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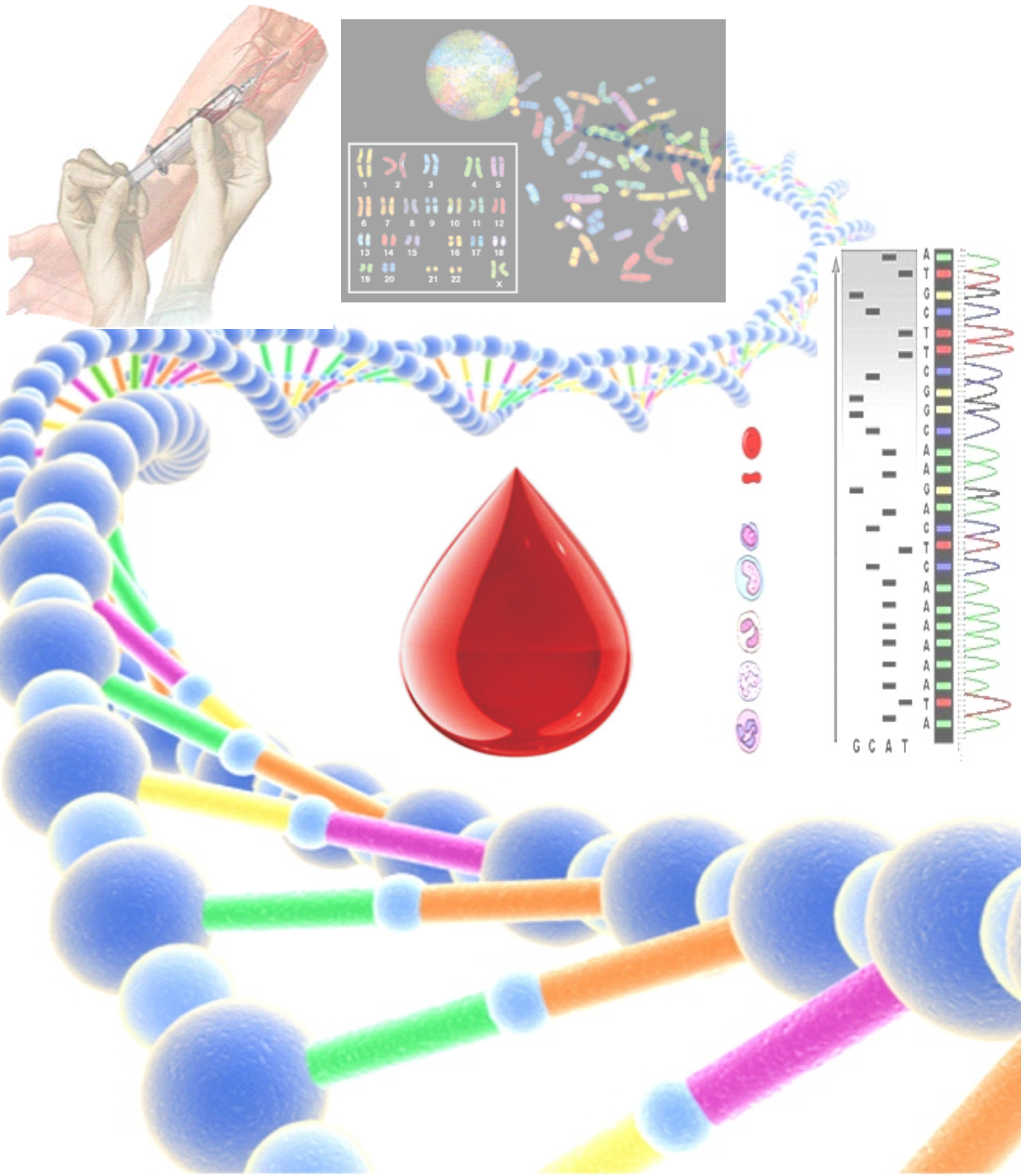

Workshop on Flow Cytometry

EQAS Workshop on HIV

Workshop on Diagnosis and management of Thalassemia, Sickle Cell Anemia and Hemophilia

RED CELL MEMBRANE PROTEIN DISORDERS

Prashant Warang

The red cell membrane is an elegant system and has many important functions. The red cell membrane and its skeleton provides the erythrocyte it's flexibility, durability and tensile strength to undergo large deformations during repeated passage through narrow microcirculatory channels. The red cell membrane maintains a slippery exterior so that the erythrocytes do not adhere to the endothelial cells or aggregate and occlude the micro-circulation. At the level of the organism, the membrane participates in the maintainace of pH homeostasis, participating in the exchange of chloride and bicarbonate (1).

