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Raised Haemoglobin F (HbF) Level in Haemoglobinopathies: an Indicator of Polymorphism

Bijeta Ghose Guha[1], S.K.Sharma[2]

[1]Research Scholar, Dibrugarh University, Dibrugarh-786001, Assam, India

[2]Scientist-E in Regional Medical Research Centre, North east region (Indian Council Of Medical Research), Post Box # 105, Dibrugarh-786001, Assam, India.

Abstract: ***Hemoglobinopathies are the most commonly encountered monogenic disorders of blood posing a major genetic and public health problem in Southeast Asia and the Indian subcontinent. Of the several abnormal hemoglobins so far identified, there are three variants – sickle cell (Hb S), hemoglobin E (Hb E) and hemoglobin D (Hb D), which are predominantly prevalent in India. Among these clinically important hemoglobinopathies, hemoglobin E (Hb E) is mostly restricted to the North eastern states of India with high gene frequency. Hb E disorders may be found in heterozygous (AE), homozygous (EE) and compound heterozygous states (e.g., Hb E with other abnormal hemoglobins or thalassemias) with widely variable clinical phenotypes. Studies suggest that there is a positive correlation of the βE-globin gene with malaria endemicity. Since northeast India is a holoendemic area for Plasmodium falciparum and other types of malaria, it is reasonable to suspect that malaria might be acting as a selective factor for this gene. Increased levels of fetal hemoglobin (HbF, α2γ2) are of no consequence in healthy adults, but confer major clinical benefits in patients with sickle cell anemia (SCA) and β thalassemia, diseases that represent major public health problem. Higher level of Hb F is also associated with Hb E. The most significant genetic factor associated with high HbF is Xmn I polymorphism located at −158 upstream to the Gγ globin genes. Thus Hb E, a haemoglobin variant which is clinically not very severe and associated with Xmn I polymorphism may be protective against malaria via production of high Hb F level.***

Keywords: Haemoglobinopathies, Northeastern India, Hb E, Hb F.

1. Introduction

Haemoglobinopathies are the worldwide prevalent monogenic genetic disorders with variable geographic distribution [1]-[5].Although over 700 structural haemoglobin variants have been identified, only three (Hb S, Hb C, and Hb E) reach high frequencies [6]-[8]. Three variants – sickle cell (Hb S), hemoglobin E (Hb E) and hemoglobin D (Hb D), are predominantly prevalent in India. Haemoglobin E, is the commonest structural haemoglobin variant globally [9] and also in northeastern India. High gene frequency for Hb E is prevalent in autochthonous inhabitant, having linguistic and cultural affiliation with the population of Southeast Asian countries, of the northeastern part of India [10].This haemoglobin variant is innocuous in its heterozygous and homozygous states but, because it is synthesized at a reduced rate, it can interact with β thalassemia to produce a condition called Hb E β thalassemia, which is extremely common and is presenting an increasingly important health problem in many parts of Asia [6].

The Hb E gene is a mutant form of the β-globin (HBB) gene that encodes lysine instead of glutamate at position 26[9].This β-E chain is inefficiently produced because of a novel cryptic messenger RNA splice site, leading to thalassemic RBC indices[11]-[12]. Furthermore, Hb E has somewhat enhanced sensitivity to oxidant stress [12]-[13].

Inheritance of Hb E results in a spectrum of clinical phenotypes, depending on dosage, coinheritance of other hemoglobin variants, and environmental modifiers [14]. Heterozygosity (Hb E trait) and homozygosity (Hb EE disease) are clinically mild, whereas compound heterozygosity for Hb E and Hb S (Hb SE) and compound heterozygosity for Hb E and β-thalassemia (Hb E-β-thalassemia) are clinically severe [15].

It is hypothesized that the prevalence of Hb E results from protection of red blood cells (RBCs) from invasion by *Plasmodium falciparum* [16]-[17]. The unique geo-climatic conditions of the northeastern part of India facilitate transmission of malaria in this part of the country [18]. Malaria in this region is predominantly contributed by *Plasmodium falciparum(P. falciparum*) [18]-[19].Overlapping of haemoglobinopathies and *P. falciparum* malaria has been reported from northeast India. It was further confirmed that there is a positive correlation of 0.703 of β E globin gene frequency and mean incidence of *Plasmodium falciparum* infection (Pf %)[3].

