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Assessment of Lipoprotein (a) In Blood Samples of Sudanese Diabetic Patients Correlated With Glycosylated Heamoglobin

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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 HbA_{1C} 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 HbA_{1C} and Lp (a), respectively. Results were analyzed statistically using student-'t' test, compared as mean and standard deviation and considered significant when (P <0.05). In this study Lp (a), and HbA_{1C} 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 HbA_{1C} 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.

المستخلص

إن هذه الدراسه تولي أهمية في التعرف علي معدلات الدهون في الدم بالنسبة للسودانيين المصابين بمرض السكري وعلاقتها ب(**HbA_{1C}**). في هذه الدراسة و باستخدام الطريقة المقطعية (**Cross Sectional**) تم اختيار عدد 150شخصاً مصاباً بمرض السكري من مجتمع الدراسة بعد الموافقه علي الاشتراك في البحث كتابةً او شفاهةً وذلك باستخدام استبيان تم إعداده خصيصاً لهذا الغرض. تمت مقارنة نتائج مستويات البروتين الدهني من النوع (أ), الهيموقلوبين من النوع **HbA_{1C}** في الدم لهؤلاء المرضي بنتائج مستويات نفس المواد لعدد 100 شخصاً غير مصاباً بالمرض. جميع التحليلات المعملية المطلوبة تم إجرائها باستخدام الطرائق التحليلية المتوفرة. كما تم تحليل النتائج إحصائيا باستخدام **SPSS** الإصدارة 16. متوسط البروتين الدهني (أ) و **HbA_{1C}** مرتفعه لدي المرضي مقارنةً بالأصحاء حيث أن (**P <0.01**) و معدل البروتين الدهني (أ) يزيد عن **30mg/dl** بالنسبة لجميع المرضي بينما تصل نسبة الذين لديهم تركيز يزيد عن **100mg/dl** الي ثلث المرضي, هذا يعتبر مؤشراً للارتفاع من خطر

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الإصابة بإمراض تصلب الشرايين لهؤلاء المرضي. كما وأن هناك نسبة عالية من المرضي تصل إلي أعلي من 40% مستوي **HbA_{1C}** لديهم يصل إلي أكثر من **9.0%**, هؤلاء يتم وصفهم بان لهم تحكم سئ لمستوي سكر الجلكوز في الدم لذلك فهم أكثر عرضة للإصابة بإمراض تصلب الشرايين. هذه النتائج تؤيد الفكرة التي تنادي بإضافة تحليل البروتين الدهني (أ) إلي التحليل الروتيني لدهون الدم لدي مرضي السكري مما يزيد من احتمالية التعرف علي الاعتلال الوظيفي في هذه الدهون و بالتالي المعالجة المبكرة للإخطار التي يمكن ان تنجم عن ذلك.

KEYWORDS*: Lp (a), HbA_{1C}, DM, Cardiovascular disease (CVD), Dyslipidaemia*

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 (HbA_{1C}) is directly proportional to the plasma glucose level. HbA_{1C} assay, a measure of chronic glycaemia, is critical to the study of diabetic control and complications. [4] The benefits of measuring HbA_{1C} 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) [(15,8,16)]. 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 HbA_{1C} 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 HbA_{1C} 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 HbA_{1C} levels. Means were compared with those of diabetic patients. Five milliliters (5ml) of venous blood sample sufficient for analysis of Lp (a) and HbA_{1C} 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 Eppindorffs' tube and kept at -20°C for measurement of Lp (a), the other part was put in an EDTA container for HbA_{1C} measurement. All parameters were analyzed using commercially available test methods. HbA_{1C} 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 HbA_{1C} 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 HbA_{1C} mean levels were increased significantly in diabetic patients when compared to controls (P <0.01), (Table, 1).

Table 1: Lp (a) and HbA_{1C} obtained levels in diabetic patients and controls

	Diabetics (n=150)	Controls (n=100)	*P* value
Age	55.7±12.6years	41.6± 11.14 years	0.79
Lp (a)	82.5±34.2mg/dl	16.4± 5.8 mg/dl	0.00
HbA_{1C}	10.4±4.5%	4.3± 0.7%	0.00

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

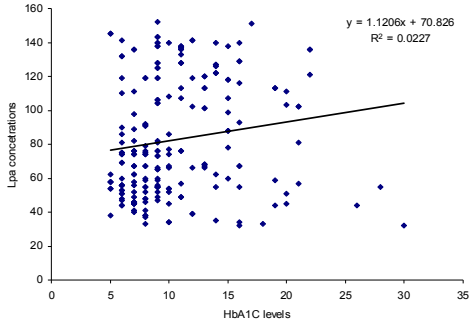

Figure 1: Correlation plot of Lp (a) and HbA_{1C} in diabetic patients (P< 0.05).

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 ± 13years	55.2 ± 12.2years	0.45
Duration of DM	11.2±6years	9.8±4.9years	0.06
Lp (a)	79±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±12.9years	57.4±12years	0.10
Duration of DM	10.3±5.5years	10.5±5.2years	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±13.1years	55.9±12.3years	0.82
Duration of DM	10.5±4.6years	10.4±5.8years	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_{1C} 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_{1C} 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_{1C} 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_{1C} 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.

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