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ORIGINAL RESEARCH

A Study on Serum Myeloperoxidase, Apolipoprotein B and Glycated Hemoglobin Levels in Type 2 Diabetes Mellitus

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ABSTRACT

Background: Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. Type 2 diabetes (T2D) occurs due to a progressive decline in the ability of the pancreas to secrete enough insulin as well as insulin resistance in insulin target tissues. The pathophysiology of T2D is characterized by excessive accumulation of ectopic fat in the liver, pancreas, and skeletal muscles, eventually manifesting as insulin resistance in these tissues and pancreatic beta cell dysfunction that ultimately leads to hyperglycemia.Metabolic abnormalities such as dyslipidemia, hyperinsulinemia, or insulin resistanceand obesity play key roles in the induction and progression of type 2 diabetesmellitus (T2DM). Objectives: To estimate the levels of serum myeloperoxidase, apolipoprotein B and glycated hemoglobin in type 2 diabetic patients and also in healthy controls, to observe the relationship between serum MPO with apo B and glycated hemoglobin and also between glycated haemoglobin with apo B in type 2 diabetic patients and healthy controls.

Materials and Methods: Case control study was done taking 30 cases of type 2 diabetes mellitus and 30 age and sex matched healthy controls. In all the subjects, concentrations of HbA1c, serum apo B and serum MPO were estimated. HbA1c was measured by turbidimetric method and serum Apo B by immune turbidimetric method using semi auto analyser CHEM 5 Plus. Serum MPO was measured by ELISA method using ELISA reader.

Results: The mean concentrations of HbA1c, serum Apo 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 Apo B and serum MPO in type 2 diabetic cases but there is no significant correlation between serum MPO and serum Apo B levels.

Conclusion: The present study suggests that chronic hyperglycemia and endothelial dysfunction are the major causal factors for pathogenesis of macrovascular complications in type 2 DM. The future risk of CVD can be detected by evaluating the levels of Apo 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] Type 2 diabetes (T2D) occurs due to a progressive decline in the ability of the pancreas to secrete enough insulin as well as insulin resistance in insulin target tissues. The pathophysiology of T2D is characterized by excessive accumulation of ectopic fat in the liver, pancreas, and skeletal muscles, eventually manifesting as insulin resistance in these tissues and pancreatic beta cell dysfunction that ultimately leads to hyperglycemia.Metabolic abnormalities such as dyslipidemia, hyperinsulinemia, or insulin resistanceand obesity play key roles in the induction and progression of type 2 diabetesmellitus (T2DM). The field of immunometabolism implies a bidirectional link betweenthe immune system and metabolism, in which inflammation plays an essential rolein the promotion of metabolic abnormalities (e.g., obesity and T2DM), and metabolic factors, in turn, regulate immune cell functionsChronic hyperglycemia causes increased glycation of proteins including hemoglobin, resulting in the formation of Advanced Glycated End products (AGE). Glycated hemoglobin (HbA1c) is formed by a post-translational, non-enzymatic process.[2] The increased level of HbA1c in diabetes is used as a reliable index of glycaemic control over the preceding weeks and months.3 The diabetes control and complications trail showed that 1% increase in HbA1c, corresponded to an increase of average blood glucose by 30 mg/dl.[2] Type 2 diabetes is characterized by a two-to-four-fold increased risk of cardiovascular disease. This is generally attributed to the adverse effects of hyperglycemia and oxidative stress. Even patients with pre diabetic conditions, such as impaired fasting glucose and impaired glucose tolerance, are also at increased risk of cardiovascular disease. Hence to assess the cardiovascular risk in type 2 diabetic patients, parameters like serum MPO, Apo B and HbA1c can be used.

MPO plays an important role in the initiation, progression and the complications of atherosclerosis.[3]

Myeloperoxidase, a pro-oxidant enzyme, released from the granules of leukocytes, monocytes and macrophages from the inflammatory sites can stimulate increased production of reactive oxygen species which can cause oxidative damage to the endothelium and vessel wall.[3]

HbA1c measures chronic glycaemic exposure rather than an acute value, therefore providing a more relevant view of long term glycaemia and future risk of complications.[4]

By measuring the serum levels of MPO, apo B and HbA1c, we can assess the future risk of cardiovascular disease in type 2 diabetic patients at an early stage and thereby we can prevent cardiovascular complications in type 2 diabetes patients by an adequate control of glycemia to the near normal levels.

