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Compositional alterations in erythrocyte membranes in Type II diabetes

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Loss of erythrocyte membrane deformability is one of the most crucial factors in developing complications associated with Type II diabetes. The observed loss of erythrocyte membrane deformability could be related to structural changes in the membrane. In this context, here, we have made an attempt at gaining a better insight (quantitative as well as qualitative) into the protein and lipid contents in erythrocyte membranes and their interrelationships in Type II diabetes. Age matched control (n=12) and Type II diabetic subjects (n=22) were selected for this study. Morphological characteristics were studied by atomic force microscopy (AFM). AFM study confirmed remarkable alterations in morphology of the diabetic erythrocytes. In diabetic erythrocytes following changes were noted: (i) Significant increase in membrane as well as cytosolic proteins with a marginal increase in phospholipids content; (ii) The membrane total lipids:protein, phospholipids:protein, cholesterol:protein and phospholipids:cholesterol (mole:mole) ratios decreased significantly; (iii). A reproducible decrease in docosahexaenoic acid (DHA) and Omega-3 index with increase in Omega-6:Omega-3 ratio in membrane fatty acids; and (iv) The SDS-PAGE analysis indicated that all membrane proteins increased in almost equal proportion leading to increased membrane protein content. The observed compositional and stochiometric changes in lipids, proteins and their ratios may underlie morphological alterations and loss of deformability.

Keywords: Atomic force microscopy (AFM), Diabetic erythrocyte deformability, Erythrocyte membrane lipids, Erythrocyte membrane proteins

Growing worldwide incidence of diabetes poses a serious challenge. According to 2019 survey of International Diabetes Federation, around 463 million adults of world population[1] are currently living with Diabetes Mellitus (DM) and India is the second topmost country with the largest numbers of adults with diabetes i.e. 77 million[2]. The numbers is expected to increase significantly by 2030[3]. The primary physiological defect characterizing pathogenesis of Type II diabetes is hyperglycemia which is attributed to resistance to the biological action of insulin due to alterations in signalling downstream to the insulin receptor[4]. Eventually, Type II diabetes leads to the development of many serious complications such as nephropathy, retinopathy, cardiomyopathy, peripheral and cerebral neuropathy, starting with initiation of micro- and macro-angiopathy[5]. More recently, it has been recognized that loss of deformability of erythrocytes is a characteristic feature of Type II diabetes[6,7]. Deformability is an essential and crucial attribute of the erythrocytes which enables them to squeeze through capillaries and regain their biconcave shape and size when they come out of capillaries which is essential to maintain the flexibility/ physiological function of the erythrocytes[8]. recent Scanning electron microscope (SEM) and a few Atomic force microscope (AFM) studies have demonstrated changes in structure, size and shape of Type II diabetes erythrocyte membranes in terms of surface deformation[9,10]. AFM is the widely used technique because of its higher resolution capacity in cytobiology to study the topographical structure of biological samples. AFM measures the surface roughness, stiffness and morphology of an individual cell[10].

The deformability of cell membrane is a composite function of two main components: the outer lipid bilayer leaflet composed of phospholipids and cholesterol with embedded proteins, and a spectrin-based cytoskeleton which lies underneath the inner leaflet[11]. Any change in one of these components may alter the deformability and ultimately the flexibility of the cell membrane. There are reports which indicate

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that loss of deformability could lead to fragmentations of the erythrocytes[12,13]; indeed, presence of erythrocyte fragments has been shown in kidney glomeruli of diabetic nephropathy patients[14]. It has been suggested that possibly the biochemical changes in lipid and protein composition can affect the physico-chemical properties of the erythrocyte membrane. However, the reports are contradictory[15,16].

With this background in mind we tried to carry out qualitative and quantitative analysis of diabetic erythrocyte cell membrane components *viz.* proteins and lipids and their interrelationships. In the first place we characterized the Type II diabetic erythrocytes using AFM technique. In the next set of experiments we studied the quantitative aspects of proteins and lipids components of cell membrane and their interrelationships.

