East African Medical Journal Vol. 78 No. 8 August 2001
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E. O. Adewoye, MSc, PhD., Department of Physiology, K. S. Akinlade, MBBS, FMCP, Department of Chemical Pathology and O. O. Olorunsogo, MSc, PhD, Department of Biochemistry, College of Medicine, University of Ibadan Ibadan, Nigeria.

Request for reprints to: Dr. E. O. Adewoye, Department of Physiology, University of Ibadan, Ibadan, Nigeria.

ERYTHROCYTE MEMBRANE PROTEIN ALTERATION IN DIABETICS

E. O. ADEWOYE, K. S. AKINLADE and O. O. OLORUNSOGO

ABSTRACT

Objectives: **To study the protein components of the erythrocyte membranes of diabetic Nigerians and to compare the results with the erythrocyte membrane protein components of normal healthy Nigerians.**

Design: **Laboratory based cross-sectional study.**

Setting: **Department of Medicine, University College Hospital (UCH), Ibadan and Biomembrane Research Laboratory, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.**

Subjects and methods: **The study was carried out in patients with insulin-dependent diabetes mellitus - IDDM (Type 1 diabetes), non-insulin dependent diabetes mellitus - NIDDM (Type 2 diabetes) and healthy human volunteers (HHm), which served as controls. The subjects were aged 30-65 years. There were 12 subjects in each of the IDDM and NIDDM) and 18 subjects in the HHm group. Blood samples (20 ml per subject) were obtained from each subject and erythrocyte ghost membranes were isolated separately from each sample. Total erythrocyte membrane protein concentration of each sample was determined using bovine serum albumin (BSA) as standard. The protein components of the erythrocyte ghost membranes were determined using sodium dodecyl sulphate polyacrylamide gel electrophoresis.**

Statistical method: **All values given are the mean±standard deviation (±SD) of the parameters measured. Significance was assessed using Student's t-test and P values of 0.05 or less were taken as statistically significant.**

Results: **The total protein concentration of HHm was 5.5±0.01 μg/ml, total protein concentration of IDDM was 4.5±0.01 μg/ml while NIDDM was 5.1±0.02 μg/ml. The spectrin alpha and beta-chain bands are heavily present in the healthy human erythrocyte membranes and are absent in the insulin dependent diabetic membranes. The ankyrin band, band six and below are more pronounced in IDDM and NIDDM but are relatively absent in the healthy humans.**

Conclusions: **The results obtained provide evidence of profound quantitative and qualitative alteration of the erythrocyte membrane proteins in diabetic Nigerians. This may likely have serious functional implications on the diabetic patients.**

INTRODUCTION

Several workers have studied the structure and function of biological cell membranes and have reported various components of membranes depending on the tissue studied. Membrane protein concentration has been reported to be closely related to its function(1,2). Membrane proteins vary with the type of cell and they are responsible for most of the dynamic processes carried out by membranes. Specific proteins mediate distinctive membrane functions such as transport, communication and energy transduction(3). Reports on human erythrocyte membrane show a variety of structures, which depend largely on the sensitivity of the technique used for the study(4). Thus, reports on the structure of healthy human erythrocyte membrane vary slightly but have in common a number of major features like spectrin, actin, ankyrin, band 3 and band 4.1 proteins(3-6). Previous studies have reported the importance of membrane components and structural organisation to normal functioning of cells and tissues(7-10). Although there are reports on the locations of protein molecules in the erythrocyte membrane of healthy humans(1,2), there is no information on protein component of erythrocyte membranes in diabetic Africans. In view of the relevance of a healthy cell membrane to cellular function and the lack of information of protein component of diabetic cell membrane in Africans, the study was carried out to investigate the protein content of the erythrocyte membranes of insulin dependent and non-insulin dependent diabetic Nigerians. The protein concentrations of the groups studied were estimated using the method of Lowry *et al*(11) using bovine serum albumin

(BSA) as a standard, while the protein molecules were identified by their molecular weights using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

MATERIALS AND METHODS

Experiments were carried out on blood samples (20 mls per subject) collected from healthy human volunteers (HHm), patients from insulin dependent diabetes mellitus (IDDM) and patients suffering from non-insulin dependent diabetes mellitus (NIDDM) attending the Diabetic Clinic, University Hospital, Ibadan. The HHm group served as control. There were 12 subjects in each of the IDDM and NIDDM groups and 18 subjects in the HHm group. The subjects were aged 30-65 years. Erythrocyte ghost membranes were isolated from the three groups, that is, HHm, IDDM and NIDDM using the method of Niggli *et al*(12). The total protein concentration of HHm, IDDM and NIDDM were estimated using the method of Lowry *et al*(11). 200 µg/ml bovine serum albumin (BSA), (Sigma Chemical Co. London) was used to prepare standard solutions and the absorbance were read at 750 nm. A standard curve of absorbance against concentration was obtained from these results and the curve was used for estimating the total protein concentration in HHm, IDDM and NIDDM.

The erythrocyte ghost membranes from each group were separated into their component polypeptides using sodium dodecyl sulphate gel unit. Membrane samples for electrophoresis were prepared by mixing 20-50 µl of sample to be analysed with 150-180 µl of the sample buffer and were incubated in a boiling water bath for three minutes to ensure breakage of the disulphide bonds in the proteins. Equal amount of these samples (40-50 µl) were then applied gently to the bottom of the sample wells with Hamilton syringe. Electrophoresis was run at constant voltage of 60 volts and a constant current of 25 mA. After the separation, the gel was removed and fixed in a solution of 50% methanol and 7.5% acetic acid for 30 minutes and then stained for three hours with coomasie brilliant blue solution (0.25% coomasie brilliant blue, 50% C_3OH and 7.5% acetic acid). The gel was then destained by rinsing several times in a solution of methanol; acetic acid; and water in a (10:7:83 v/v) ratio. It was later drained and photographed.

