The erythrocyte membrane skeleton biology essay

Science, Biology

\n[toc title="Table of Contents"]\n

\n \t

\n[/toc]\n \n

Introduction:

The erythrocyte membrane skeleton is an extensive network of structural proteins that underlies the plasma membrane and appears to regulate its deformability and mechanical stability [1]. When the integrity of the membrane skeleton is disrupted by structural abnormalities or deficiencies of its protein components, the membrane becomes susceptible to fragmentation and loses its characteristic discoid shape [2, 3]. These proteins intervene not only in the flexibility of the membrane, but also in the surface to volume relation and through transmembrane exchanges, in the internal viscosity of the red cells. These properties depend essentially on the molecular composition of membrane proteins and on their interactions, whose main components are spectrin, actin, and protein band 4. 1[4]. The failure of surface area caused as a result of increased membrane feebleness due to imperfection in quite a lot of membrane proteins, including ankyrin, protein 3, α-spectrin, β-spectrin and protein 4. 2 of the erythrocyte membrane comprising an important group of innate disorders including

hereditary spherocytosis (HS), hereditary elliptocytosis (HE), hereditary pyropoikilocytosis (HPP), and the hereditary stomatocytosis (HSt) syndromes [5]. Mutations in the gene for the globin protein results in a group of hereditary disorders of haemoglobin: (i) thalassaemia and (ii) haemoglobinopathies, representing a key public health problem covering most areas of the globe, including Southeast Asia [6, 7]. The structural alterations, referred in case of haemoglobinopathies, are mostly due to the substitution of single amino acid such as haemoglobins S, C, D, E, etc. However, substitution of two amino acids, insertion or deletion of amino acids including fusion of two polypeptide chains are also been reported [8]. Haemoglobinopathies are the worldwide prevalent monogenic disorders with variable geographic distribution [9]. Among the variant haemoglobins, Haemoglobin E (HbE, α2β226glu-lys) is the most common β-thalassaemic haemoglobinopathy in Asian population affecting about 30 million inhabitants of Southeast Asia [6]. HbE was suggested as a marker for the Mongoloid element in Northeast Indian populations [10], the prevalence of which is widely variable among the different ethnic groups [11]. In some of the ethnic groups of the region, the gene frequency for E-globin gene is as high as 0. 6 [12]. The subjects carrying E-globin gene are also reported to be minimally anemic with slightly decreased RBC survival [13]. This variant haemoglobin behaves like a mild thalassaemic gene associated with the production of lower level globin mRNA, due to the activation of cryptic donor splice site by the GAG to AAG mutation in Exon I of E globin gene [14, 15]. Therefore, an attempt was made to explore the possible changes of the membrane proteins of red blood cells carrying E-globin gene.

Methods and Materials:

Considering 30% prevalence of βE-globin gene with a precision of 5% and 99% confidence interval estimated sample size would have been 558. However, in view of prevalence of 5% homozygous state of βE-globin gene as well as about 3% of β-thalassaemia and HbE/ β-thalassaemia carrier state in the population of Assam, we have included 756 subjects (male = 260 & female = 496) for better representation and to register maximum numbers of cases. Finally, adopting non-probability sampling method, unrelated subjects volunteered to participate in the study were included for screening of haemoglobinopathies. Seven hundred fifty six blood samples collected randomly from the volunteer's intravenous blood (3ml) in aseptic conditions in K3EDTA coated vials. Presence of variant haemoglobins was evaluated by Cation exchanger HPLC based Variant haemoglobin Testing SystemTM (Bio Rad) using β-thalassaemia Short Program [16, 17]. Red blood cell membrane proteins extracted adopting standard protocol [18] from individual blood samples with heterozygous, homozygous state for HbE and normal haemoglobin pattern (HbAA). Membrane proteins, thus extracted were quantified adopting standard protocol [19]. Finally, 15 µl of the membrane protein sample mixed with sample buffer (3: 1 ratio) was loaded in 10% polyacrylamide gel containing SDS as suggested by Fairbanks et al. 1971[20]. The electrophoregram was visualized after staining by Coomassie brilliant blue in Gel documentation system and images were recorded.