Materials and Methods

Sample selection

For the present study, 12 age- and sex- matched healthy nondiabetic controls and 22 Type II diabetic subjects were included. The major criteria for inclusion in diabetic group were: glycated hemoglobin (HbA_{1c}) >7.0% or fasting blood glucose (FBG) >126 mg/dL, BMI >22 kg/m^2, and age 40-60 years. The mean age was 47.62±1.19 and 50.58±2.29 years respectively for control and diabetic groups. The values of FBG levels were 99.13±4.11 and 236.86±13.10 mg/dL respectively for the two groups. The corresponding values of HbA_{1c} were 4.8±0.19 and 7.8±0.16 %. The plasma triglycerides levels for control and diabetic groups were respectively 146.13±10.69 and 182.75±11.5 mg/dL. The corresponding values for plasma cholesterol were 143.61±5.81 mg/dL and 174.13±6.58 mg/dL (Table 1). The Type II diabetic subjects continued their treatment with prescribed anti-diabetic agents.

Fasting blood samples were collected after informed written consent. The study was conducted following Helsinki Convention Guidelines and was approved by Institutional Ethics Committee (Ethics Approval Number: DCGI Reg. No. ECR 518) of Bharati Vidyapeeth Deemed University Medical College, Pune, India.

Collection of blood sample

A volume of 5.0 mL fasting blood samples were collected by vein puncture in EDTA tubes, and aliquots were used for measurements of erythrocyte count, blood glucose level, total Hb, HbA_{1c} and, plasma triglycerides and cholesterol.

Atomic force microscopy (AFM)

For AFM studies washed erythrocytes (details given below) were diluted 1:10 using 0.9% NaCl solution. A small portion of erythrocyte suspension was spread evenly on sterilized round silicone plate to form a monolayer. The sample was completely air dried for about 30 min[17,18] and then subjected to AFM (Breuker, AFM multimode 8.0) measurements. A silicone probe with aluminium reflective coating on its backside (TEPSA, Bruker) was used for AFM imaging. The probe has a spring constant of 20-80 N/m, a tip curvature with a radius of 15 nm, and a resonance frequency of 70 KHz. The AFM probe and optical microscope were used to visualize the top view of anticipated cells. Eight to ten cells from different areas from each smear were randomly selected and scanned at the field (10×10 μm) to analyze the alteration in the morphology of cell membrane. Determination of AFM images and morphological parameters and surface roughness measurements were carried out using Bruker Nanoscope Analysis software 1.3.

Isolation of membrane and cytosol protein from erythrocytes

Erythrocytes were sedimented by centrifugation at 400 g for 10 min and the plasma was carefully decanted and saved for further investigations. The buffy coat was discarded following gentle swirling with 0.9% NaCl solution. The packed cells were repeatedly washed and resuspended to original volume using 0.9% NaCl solution and the cell count

Table 1 — Baseline characteristics of the subjects

Parameter	Control (n=12)	Diabetic (n=22)
Mean Age, (Years)	47.62±1.91 (range: 40 to 54)	50.58±2.29 (range: 41 to 60)
Gender (Male/Female)	6/6	16/6
Fasting blood sugar level (mg/dL)	99.13±4.11 (range: 84 to 115)	236.86±13.10 (range: 145 to 314)
Erythrocyte Count (million/mm^3)	4.9±0.23 (range: 4.16 to 5.7)	5.15±0.15 (range: 4.02 to 6.11)
Hb (g /dL)	12.4±1 (range: 9.13 to 16.2)	13.1±0.6 (range: 9.3 to 17.6)
HbA_{1c} (%)	4.8±0.19 (range: 4.06 to 5.7)	7.8±0.16 (range: 7.06 to 9.7)
Plasma Triglycerides (mg/dL)	146.13±10.69 (range: 108.5 to 186)	82.75±11.5 (range: 116.43 to 277.6)
Plasma Cholesterol (mg/dL)	143.61±5.81 (range: 122.2 to 164.3)	174.13±6.58 (range: 135.7 to 233.3)

[Results are given as mean ± SEM of number of observations indicated in parentheses]

was recorded. The resuspended erythrocytes were lyzed (1:10) in 10 mM sodium phosphate buffer, pH 7.4. Aliquots were used for estimation of total lysate proteins. The lysate was subjected to further centrifugation at 24700 g for 40 min to sediment membrane fraction and the supernatant was carefully separated for determination of total protein and hemoglobin contents. The membrane pellet was carefully resuspended in 14 mM Tris-HCl buffer pH 7.4 and washed repeatedly using the same buffer and re-sedimented at 24700 g for 40 min. Finally, the pellet was suspended in 14 mM Tris-HCl buffer pH 7.4 to a known volume. All operations were carried out at 0-4ºC.