The present study has been undertaken to evaluate the serum levels of MPO, apo B and HbA1c as early markers of cardiovascular risk in type 2 diabetic patients in turn helping in the early intervention and prevention of cardiovascular diseases in type 2 diabetic patients. The relationship between the levels of MPO and apo B is also assessed with the levels of glycated hemoglobin.

Aim of the study:

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.

Objectives of the study:

1. To estimate the levels of serum myeloperoxidase, apolipoprotein B and glycated hemoglobin in type 2 diabetic patients and also in healthy controls.

2. To observe the relationship between serum myeloperoxidase with apolipoprotein B and glycated hemoglobin in type 2 diabetic patients and healthy controls.
3. To observe the relationship between glycated hemoglobin with serum apolipoprotein B in type 2 diabetic patients and healthy controls.

MATERIALS & METHODS

A 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 a period of one year from 2019 – 2021. Patients were selected from TRR Medical College & Hospital.

Each patient gave an informed consent and the study is approved by the ethical and research committee of TRR Medical College & Hospital. The patients and controls voluntarily participated in the study.

Inclusion criteria

Cases:

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

Controls:

30 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 physician with FBS and PPBS according to American Diabetes Association criteria (FBS ≥ 126 mg/dl &2-hour PPBS ≥ 200 mg/dl).

Exclusion criteria

- Congenital Heart Diseases
- Hypertension
- Diabetic complications
- Systemic diseases
- Endocrinal disorders
- Malignancies
- Hemoglobinopathies
- Drugs which interfere with serum levels of myeloperoxidase, apolipoprotein B and glycated hemoglobin.

Collection of blood sample

After obtaining informed consent, about 6ml of fasting venous blood samples is drawn under aseptic precautions in to a sterile bulb from selected subjects.

- 4 ml of blood is taken into plain vaccutainer and Serum is separated by centrifugation, which is used for estimation of serum myeloperoxidase and apolipoprotein B.
- 2 ml blood is taken into EDTA containing vaccutainer and used for estimation of HbA1c.

Parameters measured

1. Myeloperoxidase
2. Glycated hemoglobin (HbA1c)
3. Apolipoprotein B

- Based on the inclusion and exclusion criteria, age and sex matched controls and cases are included in the present study after obtaining informed consent.
- A proforma is used to record the relevant information and patient‟s data.

RESULTS

Table 1: Age and sex wise distribution of controls and type 2 diabetic cases.

		Controls	Cases	p Value
Number of subjects		40	40	
Age (years)	Mean ± S.D	45.3 ± 8.2	48.2 ± 7.01	
	Range	28 – 58	31 – 57	> 0.05 NS
Gender	Male	14	13	
	Female	16	17	> 0.05 NS

NS- Not significant

Among 30 controls, 14 were males and 16 were females with a mean age of45.3 ± 8.2 years. Among the 40 diabetic cases, 13 were males and 17 were females with a mean age of 48.2 ± 7.01 years. There is no significant difference among controls and cases for the age ($p > 0.05$).

Table 2: Levels (mean ± SD) of HbA1c, Serum Apo B, Serum MPO in healthy controls and patients with type 2 diabetes mellitus.

Groups		HbA1c (%)	Serum Apo B (mg/dl)	Serum MPO (pg/ml)
Controls	Mean ± SD	4.96 ± 0.57	120.60 ± 29.76	10103 ± 2952.4
	Range	3.8 – 6.00	57.0 – 181.0	4000 – 15000
Cases	Mean ± SD	7.00 ± 1.11	234.51 ± 51.05	21223 ± 7885.5
	Range	5.0 – 8.6	123.0 – 373.1	4800 – 36600
Controls and Cases	Meandifference	2.01	111.8	11118
	t value*	10.05	12.16	7.42
	p value	< 0.001	< 0.001	< 0.001

[Table 2] shows comparative analysis of Glycated hemoglobin, serum Apo B and serum MPO levels between healthy controls and type 2 diabetic cases. It is observed from the table that the mean levels of HbA1c, serum Apo B and serum MPO in controls are in the range of 4.96 ± 0.57, 120.62 ± 29.76 mg/dl and 10103 ± 2954.4 pg/ ml respectively.
The mean levels of HbA1c, serum Apo B and serum MPO in cases are in the range of 7.00 ± 1.11 %, 234.51 ± 51.05 mg/dl and 21223 ± 7885.5 pg/ml respectively, which are significantly higher than controls ($p < 0.001$).

Table 3: HbA1c levels in controls and type 2 diabetic cases.

Groups	No. of subjects	HbA1c (%) Mean ± S.D	t value	p value
Controls	30	4.96 ± 0.57	10.05	< 0.001
Cases	30	7.00 ± 1.11		

A mean HbA1c levels in controls is 4.96 ± 0.57 % and in cases is 7.00 ± 1.11%. There is a significant increase in HbA1c levels in cases as compared to controls ($p < 0.001$).