The red cell membrane is one of the best known membranes in terms of structure, function and genetic disorders. The erythrocyte membrane is composed of three major structural elements: a lipid bilayer primarily composed of phospholipids and cholesterol that provides a permeability barrier between the external environment and the red cell cytoplasm; integral proteins embedded in the lipid bilayer that span the membrane; and a membrane skeleton on the internal side of the red cell membrane that provides structural integrity to the cell.(Fig.1)

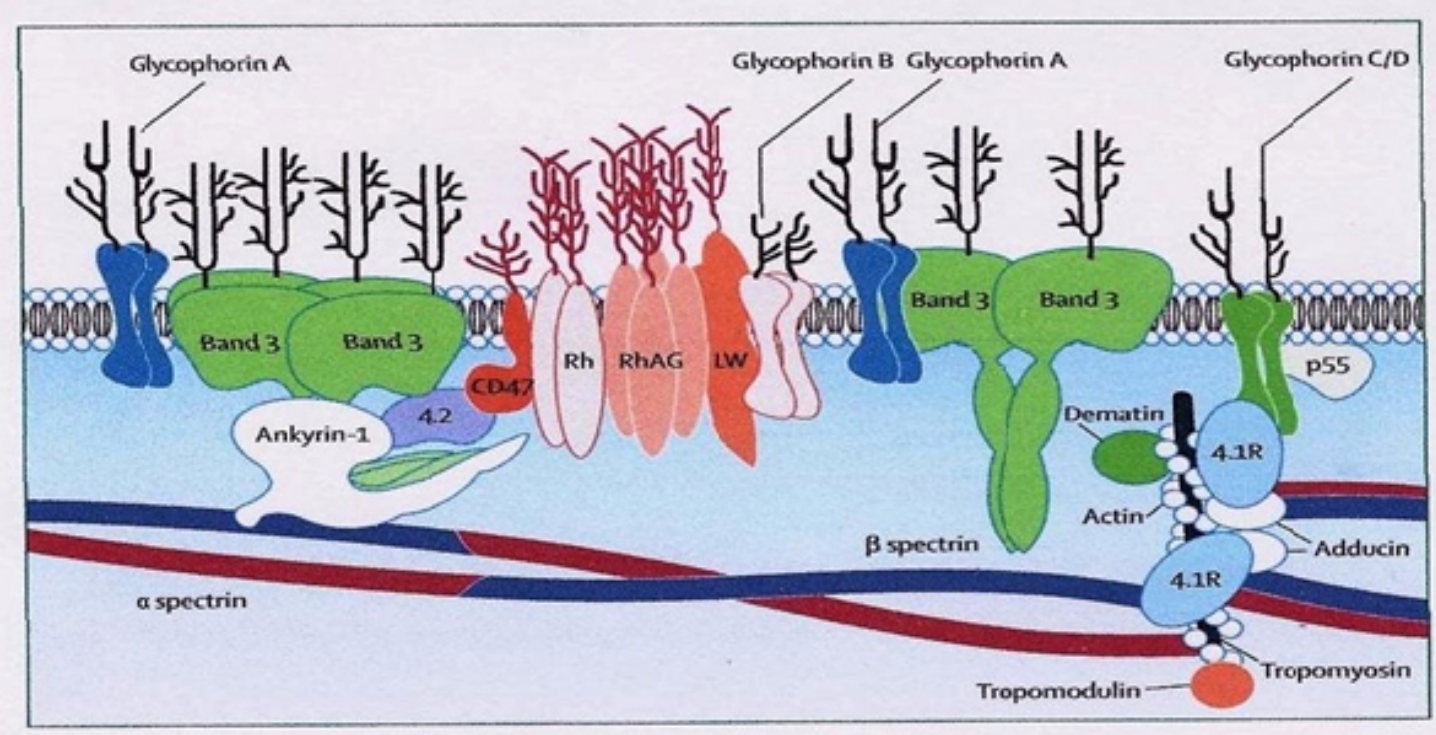

Fig.1- A simplified cross-section of the red blood cell membrane. [Adopted from: Perrotta et al 2008. (2)]

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Red cell membrane disorders

Hereditary Spherocytosis: Hereditary spherocytosis (HS) is a common inherited hemolytic anaemia in which defects of spectrin or proteins that participate in the attachment of spectrin to the membrane, ankyrin, protein 4.2, or band 3 lead to spheroidal, osmotically fragile cells that are selectively trapped in the spleen resulting in a shortened red cell life span. HS occurs in all racial and ethnic groups. It is particularly common in northern European people, where it affects approximately 1 in 5000 people. HS has also been frequently described in other populations, notably in Japan (1). There are no good estimates of the prevalence in other populations but clinical experience suggests it is less common in Africans, Americans and Southeast Asians.

HS exhibits both dominant and non-dominant phenotypes. About two thirds of patients have a typical autosomal dominant disease with the remaining patients exhibiting non-dominant inheritance (1, 3). The clinical manifestations of the spherocytosis syndromes vary widely. The typical clinical picture of HS includes anemia, jaundice (mainly indirect hyperbilirubinemia), reticulocytosis, gallstones and splenomegaly with spherocytes seen in the blood film and a positive family history. Mild, moderate and severe forms of HS have been defined according to differences in hemoglobin levels, bilirubin concentrations, and reticulocyte counts which can be correlated with the degree of compensation for the hemolysis. In severe cases of HS, red cell transfusions may be required (4).