Protein estimation was carried out by the method of Lowry *et al*.[19]. Estimation of hemoglobin was carried out by cyanmethemoglobin method[20]. For protein estimation the whole lysate and membrane proteins were solubilized using 10% SDS solution. The content of cytosolic protein was calculated by subtracting hemoglobin protein content from the protein content of membrane free supernatant.

Extraction of Lipids

Known aliquots of erythrocyte membrane suspension and plasma were used for extraction of lipids essentially according to the procedure of Folch *et al*.[21]. Aliquots were used for estimation of total lipids, phospholipids, cholesterol and for fatty acid analysis.

Lipid analysis

Total lipid determination was carried out by phosphovanillin method[22]. Cholesterol estimation was according to method of Zlatkis *et al*.[23]. Aliquots of extracted lipids were digested with H_2SO_4 followed by digestion with 70% perchloric acid to liberate inorganic phosphate[24,25]. The phospholipids estimation was essentially according to the method of Katewa & Katyare[25]. Phospholipids content was also computed from the phosphovanillin values as described by Rouser *et al*.[26]. The values obtained by the two methods were in close agreement and were pooled for data presentation.

Fatty acid analysis

Fatty acid methyl esters (FAME) were prepared essentially according to the method of Ichihara *et al*.[27]; 0.1 mL of internal standard (C17:0, 2 mg/mL in methanol) was included. The separation of FAME was carried out using 30 m fused silica capillary column with an internal diameter of 0.25 µm in an Agilent 7820A gas chromatography system with flame ionization detector (FID). Helium was used as carrier gas and nitrogen as a make-up gas. The split ratio was 25:1. The injection port and detector temperature was 250ºC. The initial column temperature was 110ºC and raised to 240ºC with an increment of 3ºC/min. The fatty acid peaks were identified based on retention time of the standard FAME mixture. The relative proportion of individual fatty acid (% area of total fatty acid) was quantified by integrating the area under peak and dividing the result by total area for all fatty acids.

SDS-PAGE

The erythrocyte membranes were solubilized in loading buffer containing 5% w/v SDS, 5% v/v 2-mercaptoethanol, 10% v/v glycerol and 0.00125% w/v bromophenol blue in 0.0625 M Tris-HCl buffer, pH 6.8. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed in 10% polyacrylamide gels according to the method of Laemmli[28]. The gel was stained using 0.5% w/v Coomassie Brilliant Blue R. The apparent molecular masses were calculated based on R_f values of the standard protein markers (Hi Media). After distaining, the gels were scanned in Bio-Rad Molecular Imager , ChemiDoc XRS+ with Image Lab Software. The instrument gives gel image, gel scan, R_f values with molecular weights and percent distribution of the components.

Statistical analysis

Statistical analysis of the data was carried out by Student's t-test using GraphPad Prism version 5. The results are given as mean ± SEM of the number of independent observations indicated. *P* values <0.05 were considered statistically significant.

Results

The baseline characteristics of the normal healthy controls and the diabetic group are given in Table 1 which include value of mean age, gender, and levels of fasting blood sugar. It is also apparent that the erythrocyte count and the Hb levels were generally comparable in the two groups. However, as expected, the HbA_{1c} levels were significantly high in the diabetic group.