Haldane (1949) hypothesized that the high gene frequencies of hemoglobinopathies in malaria-endemic areas may have resulted from protection conferred against malaria—a major cause of death in the tropics. This resulted in an equilibrium or "balanced polymorphism" in which the homozygote hematologic disadvantage was balanced by the heterozygote advantage of protection from malaria [20]-[26].Abundant epidemiologic evidence suggests that certain genetic disorders of RBCs have been selected because they confer protection from malaria[27]-[33] . This hypothesis has been supported by many studies and is now generally accepted. Erythrocytes containing sickle cell hemoglobin retard parasite maturation and thus reduce multiplication. Some laboratory studies suggested that hemoglobin E (Hb E)

retarded intraerythrocytic growth of *Plasmodium falciparum*[20]-[34]. Recently, in a hospital-based study in Thailand, the presence of Hb E trait (Hb AE) was associated with reduced disease severity in acute *P. falciparum* malaria[17]-[20]. Some studies however reveal that *P. falciparum* grows slowly in homozygous Hb E RBCs, but not in heterozygous RBCs[34]-[35]. Epidemiologic studies have been divided as to whether hemoglobin E protects from malaria infection although recent evidence from clinical studies indicates that this hemoglobin type does confer protection from severe malaria.

The mechanisms underlying malaria protection by these genetic RBC variants have been studied extensively and can be classified into several broad groups: (1) reduced probability of merozoite invasion into the variant RBCs, (2) impairment of parasite growth within the variant RBCs, (3) enhanced removal of the parasitized variant RBCs, and (4) enhanced probability of infection early in life, particularly with *P. vivax*, which protects against subsequent severe *P. falciparum* malaria[36]. Nevertheless despite considerable research, the relative roles of these processes and the cellular mechanisms that mediate resistance of many of these genetic RBC variants to malaria parasites remain unclear. Protection provided by the Hb AE heterozygote cells may be because of their membrane differences. Previous studies showing increased human monocyte phagocytosis of Hb AE cells infected with *P.falciparum* also led to a suggestion of a membrane abnormality in these cells. Potential factors that reduce the probability of invasion by *P.falciparum* include differences in membrane rigidity and in particular in glycophorin and sialic acid content [20]. It would be of interest to determine whether RBC age is the factor determining invasion in Hb AE cells. Some studies suggest that the genetics of hemoglobin E may be explained by a balanced polymorphism in which the deleterious effects of the homozygote condition are offset by the protection against high parasitemias and thus severe malaria conferred by the heterozygote. The increased amounts of haemoglobin F present in patients with some haemoglobinopathies is also associated with impaired malaria parasite growth [37]-[39].

Hemoglobin molecules are composed of two pairs of globin chains, each containing a heme group at its core. In adults, the predominant hemoglobin molecule, hemoglobin A (Hb A) contains two alpha chains and two beta chains. The normal adult also has between 2.3 and 3.5% of hemoglobin A2 (HbA2), and may have up to 2% HbF in circulating erythrocytes[40]. During fetal life, the most common molecule is hemoglobin F (HbF) that contains two alpha chains and two gamma chains.

In Fetal hemoglobin (HbF) is the main hemoglobin component throughout fetal life and at birth, accounting for approximately 80% of total hemoglobin in newborns. HbF is produced from the sixth week of gestation and during the rest of fetal life, replacing the embryonic hemoglobins Gower I, Gower II and Portland. After birth, HbF synthesis rapidly declines and HbF is gradually substituted by Hb A in the peripheral blood, so that within the first two years of life, the characteristic hemoglobin phenotype of the adult with very low levels of HbF (less than 1%) is found [41]-[43]. In normal adults, HbF is heterogeneously distributed among erythrocytes though its synthesis is restricted to a small population of cells, termed F-cells. Approximately 3–7% of red blood cells are F-cells, containing 20–25% of HbF [41],[44]. HbF ($\alpha 2\gamma 2$) is formed by two α- and two γ-globin chains consisting of 141 and 146 amino acid residues, respectively. Changes in this ratio were observed in some hemoglobin disorders[45]-[46].

