Summary and Review of the Abstracts on Disorders of Red Cells and Erythropoiesis Presented at Haematocon 2016–2017

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Abstract Disorders of red cells and erythropoiesis encompass a vast array of inherited and acquired conditions, several of which are important not just medically but are also concern public health and policy making. Research in India in this area has been plentiful and has, in the past, focused on the laboratory diagnosis and clinical management of these disorders as well as their basic underlying pathogenesis. In the last 2 years too, papers presented at the annual national conferences of the Indian Society of Haematology and Blood Transfusion, ISHBT (Haematocon 2016 and 2017) presented a snapshot of the contemporary research activities that are ongoing in various academic and other centres. This mini-review analyzes and summarizes the salient findings of the original research abstracts presented. The papers are divided into broad subsections of α - and β -thalassemias with the hemoglobinopathies, iron deficiency and overload states, red cell membranopathies and enzymopathies, and acquired hemolytic anemias.

Keywords Acquired hemolytic anemia · Enzymopathy · Hemoglobinopathy · Nutritional anemia · Red cell membranopathy · Thalassemia

Introduction

Disorders of red cells and erythropoiesis include a vast array of inherited and acquired medical conditions. Research in India has, for many decades now, focused on the laboratory diagnosis and clinical management of these disorders as well as their basic underlying pathogenesis and relationships with public health and policy. In the last 2 years too, papers presented at the annual national conferences of the ISHBT (Haematocon 2016 and 2017) focused on ongoing contemporary research activities in various academic and other centres. This mini-review analyzes and summarizes the salient findings of the original research abstracts presented.

Methodology

Published abstracts as well as salient points from personally attended sessions by the author (wherever feasible) for all oral and poster presentations at Haematocon 2016 (Jaipur) and 2017 (Guwahati) were reviewed. Neoplastic disorders of red cells, for example: myelodysplasia with refractory anemia, polycythemia vera etc. were excluded along with inherited bone marrow failure syndromes involving non-erythroid lineages. Two other abstracts were initially rejected due to lack of author names and affiliations in the citation. One of these was later re-incorporated after the same were obtained. Key findings are being highlighted after dividing the topic into broad themes.

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Beta-Thalassemias and Its Combinations with the Hemoglobinopathies

Studies in this area attempted to unravel the complex genetic and environmental influences that determine the varied phenotypes of these diseases. Shaw et al. [1] demonstrated that intracellular iron overload in symptomatic thalassemia patients but not normal subjects caused oxidative damage to lymphocyte DNA. Iron chelators partially inhibited this damage and lowered reactive oxygen species (ROS) levels. The authors postulated that this DNA damage contributed to the increased susceptibility to infections and the immune dysfunction observed in thalassemia. The same center, in another study, evaluated 245 cases of HbE/ β -thalassemia and found a high frequency of asymptomatic malarial infection (35.6%) using PCR-based diagnosis. Genetic determinants of reduced severity in this cohort were the *XmnI*- C_{γ} polymorphism, co-inherited α -thalassemia and heterozygous hereditary persistence of fetal hemoglobin [2]. More recently described single nucleotide polymorphisms that modulate fetal hemoglobin (HbF) levels in thalassemia intermedia were the subject of another paper [3], which too found the *XmnI*- C_{γ} polymorphism to outweigh the *BCL11A* (rs11886868) and *HBS1L-MYB* intergenic-region (rs4895441) quantitative trait loci in upregulating HbF. Other diagnostic/prognostic studies reported that NT-proBNP levels correlate significantly with diastolic dysfunction in β -thalassemia major patients [4] and found a 69% frequency of low bone mineral density (BMD) as well as low bone mineral content (BMC) on dual-energy X-ray absorptiometry (DEXA) scans [5] in thalassemics. This frequency reduced to only 38% after 1 year of oral vitamin D and calcium supplementation, leading the authors to speculate that other underlying defects may play a role in thalassemic osteopathy. In one paper [6], serum hepcidin was threefold higher in multi-transfused β -thalassemia major patients vis-à-vis controls. Heparin correlated negatively with serum iron, total iron binding capacity and percentage transferrin saturation, but not with the serum ferritin or transferrin receptors, suggesting that multiple regulatory pathways regulate iron stores, erythropoiesis and iron absorption.

