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CARDIOVASCULAR DISEASE RISK ASSESSMENT IN TYPE 2 DIABETES: ROLE OF MYELOPEROXIDASE AND APOLIPOPROTEIN B

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ABSTRACT

Introduction: Cardiovascular death accounts for more than 75% of all deaths among persons with diabetes mellitus. This is generally attributed to the adverse effects of hyperglycaemia and oxidative stress on vascular biology. By measuring the serum levels of MPO, apolipoprotein B, and HbA1c we can assess the future risk of cardiovascular disease in type 2 diabetic patients at an early stage and initiate glycaemia control measures to prevent cardiovascular complications in type 2 diabetes patients. This study was undertaken to observe the relationship between serum myeloperoxidase with apolipoprotein B and also with that of serum glycated hemoglobin in type 2 diabetic patients and healthy controls.

Materials and Methods: A case-control study was done taking 40 cases of type 2 diabetes mellitus and 40 age and sex-matched healthy controls. In all the subjects, concentrations of HbA1c, serum apolipoprotein B, and serum MPO were estimated. HbA1c was measured by turbidimetric method and serum apolipoprotein B by an immune turbidimetric method using semi-auto analyzer CHEM 5 Plus. Serum MPO was measured by ELISA method using ELISA reader.

Results: The mean concentrations of HbA1c, serum apolipoprotein B and Serum MPO are significantly increased in type 2 diabetic cases when compared with healthy controls. HbA1c concentration is significantly positively correlated with serum apolipoprotein B and serum MPO in type 2 diabetic cases but there is no significant correlation between serum MPO and serum apolipoprotein B levels.

Conclusion: The present study suggests that chronic hyperglycemia and endothelial dysfunction are the major causal factors for the pathogenesis of macrovascular complications in type 2 DM. The future risk of CVD can be detected by evaluating the levels of apolipoprotein B and MPO in type 2 diabetic patients which can be prevented by adequate control of glycemia.

Keywords: Diabetes Mellitus; Endothelial dysfunction; dyslipidemia; CVD; hyperglycemia.

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INTRODUCTION

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin.[1] In the presence of chronic hyperglycemia haemoglobin undergoes glycation by the non-enzymatic process to form glycated haemoglobin. In diabetic patients, HbA1C indicates glycemic control over the previous few months. Increase in blood sugar by 30 mg/dl increases the HbA1C by 1%. approximately[2,3] Hyperglycemia and oxidative stress increase the risk of cardiovascular disease in diabetic patients. Myeloperoxidase (MPO), which is present in the granules of leukocytes, macrophages and monocytes is released at the inflammatory sites. It stimulates the increased production of reactive oxygen species. These reactive oxygen species cause endothelium

dysfunction. MPO is implicated in the initiation, progression, and the complications of atherosclerosis.[4] Total mass of atherogenic particles like VLDL, IDL, and LDL is indicated by apolipoprotein B. Its levels will increase in cardiovascular disease[5]

By measuring the serum levels of MPO, apolipoprotein B and HbA1c we can assess the future risk of cardiovascular disease in type 2 diabetic patients at an early stage and initiate glycemia control measures to prevent cardiovascular complications in type 2 diabetes patients. Therefore the present study aims to evaluate the serum levels of MPO, apolipoprotein B and HbA1c as early markers of cardiovascular risk in type 2 diabetic patients. In addition, MPO and apolipoprotein B levels will be correlated with glycated hemoglobin levels. This study was undertaken to observe the relationship between serum myeloperoxidase with apolipoprotein B and also with that of serum glycated hemoglobin in type 2 diabetic patients and healthy controls.

MATERIAL AND METHODS

This study is carried out to estimate the levels of serum Myeloperoxidase (MPO), glycated hemoglobin (HbA1c), apolipoprotein B in type 2 diabetic patients and healthy controls, for one year. Patients were selected from the district hospital. Each patient gave an informed consent and the study was approved by the ethical and research committee. The patients and controls voluntarily participated in the study.

Inclusion Criteria:

Cases: 40 proven cases of type 2 diabetic patients without complications, on treatment with no time duration, in the age group of 30 - 80 years.

Controls: 40 cases of age and sex-matched healthy controls will be compared.