Table 4: Serum Apo B levels in controls and type 2 diabetic cases.

Groups	No. ofsubjects	Serum MPO (pg/ml) Mean ± S.D	t value	p value
Controls	30	10103 ± 2952.4	7.42	< 0.001
Cases	30	21223 ± 7883.5		

A mean serum MPO levels in controls is 10103 ± 2952.4 pg/ml and in cases is 21223 ± 7883.5 pg/ml. There is a significant increase in serum MPO levels in cases as compared to controls ($p < 0.001$).

Table 6: Pearson's correlation between biochemical parameters in type 2 Diabetes Mellitus patients.

Correlation Analysis		
Relationship between	r value	p value
Serum MPO and HbA1c	+ 0.50	< 0.05*
Serum MPO and Apo B	+ 0.31	> 0.05
HbA1c and Apo B	+ 0.50	< 0.05*

Pearson‟s correlation coefficient analysis was used to find the correlation of serum MPO with HbA1c, MPO and HbA1c with serum Apo B among diabetic cases. The Pearson‟s correlation analysis shows statistically significant positive correlation between serum MPO and HbA1c with r value + 0.50 with ($p < 0.05$).

DISCUSSION

Diabetes mellitus is associated with low grade inflammation resulting in increase in inflammatory markers. Persistent hyperglycemia in uncontrolled diabetics can cause inflammation and increased production of reactive oxygen species from glucose auto oxidation which can predispose to detrimental consequence in diabetes mellitus.

Chronic hyperglycemia is associated with significant long-term complications particularly damage to the heart, blood vessels and nerves. The importance of protecting the body from hyperglycemia cannot be overstated; the direct and indirect effects on the human vascular tree are the major source of morbidity and mortality in type 2 Diabetes. The injurious effects of hyperglycemia are separated into macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy).[5,6]

Type 2 diabetes is characterized by a two-to-four-fold increased risk of cardiovascular disease. Cardiovascular death accounts for more than 75 % of all deaths among persons with diabetes mellitus. This is generally attributed to the adverse effects of hyperglycemia and oxidative stress on vascular biology.[7,8]

The aim of this study is to evaluate long term glycemic control, endothelial dysfunction and dyslipidemic status by measuring glycated hemoglobin, serum myeloperoxidase and serum apolipoprotein B levels in cases of type 2 DM and compare it with healthy controls.

In the present study a total of 80 subjects, of which 40 type 2 diabetes mellitus patients and rest 40 healthy controls were included. The glycated hemoglobin, serum apolipoprotein B and serum myeloperoxidase levels were estimated in all these subjects.

HbA1c:

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) with the mean value of 4.96 ± 0.57 % in controls and 7.00 ± 1.11 % in cases. This is in accordance with the studies of SelvinE et al.[8]

The level of HbA1c value ≤ 7.0% was said to be appropriate for reducing the risk of cardiovascular complications in type 2 diabetes mellitus. Estimated risk of CVD has shown to be increased by 18% for each 1% increase in absolute HbA1c value in diabetic population.[5,8]

Further correlation was done between HbA1c level and serum apo B level. It was found out that there is a positive correlation between HbA1c level and serum Apo B (r value + 0.52), which is statistically significant (p value < 0.05).
This suggests that there was a significant positive correlation between HbA1c and serum apo B. Glucose and fatty acids are the two major fuels in the body. Disorder involving one is associated with altered metabolism of the other. Diabetes is associated with altered glucose metabolism and dyslipidemia. Researchers suggest that triglyceride-rich lipoproteins accumulate in the insulin-resistant state, causing the decreased activity of lipoprotein lipase, increased lipolysis in adipose tissue and increased output of very low-density lipoprotein (VLDL) particles from the liver.[9]

Serum Apolipoprotein B

In our study mean serum apo B levels of 120.60 ± 29.76 mg/dl and 234.51 ± 51.05 mg/dl are noted in healthy controls and type 2 diabetes mellitus respectively. The differences in mean values are found to be statistically significant with p value (< 0.001). These results are in accordance with the studies done by Sniderman AD et al,Martin SS et al.[10-13]
Type 2 diabetes is associated with cardiovascular disease, which might be due at least in part, to abnormalities in lipid and lipoprotein metabolism. Apo B is the principal protein moiety of LDL, IDL, and VLDL and its concentrations are a good estimate of the total mass of the atherogenic particles. High apo B concentration is associated with increased cardiovascular disease, independently of LDLc levels in type 2 Diabetes mellitus.[14]
Furthermore correlation was done between serum Apo B and serum MPO levels in type 2 diabetic cases. And we found out that there is no significant correlation between serum Apo B and serum MPO levels in type 2 diabetes patients (r value + 0.33) with p value (> 0.05).