The representative AFM images of control and diabetic erythrocytes are shown in Fig. 1 (A & B), respectively which are in conformity with previously published reports[10,29]. Thus, in the control group, the erythrocytes show a typical concave shape with a diameter of 7.73 µm±0.15 µm and height of 2.32 µm±0.12 µm (Fig. 1C), whereas the diabetic

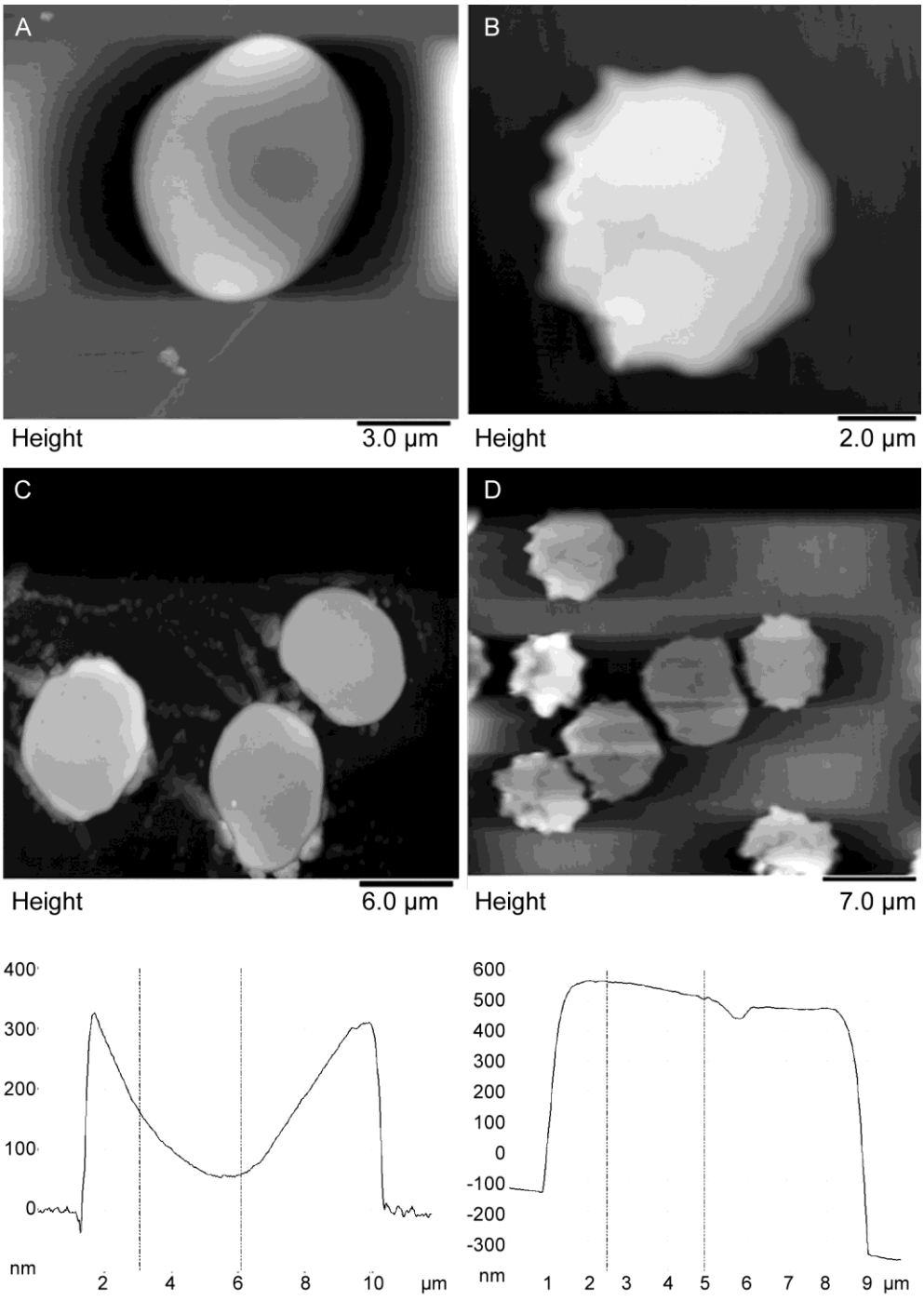

Fig. 1 — The AFM image of dry sample prepared from erythrocyte of (A) control subjects; and (B) diabetic patients; Image of a single erythrocyte of (C) control subjects; and (D) diabetic patients; and Geometrical profiles of (E) control erythrocyte; and (F) diabetic erythrocyte.

erythrocyte shows atypical shape with extended projections on membrane (Fig. 1D). Diabetic erythrocytes show decrease in size with a diameter of 6.71 µm±0.05 µm and height of 1.35 µm±0.028 µm. Figure 1E shows that the concave depth of control erythrocyte is 263.13 nm±7.56 nm. By contrast, diabetic erythrocyte perceptibly shows a well-developed balloon like convex shape (Fig. 1F). The surface roughness of erythrocyte membrane for control group is 2.37 nm ± 0.031 nm which decreases in diabetic group by 27% to 1.71 nm ± 0.027 nm (Table 2).