All patients suffering from type 2 diabetes without complications, on treatment with no time duration diagnosed and confirmed by a physician with FBS and PPBS according to American Diabetes Association criteria (FBS ≥ 126 mg/dl & 2-hour PPBS ≥ 200 mg/dl)

Exclusion Criteria: Patients with congenital heart diseases, hypertension, diabetic complications, systemic diseases, other endocrinal disorders, malignancies, hemoglobinopathies, drugs that interfere with serum levels of myeloperoxidase, apolipoprotein lipoprotein B, and glycated hemoglobin.

Collection of Blood Sample:

After obtaining informed consent, about 6ml of fasting venous blood samples were drawn under aseptic precautions into a sterile bulb from selected subjects. 4 ml of blood was taken into a plain vacutainer, and serum is separated by centrifugation, which was used for estimation of serum myeloperoxidase and apolipoprotein B. 2 ml blood was taken into EDTA containing vacutainer and used for estimation of HbA1c. Glycated hemoglobin was estimated by immunoturbidometric method[6], Serum apolipoprotein B was estimated by Immunoturbidometric method,[7] serum myeloperoxidase by Enzyme Linked Immuno Sorbent Assay[8] .

Statistical analysis was done using SPSS software, version 17.0. Descriptive data were presented as mean ± SD and range values. Results were subjected to appropriate statistical analysis. An unpaired t-test was used to compare the various parameters between cases and controls. For all the tests, a probability value (p-value) of less than 0.05 was considered statistically significant. Correlation analysis was done to assess the relationship between different variables. (Pearson‟s correlation coefficient). Receiver Operating Curve (ROC) analysis was done to obtain Area Under Curve (AUC) and optimum cut-off value for Myeloperoxidase.

RESULTS:

In the present study total of 80 subjects were included. They were divided into 2 groups.

Controls: It consisted of 40 age and sex-matched, healthy subjects.

Cases: 40 proven cases of Type 2 DM disease patients without complications, on treatment with no time duration in the age group of 30-80 years attending the medicine department of district general Hospital were included in this study. The parameters were estimated were Glycated hemoglobin (HbA1c), Serum apolipoprotein B, Serum Myeloperoxidase

Table 1: Age and gender-wise distribution of controls and type 2 diabetic cases

Number of subjects		Controls	Cases	p-Value
		40	40	
Age (years)	Mean ± S.D	47.5 ± 8.4	48.4 ± 7.02	> 0.05 NS
	Range	33 – 63	36 – 62	
Gender	Male	19	18	> 0.05 NS
	Female	21	22	

NS- Not significant

Among 40 controls, 19 were males and 21 were females with a mean age of 47.5 ± 8.4 years. Among the 40 diabetic cases, 18 were males and 22 were females with a mean age of 48.4 ± 7.02 years. There is no significant difference among controls and cases for the age ($p > 0.05$).

Table 2: Levels of HbA1C, apolipoprotein B, Myeloperoxidase in healthy controls and diabetic cases

Groups		HbA1c (%)	Serum apolipoprotein B (mg/dl)	Serum MPO (pg/ml)
Controls	Mean ± SD	4.98 ± 0.59	120.62 ± 29.78	10105 ± 2954.6
	Range	4.0 – 6.01	57.2 – 183.0	4000 – 15000
Cases	Mean ± SD	7.01 ± 1.12	234.53 ± 51.07	21225 ± 7885.7
	Range	5.0 – 8.9	125.0 – 373.3	4800 – 36600
Controls and Cases	Mean Difference	2.03	113.9	11120
	t value*	10.07	12.18	7.44
	p value	< 0.001	< 0.001	< 0.001

* Unpaired student,, t' test

p value: > 0.05 not significant, < 0.05 significant, < 0.001 highly significant.

The mean levels of HbA1c, serum apo B and serum MPO in cases are in the range of 7.01 ± 1.12 %, 234.53 ± 51.07 mg/dl and 21225 ± 7885.7 pg/ml respectively, which are significantly higher than controls ($p < 0.001$).