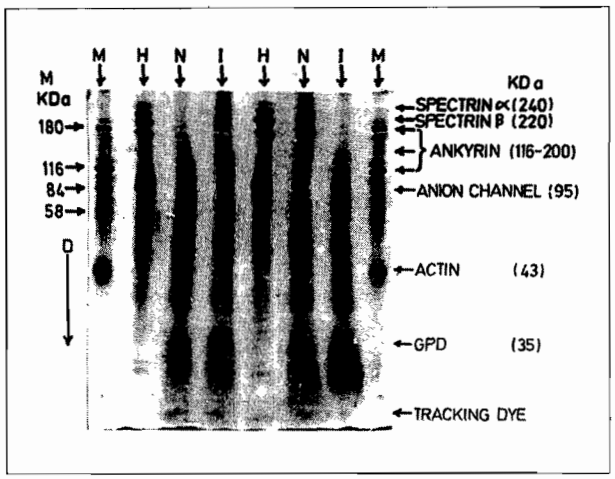
Protein values were compared for statistically significant difference using Student's-t-test. P values of 0.05 or less were taken as statistically significant.

RESULTS

The erythrocyte membrane protein concentration of HHm was highest, being 5.5±0.01 µg/ml while protein concentration of IDDM was lowest (4.5±0.01 µg/ml) the erythrocyte membrane protein concentration of NIDDM lies between the values of HHm and IDDM (5.1±0.02 µg/ml). The protein concentrations in IDDM and NIDDM were significantly different from one another and also from HHm ($P<0.05$). The results of the electrophoretic separation is shown in Figure 1. Membranes from each group were separated into their polypeptide components.

Figure 1

Electrophoretic separation of erythrocyte ghost membrane proteins of HHm, IDDM and NIDDM with SDS polyacrylamide gel



These results show that there are differences in the polypeptide components of IDDM and NIDDM compared with HHm. Some polypeptide bands are absent in the IDDM and NIDDM groups when compared with HHm. The spectrin alpha-chain (240 kDa) and spectrin beta-chain (220 kDa) bands are heavily precipitated in HHm while they are almost absent in the NDDM and markedly reduced in IDDM. The lower portion of ankyrin band (116 to 200 kDa) is more pronounced in the two diabetic groups. Also, the actin band (43 kDa) is more pronounced in the IDDM and NIDDM than in HHm. Band 6 (35 kDa) and below are pronounced in IDDM and NIDDM but absent in HHm.

DISCUSSION

The significantly higher protein concentration in HHm compared with protein concentration in IDDM and NIDDM observed in this study is consistent with the reports of Weed *et al*(13), Eaton *et al*(14) and Olorunsogo *et al*(15). Previous studies on erythrocyte membrane (EM) protein concentration in sickle cell anaemia(14) and hypertension(15) showed reduction when compared with normals. The marked reduction in EM protein concentration observed in this study shows that EM proteins and most probably other body proteins are considerably affected in diabetes mellitus. Oli(16) and Zimmet and King(17) reported more severe symptoms and a higher rate of wasting in IDDM whereas NIDDM presents with milder symptoms and less wasting(18). The differences in the presentation and severity of IDDM and NIDDM are most probably related to the differences in erythrocyte membrane protein concentration and composition observed in the

two groups. That is to say, the much lower EGM protein in IDDM than in NIDDM is highly suggestive of a higher degree of wasting in IDDM than NIDDM. Also, in the present study, the results of protein concentration in EGM of HHm, IDDM and NIDDM provide evidence of profound alteration of EGM proteins in diabetes mellitus. The heavy precipitation of spectrin-alpha and beta-chain bands in HHm and their absence in IDDM and NIDDM are indications of alterations in the structure of EGM proteins in diabetics. Spectrin and other structural proteins stabilize the shape of the red cell(1,2). The almost total absence of spectrin in diabetics in this study suggests that the structures of the erythrocyte altered. The heavy precipitation of ankyrin in IDDM and NIDDM and its relative absence in HHm is also suggestive of marked structural alteration in EGM polypeptides in diabetes mellitus. The altered polypeptides could have attained different conformations and probably a change in molecular weight since differences in molecular weights affect distances travelled by polypeptides in an electric field(4). However, the similarities observed in the electrophoretic separation of proteins in IDDM and NIDDM may well be that the proteins in these disease groups were similarly altered by factors(s), which may be common to both disease conditions. Glycosylation of membrane protein would not be unexpected in high blood glucose condition such as in diabetes mellitus. The prolonged interaction of glucose with cellular proteins could have altered the structure of cell proteins(19,20). This possible alteration would probably result in new conformers with different molecular weights. It follows therefore, that such new conformers would migrate in an electric field according to the new charge and molecular weight(4). The heavy precipitate observed below 36 kDa bands in NIDDM and IDDM but not in HHm provide further evidence of a profound alteration in EGM proteins in diabetes mellitus.

In conclusion, the results of this study showed that there are profound alterations of erythrocyte ghost membrane proteins in the diabetic state in Nigerians. The functional implications of these alterations in membrane proteins are unknown. It is also conceivable that other body cells, apart from the erythrocyte membrane, have similar alterations in their proteins in diabetes mellitus. There is need for further studies to clarify these issues.

ACKNOWLEDGEMENTS

We are grateful to Mrs. Adefunke Olawuni for her secretarial assistance. This study was supported in part by Senate Research Grant E.O.A.

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