia, which contribute to accelerated coronary atherosclerosis. This study aims to assess Lp (a) and HbA1C in blood samples of Sudanese diabetic patients. In this cross-sectional prospective study, blood samples of 150 Sudanese diabetic patients were collected. Diabetic patients were informed and consented to participate in this study. Results of 100 non-diabetics were compared with patient's results. Chromatographic spectrophotometric ion-exchange method and turbidimetric spectrophotometric method were applied to measure HbA1C and Lp (a), respectively. Results were analyzed statistically using student's t test, compared as mean and standard deviation and considered significant when ($P <0.05$). In this study Lp (a), and HbA1C mean levels were increased significantly in diabetic patients when compared to control ($P<0.01$). All diabetic patients participated in this study had Lp (a) concentrations >30mg/dl exceeding the cut-off value of Lp (a). However, Lp (a) concentration at the level ≥100mg/dl represent 33.3% of the total diabetic cases. This indicates a high risk for those patients. Greater than 40% of diabetic patients were having HbA1C level >9.0%, hence they were at increased risk of cardiovascular complications because they were having poor glycaemic control. These results conclude addition of Lp (a) to the routine lipid profile to assess cardiovascular risk in diabetic patients which may enhance management of diabetes mellitus.

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المستكشف
إن هذه الدراسة تولى أهمية في التعرف على معدلات الدهون في الدم بالنسبة للمصايبين بمرض السكري وعلاقتها بـ (HbA1c). في هذه الدراسة و باستخدام الطريقة المقطعة (Cross Sectional), تم اختيار عدد 150 شخصًا مصابًا بمرض السكري من مجتمع الدراسة بعد الموافقة في البحث كتابةً أو شفاهًا وذلك باستخدام استبيان تم إعداده خصيصًا لهذا الغرض. تم إجراء مقارنة النتائج مستويات البروتين الدهني من النوع (أ) في الدم لـ 100 شخصًا غير مصابًا في المرضى من النوع (أ) في الدم للـ 100 شخصًا غير مصابًا في المرضى من النوع (أ). تم تحليل النتائج إحصائيًا باستخدام SPSS تكرار الدراسة 16. متوسط البروتين الدهني (أ) و (ب) في المرضى، بينما يزيد عن 30mg/dl لdegree مع المستكشف بالاضافة إلى 100mg/dl لdegree مع المستكشف. هذا يعتبر مؤشراً لارتفاع من خطر التصل نسبة الذين لديهم تركيز يزيد عن 100mg/dl للطلق من خطر
INTRODUCTION

Diabetes mellitus (DM) is a significant worldwide health burden with a growing prevalence globally (1). Nearly 80% of diabetic patients die as a result of cardiovascular disease (CVD). The cause of the increased risk of CVD is multi-factorial; important factors include dyslipidaemia and poor glycaemic control (2,3). The rate of formation of glycosylated haemoglobin (HbA1c) is directly proportional to the plasma glucose level. HbA1c assay, a measure of chronic glycaemia, is critical to the study of diabetic control and complications. (4) The benefits of measuring HbA1c is that it gives more reasonable and stable view of what is happening concerning the glycaemia over a course of time (i.e.; three months) (5). Lipids disorders are common in patients with DM, and play crucial roles in the development of diabetic cardiovascular complications. (6)

Patients with diabetes have lipids abnormalities that placed them at high risk for cardiovascular and cerebrovascular events. (7) Atherosclerosis, a chronic condition characterized by the formation of lipid-rich plaques within the walls of medium and large arteries, underlies many forms of vascular disease (8). Atherosclerosis is an inflammatory disorder that may be initiated by several factors (9). Lipoprotein (a) Lp (a) which was first described more than 40 years ago, is an low density lipoprotein (LDL) like molecule synthesized by the liver and is composed of protein, lipid, and carbohydrate. (10) It is a macromolecular complex found in human plasma that combines structural elements from the lipoprotein and blood clotting systems associated with premature CHD. (11) It consists of an apolipoprotein B (Apo B-100) particle attached by a disulfide bridge to apolipoprotein (a) (12,13). Lp (a) is involved in lipids transport. (14) It is an independent risk factor for the development of coronary heart disease (CHD) (15,16,17). Increased Lp (a) concentration is predictive for coronary artery disease (CAD), the major cause of morbidity and mortality (17,18).