Hereditary Elliptocytosis: Hereditary elliptocytosis (HE) is characterized by the presence of elliptical, cigar-shaped or oval erythrocytes on the peripheral blood smear. In humans, the presence of elliptocytosis indicates a defect in the erythrocyte membrane skeleton. The worldwide incidence of HE has been estimated to be 1:2000-4000 in the population. HE is common in individuals of African and Mediterranean descent, presumably because elliptocytes confers some resistance to malaria. The true incidence of HE is unknown because it's clinical severity is heterogenous and many patients are asymptomatic and do not have anemia. These patients are diagnosed incidentally during testing for unrelated conditions and they have a normal erythrocyte life span. However, many patients are severe with life threatening anemia. A symptomatic HE patient may experience hemolysis in association with infections, hypersplenism or vitamin B12 deficiency. A few cases of hydrops fetalis accompanied by fetal or early neonatal death due to unusually severe forms of HE have been described. Complications associated with HS and HE includes gallstones, aplastic crises, leg ulcers, extramedullary hematopoiesis and hemochromatosis (5).

Southeast Asian Ovalocytosis: Southeast Asian Ovalocytosis (SAO) also known as Melanesian elliptocytosis or stomatocytic elliptocytosis, is a dominantly inherited trait characterized by the presence of oval red cells, many of which contain one or two transverse ridges or a longitudinal slit. This condition is widespread in certain ethnic groups of Malaysia, Papua New Guinea, the Phillippines, Indonesia and other parts of Southeast Asia. Most SAO patients are asymptomatic, although a few experience mild hemolysis. Compared to other membrane defects, SAO red cells are rigid and hyperstable, rather than unstable.

In vivo, there is evidence that Southeast Asian Ovalocytosis provides some protection against all forms of malaria, particularly against heavy infections and cerebral malaria. The mechanism of malaria resistance of Southeast Asian Ovalocytosis red cells is speculative. Band 3 serves as one of the malaria receptors, as evidenced by inhibition of invasion in-vitro by the band 3- containing liposomes (6). It should be noted that the SAO red cells are osmotically resistant rather than fragile. In addition, in the SAO red cells, many blood group antigens are poorly expressed or even missing such as LW, D, C ,e, S, s, U,

Kp^b, Jk^a, Jk^b, Xg^a Wr^b,Scl and En^a (7).

Hereditary Stomatocytosis : Hereditary stomatocytosis, the name given by Lock and co-workers who were the first to report a a case which the first characterized by erythrocytes with a mouth-shaped (stoma) area of central pallor on peripheral blood smears. The clinical severity of hereditary stomatocytosis is variable; some patients experience hemolysis and anemia whereas others are asymptomatic (8). Stomatocyte membranes are remarkably permeable to K^+ ions and particularly to Na^+ ions. Intracellular Na^+ is increased and K^+ is decreased, but the total monovalent cation content (Na^+, K^+) is high, which leads to an increase in cell water and cell volume. As a consequence, the "edematous cells" are sometimes called hydrocytes or overhydrated stomatocytes. Because the influx of Na^+ exceeds the loss of K^+, stomatocytic red cells progressively gain cations and water and swell. As a result their average density is less than normal and the swollen stomatocytes are osmotically fragile. In many patients, stomatocytes are also moderately deficient in 2,3Bisphosphoglycerate (2,3 BPG) (9). Perhaps a portion of the 1, 3 BPG normally used for 2,3 BPG synthesis is diverted through phosphoglycerate kinase to provide extra ATP for cation transport. The 2, 3 BPG deficiency mildly enhances oxygen affinity and causes additional water entry and cell swelling.

Hereditary stomatocytosis is probably more heterogeneous than suggested earlier. Some patients with severe permeability defects have little or no hemolysis. In addition, studies of 44 Japanese patients with stomatocytosis showed that the proportion of stomatocytes and the degree of Na^+ influx do not correlate with each other and neither correlate with the amount of hemolysis or anemia (10). Furthermore, stomatin deficiency was only present to a mild degree in 5 of 9 patients with more moderate Na_+ leaks. This suggests that hereditary stomatocytosis is a complex mixture of diseases or that factors other than Na_+ leak and stomatin content are critical to the demise of the stomatocyte.

Hereditary Pyropoikilocytosis: This uncommon disorder presents in infancy or early childhood as a severe hemolytic anaemia (Hemoglobin level of 4 to 8 g/ dl). It is characterized by extreme poikilocytosis with budding red cells, fragments, spherocytes, triangulocytes and other bizarre-shaped cells and in some patients, few or no elliptocytes. Complications of severe anemia including growth retardation, frontal bossing, and early gallbladder disease have been reported. Another characteristic feature of these cells is their remarkable thermal sensitivity. Pyropoikilocytes fragment at 45°C to 46°C (normal 49°C) after short periods of heating (10 to 15 minutes). After splenectomy, hemolysis is markedly decreased, but not eliminated. (9, 11).

Molecular pathology of red cell membrane protein disorders.

The genetic characteristics of membrane protein genes in human erythroid cells are shown in table 1.

