Original Article

Type-2 diabetes mellitus with or without metabolic syndrome and their associated critical factors: A study from Northern India

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Abstract

Background: Diabetes mellitus is associated with carbohydrate, lipid and protein metabolism abnormalities. Uncontrolled hyperglycaemia can result in dysfunction of various organs such as eyes, kidneys, nerves, and heart and blood vessels leading to long-term complications like nephropathy, neuropathy, retinopathy, stroke and ischaemia. The main objective of the study was to identify critical factors in Type 2 diabetes mellitus (Type 2 DM) with metabolic syndrome (mets) compared with Type 2 DM without mets and their association in the development of Type 2 DM to Type 2 DM with mets and cardiovascular complications. This can aid in improving the clinical management and the consequences of the disease. **Materials and Methods:** The present study was conducted in the Department of Biochemistry, a tertiary care centre in Northern India. All patients who were aged between 35 and 65 years of age were enrolled. Enrolled subjects were divided into three groups, Group I: 50 healthy people; Group II: 50 Type 2 DM without mets; and Group III: 50 Type 2 DM with mets. These patients were subjected to Anthropometric and biochemical parameter assessment. **Results:** On comparing Group III with control and Group II significant difference was observed in these parameters, that is, elevated TGs ($P = 0.001$), reduced high-density lipoprotein (HDL) level ($P = 0.001$), elevated high-sensitivity C-reactive protein (hs-CRP) (0.011), high serum insulin fasting ($P = 0.010$), weight ($P = 0.021$), waist circumference ($P = 0.001$) and BMI ($P = 0.001$). In the control group, head circumference was significantly lower compared to Group II ($P = 0.001$) and Group III ($P = 0.001$). **Conclusion:** On the basis of observed observation, it has been suggested that low enzymatic activity with poor glycaemic control may further progress Type 2 DM into Type 2 DM with metabolic syndrome and cardiovascular complications. High hs-CRP concentration and high fasting insulin can be independent predictor of cardiovascular complications.

Keywords: Diabetes mellitus, hs-CRP, metabolic syndrome

Introduction

Diabetes mellitus has become a global burden representing 415 million people with Diabetes, accounting for 8.8% of adults aged 20-79. Around 75% of the people, and more than 80% of the population in low- and middle-income countries, are undiagnosed Diabetes.[1] Type 2 diabetes mellitus (Type 2 DM) is the commonest form of Diabetes, representing 90–95% of the population with Diabetes.[2] Diabetes mellitus is associated with carbohydrate, lipid and protein metabolism abnormalities. Uncontrolled hyperglycaemia can result in dysfunction of various organs such as eyes, kidneys, nerves, heart and blood vessels leading to long-term complications like nephropathy, neuropathy, retinopathy, stroke and ischaemia.[1,2]

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An impairment of insulin secretion leading to a defect in the action of insulin on target tissues is the basis of abnormalities in Type 2 DM. Reduced physiological response of the peripheral tissues to the action of the insulin, representing insulin resistance, is the major finding in Type 2 DM and metabolic syndrome (mets).[3,4] Insulin resistance is involved in the physiology of dyslipidemic Diabetes and metabolic syndrome (mets).[5] Metabolic syndrome is a combination of risk factors such as insulin resistance, abdominal obesity, hyperglycaemia, hypertriglyceridemia, decreased high-density lipoprotein (HDL) and hypertension.[6] Diabetes and cardiovascular disease (CVD) are important complications of metabolic syndrome. Recent studies have shown that alteration in Na+/K+ -ATPase enzyme, a high-sensitivity C-reactive protein (hs-CRP), can play a vital role in an increased incidence of Diabetes and cardiovascular complication.[7] Therefore, it is essential to understand the critical factors and their pathophysiological roles in Diabetes, metabolic syndrome and cardiovascular outcomes. The main objective of the study was to identify critical factors in Type 2 DM with mets compared with Type 2 DM without mets and their association in the development of Type 2 DM to Type 2 DM with mets and cardiovascular complications. This can aid in improving the clinical management and the consequences of the disease.