Problem of Study:

Cause of the increased risk of CVD in diabetes mellitus is multi-factorial. Appropriate interventions to address each of these risk factors are imperative to lower the risk of CVD in people with diabetes mellitus. Therapeutic strategies for management of diabetic patients should give equal emphasis to the control of hyperglycaemia and dyslipidaemia.

Objective of study:

This study aimed to determine Lp (a) and HbA1c addressing CV risks so that therapeutic strategies could control CV diseases in Sudanese diabetic patients.

MATERIAL and METHODS:

This study was designed as cross-sectional prospective study. Samples were collected in the internal medicine unite, Kosti teaching hospital, Kosti, White Nile state, in the period October, 2008 – April, 2009. Blood samples of one hundred and fifty known diabetic patients both types (type 1 and type 2 diabetes mellitus) defined by history from different ages and sexes, were
collected. Diabetic patients were informed and consented to participate in this study. Each patient was asked for his/her age, duration of disease, smoking and hypertension. The Lp (a) and HbA1C levels in samples of those patients were measured. Also blood samples of apparently 100 normal subjects, with no personal or family history of diabetes, were examined for Lp (a) and HbA1C levels. Means were compared with those of diabetic patients. Five milliliters (5ml) of venous blood sample sufficient for analysis of Lp (a) and HbA1C were obtained from patients and controls. Each sample was divided into two parts, one part was put in a heparized container, centrifuged and then serum was collected in an Eppendorf's tube and kept at -20°C for measurement of Lp (a), the other part was put in an EDTA container for HbA1C measurement. All parameters were analyzed using commercially available test methods. HbA1C was measured using chromatographic spectrophotometric ion-exchange method purchased from Cypress Diagnostic, Belgium. Colorimeter from Lab Tech, India was applied. Measurement of Lp (a) was performed using latex enhanced turbidimetric quantitative technique (antigen antibody reaction) obtained from Human Gesellschaft for Biochemica and diagnostica mbH, Germany. Hitachi photometer 4020 from Boehringer Mannheim, Japan was used. Statistical analysis of data was carried out using statistical packages of social studies (SPSS) program for windows, version 16.0. Results were expressed as mean, standard deviation and coefficient of variation. Differences in means were tested using the Student t-test and results were considered significant when $p<0.05$. Analysis of variance (ANOVA test) to estimate the regression between Lp (a) and HbA1C was applied. Control sera that obtained from Human Gesellschaft for Biochemica and diagnostica mbH, Germany, were applied for quality control purposes.

**RESULTS:**

In this study the Lp (a) and HbA1C mean levels were increased significantly in diabetic patients when compared to controls ($P <0.01$), (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Diabetics (n=150)</th>
<th>Controls (n=100)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.7±12.6 years</td>
<td>41.6± 11.14 years</td>
<td>0.79</td>
</tr>
<tr>
<td>Lp (a) mg/dl</td>
<td>82.5±34.2</td>
<td>16.4± 5.8</td>
<td>0.00</td>
</tr>
<tr>
<td>HbA1C %</td>
<td>10.4±4.5</td>
<td>4.3±0.7%</td>
<td>0.00</td>
</tr>
</tbody>
</table>

In this study there was significant correlation of Lp (a) with HbA1C ($P<0.05$) in all diabetic patients (figure, 1).

![Correlation plot of Lp (a) and HbA1C in diabetic patients ($P< 0.05$).](image-url)
The Lp (a) and HbA_1C mean levels were slightly non significantly increased in female than male diabetic patients (Table 2).

| Table 2: Lp (a) and HbA_1C levels in diabetic patients associated with sex |
|-----------------|-----------------|-----------------|-----|
|                | Diabetic Male (n = 67) | Diabetic Female (n = 83) | P value |
| Age            | 56.4 ± 13 years      | 55.2 ± 12.2 years | 0.45 |
| Duration of DM | 11.2 ± 6 years       | 9.8 ± 4.9 years   | 0.06 |
| Lp (a)         | 79.3 ± 35 mg/dl      | 85.3 ± 33.3 mg/dl | 0.18 |
| HbA_1C (%)     | 10 ± 4.5%            | 10.7 ± 4.6%       | 0.22 |