Results:

Screening of the blood samples by Variant Hb Testing SystemTM (Bio Rad) indicates Hb E is the only variant haemoglobin observed in the present

study. The mean (± SD) HbE level in heterozygous and homozygous state of Hb E was 29. 22 ± 2. 03 and 77. 47 ± 5. 29% respectively. Thus 15. 34% and 40. 74% of the subjects were detected with heterozygous and homozygous state of HbE respectively with a gene frequency for βE-globin gene of 0. 3571. The remaining 43. 92% of the samples was with normal haemoglobin pattern. We have observed a significant difference of HbF level in subjects carrying heterozygous (P= 0. 026) and homozygous (P= 0. 000) state of HbE. The mean (±SD) HbF level of subject carrying heterozygous and homozygous state of HbE was 0. 93 ± 0. 79 and 4. 20 ± 3. 30% respectively. While, the HbF level of subjects with normal haemoglobin (Hb AA) was 0. 23 ± 0. 15%. SDS-PAGE electrophoretogram of the RBC membrane proteins of Hb AA, HbE heterozygous and homozygous state are depicted in Figure. The differences of the membrane proteins especially in the α-spectrin and protein 4. 1 were found to have in the electrophoretogram of RBC's carrying heterozygous and homozygous for HbE in comparison to HbAA. The other major membrane proteins of RBC (i. e., β-spectrin, protein 4. 2, F-actin, ankyrin) were also found to be slightly decreased in RBC carrying βE-globin gene (Fig.).

Discussion:

Using the latest proteomic technique, the current erythrocyte protein count stands at 1989 along with around 850 interaction pathways in which those proteins could take part [21]. However, even after this large data explosion, the indepth understanding of the roles of erythrocyte proteins has not improved much. The Red blood cells (RBC) membrane skeleton proteins network comprising mainly of α- and β-spectrin, protein 4. 1, F-actin, ankyrin

that determines the structural stability of the red cell membrane. The quantitative and functional abnormalities of either of these membrane proteins of red cells are responsible for the hemolytic anemia1. Wong et al., (2000) suggested that the defect of surface area is caused by increased membrane fragility due to fault in proteins of the erythrocyte membrane. Mohandas and Evans (1994) [22] reported that the spectrin protein, a major component of the erythrocyte membrane skeleton is responsible for maintaining erythrocyte membrane integrity and mechanical stability. Hassoun and Palek (1996) [23] observed unusually high mechanical fragility of the erythrocyte membranes in patients suffering from hereditary spherocytosis and hereditary elliptocytosis were known to be the result of defects in spectrin. The findings of Takakuwa et al., (1986)2 on erythrocyte membranes, deficient in protein 4. 1, exhibiting marked mechanical instability in vivo and in vitro, who suggested that the normal membrane stability can be restored to these membranes in vitro by reincorporation of exogenous purified protein 4. 1. Quantitative changes in the red blood cell membrane proteome in sickle cells disorder were analyzed using the 2D fluorescence difference gel electrophoresis (2-D DIGE) technique by Goodman’s group. They found 49 protein spots whose content in sickle cell membranes were changed considerably as compared with normal cells [24]. Kakhniashvili et. al., (2005) evaluated the sickle cell erythrocyte membrane proteins using 2D-DIGE, trypsin digest, HPLC and tandem MS. They found to have 22 different proteins (44 protein forms, including post-translational modifications) in sickle cell erythrocyte membrane having a 2. 5-fold or greater disparity from control erythrocyte membranes [25]. Chakrabarti et.

al. (2011) [26] discussed the role of red cell proteome on sickle cell disease and thalassaemia, emphasizing on the differential expression of redox regular proteins. These findings were corroborated with our result on membrane proteins of red blood cells carrying βE-globin gene, both heterozygous and homozygous state, indicating differences in the pattern of bands, especially α-spectrin and protein 4. 1.

Acknowledgement:

Researchers are thankful to Dr. J. C. Mahanta, Director, RMRC (N. E. Region), ICMR, Dibrugarh for his kind help and support in the successful completion of the entire programme. Thanks are also due to Mr. S. Gogoi and Mr. R. Dutta for technical assistance.