Materials and Methods

Study area, population and period

The present study was conducted in the Department of Biochemistry, a tertiary care centre in Northern India. All patients who were aged between 35 and 65 years of age were enrolled. Enrolled subjects were divided into three groups, Group I: 50 healthy people; Group II: 50 Type 2 DM without mets; and Group III: 50 Type 2 DM with mets. Diabetes is a clinical syndrome diagnosed based on the criteria (fasting glucose ≥126 mg/dl or 2 h postprandial glucose ≥200 mg/dl) as proposed by the American Diabetes Association.[8] According to ATP (Adult Treatment Panel) III criteria, metabolic syndrome is defined when three or more of the following critical determinants are present: Hyperglycaemia (fasting plasma glucose ≥100 mg/dl), hypertriglyceridemia (triglyceride ≥150 mg/dl), low HDL cholesterol (HDL, 40 mg/dl for men and 50 mg/dl for women), blood pressure elevation (≥130 / 85 mmHg) and increased waist circumference (≥102 cm for men and ≥88 cm for women). Patients on medication like glucocorticoids, calcium blockers, thyroxin, digitalis, lipid-lowering agents and mineralocorticoids interfering with e Na+/K+ -ATPase enzyme activity were excluded from the study.

Data collection

A total of 150 patients who met the inclusion criteria were enrolled in this study. All patients underwent Waist (WC) and hip (HC) circumference measurements to calculate BMI (Body Mass Index) calculation. Systolic and diastolic blood pressure measurement was done in all the patients.

Collection and processing of the samples

All enrolled patients underwent investigations like fasting blood sugar (FBS), triacylglycerides (TGs), total cholesterol (TC), low-density lipoprotein (LDL), HDL, serum insulin fasting (SIF), hs-CRP, and haemoglobin A1c (HbA1c). Venous blood samples were collected in sodium fluoride, ethylenediaminetetraacetic acid (EDTA) and plain test tubes. Serum and plasma were separated as per standard protocol. Serum TGs, TC, LDL, and HDL were determined by enzymatic methods using an automated analyzer. FBS was measured by enzymatic methods using an automated analyzer. SIF was measured by enzymatic methods using an automated analyzer. Both HbA1c and hs-CRP were measured by an immunoturbidimetric method using an automated analyzer. We have also categorized serum hs-CRP into low level (<1 mg/l), moderate level (1–3 mg/l), and high level (≥3 mg/l).[9]

Isolation of erythrocyte membrane

The erythrocyte membrane was prepared as per the previously published protocol by M DeLuise *et al.*[10] Washed-packed erythrocyte cells were lysed by 10 volumes of ice-cold 5 mmol/l Tris and 0.1 mmol/l Na2EDTA at pH 7.6, then centrifuged at 20000 g for 20 min at 4°C. The supernatant was discarded, and the pellet was washed three times with 20 ml of 5 mmol/l Tris-HCl and 17 mmol/l NaCl of pH 7.6 and then with 20 ml of 10 mmol/l Tris-HCl (pH 7.5). The erythrocyte membrane was resuspended in 10 mM Tris-HCl buffer (pH 7.5). Furthermore, erythrocyte membrane protein and erythrocyte Na+/K+ -ATPase activity were estimated as per previously published standards by M A Markwell *et al.*[11]

Na+/K+ -ATPase activity assay[11]

A 50 µl of the suspended erythrocyte membrane was mixed with 5 mmol/l ATP, 25 mmol/l KCl, 75 mmol/l NaCl, 5 mmol/l MgCl2, 0.1 mmol/l EGTA and 25 mmol/l Tris-HCl at pH 7.5 in a total volume of 500 µl and incubated for 90 min at 37°C. Further, trichloroacetic acid to a final concentration of 5% (w/v) was added to stop the reaction. According to Fiske and Subbarow reaction total amount of inorganic phosphorus was measured in supernatant after centrifugation for 20 min at 1500 g. An inhibitor of Na+/K+-ATPase activity, that is, 1 mmol/l Ouabain is used, and this assay procedure was repeated. The amount of inorganic phosphorus (nmol) liberated per mg of membrane protein per hour was expressed as total ATPase activity. By subtracting the ATPase activity in the presence of Ouabain from the total Na+/K+-ATPase activity in the absence of Ouabain, the activity of Na+/K+-ATPase was determined.

Data analysis

The data collected were analyzed using the STATA/SE version 14.0 statistical software (Stata Corp, Texas, USA). Categorical data were described using numbers and percentages. Data generated from the present study have been presented in the form of tables, and all descriptive analyses have been shown in percentages. Comparisons between categorical variables were performed with the χ^2 – test. An independent *t*-test was used

to evaluate differences in continuous variables between the two groups. The correlations between quantitative variables were studied with Pearson's correlation for parametrical variables and the Spearman test for nonparametric variables. *P* value has been calculated to analyze statistical significance.

