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Erythrocyte Membrane Lipid Alteration in Type 2 Diabetic Subjects

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Abstract

The lipid components present in biological membranes including erythrocyte membrane are significant in regulation of the membrane fluidity as well as transport across the membranes. The conflicting results of either increase or decrease or no change have been reported in erythrocyte membrane fluidity in type 2 diabetic subjects. The diabetes induced dyslipidemia possibly lead to an alteration in erythrocyte membrane lipid composition. Hence a study was undertaken to assess erythrocyte membrane lipid alterations in type 2 diabetic subjects. The blood samples from randomly selected type 2 diabetic subjects, attending Medical OPD of Basaveshwara Medical College Hospital, Chitradurga, were collected with heparin as an anticoagulant. Erythrocyte membrane total cholesterol (mTC), Erythrocyte membrane phospholipids (mPL), Erythrocyte membrane phosphatidyl choline, Erythrocyte membrane sphingomyelin and Erythrocyte membrane phospholipids/ Erythrocyte membrane cholesterol ratio were studied. The results suggests a significant rise in erythrocyte membrane cholesterol (mTC, N=1.25±0.31, T2DM= 1.54± 0.14, p<0.001), erythrocyte membrane phospholipid contents (mPL, N=6.99±1.84, T2DM=23.44±10.31, p<0.001), Erythrocyte membrane sphingomyelin, (N=9.28± 1.37, T2DM=19.32± 2.37), Erythrocyte membrane phosphatidyl choline (N=27.08± 1.58, T2DM=26.09±3.04, p<0.001), as well as phospholipid/cholesterol ratio (mPL/mTC, N=6.08±2.65, T2DM=11.76±5.31, p<0.001.) in type 2 diabetic subjects was observed. Though, there is a significant alteration seen between normal and diabetic groups, but there was no significant change was observed between male and female subjects of both normal as well as diabetic groups.

Index terms— membrane cholessterol, membrane phosplipids, membrane Phospholipids/ cholesterol ratio.

Erythrocyte Membrane Lipid Alteration in Type 2 Diabetic Subjects G. Rudrappa , Basavaraj Aski , Kashinath R.T.

Abstract -The lipid components present in biological membranes including erythrocyte membrane are significant in regulation of the membrane fluidity as well as transport across the membranes. The conflicting results of either increase or decrease or no change have been reported in erythrocyte membrane fluidity in type 2 diabetic subjects. The diabetes induced dyslipidemia possibly lead to an alteration in erythrocyte membrane lipid composition. Hence a study was undertaken to assess erythrocyte membrane lipid alterations in type 2 diabetic subjects. The blood samples from randomly selected type 2 diabetic subjects, attending Medical OPD of Basaveshwara Medical College Hospital, Chitradurga, were collected with heparin as an anticoagulant. Erythrocyte membrane total cholesterol (mTC), Erythrocyte membrane phospholipids (mPL), Erythrocyte membrane phosphatidyl choline, Erythrocyte membrane sphingomyelin and Erythrocyte membrane phospholipids/ Erythrocyte membrane cholesterol ratio were studied. The results suggests a significant rise in erythrocyte membrane cholesterol (mTC, N=1.25±0.31, T2DM= 1.54± 0.14, p<0.001), erythrocyte

membrane phospholipid contents (mPL, N=6.99±1.84, T2DM=23.44±10.31, p<0.001), Erythrocyte membrane sphingomyelin, (N=9.28± 1.37, T2DM=19.32± 2.37), Erythrocyte membrane phosphatidyl choline (N=27.08± 1.58, T2DM=26.09±3.04, p<0.001), as well as phospholipid/cholesterol ratio (mPL/mTC, N=6.08±2.65, T2DM=11.76±5.31, p<0.001.) in type 2 diabetic subjects was observed. Though, there is a significant alteration seen between normal and diabetic groups, but there was no significant change was observed between male and female subjects of both normal as well as diabetic groups.