Among the clinical studies, one abstract on 50 non-splenectomized HbE/ β -thalassemics by Vinodhini et al. [7] showed that the severity of pulmonary hypertension and hepatic fibrosis (present in 42% and 05% patients respectively) increased with age and iron overload, and was also associated with minimal transfusion requirement in this group. Biswas et al. [8] reported that transfusion requirements reduced significantly and HbF levels (assessed by high performance liquid chromatography, HPLC) as well

as F-cell percentage (by flow cytometry) increased in 47 out of 60 severely affected HbE- β thalassemia patients. Adopting a curative approach, Balar et al. [9] from Pune reported their experiences with 55 consecutive family-related allogeneic-hematopoietic stem cell transplants (HSCT) for β -thalassemia major patients, yielding 94.7 and 80% overall survival at a median follow-up of 6.6 years for Pesaro classes I and II patients respectively. Of the 12 deaths in their cohort, 11 were attributed to acute graft-versus-host disease with sepsis.

Rare hemoglobin variants continued to be detected and reported, with papers describing hemoglobins O-Indonesia [10], Burke [11], Tsukumi, J-Rajappen, Le-Lamentin and South Florida [12]. A high-throughput multiplexed reverse dot-blot strip for PCR-based assessment of 22 *HBB* (β globin) mutations was found to be highly accurate and rapid, hence suitable for prenatal diagnosis, when compared against conventional amplification-refractory mutation system (ARMS)-PCR [13]. Iso-electric focusing (IEF) was evaluated as a tool to screen 4200 neonates, yielding 213 babies with hemoglobinopathies, 28 harboring diagnoses with major clinical implications. HPLC in comparison, missed three IEF-positive cases, including alpha thalassemia [14]. Bafna et al. [15] compared serum ferritin with T2* magnetic resonance imaging (MRI) and liver iron concentration (LIC) by atomic absorption spectrometry on liver biopsies in thalassemic patients undergoing HSCT and found good correlations of the LIC by all three techniques but with high individual-level variance in ferritin values at a given LIC. Cardiac iron did not correlate with liver deposits on T2* MRI, but the technique had the major advantages of being convenient, non-invasive and safe.

Low-cost diagnostics continue to retain their importance in resource-constrained settings, and a paper from Sri Lanka evaluated the single-tube osmotic fragility test as a screening tool for β -thalassemia trait to find a 98.1% sensitivity, although the interferences from other hemoglobin disorders and iron deficiency were seen, as expected [16]. Two papers examined the role of red cell indices and their calculated discriminant indices to distinguish β -thalassemia trait from iron deficiency [17, 18]. One found the Mentzer quotient to be a reasonable screening tool [17] while the other, although declaring the Kerman-1 and RDW indices to be most successful, conceded that none had a 100% diagnostic accuracy [18].

Alpha Thalassemia

Kumar et al. [19] reported genotypic findings in 100 patients with HbH disease assessed using multiplex gap PCR (for deletions), Sanger sequencing (for point mutations) and gene dosage multiplex fluorescent PCR (copy

number analysis). Deletional and non-deletional HbH comprised 28 and 22% of all cases, with the former having higher Hb with lower reticulocyte counts and lower rates of transfusion dependency. Saluja and coworkers [20] applied multiplex ligation-dependent probe amplification (MLPA) in 20 cases and found it to be comparable in accuracy and less laborious as compared to multiplex gap-PCR, provided high-quality DNA was available. As a modifier of sickle cell anemia (SS-state), concomitant α -thalassemia increased the hemoglobin levels. An intriguing group with MCV < 75 fl without α -gene deletions had much lower Hb, possibly due to iron deficiency [21]. Screening for α -triplications in 75 healthy blood donors, one paper found a frequency of 21.3%. All persons with triplicated- α genes had normal hematological parameters and HPLCs, indicating that molecular analysis is the only recourse when the condition is suspected to be aggravating a β -thalassemia carrier state [22].

Iron Deficiency, Overload and Other Nutritional Anemias

Three papers evaluated the role of serum transferrin receptors (sTfr) in the diagnosis of iron deficiency in intriguingly different settings. Pathre et al. [23] studied 90 anemic children aged ≤ 12 years, Manu et al. [24] studied 68 patients with chronic renal failure and 100 with classical iron deficiency, and Surekha et al. [25] compared maternal and cord blood values in 96 cases. The former two found the sTfr/ferritin ratio or its log as the best indicator for assessing iron status, while the last study found that neonatal iron stores were largely unaffected by maternal iron deficiency. Bhatia et al. [26] analyzed all 18 exons of the *TMPRSS6* gene by multiplex PCR and Sanger sequencing in 20 children with iron-refractory iron deficiency anemia with the IRIDA phenotype and could genetically define and predict IRIDA phenotype in nearly 11/20 cases (55%) after finding effective exonic SNPs in 3 and functional mutations in non-coding/intronic regions in 11 cases.