Table 1. Genetic characteristics of red cell membrane protein genes. [Adopted from: Yawata 2003. (1)]

Protein	Gene symbol	Chromosome location	Amino acids	Exons	Related diseases
α– Spectrin	SPTA1	1q22-q23	2429	52	HE, HPP,HS
β– Spectrin	SPTB	14q23-q24.2	2137	32	HE, HPP,HS
Ankyrin	ANK1	8p11.2	1881	42	HS
Band 3(AE 1)	EPB3 (SLC 4A1)	17q21-q22	911	20	HS, SAO, dRTA
Protein 4.1	EPB41	1p36.1	588	23	HE
Protein 4.2	EPB42	15q15	691	13	HS variant
Glycophorin A	GYPA	4q28.2-q31.1	131	7	None

The genotypes of red cell membrane protein disorders are shown in fig.2. Several mutations have been described in α and β spectrin, ankyrin, band 3 and protein 4.2. Majority of them are frameshift, missence, nonsence or splicing mutations. In western countries, a combined deficiency of spectrin and ankyrin is the most common feature in HS. Frameshift mutatios and nonsense mutations of the ankyrin gene were found mostly in HS patients with autosomal dominant (AD) inheritance and missense mutations tended to be detected mostly in HS patients with autosomal recessive inheritance. In western countries ankyrin gene mutations are found in 55-60% of all HS patients (12). The incidence of band 3 deficiency in red cells of Caucasian HS patients has been reported to be up to 30%. In Japanese HS patients, the incidence of band 3 deficiency was also around 20-30 % (13).

HE is inherited in an autosomal dominant manner with rare cases of de novo mutations. Abnormalities of either α or β spectrin associated with the majority of cases of HE are due to mutations in the spectrin heterodimer self association site. Most of these mutations are missense mutations at, or very near, highly conserved residues of spectrin (5).

The Southeast Asian ovalocytosis phenotype due to heterozygosity for two band 3 mutations in cis: the deletion of 27 bp encoding amino acids 400 to 408 located at the boundary of the cytoplasmic and membrane domains of band 3 and the amino acid substitution Lys 56 Glu. has been described (14).

Status of RBC membrane defects in India.

Hereditary spherocytosis cases are being reported since a long time from India. Studies with a large number of cases have been reported (15, 16, 17, 18) and the findings suggest that both autosomal dominant and recessive patterns of HS are seen in India and the clinical profile of the Indian HS patients is similar to that described in other populations. HS presenting in childhood is also not uncommon. Association of red cell membrane defects like HS and HE with G6PD deficiency and β-thalassemia trait respectively have been reported earlier from western India (19, 20, 21). Elevated level of ATP and high PK activity have also been reported along with hereditary spherocytosis from eastern India (22). However, the predominant underlying protein defect in Indian patients has not been characterized.

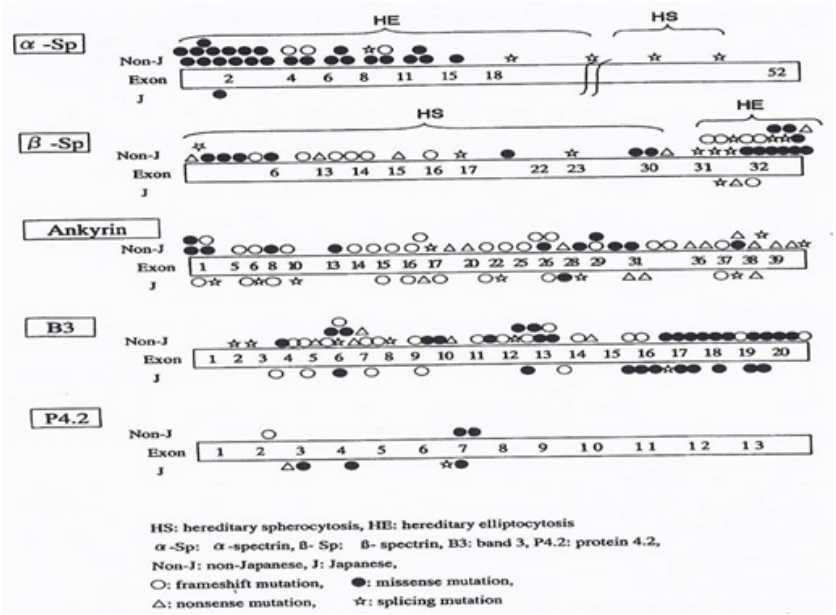

Fig.2- The nature of mutations described in red cell membrane protein disorders. [Adopted from: Yawata 2003. (1)]