1 INTRODUCTION iabetes Mellitus (Type 2)

is a metabolic syndrome characterized by chronic hyper glycemia and disturbances of carbohydrate, protein and lipid metabolism due to underlying insulin lack or subnormal functioning of insulin. In diabetic patients, a reduction of erythrocyte deformability and an increase in whole blood viscosity were correlated with microangiopathy (1). The membrane surrounding the erythrocytes forms a boundary between the interior of the cell and the plasma surrounding it, and severs as a phospholipids are asymmetrically arranged in to a lipid bilayer two molecules thick. Cholesterol is intercalated between the phospholipid molecules. The relative amounts of phospholipids and cholesterol are responsible for the fluid properties of the erythrocyte membrane (19). It is also responsible for the biconcave shape and basic structural integrity of the erythrocyte.

The changes of erythrocyte membrane properties induced by high levels of free fatty acids or aldehydes which are produced in membrane during peroxidative processes, may be responsible for long-term complications in a number of diseases, such as diabetes mellitus (2). It has been also suggested that the ability of red blood cells to change their shape is decreased in diabetic patients. Such an impairment of the red blood cells deformability might be another contributing factor to the reduction of blood flow in the capillaries (7).

2 II.

3 MATERIALS AND METHODS

4 D ? ?

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5 Medical Research

The diabetic type 2 subjects (male and female) in the age group of 30-60 years attending Medical OPD of Basaveshwara Medical College Hospital and Research Center, Chitradurga, were randomly selected. The normal subjects (male and female) were randomly picked among medical students, house surgeons and employees of the college as well as Hospital, who were in the age group of 30-60 years. The normal subjects include 30 males and 06 females; whereas diabetic subjects include 59 males and 31 females.

Blood samples (6-7ml) from the selected normal subjects and type 2 diabetic subjects were collected, in the fasting state, with heparin as an anticoagulant by obtaining informed consent. Plasma was separated by centrifugation at 3500 rpm, for 10 minutes. Erythrocytes were washed three times with an aliquot of 5 ml normal saline and were preserved for further use.

The differences of results in the literature led us to further investigations of the lipid composition of erythrocyte membrane in type2 diabetes. membrane cholesterol, membrane phospholipids, membrane Phospholipids / cholesterol ratio. lyse the erythrocytes. This was centrifuged at 3500 rpm for 5 minutes. Supernatant was discarded. The sedimented membranes were washed 3 times with 3ml aliquots of normal saline. The washed erythrocyte membranes were employed for membrane lipid phosphorous profile studies.

One part of washed membranes was homogenized with 9 parts of chloroform: methanol (1:1 v/v) mixture for 8 minutes using Potter-Elvejham tissue homogenizer. The extracts were used for the estimation of membrane total cholesterol (mTC) (12) , Membrane total lipids (mTL) (8), and membrane total phospholipids (mPL) (17). The phospholipid profilemembrane phosphatidyl choline (PC), membrane sphingomyelin (SM) was estimated in the chloroform: methanol extract, using quantitative thin layer chromatography procedure (10). Another part of washed erythrocyte membranes were homogenized with 4 parts of normal saline for 10 minutes and the extracts were employed for membrane free and total phosphorous estimation (10).

IV.

6 RESULTS

In the present study, a total number of 126 subjects were employed which includes 36 normal subjects and 90 diabetic subjects. The normal subjects subjects. Further the diabetic group consisted of 59 male diabetic subjects and 31 female diabetic subjects. The results of the present study are narrated in table **??** and 2.

