RBC MEMBRANE COMPOSITION IN INSULIN DEPENDENT DIABETES MELLITUS IN CONTEXT OF OXIDATIVE STRESS

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ABSTRACT

Glyco-oxidation is considered as a source of permanent, cumulative, oxidative damage to long lived proteins in ageing and in diabetes. Although RBC depends solely on glucose for energy purpose, hyperglycemic state glycosylates hemoglobin, creates oxidative stress and puts the cellular components at risk. In view of this, RBC membrane composition was analyzed in diabetic patients. The results were compared with healthy age and sex matched control groups. When RBC membrane components such as protein, sialic acid, phospholipids and cholesterol were determined in insulin dependent diabetes mellitus, a significant rise in phospholipids and cholesterol and significant fall in sialic acid and protein content was noted. RBC membrane composition showed pronounced alterations in insulin dependent diabetes mellitus. These changes were accompanied by higher levels of lipid peroxidation products like Malondialdehyde.

KEY WORDS

Insulin dependent diabetes mellitus, RBC Membrane composition, Protein and Lipids, Lipid peroxidation.

INTRODUCTION

Insulin dependent diabetes mellitus (IDDM) is a complex, multifactorial disease involving autoimmune destruction of insulin producing β -cells (1, 2). Although the multiple risk factors such as genetics, environmental stress, viral infections and diet can trigger the development of IDDM, it is likely that Reactive Oxygen Species (ROS) play a central role in β -cell death and disease progression (3). There are two mechanisms of free radical induced β -cells destruction. a) β -cells are prone to be destroyed by free radicals such as superoxide and hydroxyl radicals. These radicals attack cellular membrane and cause DNA breaks and lead to cell death (4). b) Cytokines that are released by immune cells (macrophages, T cells) induce the formation of intracellular ROS such as superoxide and Nitric oxide (NO). NO results in direct inhibitory effect on β -cells mitochondrial function and thus NO can directly destroy

β -cells (5). The occurrence of free radical-induced lipid peroxidation causes considerable changes in structural and functional organization of cell membrane and makes the membrane leaky (6). Indeed, alterations in proteins and lipids may often be more important in oxidative stress situation *in vivo*, since they provide viscoelastic properties necessary for membrane deformability (7, 8). While the length and the degree of unsaturation of phospholipids influence the lipid bilayer fluidity (9), the proteins play a crucial role in maintaining erythrocyte integrity and antigenicity (10). Although the studies have described modifications in the RBC membrane components in IDDM, few and rather conflicting data are available on the Indian population. We therefore investigated the erythrocyte membrane composition that may contribute to erythrocyte pathology in IDDM.

MATERIALS AND METHODS

Blood samples were collected from patients attending the endocrinology Out Patient Department (OPD) of B. Y. L. Nair Charitable Hospital, Mumbai. Based on the clinical and laboratory data, 30 patients of IDDM (29-62yrs) were included in the study with their informed consent. Table 1 shows number of male and female subjects included in the study along with the duration of the diabetes and body mass index (BMI). None of the patients were overweight. Fasting and post-prandial

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Table 1 : Age and sex distribution alongwith the duration of disease and BMI in IDDM

Sr.No.	Subjects	Sex	Mean Age & Range	Duration of Disease	Body Mass Index	
					M	F
1.	Controls (n=80)	M-55 F-25	33.93±5.07 (28-52)	---	24.90±3.10 (18.08-28.90)	23.50±3.02 (18.00-24.58)
2.	IDDM (n=30)	M-20 F-10	53.76± 6.24 (29-62)	5 – 8 yrs.	24.99 ±3.07 (18.84-29.20)	23.88 ±3.19 (17.39-30.00)

M- Male; F-Female

blood samples were collected from diabetic patients. The patients suffering from hepatic disease, Cardiovascular Disease (CVD) and any chronic or acute inflammatory illness, cancer of all types, pulmonary tuberculosis, alcoholics and smokers were excluded from the study. The protocol was approved by the ethical committee of Nair Hospital. The control group comprised of age and sex matched (n=80) volunteers. Patients selected for the study were controlled diabetics. They were on regular medication prescribed by their clinician. Patients were stabilized with insulin injections (long acting and short acting dosage) and did not show any insulin resistance and antihypertensive drugs as required. Ghosts were prepared from red blood cells according to the method of Kuross et al (11). Two ml of washed cells were hemolysed in 5mM Sodium Phosphate buffer (pH 8.0) containing 0.5mM/L EDTA and centrifuged at 15,000 rpm at 4°C for 30 min by adding 30 ml lysing buffer forcefully to get ghost pellet. The ghost pellet was washed two more times with the lysing buffer in order to get colourless (white) ghost. The final volume of the erythrocyte membrane suspension was made to 3ml with 5mM phosphate buffer. Lipids were extracted from 2ml of the erythrocyte membrane suspension following a modification in the procedure of Folch et al (12), using a chloroform-methanol mixture in the proportion 2:1(v/v) containing 15mg of butylated hydroxy toluene (BHT). This lipid extract was used for the estimation of the total cholesterol and phospholipids by using the method of Allain et al (13) and Takagama et al (14) respectively. Total RBC membrane protein content and Sialic acid content were estimated from the erythrocyte suspension by using the method of Lowry et al (15) and Warren (16) respectively. RBC MDA was estimated by Stock et al (17) method. All the chemicals used in the study were of analytical grade.