Table 3: Pearson's correlation between biochemical parameters in type 2 diabetic cases

CORRELATION ANALYSIS		
Relationship between	r value	p value
Serum MPO and HbA1c	+ 0.52	< 0.05*
Serum MPO and apolipoprotein B	+ 0.33	> 0.05
HbA1c and apolipoprotein B	+ 0.52	< 0.05*

r: Pearson‟s correlation coefficient; < 0.05*- Significant; > 0.05- not significant

The Pearson‟s correlation analysis shows statistically significant positive correlation between serum MPO and HbA1c with r value + 0.52 with ($p < 0.05$), no significant correlation between serum MPO and serum apo B with r value 0.33 with ($p > 0.05$) statistically significant positive correlation between HbA1c and serum apo B with r value + 0.52 with ($p < 0.05$).

Table 4: ROC curve to determine the cut off level MPO activity (pg/ml) in diabetics

Cut-off	Sensitivity	Specificity	AUC	p-Value
15200	80	100	0.88	<0.001

The area under the curve at the cut off level of 15200 pg/ml is about 0.88 ($p < 0.001$) with a sensitivity of 80% and specificity of 100%.

DISCUSSION

Diabetes mellitus is a multisystem disorder characterized by a relative or absolute insufficiency of insulin secretion or resistance to the metabolic action of insulin on the target tissue. Recently diabetes mellitus has become a major health burden. [4] Every year about 5% of the patients die due to diabetes worldwide. [9]

Persistent hyperglycemia in uncontrolled diabetics can cause inflammation and increased production of reactive oxygen species from glucose auto-oxidation which can predispose to detrimental consequences in diabetes mellitus.

Cardiovascular complications are due to the adverse effects of hyperglycemia and oxidative stress on vascular endothelium. Approximately about 75% of deaths in diabetic patients are caused due to these cardiovascular complications.[10, 11] In the present study, it is found that the concentration of HbA1c is increased in cases when compared with healthy controls, which is statistically highly significant (p-value < 0.001). This is in accordance with the studies of Pasupathi Pet al.[12], Mohsen AF et al.[13]and Silbernagel G et al.[14] HbA1C is produced by the covalent binding of glucose with haemoglobin. Since the red blood cell membrane is highly permeable to the glucose, the quantity of HbA1C formed is directly proportional to the average blood sugar. There is little effect on the HbA1C levels by short-term elevation of plasma glucose. [15] HbA1C levels also help in the predicting development and progression of macrovascular complications.[16]. Advanced glycation end products lead to the long term complications. They also cause endothelial dysfunction, plaque formation and finally atherosclerosis. HbA1C level should be less than 7 % for reducing the risk of cardiovascular complications in type 2 diabetes mellitus. Approximately 1% increase in the HbA1C increase the estimated risk of CVD by 18%. [17, 11] It was found out that there is a positive correlation between HbA1c level and serum apolipoprotein B which is statistically significant (p-value < 0.05). Insulin resistance in diabetes leads to the accumulation of triglyceride rich lipoproteins. Altered glucose metabolism and dyslipidemia are the two important features of diabetes. [18, 19]. The severity of the dyslipidemia increases with an increase in the HbA1C. [20] There is a significant reduction in cardiovascular risk by improved glycemic control. Reduction in the HbA1C levels by 0.2% could decrease the mortality by 10%.[17] In the present study it was found that the concentration of serum apolipoprotein B is increased in cases when compared with healthy controls, which was statistically highly significant (p-value < 0.001). These results are in accordance with the studies done by Sniderman AD et al,[21] Martin SS et al [22] , Ley SH et al,[23] and Kanani FH et al.[24] Dyslipidemia in diabetes consists of an increase in triglycerides, triglyceride-rich lipoproteins, decreased HDL, increased LDL and cholesterol depleted small dense LDL. The main component of atherogenic lipoproteins is apolipoprotein B.[5] Insulin resistance is the cause for the increase in triglyceride concentration and apolipoprotein B in diabetes mellitus.[23] Apolipoprotein B is required for secretion of VLDL from the liver and it is bound to it until VLDL is cleared from the circulation as IDL or LDL.[5] Furthermore, correlation was done between serum apolipoprotein B and serum MPO levels in type 2 diabetic cases. And we found out that there is no significant correlation between serum apolipoprotein B and serum MPO levels in type 2 diabetes patients (r-value + 0.33) with p-value (> 0.05). In the present study, it is found that the concentration of MPO is increased in cases when compared with healthy controls, which is statistically highly significant (p-value < 0.001). It is in accordance with the studies carried out by Shetty S et al.[4] Wiersma JJ et al.[63] and Vit JA et al.[26] Endothelial dysfunction precedes clinically detectable atherosclerosis. Decreased levels of nitric oxide and oxidative stress are the major contributing factors for the development of endothelial dysfunction and atherosclerosis. Increased insulin levels stimulate the activity of the neutrophils. Myeloperoxidase is released from the granules of activated neutrophils, monocytes, macrophages at the inflammatory sites.[25] Myeloperoxidase oxidizes chloride in the presence of H2O2 to form HOCl. Compounds like Chlorinated L-arginine, chlorotyrosine, and HOCl-modified LDL are formed by the reaction of HOCl with amino acids and proteins. Increased levels of these compounds are found in the atherosclerotic lesions.[4] Myeloperoxidase stimulates the formation of oxidized LDL which promotes atherosclerosis. It also oxidizes HDL which in turn decreases its capacity for the reverse cholesterol transport. It also decreases the bioavailability of endothelial derived nitric oxide. [27,28] We found out that there is a significant