Serum Myeloperoxidase (MPO)

In the present study the mean level of serum MPO in controls is 10103 ± 2952.4 pg/ml and in cases is 21223 ± 7885.5 pg/ml. Statistical analysis by Unpaired student‟s t test has shown that the level of serum MPO in type 2 diabetic patients is significantly increased as compared to controls (p < 0.001). It is in accordance to the studies carried out by Vit JA et al.[15]
Furthermore correlation was done between HbA1c and serum MPO. And we found out that there is a significant positive correlation between HbA1c and serum MPO levels in type 2 diabetes patients (r value + 0.50) which is statistically significant p value (< 0.05).
This suggests that the as the HbA1c values were increasing, there is increase in the serum MPO values in diabetic cases.MPO can use high-glucose stimulated, vascular non-leukocyte derived H2O2 to induce diabetic endothelial dysfunction by reducing nitric oxide bioavailability.

CONCLUSION

The results of our present study supports the concept that increase in HbA1c, serum Apo B and MPO may play an important role in the development of macrovascular complications in type 2 DM.
The present study also suggests that chronic hyperglycemia and endothelial dysfunction are the major causal factors for the pathogenesis of macrovascular complications in type 2 DM.
There is a significant increase in HbA1c levels in type 2 diabetes mellitus patients when compared to healthy controls showing a state of hyperglycemia in DM patients.
HbA1c is the most valuable index of metabolic control for diabetic patients as well as a measure of risk for development of macrovascular complications.
Serum MPO is also significantly increased in type 2 diabetic patients as compared to healthy controls in the present study. MPO is the pro-oxidant enzyme that is released from granules

of activated leukocytes, monocytes and macrophages. The increase in the MPO levels in diabetes is explained by the fact that adhered neutrophils evaluated by MPO activity, are enhanced in diabetes patients which might be related to hyperinsulinemia. MPO plays an important role in the leukocyte mediated vascular injury responses in inflammatory vascular diseases such as atherosclerosis. MPO is an independent predictor of CVD in type 2 diabetes mellitus patients. By evaluating the levels of Apo B and MPO we can assess the future risk of cardiovascular disease in type 2 diabetic patients at an early stage and thereby we can prevent cardiovascular complications in type 2 diabetes patients by an adequate control of glycemia to the near normal levels. Limitations of the study are large scale study is required to confirm the conclusion.

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. Shankar Shetty, Sucheta Kumari N, 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.
4. Goldenberg RM, Cheng AY, Punthakee Z, Clement M. Use of Glycated Hemoglobin (A1c) in the Diagnosis of Type 2 Diabetes Mellitus in Adults, Canadian Journal of Diabetes: 247-249.
5. Singh G, Kumar A. Relationship among HbA1c and lipid profile in Punjabi type2 diabetic population, Journal of Exercise science and physiotherapy 2011;7(2):99-102.
6. Fowler MJ. Microvascular and macrovascular complications of diabetes. Clinical Diabetes 2008;26(2):77-82.
7. Avogaro A, Kreutzenberg SD, Albiero M, Menegazzo L, Fadini GP. Endothelial dysfunction in Diabetes. Diabetes Care 2011;34(2):S285-S290.
8. 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.
9. Onal ZE, Atasayan V, Akici N et al. The Relationship between Glycosylated Haemoglobin (HbA1c) Levels and Serum Lipid Profiles in Insulin Resistant Children. HK J Paediatr 2014;19:22-27.
10. Ley SH, Harris SB, Connelly PW, Mamakeesick M, Gittelson 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.
11. 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.
12. Sniderman AD, Lamarche B, Tilley J, Secombe D, Frohlich J. Hypertriglyceridemic hyperapo B in type 2 diabetes, Diabetes Care 2002;25(3):579-582.
13. 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.
14. Wagner AM, Perez A, Calvo F, Bonnet R, Castellvi A, Ordonez J. Apoliopoprotein (B) Identifies Dyslipidemic Phenotypes Associated With Cardiovascular Risk in Normo cholesterolimic Type 2 Diabetic Patients. Diabetes Care 1999;22(5): 812-17.
15. Vita JA, Brennan ML, Gokce N et al. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. Circulation 2004;110:1134–1139.