Table **??** gives, membrane total lipids (mTL), membrane total cholesterol (mTC), membrane total phospholipids (mPL) and calculated ratios of mPL/mTC in erythrocytes of normal subjects (group 1), in erythrocytes of diabetic subjects (group 2), in erythrocytes of normal male subjects (group 3), in erythrocytes of diabetic male subjects (group 4), in erythrocytes of normal female subjects (group 5), and in erythrocytes of diabetic female subjects (group 6). As seen from the table 1 there is a significant elevation observed in mTC

level (p< 0.001) , in mPL levels (p< 0.001) as well as in mPL/mTC ratio (p< 0.001) in group 2 as compared to group1, in group 4 as compared to group 3 and in group 6 as compared to group 5. However no significant alterations observed in mTL levels between group 2 as compared to group 1, group 4 as compared to group 3 and in group 6 as compared to group 5. It is also evident from the table that there is no significant alterations observed in mTL levels, mTC levels, mPL levels and mPL/mTC ratio between group 4 and group 6, showing that diabetes mellitus induced alterations are common in diabetic male and diabetic female subjects Table **??** depicts membrane levels of lipid phosphorous profile -free phosphorous, bound phosphorous, total phosphorous as well as membrane phosphatidyl choline (PC) and membrane sphingomyelin (SM) in erythrocytes of normal subjects suspension 4 ml distilled water were added and the mixture was stirred vigorously with a clean glass rod to were consisted of 30 male subjects and 06 female III.

7 PREPARATION OF ERYTHROCYTE MEMBRANE

To 1 ml of 50% saturated erythrocyte (group 1), in erythrocytes diabetic subjects (group 2), in erythrocytes of normal male subjects (group 3), in erythrocytes of diabetic male subjects (group 4), in erythrocytes of normal female subjects (group 5), and in erythrocytes of diabetic female subjects (group 6). It is evident from the table that there is a significant elevation observed in membrane free phosphorous (p<0.001), membrane bound phosphorous (p<0.001), membrane total phosphorous (p<0.001), and membrane sphingomyelin (p<0.001) in group 2 as compared to group 1, in group 4 as compared to group 3, and in group 6 as compared to group 5. However, there is less striking change observed in membrane phosphatidyl choline in group 2 as compared to group1 (p<0.01), and in group 6 as compared to group 5. Further it is evident from the table **??** that there is a significant change observed in membrane bound phosphorous (p<0.001) and in membrane total phosphorous levels (p<0.001) levels in group 4 as compared to group 6, however there is no significant alteration observed in membrane free phosphorous, membrane SM levels and membrane PC levels between these two groups.

V.

8 Discussion

Diabetes Mellitus (DM) is a chronic metabolic disorder which is widespread and is associated with substantial morbidity and mortality. Type 2 DM is a chronic disease characterized by hyperglycemia and dyslipidemia due to underlying insulin resistance (11). Apart from hyperglycemia and glucosuria in diabetes Table **??** : Table **??**howing the membrane levels of membrane lipid phosphorous (Free, bound and total), PC as well as SM in erythrocytes of normal male/female and diabetic male/diabetic female subjects. (22,25,28). The Dyslipidemia in diabetes mellitus is related to the type of diabetes as well as to the level of glycemic control achieved in these subjects. It is known that there exists a symmetrical bilayer distribution of lipids in biological membrane including erythrocyte membrane. Normally amine rich lipids are on the innerline of cytoplasmic side of the membrane where as choline rich sphingolipids are on outer surface. It has been shown by previous workers that erythrocyte may be related to membrane lipid composition (5,14,16).

9 Membrane free

10 membrane fluidity as well as deformability

In the present study a significant elevation as been observed in mTL, mTC, mPL as well as mPL/mTC ratio in diabetic erythrocytes as compared to normal erythrocytes (group 2 X group 1), (group 4 X group 3), and (group 6 X group 5). This is in agreement with the reports of Bryzewska et. al. (6), and other workers (9,15,18,27). The erythrocyte membrane principally consisting of different phospholipid and cholesterol. Altered membrane cholesterol, as well as membrane phospholipids, including mPL/mTC ratio was observed in type 2 diabetic erythrocytes in the present study. Though there is an increase in both erythrocyte membrane cholesterol (p<0.001) and in erythrocyte membrane phospholipids (p<0.001), the increase in phospholipids in diabetic erythrocyte seem to be higher as there is a significant elevation seen in mPL/mTC ratio (p<0.001), (ref. **??**able 1).