The data in Table 3 show the protein content of whole erythrocytes, erythrocyte membrane and cytosol, and Hb from control and diabetic subjects. The values are given as µg per 10^6 erythrocyte as well as mg per mL erythrocyte. It can be noted that the protein content of erythrocyte membranes and cytosol is more than doubled in the diabetic group, whereas the Hb content was comparable in both the groups.

The data on protein and lipid content of erythrocyte membranes from control and diabetic subjects are given in Table 4. It is interesting to note that while the total lipid content was generally comparable in the two groups, the phospholipid content was 40% higher in the diabetic group. By contrast, the cholesterol content was somewhat lower although the observed decrease was not statistically significant.

Based on these values given as µg/10^6 erythrocyte (Table 4), we tried to compute the lipid:protein ratios as well as cholesterol:phospholipid ratios in erythrocyte membranes from the control and the diabetic subjects. These data are shown in Fig. 2. It is apparent that in the diabetic group the Total lipids:protein ratio decreased almost by 50 %. Similarly, phospholipid:protein ratio also decreased by 43%. Interestingly, the cholesterol:protein ratio decreased drastically by 62% in the diabetic group. this was also reflected in the mole: mole ratio of cholesterol:phospholipid. In the control group it was 0.91 which is in close agreement with the values reported for human and rat erythrocytes[30,31]. However, in the diabetic group there was a 32% reduction in the ratio. Identical results were obtained when the data were presented on the basis of µg content per mL of erythrocyte (Fig. not shown).

In view of the observed changes in the phospholipids and cholesterol contents, it was of interest to find out if diabetic state also affected the membrane fatty acid composition. These results are given in Table 5 which shows that in the diabetic state the proportion of docosahexaenoic acid (DHA) decreased resulting in lowering of the omega-3 index. Consequently, the Omega-6/Omega-3 ratio increased. Almost parallel changes were noted for plasma fatty acid composition except that the proportion of palmitic acid increased marginally which was statistically significant (Table 5).

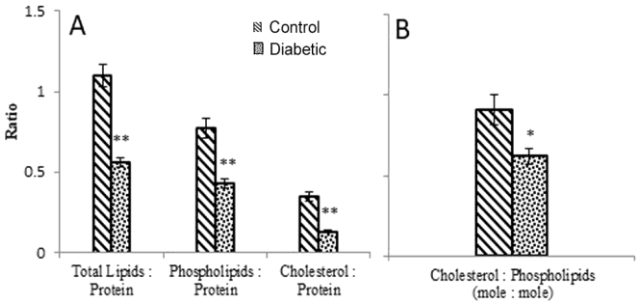

Fig. 2 — Relative proportions of lipids and proteins in erythrocyte membranes from control and diabetic subjects. [(A) Total lipids; and (B) Cholesterol. *P <0.05; **P <0.0001]

Table 2 — Morphological measurements of control and diabetic erythrocytes

	Control (n=12)	Diabetic (n=22)
Diameter	7.73 µm±0.15 µm	6.71 µm±0.05 µm[a]
Height	2.32 µm±0.12 µm	1.35 µm±0.028 µm[a]
Concave Depth	263.13 nm±7.56 nm	convex
Surface Roughness	2.37 nm±0.031 nm	1.71 nm±0.027 nm[a]

[Results are given as mean±SEM of number of observations indicated in parentheses, [a]P <0.0001]

Table 3 — Recovery of erythrocyte cytosolic and membrane proteins from control and diabetic subjects