Diagnosis of red cell membrane defects

Diagnosis of moderate to severe forms of hereditary spherocytosis and other membrane disorders like hereditary elliptocytosis (HE) and hereditary pyropoikilocytosis is conventionally done by a series of investigations which include clinical features (anemia, jaundice, splenomegaly), peripheral smear examination, reticulocyte count, red cell indices, osmotic fragility, autohemolysis test and acidified glycerol lysis test after exclusion of other hemolytic conditions like hemoglobinopathies, red cell enzymopathies, and autoimmune hemolytic anemia . As these tests do not have a high degree of sensitivity and specificity, milder and atypical cases of HS may be missed (23). Flow cytometric analysis of red cell membrane proteins using eosin-5-maleimide (E5'M) dye is a reliable screening test and gives quantitative results with greater sensitivity. However E5'M is expensive and very unstable (24). The confirmation of diagnosis requires sodium dodecyl sulfate polyacylamide gel electrophoresis (SDS-PAGE) analysis of red cell membrane proteins to identify the specific protein deficiency, but this technique reveals abnormalities in only 70-80% patients. The amount of spectrin and Ankyrin are best assessed by RIA or EIA. However, these methods are available only in few specialized laboratories. Recently an automated capillary gel electrophoresis system has been used to separate and quantify the erythrocyte membrane proteins (25). Molecular techniques have been applied for the detection of mutations leading to red cell membrane defects. As most of the genes that cause the membrane defects are large and contain many exons, hence this approach is difficult. A simple flow-cytometric screening approach for red cell membrane disorders based on osmotic fragility (FCM-OF) by measuring the % residual red cells after spiking the RBCs with distilled water was standardized by us (26). This was validated on a large number of samples. The FCM-OF test could be a valid alternative as a first line screen for red cell membrane disorders due to its simplicity and cost effectiveness compared to the E5'M dye test in hematology laboratories where a flow cytometer is available. The FCM OF test also has an excellent discriminatory power between the normal group and the β thalassemia trait group.

Clinical management

Folate supplementation is recommended in severe and moderate HS but is not necessary in mild cases. Blood transfusions may be required in severely anemic cases, particularly in the first years of life, the hemoglobin then tends to stabilize in many cases at about 6-8 g/dl and transfusions are no longer necessary unless the anemia is exacerbated by infections, pregnancy or other conditions.

Splenectomy is very effective in reducing hemolysis, leading to a significant prolongation of the red cell life span. The clinical manifestations and complications are much reduced in severe HS and abolished in milder case but there is an increased risk of life-threatening sepsis from encapsulated organisms, particularly streptococcus pneumonia. Recent studies showed that splenectomy for HS in children is very safe in the short term with no deaths and infrequent complications (< 1% in 1657 splenectomies) (28).

NIIH Exprience

In the last five year, total 167 cases that were suspected to have a red cell membrane defects were investigated. After looking at the peripheral smear all cases were screened by E5'M fluorescence dye test by flow cytometry. Out of 167 cases, 115 (68.8%) showed the presence of RBC membrane protein defects (110 HS, 2 HE, 3 Hst with dRTA). The most common features observed in our 110 HS patients were splenomegaly, gall stones, anemia, increased mean corpuscular hemoglobin concentration (MCHC), increased reticulocyte counts and indirect hyperbilirubinemia which is comparable to previous studies from India (16,17,18) as well as from Italy which included clinical and hematological features of 300 patients of hereditary spherocytosis (27). Splenectomy was done in 10 of our patients (10.86%) and after splenectomy the patients have been doing well. Most of the patients had mild to moderate hemolytic anemia except two patients who received multiple blood transfusions.

Three cases of distal renal tubular acidosis with hereditary stomatocytosis were also identified. All three cases had reticulocytosis and two of them had anemia and indirect hyperbilirubinemia. Abdominal

sonography detected hepatosplenomegaly and nephrocalcinosis. Metabolic acidosis with alkaline urine (pH 7.2-8.0) was also observed. All three cases had history of receiving blood transfusions.

Hereditary elliptocytosis is a comparatively rare red cell membrane protein defect. We identified only 2 cases. Of the two cases, one had indirect hyperbilirubinemia, reticulocytosis and hepatosplenomegaly whereas other case only mild anemia. Characterization of red cell membrane proteins was done in ten cases using SDS PAGE and automated electrophoresis and this showed the presence of band 3, Ankyrin, α spectrin and protein 4.2 defects. Molecular characterization was done in two cases of band 3 protein defect and showed the homozygous A858D mutation of the AE1/SLC4A1 gene.

Conclusion and future direction

Although E5'M dye test and flow cytometric osmotic fragility test pick up cases of hereditary RBC membranopathies, however mild forms may be difficult to identity and could be missed.

SDS PAGE and molecular analysis need to be done in all our cases of suspected RBC membranopathies that remained undiagnosed. Recently chip-based DNA technologies are being developed for mutation analysis of the major RBC membrane and cytoskeleton genes (29) which will increase the diagnostic repertoire of membrane protein disorders

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A TWO TUBE ASSAY FOR DETECTING MILD TO MODERATE DEFICIENCY OF FACTOR XIII

Sharda Shanbhag

Deficiencies of coagulation factors other than factor VIII and factor IX that cause bleeding disorders are inherited as autosomal recessive traits and are rare. As a consequence of the rarity of these deficiencies, the type and severity of bleeding symptoms, the underlying molecular defects, and the actual management of bleeding episodes are not as well established as for haemophilia A and B.