Analysis of variance (ANOVA test) to estimate the regression between Lp (a) and HbA_1C of diabetic patients was applied. The r^2 and P value were (0.02) and (<0.05), respectively. In this study there was 58% of diabetic patients were having Lp (a) mean level of 91±35mg/dl when Lp (a) was correlated (P<0.01) with the pathological level of HbA_1C ≥9% (Table 3).

| Table 3: Lp (a) associated with pathological value of HbA_1C in diabetic patients |
|-----------------|-----------------|-----|
|                | HbA_1C ≥ 9% (n=88) | HbA_1C <9% (n=62) | P value |
| Age            | 54.6±12.9 years  | 57.4±12 years    | 0.10 |
| Duration of DM | 10.3±5.5 years   | 10.5±5.2 years   | 0.82 |
| Lp (a)         | 91±35mg/dl       | 69.7±27mg/dl     | 0.00 |

Also there were 34% of diabetic patients their HbA_1C mean level was 11.9±4.3%. This level was found significant (P<0.01) when HbA_1C was correlated with the pathological level of Lp (a) (≥100mg/dl) (Table 4).

| Table 4: HbA_1C associated with pathological value of Lp (a) in diabetic patients |
|-----------------|-----------------|-----|
|                | Lp(a)≥100mg/dl (n=51) | Lp(a) <100 mg/dl (n=99) | P value |
| Age            | 55.4±13.1 years  | 55.9±12.3 years    | 0.82 |
| Duration of DM | 10.5±4.6 years   | 10.4±5.8 years     | 0.87 |
| HbA_1C %       | 11.9±4.3%        | 9.6±4.5%            | 0.00 |

DISCUSSION
In this study Lp (a), and HbA_1C mean levels were increased in diabetic patients when compared to controls. These findings agreed with results of a study conducted by Valabhji, et al [19] in 2003. However, Imani, et al [20] in 2006 found that means of Lp (a) was lower in diabetic children than in control group in Isfahan. Lp (a) mean level in this study was significantly higher in diabetic patients as compared to controls (P<0.01). Our results disagreed with results of study done in Tunisian population. [21] Ben Hamda, et al. 2002. [21] reported that Lp (a) mean level was not significantly, higher in diabetics as compared to controls, study done in Tunisia. In this study all diabetics had Lp (a) level >30mg/dl. Cantin et al [22] in 2002 reported Lp (a) cut-off value of 30mg/dl. One third of diabetic patients in this study had Lp (a) exceeded 100mg/dl. This finding indicated high risk for those diabetics. High levels of Lp (a) was the
suggested risk factors for CHD morbidity and mortality. [23,10]

Concentration of Lp (a) in human plasma vary from 0 to 30mg/dl.[8] Lp (a) levels ≥20mg/dl, were associated with an increased risk of sudden death. [24] In this study HbA<sub>1C</sub> mean level was 10.4±4.5% for the diabetic patients under study and 4.3±0.7% for the non-diabetic controls. These findings were comparable to other study results; mean level of HbA<sub>1C</sub> was 9.9±1.40% and 6.4±0.07% for diabetic patients and healthy controls respectively, a study done in Khartoum State, Sudan. [25] 42.5% of diabetic patients were having HbA<sub>1C</sub> level >9.0%, hence they were suggested at increased risk of cardiovascular complications, because they were considered having poor glycaemic control. In patients with DM the risk of diabetic complications was strongly associated with previous hyperglycaemia. [26]

CONCLUSION

Lp (a) seen to be determinant risk factor of all diabetic patients. HbA<sub>1C</sub> remains a suitable measure to assess hyperglycaemic control in diabetic patients. The diabetic patients under study were at poor glycaemic control. Therapeutic strategies will be needed addressing hyperglycaemia and dyslipidaemia to control diabetic complications. Addition of Lp (a) to the routine lipid profile to assess cardiovascular risk in diabetic patients may enhance management of diabetes mellitus. Measurement of Lp (a) will be sufficient for the assessment of the lipid profile.

REFERENCES


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