Parameter/Fraction	µg/10^6 erythrocyte		mg/mL of erythrocytes	
	Control (n=12)	Diabetic (n=22)	Control (n=12)	Diabetic (n=22)
Whole erythrocyte	38.40±2.40	41.40±3.40[NS]	189.50±9.50	214.0±11.0[NS]
Erythrocyte membrane	0.61±0.09	1.41±0.05[b]	2.79±0.32	6.88±0.56[b]
Cytosol with Hb	29.42±1.01	30.25±1.62	138.68±7.18	144.3±7.77
Hb	28.01±1.00	27.175±1.48	132.08±7.24	130.4±7.65
Cytosol	1.41±0.13	3.076±0.31[a]	6.61±0.42[a]	13.89±0.75

[Results are given as mean±SEM of number of observations indicated in parentheses, [a]P <0.02, [b]P <0.0001; [NS]not significant]

Table 4 — Protein and lipid content in erythrocyte membranes from control and diabetic subjects

Parameter	µg/10^6 erythrocyte		mg/mL of erythrocyte	
	Control (n=12)	Diabetic (n=22)	Control (n=12)	Diabetic (n=22)
Protein	0.61±0.09	1.41±0.05[b]	2.79±0.32	6.88±0.56[b]
Total Lipids	0.65±0.06	0.79±0.05 NS	3.03±0.21	3.79±0.32 NS
Phospholipids	0.45±0.05	0.61±0.05[a]	2.08±0.14	2.95±0.30[a]
Cholesterol	0.20±0.02	0.18±0.01 NS	0.95±0.10	0.85±0.06 NS

[Results are given as mean±SEM of number of observations indicated in parentheses, [a]P <0.05, [b]P <0.0001; [NS]not significant]

Table 5 — Fatty acid composition of erythrocyte membrane and plasma

Fatty acid	Erythrocyte membrane		Plasma	
	Control (n=12)	Diabetic (n=22)	Control (n=12)	Diabetic (n=22)
Palmitic acid (C16:0)	25.96±1.52	24.55±0.95^{NS}	23.30±0.65	25.45±0.52^{b}
Palmitoleic acid (C16:1)	1.75 ±0.37	1.73±0.48^{NS}	1.19±0.20	1.8±0.44^{NS}
Stearic acid (C18:0)	17.28 ±0.63	16.85±0.90^{NS}	9.33±0.66	9.70±0.77^{NS}
Oleic acid (C18:1)	17.2±0.99	21.06±1.66^{NS}	21.53±1.02	21.03±0.69^{NS}
Linoleic acid (C18:2n6)	14.53±1.02	18.84±1.56^{NS}	33.39±1.05	33.18±1.45^{NS}
Alpha linolenic acid (C18:3n3)	2.24±0.42	2.71±0.49^{NS}	1.50±0.18	1.22±0.16^{NS}
Arachidonic acid (C20:4n6)	12.22±0.81	10.72±0.61^{NS}	6.80±0.37	6.92±0.48^{NS}
Eicosapentaenoic acid (C20:5n3)	0.91±0.24	0.53±0.19^{NS}	0.65±0.48	0.22±0.04^{NS}
Docosahexaenoic acid (C22:6n3)	3.62±0.94	0.90±0.25^{e}	1.39±0.52	0.43±0.09^{b}
SFA	43.24±1.9	41.39±1.60^{NS}	32.62±1.08	35.15±0.94^{NS}
MUFA	18.91±1.42	22.79±1.49^{NS}	22.73±1.14	22.83±0.60^{NS}
PUFA	34.61±1.7	33.70±1.41^{NS}	43.74±1.40	41.96±1.11^{NS}
O-6 PUFA	27.35±1.32	29.62±1.41^{NS}	40.19±1.10	40.10±1.18^{NS}
O-9 MUFA	17.20±0.99	21.06±1.66^{NS}	21.53±1.02	21.03±0.69^{NS}
O-3 PUFA	7.25±1.16	4.22±0.75^{a}	3.54±0.92	1.87±0.20^{a}
O-3 Index (ALA+EPA+DHA)	5.01±1.16	1.51±0.44^{d}	2.04±0.97	0.65±0.11^{NS}
O-6: O-3 ratio	4.57±1.04	9.61±1.06^{c}	17.47±1.51	30.88±4.18^{a}

[SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated acid; O-6 PUFA, ω-6 Polyunsaturated acid; O-9 MUFA, ω-9 Monounsaturated acid; O-3 PUFA, ω-3 Polyunsaturated acid; O-3 index, ω-3 index; O-6:O-3 ratio, ω-6 : ω-3 ratio. ALA, Alpha linolenic acid; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid. Results are given as mean±SEM of number of observations indicated in parentheses. ^{a}P <0.05, ^{b}P <0.02, ^{c}P <0.005, ^{d}P <0.002 and ^{e}P <0.001 compared to control; and ^{NS}Not significant]

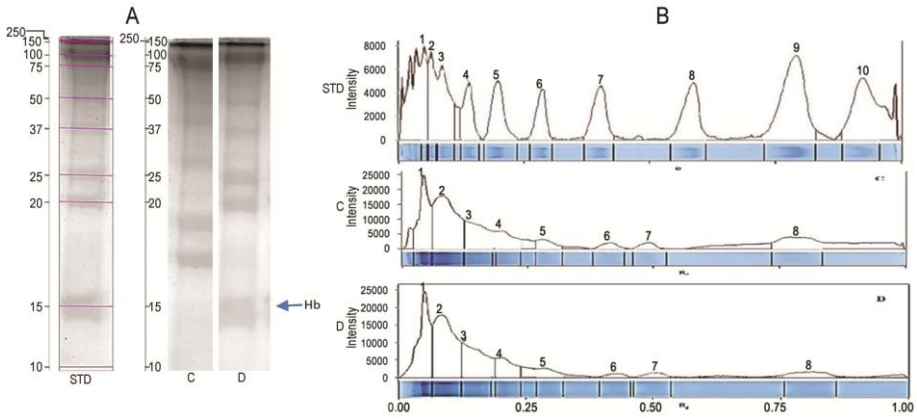

Fig. 3 — (A) SDS−PAGE pattern; and (B) densitometric scan profile of the erythrocyte membrane proteins. [STD, Standard protein marker with respective molecular weight; C, representative sample from control subject; and D, diabetic patient; and Hb, Hemoglobin]

In the next set of experiment, we examined the SDS polyacrylamide gel profiles of the erythrocyte membrane proteins since the membrane proteins had more than doubled in the diabetic group (Table 3). In particular, we wanted to find out if there was uniform or disproportionate increase in the membrane protein components. The typical SDS-PAGE profiles of erythrocyte membrane proteins and the corresponding densitometric traces from the control and the diabetic subjects are shown in Fig. 3. As is clear, the patterns were comparable for the two groups. Also, the percent distribution of the components was practically unaltered. Thus the percent distribution values for bands 1-7 in the control group were 22.0, 36.4, 18.1, 9.5, 7.0, 2.7 and 2.5; the respective values for the diabetic group were 26.7, 36.9, 18.5, 9.5, 3.6, 2.1 and 2.6. In other words, in the diabetic group where the total membrane proteins had increased significantly (Table 3), all the protein components had increased in equal proportions.

Discussion

The results of the present studies have shown that while the content of total Hb was comparable in control and diabetic subjects, there was a significant increase in the erythrocyte membrane as well as cytosolic proteins in the diabetic group. SDS-PAGE analysis indicated that there was a uniformly