Results

The anthropometric and biochemical variables in Type 2 DM patients and the control group are summarized in Table 1. No significant differences were observed in age, blood pressure, both systolic and diastolic, total cholesterol and LDL between Type 2 DM patients and the control group ($P > 0.05$). However, FBS levels were significantly different between Group I and Group II, Group II and Group III and Group I and Group III ($P < 0.05$). There was no significant difference observed between the control group and Type 2 DM Group II for these anthropometric and biochemical parameters with regards to height ($P = 0.231$), weight ($P = 0.401$), waist circumference ($P = 0.421$), BMI ($P = 0.834$), TGs ($P = 0.457$), HDL ($P = 0.615$) and hs-CRP ($P = 0.412$). On comparing Group III with control and Group II significant difference was observed in these parameters, that is, elevated TGs ($P = 0.001$), reduced HDL level ($P = 0.001$), elevated hs-CRP (0.011), high serum insulin fasting ($P = 0.010$), weight ($P = 0.021$), waist circumference ($P = 0.001$) and BMI ($P = 0.001$). In the control group, head circumference was significantly lower compared to Group II ($P = 0.001$) and Group III ($P = 0.001$). Compared to Group III, head circumference was significantly lower in Group II patients ($P = 0.003$). A significant decrease in membrane protein content was observed in Group II ($P = 0.029$) compared to Group I. No significant difference was observed between Group II and Group III ($P = 0.256$). Compared to the control group, significantly lowered erythrocyte Na+/K+ -ATPase activity values were observed in the Type 2 DM groups ($P = 0.03$). However, no significant difference in erythrocyte Na+/K+ -ATPase activity was observed between Group II and Group III ($P = 0.368$). There was no significant difference in total Na+/K+ -ATPase activity between the Type 2 DM groups and the control group. Furthermore, no differences were observed between Group II and Group III significantly. Erythrocyte Na+/K+ -ATPase enzyme activity was positively correlated with HDL (($r = -0.231$, $P = 0.01$), and negatively correlated with TGs ($r = -0.102$, $P = 0.046$), insulin fasting ($r = -0.137$, $P = 0.056$), waist circumference ($r = -0.141$, $P = 0.085$), blood sugar fasting ($r = -0.128$, $P = 0.086$). No significant correlation was observed between blood pressure ($r = 0.058$, $P = 0.451$), BMI ($r = -0.127$, $P = 0.176$) and hs-CRP ($r = -0.015$, $P = 0.765$). A positive correlation was observed between blood sugar fasting ($r = 0.164$, $P = 0.041$), TGs ($r = 0.162$, $P = 0.036$), BMI ($r = 0.341$, $P = 0.006$) and hs-CRP. BMI was positively correlated with waist circumference ($r = 0.257$, $P = 0.015$) and negatively associated with HDL ($r = -0.102$, $P = 0.046$). There was a significant positive correlation between BMI ($r = -0.102$, $P = 0.046$), TGs ($r = -0.102$, $P = 0.046$), blood sugar fasting ($r = -0.102$, $P = 0.046$), hs-CRP ($r = -0.102$, $P = 0.046$) and serum insulin fasting ($r = -0.102$, $P = 0.046$). A negative correlation was observed between serum insulin fasting and HDL ($r = -0.172$, $P = 0.056$). Blood sugar fasting ($r = -0.312$, $P = 0.066$) and waist circumference ($r = -0.671$, $P = 0.61$) were negatively correlated with HDL. Positive correlations were observed between blood sugar fasting and TGs ($r = 0.102$, $P = 0.046$). TGs were negatively correlated with HDL ($r = -0.276$, $P = 0.001$) and a positive relation was observed with waist circumference ($r = -0.159$, $P = 0.051$).

Discussion

The study's main objective was to identify risk factors in Type 2 DM compared with Type 2 DM without mets and their association in the development of Type 2 DM to Type 2 DM with mets and cardiovascular complications. The critical factors shown in Table 1 are significantly associated with Type 2 DM with metabolic syndrome as compared to the control and Type 2 DM without metabolic syndrome group. Previous studies have shown that elevated triglyceride levels are associated with increased

Table 1: Showing anthropometric and biochemical data of Type 2DM patients and the control group.

