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Changes in Erythrocyte Membrane in Type-2 Diabetes Mellitus with and without Dyslipidemia

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Abstract

Background: In Diabetes mellitus there is decreased number of pump units on the erythrocyte membrane, altered lipid – protein interaction, depleted membrane anionic charge and enzyme glycation and peroxidation which account for many abnormal complications.

Method: Diabetes subjects with and without dyslipidemia were selected along with healthy control then we correlate the changes in serum and erythrocyte membrane.

Result: The lipid composition of membrane is significantly increased with cholesterol:phospholipid ratio and Na^+K^+-ATPase of membrane was decreased when compared to control.

Conclusion: Lipid composition disturbances along with increased hyperglycemia which causes glycation of enzymes and oxidative stress causes the decrease in activity of Na^+K^+-ATPase and other changes in erythrocyte membrane.

Keywords: ATPase; Diabetes mellitus; Dyslipidemia; Erythrocyte; Membrane

Introduction

Diabetes mellitus, one of the most prevalent diseases in developing world, is a metabolic disorder characterized by hyperglycemia and other metabolic alterations due to relative or absolute insulin deficiency [1]. Diabetes mellitus induces changes in rheological properties i.e. specific changes in mechanical properties eg. Increase in erythrocyte microviscosity, aggregation and adherisivness which causes the changes in lipid composition, dysfunctioning of membrane structure and composition [2,3]. Erythrocyte membrane being continuously exposed to free radicals and the high oxidative stress leads to damage in structural components [4]. Free radicals are not only consequence of diabetes mellitus but it can also cause diabetes by damaging beta cells of pancreas [5]. Lipid peroxidation alters the cellular structure of membrane bound enzymes by changing phospholipids and fatty acid composition [6]. In addition to this the membrane bound proteins are also glycosylated decreasing the activity of proteins [4]. One of the major enzymes which regulate balance of electrolytes is Na^+K^+-ATPase a membrane bound enzyme that energizes the Na-pump by hydrolyzing ATP is associated with action of insulin [7,8]. Lack of insulin decreases Na^+K^+-ATPase activity which can cause obesity- one of the major causes for type2 diabetes mellitus. Further, diabetes mellitus also induces a reduction in erythrocyte membrane Na^+K^+-ATPase activity, which results in hemodynamic dysfunction due to altered microvascular blood flow, rheological abnormalities precipitated by decrease erythrocyte deformability and raised fluidity and complications such as nephropathy, neuropathy, cardiovascular disorders and microangiopathy [9,10]. This study is designed to establish a correlation between erythrocyte membrane parameters with lipid level in diabetes mellitus.

Serum Components	Control (Group-I)	Group-II	Group-III
Glucose (mg%)	89.2±9	187.5±24	210.3±36
Na (mmol/L)	132.8±4	141.3±5	144.6±4
K (mmol/L)	4.5±2	5.7±3	5.7±2
TC (mg%)	167.1±29	188.8±12	290.3±42
TG(mg%)	110.9± 24	142.6±19	210.7±37
HDL(mg%)	48±5	44±4	37±8
LDL(mg%)	118±18	129±14	138±21

Table 1: Comparison of Serum components of group I, II and III subjects.

Methodology

Subjects selected between age 40-60 years; subjects were mainly volunteers and are recruited with mutual consent. They were divided into three groups namely- Group I: 25 Normal healthy control subjects with no present or past history of diabetes mellitus; Group II: 15 Diabetes with no compactions, subjects who were freshly diagnosed from type2 diabetes mellitus was selected. Subjects taking medication or has a long past history of diabetes were ruled out; Group III: 20 Diabetes dyslipidemic; obese subjects with type2 diabetes were selected in this group.

Fasting Blood Samples were taken. The Blood was drawn from the forearm is mixed with 0.1 M Sodium Citrate (0.5 ml/ 4.0 ml of blood) and Sodium fluoride:Potassium oxalate 1:3 (w/w). The sample collected were analyzed immediately for Plasma glucose, sodium, potassium, along with lipid profile was estimated by kit method on semi-automatic analyzer. The RBC was isolated and erythrocyte ghost membrane was prepared [11], briefly by ultracentrifugation in cold, the membrane changes from pink to milky white. Total membrane protein was determined [12] and lipids were extracted from erythrocyte membrane [13]. After extraction membrane cholesterol and phospholipids were

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Membrane Components	Control (Group-I)	Group-II	Group-III
Total protein (**µ**g/mL)	5.4	5.1	5.0
Na^+K^+-ATPase **(µmoles/Pi/mg erythrocyte membrane protein/60minute)**	0.40±0.02	0.29±0.02*	0.27±0.03^
Total ATPase **(µmoles/Pi/mg erythrocyte membrane protein/60minute)**	1.1±0.01	1.0±0.02	1.0±0.02
Cholesterol (**µ**g/mg erythrocyte membrane protein)	128±24	178±22	193±31
Phospholipids (**µ**g/mg erythrocyte membrane protein)	12±2	15±3	16±3
Cholesterol:Phospholipid Ratio (mol:mol)	0.8±0.02	0.9±0.01	0.94±0.02
TBARS (**n**g/mg erythrocyte membrane protein)	80±11	96±13	98±17

*^p<0.005

Table 2: Comparison of Membrane components of group I, II and III subjects.

estimated [14,15] and phospholipid:cholesterol, mol:mol ratio was calculated. Membrane total ATPase and Na^+K^+-ATPase was estimated [16,17] along with lipid peroxidation on membrane in the form of TBARS activity [18].

Results

Glucose, Sodium, Potassium and Lipid Profile (Total Cholesterol, Triglycerides, HDL-C and LDL-C) all were normal for control and were increased for both group-II and group-III, significantly much higher in group-III, as can be seen in Table 1. Total membrane protein was slightly decreased in diabetics and total ATPase were almost same when all three groups were compared. The Na^+K^+-ATPase activity was decreased significantly ($p<0.005$) (0.40 for group-I when compared to 0.29 and 0.24 for group-II and III respectively). Membrane lipid i,e cholesterol and phospholipid are also increased in group-II and group-III with significant increase in cholesterol: phospholipid ratio (Table 2).

Discussion

In Diabetes mellitus there is decrease in phosphor-inositide metabolism and thus decreased Na^+K^+-ATPase along with this glycation of proteins in co-ordination with oxidative stress on erythrocyte membrane. Changing rheological properties is also one of the main factors causing decreased activity of enzyme [4]. Increased lipid peroxidation is due to increased oxidative stress in diabetes mellitus [19] increased lipid peroxidation is indicative of membrane damage, also increased membrane cholesterol, due to increased plasma cholesterol, decreases the lipid fluidity and causes surface exposure of membrane proteins [20]. Na^+K^+-ATPase has been reported as one of the membrane proteins affected structurally and functionally in diabetes mellitus. In Diabetes there is a conformational change in the structure of the enzyme due to glycation and oxidative stress hence reducing its activity [4,21]. Altered level of RBC membrane lipid ratio decreases activity of membrane protein [2,22]. Also there is non-enzymatic glycation of membrane protein [23]. Thus this study concludes that increased lipid concentration had direct effect on decreased value of Na^+K^+-ATPase, which is one of the major factor for consequences of diabetes mellitus.

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