proportionate increase in all the membrane protein components in Type II diabetes. Additionally, it was also noted that there was only a marginal increase in the phospholipids content and a small but insignificant decrease in cholesterol content of the diabetic erythrocyte membrane. Consequently, the membrane total lipid:protein, phospholipids:protein, cholesterol:protein and cholesterol:phospholipids (mole:mole) ratios decreased drastically and significantly (Fig. 2). The total lipid:protein ratio and phospholipids: cholesterol (mole:mole) ratio of around 1.0 has been reported by other researchers including our research group following pioneering work of Davson & Danielli[11,31,32]. Thus, in the diabetic state, the erythrocyte membrane did not display normal stochiometric structural attributes. In other words, abnormalities in membrane structural composition were apparent. This was also reflected in AFM analysis (Fig. 1). The lipids constitute major structural components for stability and functional attributes of the membrane. Proper stoichiometry of lipids and protein in membrane is essential for membrane structure-function relationship. In view of this, it is more likely than not, that the observed drastic alterations in membrane composition and make-up as cited above could possibly be the underlying factors causing instability of the membrane. In other words these stochiometric changes could ultimately lead to the loss of deformability leading to the development of pathogenesis of diabetic complications ensuing from micro- and macro- angiopathies. Additionally, almost two fold increase in membrane proteins deserves some comment. It has been reported that spectrin-ATP interaction plays an important role in regaining the normal shape of erythrocyte[33,34]. In this connection, it may be manifested that the energy potential of diabetic erythrocytes is comparable to the normal erythrocytes. Thus, Kono *et al*.[35] has reported the ATP content of normal and diabetic erythrocyte is comparable. This would mean that there is an inadequate spectrin-ATP interaction in diabetic erythrocyte and therefore the diabetic erythrocyte may not be able to regain their shape. This inturn can lead to loss of deformability. In all probability, it is likely that the micro- and macro- angiopathies occur as a result of blockage of circulation in the capillaries due to accumulation of fragments of circulating erythrocytes. As has been pointed out above in the introduction section, presence of erythrocyte fragments have been shown in kidney glomeruli of diabetic nephropathy patients[14]. As far as we are aware, the disproportionate membrane protein and lipid stoichiometries, reported here in Type II diabetes, have not been reported thus far. The mechanism and significance of these changes need to be studied.

The fatty acid analysis revealed that there was reproducible decrease in DHA and omega-3 index and increased omega-6/omega-3 ratio. Parallel and similar changes were noted even for fatty acid composition of diabetic plasma except that the proportion of palmitic acid was elevated (Table 5). It has been reported that in diabetic state the levels of fatty acid elongase and desaturase decrease significantly[36]. It is possible that this condition may affect the fatty acid profile of erythrocyte membrane or plasma to some extent. The SDS-PAGE analysis revealed that all membrane proteins increased in almost equal proportion leading to increased membrane protein.

Increased non-enzymatic glycosylation leading to formation of advanced glycated end products (AGEs) is well documented[37]. This could be another factor responsible for loss of deformability. Increased incorporation of ^{3}H in diabetic membrane proteins has already been demonstrated. In the present studies we could not pursue this point for lack of sufficient membrane material.

Erythrocytes are derived in the red bone marrow from pluripotent stem cells that give rise to all types of blood cells. Nucleated erythroblasts are committed to becoming mature erythrocytes[38]. It is well recognized that in type II diabetes, altered/defective insulin receptors are present in insulin responsive tissues[39]. These changes ultimately lead to improper utilization and metabolism of glucose. Besides, the cellular and tissue physiological and biochemical functions are drastically affected. Although it is not clear at this stage if bone marrow cells have also possess insulin receptor. If so, it is likely that in the diabetic state the insulin receptor even in the bone marrow cells may be altered which could lead to cellular dysfunction and erroneous signaling. One therefore wonders, whether such a condition would lead to defective membrane formation and altered make-up of membrane in terms of lipid: protein composition. Such an assumption is also supported by the fact that even the content of cytosolic proteins increased. The hyperactivation of gene(s) for synthesis of membrane and cellular protein

components could also result in change in turgor pressure. Taken together, the results would suggest that the defective membrane make-up could be the underlying cause for loss of membrane deformability leading to fragmentation[10] which is ultimate cause for beginning of microangiopathy.

In view of the above, it may be suggested that efforts should be directed towards restoring normal process of erythropoiesis which will eliminate, to a great extent, the complications ensuing from microangiopathy.

Conclusion

The present study has shown that in erythrocytes from Type II diabetic subjects there was almost two fold increase in the membrane protein content. However, there was only marginal increase in total lipids and phospholipid contents implying disproportionate stoichiometric changes in lipid: protein interactions. This is suggestive of alterations in structural features of the membrane. Additionally the result suggests that due to increased in membrane protein content, inadequate interaction between spectrin and ATP could lead to loss of deformability in diabetic erythrocytes.

Ethical statement

Fasting blood samples were collected after informed written consent. The study was conducted following Helsinki Convention Guidelines and was approved by Institutional Ethics Committee (Ethics Approval Number: DCGI Reg. No. ECR 518) of Bharati Vidyapeeth Deemed University Medical College, Pune, India.

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Conflict of interest

Authors declare are no conflict of interests.

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