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

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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, α2β2) are of no consequence in healthy adults, but confer major clinical benefits in patients with sickle cell anemia (SCA) and 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 gene. 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 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 βE 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 homozgyote 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)
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 (α2γ2) 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 (α2γ2) to adult (α2β2) 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 (α2 γ2), 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 (α2 β2). 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].
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 γ 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 γγ 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 F 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 βE-globin 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 C8T 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 β-thalassaemia. Thus, a higher Hb F level is believed to be associated with the presence of the polymorphic site.

References


[56] Nemati H, Rahimi Z, Bahrami G. The XmnI polymorphic site 5′ to the Gγ γ gene and its correlation to the Gγ γ/ΔY ratio, age at the first blood transfusion and clinical features in β-Thalassemia patients from Western Iran. *Mol Biol Rep*. 2010; 37(1): 159-64.


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