The developmental switch from foetal ($\alpha 2\gamma 2$) to adult ($\alpha 2\ \beta 2$) haemoglobin (Hb) occurs just before birth [47]. The hemoglobin switch occurs when fetal gamma globins are replaced by beta globins. The switch is *incomplete and reversible*. The switch from Hb-F to Hb-A involves the same alpha genes. The production and assembly of hemoglobin involves *balanced alpha and beta globin production*. The beta globin gene region has "LCR" regulatory regions that mediate the switch [48].This involves physical bending of the chromatin to bring the LCR promoter first to the gamma globin gene (Hb-F) and then switching to the beta globin gene. This bending is reversible. Drugs can be used to reverse the LCR bending, re-instituting Hb-F production.

The foetal haemoglobin is replaced by adult haemoglobin because the foetal haemoglobin has high affinity for oxygen. Such a high amount of oxygen in adults might create oxygen toxicity as they get the oxygen in huge amounts from atmosphere whereas the fetus has to depend on the maternal blood to grab the oxygen to the maximum amount. This greater affinity for oxygen is explained by the lack of fetal hemoglobin's interaction with 2, 3-bisphosphoglycerate (2, 3-BPG or 2, 3-DPG). In adult red blood cells, this substance decreases the affinity of hemoglobin for oxygen. 2, 3-BPG is also present in fetal red blood cells, but interacts less efficiently with fetal hemoglobin than adult hemoglobin, due to a change in a single amino acid found in the 2, 3-BPG 'binding pocket': from Histidine, interacts well with the negative charges found on the surface of 2, 3-BPG) to serine. This change results in 2, 3-BPG binding less well to fetal Hb, and as a result, oxygen will bind to it with higher affinity than adult hemoglobin.

Fetal Hemoglobin (Hb F), formed by two α and two γ-globin chains ($\alpha 2\ \gamma 2$), is produced at high levels in the fetal period due to a high expression of γ-globin genes. The γ-globin gene originates from a 5 kilo base (kb) tandem repeat. These genes differ from one another by only one amino acid [glycine (γG) or alanine (γA)] at position 136 of the polypeptide chain. At birth, γG chains are more abundant while γA chains predominate in adulthood. In adults, the β-globin gene is predominant; approximately 98% of all hemoglobin is comprised of adult hemoglobin, Hb A ($\alpha 2\ \beta 2$). Thus, γ-globin genes are poorly expressed; less than 1% of adult hemoglobin is made up of Hb F. Hb F levels can be evaluated by counting the number of F cells, that is, adult erythrocytes that contain measurable amounts of this hemoglobin. The Hb F and F cell levels vary considerably in healthy adults but usually there is a good correlation between the two [49]-[50].

When γ-globin genes are highly expressed, higher Hb F levels in red blood cells may compensate defective β-globin products and significantly reduce the symptoms of hemoglobinopathies such as sickle cell anemia [51]-[52].

The Hb F concentration and its distribution in red blood cells are major genetic modulators of disease and high levels of this hemoglobin dilute the amount of Hb S thereby inhibiting or delaying the polymerization process, the result of which is fewer harmful events [53]. In β thalassemia, an induced increase in gamma chain production has a beneficial effect on the clinical status of homozygotes, not only by reducing the imbalance in the α-type/non-α-type chains, but also by increasing the synthesis of total hemoglobin. Thus, an increased γ-globin gene expression has clinical relevance in the treatment of diseases related to the β-globin gene. Some genetic conditions are known to influence γ-globin gene expression during adulthood, including hereditary persistence of fetal hemoglobin (HPFH) and delta-beta thalassemia (δβ-thalassemia) [54]-[55].

In a study in the assamese sikh population a significant difference in Hb F level was observed in subjects carrying homozygous state of Hb E (t = 6.31;P = 0.000) when compared with Hb F levels of subjects with normal haemoglobin pattern (0.19 ± 0.24%). The Hb F level was 0.81 ± 0.73% and 3.32 ± 1.15% in subjects with heterozygous and homozygous state of Hb E respectively [56].

The C-T substitution at position –158 of the Gy globin gene, referred to as the Xmn1-g polymorphism, is a common sequence variant in all population groups, present at a frequency of 0.32 to 0.35[57]-[58].The XmnI polymorphism is known to influence the γG gene expression, predisposing carriers to increased Hb F concentrations in particular when they are under conditions of erythropoietic stress. Clinical studies have shown that under conditions of hematopoietic stress, for example in homozygous β -thalassemia and sickle cell disease, the presence of the Xmn1- Gg site favours a higher Hb F response. This could explain why the same mutations on different β chromosomal backgrounds are associated with disease of different clinical severity[59]-[60]. The strongest association of β thalassemia was observed with the XmnI polymorphism located 158-bp upstream to the Gγ gene (p = 4.6E−12). Carriers of the T allele of XmnI were more likely to have a milder disease course and higher level of fetal hemoglobin (HbF) in both the mild (p = 0.005) and severe (p = 8.7E−06) patient groups. Haplotype analysis revealed that the T allele of XmnI was nearly always in cis with the Hb E allele. The high frequency of this haplotype may be favored by positive selection against malarial infection[61].