FXIII deficiency is also known as one of the rarest blood coagulation disorders. The incidence of severe FXIII deficiency is one in 3–5 million people and it affects people of all races wherein consanguinity is practised commonly. FXIII deficiency is associated with severe bleeding, intracranial haemorrhages , poor wound healing and spontaneous abortions. Umbilical stump bleeding is a characteristic and frequent finding along with post traumatic bleeding (1). Factor XIII (FXIII) also known as fibrin stabilizing factor is a plasma transglutaminase that cross links γ-glutamyl-ε-lysine residues of fibrinogen chains, thereby stabilizing the fibrin clot. Most cases of FXIII deficiency are associated with alterations in the gene that encodes the catalytic A subunit, than due to defects of the carrier B subunit gene.

There are contradictory reports about the clinical significance of detection of moderate or mild FXIII deficiency. While most of the authors have reported that only severe (<2 IU/ mL) FXIII deficiency results in clinically significant bleeding, few reports also indicate that mild FXIII deficiency (5–40% of normal) is also associated with bleeding complications; hence it is important that the screening tests used are sensitive enough to detect mild-to-moderate FXIII deficiency (2). Routine coagulation laboratory tests including prothrombin time, partial thromboplastin time, bleeding time, thrombin time and platelet counts are in normal range in FXIII deficiency and cannot help in diagnosis.

The most common screening test for FXIII deficiency is based on solubility of fibrin clot in a solution of 5M urea, 2% acetic acid or 1% monochloracetic acid. This solubility test is a qualitative test and is positive only if FXIII activity in the patient's plasma is very low, usually below 1% in 5M urea and below 10% in 2% acetic acid.The exact diagnosis of FXIII level and activity can be performed by quantitative photometric assays like, ammonia release assay, amine incorporation assay, ELISA colorimetric assays etc. However, they are rather cumbersome and time-consuming and difficult to standardize. It has earlier been reported that different clotting agents and different solvents show different sensitivities to different levels of FXIII in plasma (3). Hence, an attempt was made to improvise this clot solubility test by using different solvents and clotting agents in order to increase the sensitivity of the test which in turn would facilitate the diagnosis of mild-to-moderate FXIII deficiency (4) Briefly the technique is as follows.

Different combinations of clotting and lysing agents were used for the following experiment:

- Considering NPP to contain 100% FXIII (1 IU /mL), it was diluted with FXIII deficient plasma, to obtain 50%, 25%.....0.78% FXIII concentration.
- In a parallel experiment, 250 IU FXIII concentrate (Fibrogammin P, ZLB Behring AG, Marburg, Germany) was diluted to obtain the same dilutions.
- Equal volumes of the respective dilutions of NPP

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and FXIII concentrate, were allowed to react with equal volumes of 0.025 mM CaCl2/10 NIH U/mL thrombin/1:1 mixture of CaCl2 and thrombin were added separately in separate glass test tubes and these tubes were placed in a water bath at 37°C for 30 min.

- The clots so formed were then suspended in 3 mL of 5 M urea, 2% acetic acid and 1% monochloroacetic acid in separate tubes.
- Each set of these tubes were kept overnight at room temperature and at 37°C. Results were obtained at the end of 12 h and 24 h.

The different combinations of clotting and lysing agents used exhibited different patterns of clot lysis in the various dilutions of NPP and FXIII concentrate. Comparing the results obtained, it was clear that routinely used urea/monochloroacetic acid and CaCl2/thrombin for the detection of FXIII deficiency could detect only severe cases, whereas use of acetic acid and thrombin proved to be highly sensitive and specific by detecting FXIII levels of NPP and FXIII concentrate. It was noted that results obtained at different temperatures and time intervals were same.

A two-tube technique has thus been proposed (Table 1) to differentiate the severe FXIII deficient (<1 IU/mL) from the moderately deficient cases (1–18.8 IU /mL).

In conclusion, we have proposed a simple and cost effective two tube technique for the detection and differentiation of severe and mild to moderate FXIII deficiency. The study reports how this simple clot solubility test can further be used to differentiate the severe deficiency from mild-to-moderate deficiency (<18.8 IU /mL). Though they lack adequate sensitivity to detect all mild FXIII deficiency cases, these simple low cost tests are still being widely used to screen for FXIII deficiency across the laboratories all over the world.

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Table 1 : Interpretation of two-tube technique for the estimation of FXIII

Thrombin + 5M Urea	Thrombin + 2% Acetic acid	Interpretation
+	+	FXIII = 18.8 IU mL^{-1}
+	-	FXIII > 1 and <18.8 IU mL^{-1}
-	-	FXIII < 1 IU mL^{-1}

(+):- clot stable; (-):- clot lysed

NIIH Happenings

Dr. K. Ghosh, Director:

1. Attended the Pre Sac meeting of National Institute for Research in Reproductive Health, Mumbai from 5th to 6th September 2012.
2. Attended the Trasncom 2013 37th Annual Conference of ISBTI and delivered a lecture on "Expanding Dimensions of Transfusion Medicine" held at Vijaya Hospital, Chennai on 13th September 2013.
3. Attended the DBT Round Table Conference on Dengue Virus Infection and delivered a lecture on "Cytokine Storm in Dengue Virus Infections" held at New Delhi from 17th to 19th September 2013.
4. Attended the Silver Jubilee Celeberations of Hemophilia Federation of India at New Delhi on 23rd September 2013.
5. Attended the SAC meeting of Span Diagnostics, Surat on 29th September 2013.
6. Attended the 52nd Annual Conference of Natioanl Academy of Medical Sciences held at Dr. M.G.R. Medical University, Chennai from 12th to 14th October 2013.
7. Attended the SAC meeting of Surat Raktdan Kendra, Surat on 3rd November 2013.
8. Attended the 53rd Haematocon Annual Conference of Indian Society of Haematology and Blood Transfusion held at Puri, Odissha from 9th to 11th November 2013.
9. Attended the 2nd Pan Arab Haematology held at Sultan Qaboos University, Muscat from 13th to 15th November 2012 and delivered two talks entitled "Inhibitor detection in haemophilia Therapy-Therapy implications" and "Allogenic stem cell transplantation for hemoglobinopathies"
10. Invited as an external examiner to undertake MD Transfusion Medicine at PGIMER, Chandigarh from 7th to 8th December 2012.
11. Attended the SAC meeting of Regional Medical Research Centre for Tribal, Jabalpur from 11th to 12th December 2012

Department of Haematogenetics

Dr. Roshan Colah, Scientist F:

1. Invited to give a talk on "Hemoglobinopathies" in the Seminar on Fetal Medicine organized by the Mumbai Obstetrics and Gynaecology Society on 16th September, 2012.
2. Invited to give a talk on "Thalassemia syndromes-Diagnostic Issues" at the CME programme on Expanding Vistas in Pathology at AFMC, Pune on 18th September, 2012.
3. Dr Roshan Colah attended a meeting as a member of the Expert Committee for Evaluation of the Reports of Solubility Testing Kits for NRHM, Maharashtra on 10th October, 2012.
4. Dr Roshan Colah attended the Red Cross Blood Transfusion sub-committee meeting as a member on 23rd October, 2012.
5. Dr Roshan Colah attended the National Thalassemia Task Force meeting at ICMR Headquarters, New Delhi on 11th December, 2012.

Dr. Malay Mukherjee, Scientist D:

1. Attended a workshop entitled "Informatics on Clinical Research and REDCap" held at National Institute of Epidemiology, Chennai from 3rd to 5th October 2012

Students:

1. **Pooja Dabke** attended the 53rd National Conference of Indian Society of Hematology & Blood Transfusion (ISHBT) 2012, held at Puri, Odisha, India, from 9th to 11th November 2012 and presented a poster entitled "Association of genetics modulators of thalassemia linked to raised fetal Hb and its effect on clinical severity".
2. **Vrushali Pathak** attended the 53rd National Conference of Indian Society of Hematology & Blood Transfusion (ISHBT) 2012, held at Puri, Odisha, India, from 9th to 11th November 2012 and presented a poster entitled "ABO blood groups differentially allow Plasmodium falciparum growth".
3. **Ashish Chhiddarwar** attended 38th Annual conference of the Indian Society of Human

Genetics:Genomics and Community Health & International symposium on Developmental and complex disorders held at Banaras Hindu University, Varanasi from 9th to 11th December 2012 and presented a poster entitled "UGT1A1 gene promoter polymorphisms and their association with hyperbilirubinemia".

4. Stacy Colaco, presented a poster on "Masking of a thalassaemia determinant by a novel globin gene defect CD 100 C → T/ HBD.c. 301 C→T" in the 38th Annual conference of the Indian Society of Human Genetics: Genomics and Community Health & International symposium on Developmental and complex disorders held at Banaras Hindu University, Varanasi from 9th to 11th December. **This paper was awarded best poster award.**

Training programme/Workshop

Organized a training programme on "Diagnosis and management of Thalassemia, Sickle Cell Anemia and Hemophilia" for Medical Officers, Technicians, Social Workers and Physiotherapists for NRHM, Maharashtra from 10th to 14th December, 2012.

Department of Transfusion Medicine

Dr. Ajit Gorakshakar, Scientist E:

1. Attended 61st Annual conference of Indian Association of Pathology and Microbiology held at Jamnagar, Gujarat, from 14th to 16th December 2012 and presented a paper entitled "Mutations in Beta Thalassemia" in a symposium on "Hematology".

Dr. Swati Kulkarni, Scientist B:

1. Invited to deliver a lecture on "Weak D testing: Implications and protocols for testing and the problems encountered for the same" in the National Seminar on Immunohaematology held at Apollo Hospital, Hyderabad on 1st September 2012 organized by Andra Pradesh Chapter of Indian Society of Blood Transfusion and Immunohaematology.
2. Invited to deliver a lecture on "Rh Molecule - Basic and Advances" in CME on "Recent concepts in Transfusion Medicine - transfusion and beyond" organized by Hinduja Hospital, Mumbai on 13th and 14th October 2012.
3. Attended the 1st Annual conference of Indian Society of Transfusion Medicine held at Jaipur from 23rd to 25th November 2012 and presented a paper entitled "Study of RhD zygosity by serology and detection of Hybrid Rhesus box by PCR-SSP".