	Group I {Control}	Group II {Type2 DM without mets}	Group III {Type2 DM with mets}	Group II vs Group I	Group III vs Group I	Group II vs Group III
Age	51.2±3.8	52±4.9	53±5.6	0.441	0.125	0.678
Weight	78.1±19.4	79±10.7	81±11.9	0.401	0.021	0.021
HC (cm)	96±7.9	101.9±6.9	104.1±6.8	0.023	0.001	0.001
WC (cm)	89±10.8	92±11.2	99.4±9.8	0.421	0.001	0.001
BP (mmHg)	122/80±12.6	119/78±9.8	130/80±16.5	0.596	0.049	0.032
BMI (kg/m^2)	21.5±2.7	24.2±3.7	25.7±3.7	0.834	0.001	0.001
TGs	124±49.5	127±47.8	200.2±45.2	0.457	0.001	0.001
HDL	45±6.8	42.7±6.9	36.9±7.2	0.615	0.001	0.001
LDL	117.5±28.5	111.2±28.9	117.4±37.5	0.764	0.487	0.235
HbA1c	5.7±0.3	7DM .8±1.8	7.7±1.9	0.001	0.001	0.786
FBS	97.2±11.5	139.4±46.2	154.1±41.5	0.001	0.001	0.023
SIF	11.5±4.9	10.5±3.6	13.9±6.8	0.891	0.010	0.010
Hs-CRP	1.7±0.8	1.9±0.6	2.1±0.8	0.412	0.011	0.011

Data are expressed as mean±SD and *P* values are analyzed. BMI=Body mass index, BP=Blood pressure, FBS=Fasting blood sugar, SIF=Serum insulin fasting, Hb A1c=Hemoglobin A1c, HC=Hip circumference, HDL= High density lipoprotein, hs-CRP=High sensitivity C-reactive protein, LDL=Low density lipoprotein, TGs=Triacylglycerides, WC=Waist circumference, mets=Metabolic syndrome, Type2 DM=Type 2 diabetes mellitus

insulin levels, which can lead to obesity.[12] Weight gain and obesity are associated with insulin resistance, dyslipidemia, Type 2 DM, hypertension and cardiovascular complications as shown in previous studies. Many studies have shown these risk factors are a predictor of cardiovascular disease and its complication.[13,14] A similar, positive correlation was observed in our study, especially in TGs with insulin fasting, blood sugar fasting, waist circumference and hs-CRP. These risk factors were present in Type 2 DM patients with metabolic syndrome as observed in our study. As shown in several other studies, a significant positive association was observed in blood sugar fasting, TGs, waist circumference and BMI, serum insulin fasting and hs-CRP. Similar, results were observed in our studies in patients with Type 2 DM groups. hs-CRP levels are important predictors of cardiovascular diseases and complications. Type 2 DM with metabolic syndrome showed high hs-CR*P* values as compared to control and Type 2 DM without metabolic syndrome groups. Na+/K+ -ATPase enzyme activity was positively correlated with HDL and negatively correlated with TGs, insulin level, blood sugar fasting and waist circumference. The shown results explain that low erythrocyte enzymatic activity is associated with low metabolic activity, which could be involved in the pathophysiology of Diabetes and its related complications. However, more province-wise studies are warranted to explain that low erythrocyte enzyme activity is associated with the development of Diabetes- related complications. Previous studies have shown that poor glycaemic control is associated with low erythrocyte enzyme activity and an increased incidence of Diabetes-related complications.[15] Similar results were observed in our study too. In contrast to the study by Koc B *et al.* researchers have reported no significant correlation between enzymatic activity and glycemic state in Type 2 DM and control populations.[16]

The study concludes that primary care physicians should be aware of these critical factors to provide better care for patients with Type 2 DM.

Conclusion

On the basis of observed observation, it has been suggested that low enzymatic activity with poor glycemic control may further progress Type 2 DM into Type 2 DM with metabolic syndrome and cardiovascular complications. High hs-CRP concentration and high fasting insulin can be independent predictors of cardiovascular complications. This study is a reminder for clinicians that timely measurement of critical factors and assessment of cardiovascular risk should be done in the patients with Diabetes to prevent progression of diseases, which will further improve the management of the diabetic patients.

Strengths of the study

This study was done in a tertiary care hospital in north India in which patients of different age groups were enrolled This study highlights that the timely measurement of critical factors and assessment of cardiovascular risk should be done in patients with Diabetes to prevent progression of diseases.

Limitation of the study

This was a single-centric study with a small sample size and only in-patients were enrolled.

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Conflicts of interest

There are no conflicts of interest.

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