positive correlation between HbA1c and serum MPO levels in type 2 diabetes patients (r-value + 0.52), p-value (< 0.05). This suggests that as the HbA1c values were increasing, there is an increase in the serum MPO values in diabetic cases. Thus it can be concluded from our study that the future risk of CVD can be detected by evaluating the levels of apolipoprotein B and MPO in type 2 diabetic patients and can be prevented early by adequate control of glycaemia.

REFERENCES

1. John AA. Hunter, Diabetes Mellitus: B.M. Frier, A.S. Truswell, J. Shepherd, A.de Looy and R. Jung. Davidson‟s Principles and Practice of Medicine. 19th Ed; Elsevier:642-682.
2. Chandalia HB, Krishnaswamy PR. Glycated hemoglobin. Current Science 2002;83(12):1522-1532.
3. Kilpatrick ES. Hemoglobin A1c in the diagnosis and monitoring of diabetes mellitus. J Clin Pathol 2008;61:977-982.
4. Shetty S, Kumari S, Madhu LN. Variations in Serum Myeloperoxidase Levels With Respect to Hyperglycemia, Duration of Diabetes, Bmi, Sex and Aging in Type 2 Diabetes Mellitus. International Journal of Research in Pharmaceutical and Biomedical Sciences 2012;3(2):652-55.
5. Wagner AM, Perez A, Calvo F, Bonnet R, Castellvi A, Ordonez J. Apolipoprotein (B) Identifies Dyslipidemic Phenotypes Associated With Cardiovascular Risk in Normo cholesterolimic Type 2 Diabetic Patients. Diabetes Care 1999;22(5): 812-17.
6. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, Burns DE editors. Teitz fundamentals of clinical chemistry. 6th ed. Philadelphia: Saunders company; 2008. 273-401
7. Rifai N, Warnick R, Remaley AT. Lipids, Lipoproteins, Apolipoprotein, and other cardiovascular risk factors. In: Burtis CA, Ashwood ER, Burns DE editors. Teitz fundamentals of clinical chemistry. 6th ed. Philadelphia: Saunders company; 2008. 402-430
8. Santhi.T, Shaik JB , Mahendran KB. Myeloperoxidase levels predicts the vascular dysfunction in patients with Type 2 Diabetes Mellitus. IOSR Journal of Dental and Medical Sciences. 2017; 16(3): 30-34
9. Mahato RV, Gyawali P, Raut PP, Regmi P, Singh KP, Pandeya DR, et al. Association between glycemic control and serum lipid profile in type 2 diabetic patients: Glycated hemoglobin as a dual biomarker. Biomedical Research 2011; 22(3): 375- 380.
10. Avogaro A, Kreutzenberg SD, Albiero M, Menegazzo L, Fadini GP. Endothelial dysfunction in Diabetes. Diabetes Care 2011;34(2):S285-S290.
11. Selvin E, Marinopoulos S, Berkenblit G, Rami T, Brancati FL, Powe NR, et al. Meta-analysis : Glycosylated hemoglobin and cardiovascular disease in diabetes mellitus. Ann Intern Med 2004;141:421-431.
12. Pasupathi P, Manivannan P, Uma M, Deepa M. Glycated haemoglobin (HbA1c) as a stable indicator of type 2 diabetes. Int J Pharm and Biomed Res 2010;1(2):53-56
13. Mohsen AF, Hazmi EL, Swailem AL, Warsy AS, Meshari AL, Sulaimani R et al. Lipids and related parameters in Saudi type 2 diabetes mellitus patients. Ann Saudi Med 1999;19(4):304-307.
14. Silbernagel G, Kleber ME, Grammer TB, Winkelmann BR, Boehm BO, Marz W. Additional Use of Glycated Hemoglobin for Diagnosis of Type 2 Diabetes in People Undergoing Coronary Angiography Reveals a Subgroup at Increased Cardiovascular Risk. Diabetes Care 2011;34(11):2471–2473.
15. Richard F. Dods. Diabetes Mellitus. In: Kaplan L, Pesce A. Clinical Chemistry, Theory Analysis and Correlation. Hickman P, Koerbin G, Sublett K (eds).Chapter 38.2010:5th edn.USA;Mosby:749.
16. Fowler GC, Vasudevan DA. Type 2 diabetes mellitus: Managing hemoglobin A1c and beyond. Southern Med J 2010;103(9):911-916