At the other end of the spectrum of iron disorders, Madhavi et al. [27] tested 47 cases with suspected primary hemochromatosis, with partial success. *HFE* genetic variants occurred in 66% cases: homozygous C282Y in one family, homozygous H63D ($n = 1$) and H63D/S65C compound heterozygosity ($n = 1$) alongwith heterozygous E277K ($n = 1$) and heterozygous H63D ($n = 4$). Novel *HFE2* (type IIa) variants were identified in 3 cases and a heterozygous *HAMP* (type IIb) defect in 1. No *TFR2* (type III) and *SLC40A1* (type IV) variants were detected. The authors concluded that next-generation sequencing (NGS)-

based approaches might be required to fully unravel the molecular basis of hereditary hemochromatosis in India.

Red Cell Enzymopathies and Membrane Disorders

Two papers in this section discussed NGS-based diagnostics. The first, from north India used a commercial targeted resequencing-based panel in 16 patients with undiagnosed hemolytic anemias after most common entities had been excluded by conventional tests [28]. 12/16 cases were resolved by NGS: five had pyruvate kinase (PK) deficiency while two each had glucose-6-phosphate dehydrogenase (G6PD) and glucose-6-phosphate isomerase (GPI) deficiencies. Probable causative mutations were found in *RHAG*, *ABCG5* and *MTRR* genes (one patient each). The other paper, from Mumbai, also used targeted NGS in 10 patients with hereditary spherocytosis. Mutations in β -spectrin (*SPTB*) were the commonest, followed by band 3 and α -spectrin (2 patients each) and ankyrin (*ANK1*), *PIEZO* and *EPB42* [29]. Together, both these papers highlight the powerful approach NGS represents as a genetic test, along with the challenges inherent in its data interpretation and ascribing of causality.

The genetics of G6PD, PK and pyrimidine 5' nucleotidase (P5N) deficiencies were addressed in three studies. The first study, from Vellore, detected genetic defects in 12 out of 26 cases using Sanger sequencing, including 2 with novel variants, 4 with G6PD Kerala-Kalyan and only 1 patient with G6PD Mediterranean. Patients without a detected mutation (54%) tended to have less severe hemolysis [30]. The three P5N deficient cases reported by Warang et al. [31] had a wide range of transfusion requirements, while all 3 PK deficient cases [32] had required transfusions in the past. These reports suggest a need to bear these less commonly encountered enzyme deficiencies in mind in patients with chronic non-spherocytic hemolysis.

Acquired Hemolytic Anemias

Deka et al. [33] studied a 5-antibody, single tube assay for paroxysmal nocturnal hemoglobinuria (PNH) in 52 patients. The panel incorporated CD157 as a common granulocyte-cum-monocyte GPI-linked marker in addition to CD45, CD15, CD64 and FLAER. It was compared to another single tube assay that included CD14 + CD24 instead of CD157. The revised tube showed a performance agreement with the 6-antibody assay and resulted in a 15% decline in test cost. Gupta et al. [34] described a PNH patient with delayed diagnosis while Singh et al. [35] reported erythematous and violaceous plaques in a PNH

patient attributed to cutaneous thrombosis. These reports highlight the need to maintain a high index of suspicion for PNH as well as the possibility of atypical presentations in these cases.

Among the immune hemolytic anemias, Hitha et al. [36] studied 54 patients with Coombs-positive autoimmune hemolytic anemia (AIHA) and found 31.4% of them to be secondary in origin. 42 of their cases had mixed anti-IgG and anti-C3d autoantibodies. Response rate to prednisolone was 62.2% at 3 months with very little improvement at 6 months. Six patients required further immunosuppressive agents and 5 underwent splenectomy. In a blood bank-based study, Yadav et al. [37] screened 2000 antenatal women for alloantibodies, finding an overall RBC alloimmunisation rate of 1.05%. The commonest alloantibody was anti-D (71.4%), followed by anti-D + C 19.04% (4/21), anti-E 4.76% (1/21) and unspecified 4.8%. This Delhi-based study pointed to a continuing need to strengthen antenatal services with blood group testing and adequate anti-D immunoprophylaxis in Rh-negative women.

Conclusion

The papers presented in the last two national conferences of the Indian Society of Haematology and Blood Transfusion in the broad theme of disorders of red cells and erythropoiesis reflect the unique challenges and practice scenarios faced by Indian clinical and laboratory hematologists as well as the rapid scientific advances being made today. The pace of clinical/real-world translation of new discoveries is now faster than ever before, and conferences like the Haematocon help hematology practitioners keep themselves updated about the same.

Compliance with Ethical Standards

Conflict of interest The author declares that he has no conflicts of interest.

Ethical Approval Not sought as this article does not contain any studies with human participants performed by the author.

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