This polymorphism is common in many populations with frequencies as high as 32% to 35% being reported [62]. Studies by Peri et al. and Garner et al. found similar frequencies for different populations of healthy Europeans. Evaluation of polymorphic site in adults without anemia from the northwestern region of São Paulo, observed a frequency of 33.3%, with Hb F levels ranging from 2.0% to 33.3% [51]. The XmnI polymorphic site was more frequent (60%) in individuals with Hb F below 15% of total hemoglobin [51].

Increased levels of fetal hemoglobin (Hb F or a2 γ2) are of no consequence in healthy adults, but confer major clinical benefits in patients with sickle cell anemia (SCA) and β-thalassemia, diseases that represent major public health problems In patients with sickle cell disease and beta thalassemia, the presence of this polymorphism is associated with higher Hb F levels. With this polymorphic site, there is an increase in the proportion of γG chains resulting in a γG:γA ratio similar to that seen at birth (70:30)[63]-[64]. The two types of γ chains of Hb F (Gγ and Aγ) differ at position 136 (glycine versus alanine) and are produced by closely-linked genes of the β -globin gene cluster. In normal adults, red cells have less than 1% Hb F, and Gγ accounts for some 40% of total γ chain [57],[65]. Among the genetic factors known to affect HbF production are DNA sequence variations within the β -globin gene cluster, in particular, the (C-T) variation at position – 158 upstream of the Gγ globin gene, which is detectable by Xmn1. The sequence variation has been shown to increase Hb F levels in β-thalassaemia anemia. The role of the Xmn1 polymorphism as a modulating factor in Hb E and its frequency in northeastern India is yet to be evaluated.

2. Conclusion

Hb E is the most prevalent variant haemoglobin in ethnic groups affiliated to Tibeto-Burman linguistic family. Gene frequency for βE-globin gene in these groups ranged from 0.006-0.569 with an overall prevalence of 0.266[3].As north eastern region of India exhibits high gene frequency for β^E-globin gene and higher level of Hb F may also associated with variant haemoglobin,Hb E. Hb E, a haemoglobin variant which is clinically not very severe and associated with Xmn I polymorphism may be protective against malaria via production of high Hb F level. In view of the probable disadvantage of the HbE homozygote and the certain disadvantage of the double heterozygote for the HbE and β-thalassemia genes an advantage of the heterozygote has to be postulated in order to explain the high gene frequencies. There is some evidence for and against malaria protection being the factor conveying heterozygote advantage.

The g-globins of Hb are coded by a pair of non-allelic genes located in the b-globin gene cluster on the short arm of chromosome 11. They synthesize different g-globin chains which differ from each only in a single amino acid and are named as Gg and Ag. At different stages of development there is a variable rate of expression of Gg and Ag, although the ratio shows significant differences in different populations. Normally, on both sides of the g-globin genes there exists a restriction site for Xmn I, which produces an 8.1 kb fragment carrying the g-globin gene upon digestion with Xmn I. In some individuals a polymorphic site exists 153 kb 5' to Gg. In the presence of this site, a smaller 7.0 kb fragment is obtained, as a C®T mutation generates a new restriction site of Xmn I. The presence of this site is believed to influence Gg globin gene expression in patients with sickle-cell anaemia and b-thalassaemia. Thus, a higher Hb F level is believed to be associated with the presence of the polymorphic site.

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Author Profile

Mrs. Bijeta Ghose Guha presently working in Gyan Vigyan Academy is a research scholar in the Dibrugarh University. She is post graduate in Life Sciences.

Dr. Santanu K Sharma, presently working as Scientist-E in Regional Medical Research Centre, North East Region (Indian Council of Medical Research), Post Box # 105, Dibrugarh-786001, Assam, India. A post graduate in Biochemistry and subsequently obtained his Ph.D. Dr. Sharma is working on haemoglobinopathies and thalassemias for about two decades. He has published various papers both in national and international journals.