17. Singh G, Kumar A. Relationship among HbA1c and lipid profile in Punjabi type 2 diabetic population. Journal of Exercise science and physiotherapy 2011;7(2):99-102.
18. Mallick AK, Maradi R, Joshi VR, Bhat PG. et al. A study on Apo B100/Apo A1 ratio in uncontrolled type 2diabetes mellitus. IJABPT 2011;2(1):379-384.
19. Onal ZE, Atasayan V, Akici N, Gurbuz T, Nuhoglu C. The Relationship between Glycosylated Haemoglobin (HbA1c) Levels and Serum Lipid Profiles in Insulin Resistant Children. HK J Paediatr 2014;19:22-27
20. Khan HA, Sobki SH, Khan SA. Association between glycaemic control and serum lipids profile in type 2 diabetic patients: HbA1c predicts dyslipidemia. Clin Exp Med 2007;7:24-29.
21. Sniderman AD, Lamarche B, Tilley J, Secombe D, Frohlich J. Hypertriglyceridemic hyperapo B in type 2 diabetes. Diabetes Care 2002;25(3):579-582.
22. Martin SS, Qasim AN, Mehta NN, Wolfe M, Terembula K, Schwartz S et al. Apolipoprotein B but not LDL cholesterol is assosciated with coronary artery calcification in type 2 diabetic whites. Diabetes 2009;58: 1887-1892.
23. Ley SH, Harris SB, Connelly PW, Mamakeesick M, Gittelsohn J, Wolever TM et al. Association of apolipoprotein B with incident type 2 diabetes in an Aboriginal Canadian population, Clinical Chemistry 2010;56(4):666-670.
24. Kanani FH, Alam JM. Apolipoprotein B in type 2 diabetes- a cross sectional study in a tertiary care set up. J Pak Med Assoc 2010;60(8): 653-656
25. Wiersma JJ, Meuwese MC, Miert JNIV, Kastelein A, Tijssen JGP, Piek JJ et al. Diabetes mellitus type 2 is associated with higher levels of myeloperoxidase. Med Sci Monit 2008;14(8):406-410.
26. Vita JA, Brennan ML, Gokce N, Shirley AM, Goormastic M, Shishehbor MH et al. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. Circulation 2004;110:1134–1139.
27. Dougherty A, Dunn JL, Rateri DL, Helnecke JW. Myeloperoxidase, a catalyst for lipoprotein oxidation is expressed in human atherosclerotic lesions. J Clin Invest 1994;94:437-444.
28. Kataoka Y, Shao M, Wolski K, Uno K, Puri R, Tuzcu EM etal. Myloperoxidase levels predict accelerated progression of coronary atherosclerosis in diabetic patients: Insights from intravascular ultrasound. Atherosclerosis. 2014; 232(2): 377–383.

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