Statistical Analysis: Statistical comparison of data was done using student's 't' test. Values were expressed as mean ± standard deviation (SD). Sigma version 3.0 was used for statistical analysis.

RESULTS AND DISCUSSION

The analysis demonstrated that, the total RBC membrane protein and Sialic acid contents in IDDM (Table 2) were significantly lower than control group ($P < 0.001$), whereas the total cholesterol and phospholipids were significantly higher in IDDM than control group ($P < 0.001$). The concentrations of blood glucose and glycosylated hemoglobin are shown in Table 3. In IDDM, there was a fall in total hemoglobin by 7.13% and a rise in glycosylated hemoglobin (123.96%) and blood glucose fasting and post-prandial by 58.76% and 85.60% as compared to the control group respectively. Total protein and Sialic acid content fell by 34.42% and 37.84% respectively while cholesterol, phospholipids and RBC MDA increased by 13.74%, 44.52% and 71.76% respectively.

The severity of protein and lipid damage relates to the concentration of oxidants in the cells and hence on the efficiency of the lipid repair mechanisms. Hydrogen peroxide and superoxide in the red blood cells are involved in the oxidative degradation of hemoglobin, oxidation of -SH groups and lipid peroxidation (18,19). In IDDM, our findings of elevated lipid peroxidation in RBCs are in agreement with the report documented in the literature (20). Our observation of reduced membrane protein is in agreement with the report of Adewoye et al (21), however, the extent of fall does not agree with their observations. They found marginally low levels of membrane proteins in IDDM.

Table 2 : Total RBC membrane composition in IDDM along with RBC MDA

	Total protein (mg/ ml of packed cells)	Sialic Acid (μmol/ ml of packed cells)	Cholesterol (mg/ ml of packed cells)	Phospholipids (mg/ ml of packed cells)	RBC MDA (nmoles/gHb)
Control (n=80)	5.20 ± 0.80	0.33 ± 0.07	1.82 ± 0.74	2.83 ± 0.45	49.76 ± 17.14
IDDM (n=30)	3.41 ± 1.02***	0.21 ± 0.05***	2.07 ± 0.87*	4.09 ± 0.70***	85.47 ± 9.02***

*** $P < 0.001$; * $P < 0.1$

Table 3 : Total and Glycosylated hemoglobin, Fasting and post-prandial blood glucose of IDDM patients

	Total Hemoglobin (gm%)	Glycosylated Hemoglobin (%)	Fasting Blood Glucose (mg/dl)	Post-prandial Blood glucose (mg/dl)
Control (n - 80)	13.03± 1.40	4.09±1.05	89.83 ± 7.82	109.70±8.36
IDDM (n - 30)	12.10 ± 1.30***	9.16±0.99***	142.60 ± 30.60**	203.60±35.40**

*** P<0.001, ** P<0.01

A major protective mechanism against oxidative damage is the membrane integrity. Many RBC disorders with intrinsic membrane defects are more susceptible to lipid peroxidation than normal RBCs. The two major sites of RBC destruction *in vivo* are intravascular and extravascular. Oxidation induces change in the membrane permeability resulting in hemolysis would relate to the degree of intravascular RBC destruction. Extravascular mechanism(s) of RBC destruction may involve changes in cell deformability and antigenicity (22). Lipid peroxidation causes polymerization of membrane components and decreases cell deformability (23). Products of lipid oxidation, such as oxidized cholesterol and oxidized unsaturated fatty acyl groups of phospholipids, may affect membrane bilayer structure and function. Each phospholipid class consists of a variety of phospholipid molecules characterized by their different fatty acyl side chains. Alterations in the phospholipid molecular species composition as a consequence of oxidant damage can be deleterious to the RBC membrane (24). Since the RBC lacks the ability for *de novo* synthesis of proteins and lipids, the uptake of phospholipids from plasma takes place as a part of repair mechanism of oxidized membrane lipids (25). It has been observed that in intact RBCs the exchange of phospholipids with plasma is primarily confined to phosphatidylcholine (PC), since the levels of two aminophospholipids, phosphatidylethanolamine (PE) and phosphatidylserine (PS) are very low in plasma (26).

In general, the overall effect of lipid peroxidation is to decrease membrane fluidity (24). It has been shown that peroxidation of erythrocyte membranes causes formation of high-molecular-mass protein aggregates within the membrane. The surface receptor molecules that allow cells to respond to hormones and cytokines can be inactivated during lipid peroxidation, as are enzymes such as glucose-6-phosphatase, glycerol-3-phosphate acyl transferase; the Ca^{2+} -ATPase of the endoplasmic reticulum and the Na^{+} , K^{+} -ATPase which are involved in maintenance of correct ion balance within the cells (27).

The results of our studies support the findings of Mazzanti et al (28). In the literature, Forte et al (29) have reported elevated

Sialic acid concentration in serum which is attributed to the increased output of acute phase proteins by the liver. Sialidase catalyzes the removal of Sialic acid residues from the glycoprotein and glycolipids. Venerando et al (30) have reported increased sialidase activity in IDDM which could be one of the reasons for reduced Sialic acid content in RBC membrane observed in this study. The changes in Sialic acid content would further affect structure-function relationship of RBCs.

In IDDM, increased lipid peroxidation and altered RBC membrane composition was observed. This change in the composition could be due to oxidative stress, which may affect membrane fluidity and deformability.

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