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Biochemical abnormalities in erythrocyte membrane of uncontrolled type II diabetes mellitus

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ABSTRACT

Glycated haemoglobin, lipid peroxidation alternations in lipid composition and ATPase activities were analysed in erythrocyte plasma membrane of uncontrolled type II diabetes mellitus (DM) patients. Glycated haemoglobin, erythrocyte membrane cholesterol, phospholipid were significantly increased in DM patients. The molar ratio of cholesterol to phospholipid, lipid peroxidation in the form of thiobarbituric acid-reactive substances (TBARS), and Na^+-K^+-ATPase activity significantly decreased in the diabetic subjects, as compared to controls. The study suggested that biochemical changes in the erythrocyte membrane may be involved in the patho physiology of type II DM.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by high blood sugar, insulin resistance, low consciousness and obesity .Several abnormalities related to the reduced life span, increased viscosity, excessive aggregation, and increased tendency to adhere to endothelial cells have been reported in the erythrocytes of diabetes mellitus (DM) patients[1,2]. Alterations in levels of cholesterol to phospholipid molar ratio, unsaturated fatty acids and modified membrane asymmetry configuration have also been reported in the erythrocyte membranes of diabetic patients[3,4]. The modification in lipid composition which affect the physico-chemical properties of the erythrocyte membrane of diabetic patients[5,6]. The controlled level of Insulin normalizes lipid composition and maintain the stability of erythrocyte membrane in diabetic patients. It stimulates Na^+-K^+-ATPase activity, and translocation of ions and glucose to plasma membrane via phosphorylation of the α subunits by proteins kinases[7].

Glucose under physiological conditions produces oxidants that posess reactivity similar to free radicals. Increased free radical production and high levels of lipids and rapid peroxidation products have been reported in diabetic patients[8]. When erythrocytess are exposed to continuous oxidative stress in type II diabetic patients, free radicals are continuously generated by the auto-oxidation of haemoglobin continuously[9] Oxygen radicals formed over and above the detoxifying capacity of erythrocytes can cause peroxidative breakdown of phospholipid fatty acids and accumulation of malonyldialdehyde[10,]. This change brings about membrane instability high pressure and poor in oxygen transport in RBC. The present study was undertaken to determine levels of lipid peroxidation and alterations in lipid composition and ATPase activities in erythrocyte plasma membrane of uncontrolled type II DM patients[11].

MATERIALS AND METHODS

Blood sample of type II diabetic patients were collected according to the age group of 30-70 years from south of Tamil Nadu . Glycosylated haemoglobin was calculated by analytical kits. Lipid profile of each patients were determined by Auto analyzer.

Isolation of erythrocyte membranes

Erythrocyte membrane of type II diabetic patients was isolated in cold conditions. The blood was collected in 0.1 M trisodium citrate. This was centrifuged at 3000 rpm for 15min and the plasma and buffy coat was aspirated off. Saline-washed erythrocytes were lysed in of ice-cold 5.0 mM Tris-HCL (pH 7.0), 1.0 mM EDTA (pH 7.0). The resulting erythrocyte membranes were re-suspended twice in ice-cold lysis solution, centrifuged at 6000 rpm for 15min, suspended in ice-cold 0.05 M Tris-HCL (pH 7.0), 1.0 mM EDTA, 0.5M Nacl (pH 7.0) and centrifuged at 6000 rpm for 15min. The membranes were treated with high sodium chloride concentration to form pink membrane.. The faint pink membranes, thus obtained were suspended in ice-cold 5.0 mM Tris-HCL (pH 7.0), 1.0 mM EDTA (pH 7.0) and centrifuged at 6000 rpm for 15min. The resulting haemoglobin-free milky-white erythrocyte membranes were suspended in 0.05 M Tris-HVL (pH 7.0)[12]. Protein in erythrocyte membranes was estimated by the method of Lowry *et al*[13].

Assay of ATPase activity

ATPase activity in erythrocyte membranes was assayed by the membrane suspension was diluted to a volume containing ~ 150-200 µg protein/ 100µL membrane suspension for the ATPase assay. ATP neutralized to pH 7.0 by 100 mM Tris was used for the assay[14]. ATPase activity was expressed as µmoles P/mg erythrocyte membrane protein/60min.

For total ATPase activity, erythrocyte membranes (~ 150-200 µg protein/ 100µL membrane suspension) were incubated at 37°C for 60 min in 0.5 mL reaction mixture containing 2.0 mM ATP, 100 mM Nacl, 20 mM KCl, 5.0 $MgCl_2$, 1.0 mM EDTA in 50 mM Tris-HCL (pH 7.0), and for Mg^{2+}-ATPase activity, in 0.5 mL reaction mixture containing 2.0 mM ATP, 5.0 mM $MgCl_2$ in 50 mM Tris-HCL (pH 7.0). The reaction was stopped by adding 0.1 mL of 10% sodium dodecyl sulphate (SDS). Inorganic phosphorus (P_i) hydrolyzed from the reaction was measured.. ATPase activity was calculated by the difference between 0 min (reaction stopped immediately with SDS) and 60 min incubation periods. Na^+-K^+-ATPase activity was calculated from the difference between total ATPase and Mg^{2+}-ATPase activities.

Erythrocyte membrane parameters

Thiobarbituric acid-reactive substances (TBARS) in erythrocyte membranes were estimated . Lipids were extracted from erythrocyte membrane using chloroform: methanol (2:1) mixture and cholesterol was estimated by zak 's method.. Na^+ and K^+ in serum were determined using a flame photometer[15.16,17].

RESULTS AND DISCUSSION

The diabetic patients were categoried according to its age, sex and food habits. The glycated haemoglobin is a predictor of glucose level in blood. This method is most objective and reliable measure of type 2 diabetes. Male patients in the age of 40-50 were easily affected in type 2 diabetes. Diabetic patients showed in the average of > 6.5% of HbA_1 C. They showed significantly higher concentrations of cholesterol (325mg/dl) , phospholipids (224mg/dl),Triglycerides(155 mg/dl). The levels of LDL and VLDL of diabetic patients were found to be 124 mg/dl and 34.5mg/dl. The HDL level of type 2 diabetic patients was found to be 31 mg/dl. The phospholipid , cholesterol and TBARS were drastically increased in erythrocyte membrane of diabetic patients as compared to age-and sex-matched controls. Similarly, diabetic female subjects has significantly higher concentrations of cholesterol and TBARS, but phospholipids concentration was not significantly different . The cholesterol to phospholipids molar ratio in erythrocyte membranes of both male and female diabetics was found to be higher, as compared to controls.. The rapid changes in erythrocyte membrane lipid concentrations in diabetics lead to accompany poor glycaemic control in patients, presenting with apparent normo-lipidaemia.

Studies of human erythrocyte have shown that membrane lipid peroxidation results in decreased cell survival, altered membrane phospholipid asymmetry, hypercoagulability and increased adhesion to endothelial cells. The erythrocyte membrane showed sudden raise of Mg^{2+}-ATPase, and significantly lower activity of Na^+-k^+-ATPase,

as compared to age- and sex. Type II diabetic patients were easily undergo malfunctioning of erythrocyte membrane due to stress, obesity and lack of insulin resistance.. Thus, even newly diagnosed diabetics might be suffering from complications, arising due to prolonged hyperglycaemia, which may lead to cardiovascular disorders.

CONCLUSION

The present study found abnormal erythrocyte membrane characteristics in uncontrolled type II DM patients, suggesting that these changes may be involved in the pathophysiology of type II DM. This may lead to cardiovascular complications.

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