The results in table 2 show a significant raise in erythrocyte membrane free phosphorous (p<0.001), bound phosphorous (p<0.001), total phosphorous (p<0.001), mPL levels (p<0.001) as well as membrane SM levels (p<0.001), in erythrocytes of diabetic subjects (group 2) as compared to erythrocytes of normal subjects (group 1). The observed results with respect to membrane phosphorous profile may suggest a reciprocation for alteration in erythrocyte membrane phospholipids levels as well as an alteration in erythrocyte membrane phospholipid profile in diabetic subjects.

The results observed in the present studies in erythrocytes of diabetic female subjects as well as in erythrocytes of diabetic male subjects with respect to erythrocyte membrane phospholipid profile, mTC levels, mPL levels, and mPL/mTC ratios are in (both male/ female) agreement with the reports of Juhan Vagus et. al. (20).

It may be concluded from the present studies, the lipid changes in erythrocyte membrane in type 2 diabetic subjects may lead to changes in membrane fluidity resulting in possible membrane functional alteration, including transport across the membranes. However, present study does not show any significant alterations in erythrocyte membrane levels of mTL, mTC, mPL, and mPL/mTC in erythrocytes diabetic male subjects as compared to

erythrocytes of diabetic female subjects indicating that the changes observed in erythrocyte membrane of diabetic population as compared to erythrocyte membrane of normal population is common to both sexes.

The membrane lipids specifically phospholipids play a significant role in maintenance of cell shape, cell permeability as well as movement of various compounds across the membrane. This is true in case of erythrocyte membranes also. It is well established that the phospholipid distribution across the erythrocyte membrane bilayer is asymmetrical (26). The changes in erythrocyte membrane lipid and phospholipid composition observed in the present study (ref. table 2) may induce changes in the physico-chemical properties of erythrocyte membrane as well as in fluidity / rigidity (20). [1]

Figure 1:

[1]Volume XI Issue III Version I September © 2011 Global Journals Inc. (US)This page is intentionally left blank

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Erythrocyte Membrane Lipid Alteration in Type 2 Diabetic Subjects

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Erythrocyte of normal subjects (n = 36)Group-1 Erythrocyte of diabetic subjects (n = 90)Group -2 Erythrocytes of male subjects (n=30) Group-3 Erythrocytes of diabetic normal	Erythrocyte membrane total lipids mg/cc (mTL)	Erythrocyte membrane total cholesterol mg/cc (mTC)	Erythrocyte membrane total phospholipids mg/cc (mPL)	Erythrocyte membrane total phospholipids/ cholesterol ratio (mPL/mTC)
	5.02 + 1.62	1.25 + 0.31	6.99 + 1.84	6.08 + 2.7
	5.35 + 1.53	1.54*** + 0.14	10.31*** + 3.44	11.76*** + 5.31
	4.88 + 0.89	1.25 + 0.32	7.04 + 1.86	6.14 + 2.65
	5.35 + 1.53	1.52*** + 0.14	15.52*** + 7.47	11.34*** + 5.28
male subjects (n=59) Group-4	5.12 + 1.01	1.26 + 0.32	7.04 + 1.86	6.14 + 2.7
Erythrocytes of female subjects (n=06) normal Group-5	5.38 + 1.38	1.48*** + 0.12	16.03 *** + 6.85	12.84 *** + 5.30
Erythrocytes of diabetic female subjects (n=31) Group-6				

*[Note: Note: 1.The number in parenthesis shows the number of samples.2. Values are expressed as their Mean ± SD.3. p-value * p<0.05, ** p<0.01, *** p<0.001. © 2011 Global Journals Inc. (US)]*

Figure 2: Table - 1

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