Department of Cytogenetics

Dr. V Baburao, Scientist D:

1. Participated as a local surveying and evaluation of Ethical Review Practices organized by Forum for Ethical Review Committees in Asia & the Western Pacific (FERCAP) and Strategic Initiative for Developing Capacity in Ethical Review (SIDCER) held at K.E.M Hospital, Mumbai from 1st to 4th September 2012.

2. Attended 38th Annual conference of the Indian Society of Human Genetics: Genomics and Community Health & International symposium on Developmental and complex disorders held at Banaras Hindu University, Varanasi from 9th to 11th December 2012.

Ms. Anita Rao, SRF:

1. Attended 38th Annual conference of the Indian Society of Human Genetics: Genomics and Community Health & International symposium on Developmental and complex disorders held at Banaras Hindu University, Varanasi from 9th to 11th December 2012 and presented a poster entitled "Cytogenetic and Molecular study of Fanconi anemia in Indian population".

Department of Paediatric Immunology and Leukocyte Biology

Dr. Manisha Madkaikar, Scientist E:

1. Invited to deliver a lecture on "Diagnosis of Primary Immunodeficiency Disorders: Role of flowcytometry" in the International Symposium on flowcytometry organized by D Y Patil Biotechnology and Bioinformatics Institute, Pune on 4th and 5th October 2012.
2. Invited to deliver a lecture on "Application of flowcytometry for diagnosis of Primary Immunodeficiency Disorders" in the 5th Annual Meeting of The Cytometry Society, India held at Kolkata on 12th October 2012.
3. Invited to conduct flowcytometry workshop on "Primary Immune deficiency Diseases" organized by ISPID at Institute of Liver and Biliary Sciences, New Delhi on 14th October 2012.

4. Invited to deliver a lecture on "PNH Diagnosis: is CD55 and CD59 enough?" in the CME on "Expanding Vistas in Pathology and Haematology: An Update" held at AFMC Pune on 18th October 2012.

5. Invited as a faculty member in the workshop on "Flowcytometry in Paeditrics" during the 16th Annual National Paediatric Haematology Oncology Conference of the PHO Chapter of Indian Academy of Paediatrics held at Ludhiana on 16th November 2012.

Department of Hemostasis

1. **Dr. K. Ghosh, Director, Dr. S. Shetty, Scientist E and Dr. B. Kulkarni, Scientist B** were Invited as Guest Speakers in CME on Bleeding Disorders and conducted a workshop on " Diagnosis of Bleeding Disorders" at RIMS, Ranchi, on 27th and 28th December 2012.

2. **Sharda Shanbhag, Technician C** attended the 53rd National Conference of Indian Society of Hematology & Blood Transfusion (ISHBT) 2012, held at Puri, Odisha, from 9th to 11th November 2012, and presented a poster entitled "Molecular characterization in FXIII deficient patients".

3. Following students have attended the 53rd National Conference of Indian Society of Hematology & Blood Transfusion (ISHBT) 2012, held at Puri, Odisha, from 9th to 11th November 2012, and presented the posters;

Patricia Pinto:

1. Analysis of FVIII polymorphism haplotypes as risk factors for FVIII inhibitor development in Indian severe Haemophilia A patients.
2. The epidemiology of FVIII inhibitors in Indian Haemophilia A patients.

Tejasvita Gaikwad:

1. Genetic determinants of warfarin dosage in Indian patients.

Rucha Patil:

1. Procoagulant microparticles in women with recurrent fetal loss.

Shahnaz Ali:

1. Molecular pathology of Bernard soulier patients.

Priti Nair:

1. Molecular pathology of haemophilia A in Western India

Training programme/Workshop

A workshop was jointly conducted by NIIH and Dept. of Health Services, Govt. of Maharashtra, on "Diagnosis & management of Thalassaemia, Sickle Cell Disease and Haemophilia" held at NIIH, ICMR, Mumbai, from 10th to 14th December 2012.

Department of Clinical and Experimental Immunology

S. R. Shirsat, Technical Officer:

1. Attended the workshop on "Information on clinical research and redcap" held at Chennai from 3rd to 5th October 2012.
2. Attended the 1st annual workshop on National Knowledge Network held at IIT Mumbai, from 31st October to 2nd November 2012.

Department of Transfusion Transmitted Diseases

Dr. Mehul S. Rajpurkar, TO, NACO Project:

1. Attended a meeting at MDACS (Mumbai Districts AIDS Control Society) on 15th and 29th December 2012 to discuss and resolve the issues related to HIV testing.
2. Attended the second round of EQAS (External Quality Assessment Scheme) Panel distribution workshop at NARI, Pune on 27th December 2012.

Training programme/Workshop

1. Conducted a training session on HIV testing with Rapid tests, ELISA and Western Blot for the Biotechnology students of R. K. Talreja College, Mumbai on 8th November 2012.
2. Conducted the second round of EQAS Panel distribution workshop for the linked State Reference Laboratories on 31st December 2012.

Others

1. The TTD laboratory was audited on 4th October 2012 by the Auditor, Project Concern International, India for the NABL accreditation as a National Reference Laboratory for HIV testing.

Pre Scientific Advisory Committee Meeting of the Institute

Scientific Advisory Committee Metting of the Institute

Hindi Pakhwada Celebration

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