Exploring Phenotypic Alterations in Response to High Hemoglobin F Level in Egyptian Beta Thalassemia Patients

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Abstract: ß-Thalassemia (MIM 141900) displays a great deal of phenotypic heterogeneity, despite being generally thought of as simple Mendelian disease. The lack of persistent genotype/phenotype correlation poses a problem in genetic counseling and motivated the search for clarifying phenotypic variability. The reasons for this are not well understood. The level of fetal hemoglobin (HbF) was thought of as one ameliorating factor. This ameliorating effect has prompted different therapeutic approaches for the reactivation of HbF synthesis. Hence we were motivated to study the clinical effect of persistent high HbF in Egyptian beta thalassemia patients. We compared the phenotypes among 93 cases with persistent high level of HbF to 50 transfusion dependent cases with normal adult HbF level. Most of our cases with high HbF (86%) showed moderate to severe phenotype, and the phenotypic amelioration in response to the high HbF, mentioned in previous reports, was detected only in 15% of our cases. Therefore, persistence of HbF cannot be constantly regarded as a clinical modifier during genetic counseling. Also the capacity of the patient to respond to γ chain inducers cannot be predicted nor can their effect on disease progression.

Key words: phenotypic alterations, persistent Hb F, beta thalassemia.

INTRODUCTION

β thalassaemia, caused by β globin gene affection, forms one of the most common human genetic disorders world-wide (as high as 10% in Mediterranean region, Africa, and Southeast Asia and overall 1/300), and represents a major public health problem[1]. In Egypt, β-thalassemia mutations occur at high frequency with a carrier frequency of about 10%[2]. Beta thalassemia patients display a marked phenotypic heterogeneity, which could not be explained consistently by the underlying genotype[3-9]. Genetic studies have been successful in identifying some modifiers when the loci have a major clinical effect and if the genetic variants are common[10]. It has been suggested that, factors that determine the persistence of fetal hemoglobin production may ameliorate the clinical and hematologic severity in both beta thalassemia and sickle cell hemoglobinopathies[11]. Inter-individual variation in fetal haemoglobin expression was previously reported as heritable disease modifier and correlated with reduced morbidity and mortality in both diseases[11].

HbF is produced by the fetus to transport oxygen efficiently in a low oxygen environment. Production of Hb F stops at birth and decreases to adult levels by 1-2 years of age. In normal adults, the major Hb is HbA (α2β2), with 2.5–3.5% HbA2 (α2δ2) and the rest being HbF (α2γ2), generally <1%[12] confined to F cells, which constitute about 3% of the erythrocytes[13]. The switch from foetal to adult Hb production is essentially the replacement of HBG (γ-globin) with HBB (β globin) gene expression. Most of the genetic disorders associated with persistent HbF production involve alterations of the structure of the β globin cluster. Little information is known about the factors that cause elevated levels of HbF in β thalassemia and sickle cell disease. It has been suggested that the combination of erythroid expansion and selection of red blood cell precursors and/or red blood cells able to synthesize γ chains[13,14].

HbF levels show a marked variation also in normal individuals, 10–15% of whom display a moderate increase of 0.8–5% in HbF levels[14]. Twin studies have clearly demonstrated that HbF is a highly heritable trait[15]. These findings stimulate us to study the persistence of HbF as a quantitative trait and its effect on the phenotype in Egyptian beta thalassemia patients. The best combination of clinical parameters were used as a phenotypic predictor[16,17] in comparison to the
HBF level among the studied patients.

Patients and Methods:
This Study Included:
- Ninety three Egyptian beta thalassemia patients with high HBF level classified into thalassemia intermedia (group I) & thalassemia major (group II).
- Fifty transfusion dependent beta thalassemia patients with less than 10% Hb F level (group III).

Patients were selected among patients seen at the Clinical Genetics Clinic, National Research Centre, Cairo, Egypt and Hematology Clinic, Cairo University. Patients of group I & II age ranged between 4-18 and 4-10 years respectively. They were 56 males and 37 females. Cases of group III age range between 4 and 10 years comprising 28 males and 22females.

Selection criteria of group I & II included: Egyptian beta thalassemia patients with high level of hemoglobin F (>10%) persisting after the age of 4 years.

All patients were subjected to:
- Pedigree analysis, meticulous history taking and thorough clinical examination including anthropometric and pubertal evaluation compared to standard for age
- Hematological studies including; complete blood picture with Hb level and blood indices. Hemoglobin electrophoresis. Serum ferritin level.
- Clinical classification into mild moderate and severe according to phenotypic scoring system[6,16,17] with consideration to: age of disease onset, age of first transfusion, frequency of blood transfusion, hemoglobin level, hepatosplenomegaly, facial and growth affection.
- Correlation of the HBF level to clinical data in the three included groups of patients.
- Correlation of the HBF level to the different underlying beta thalassemia mutations characterized by reverse dot blot technique and to coinheritance of XmnI Ggamma polymorphism studied in 23 TI & 14TM cases (previously reported)[19,20,21].

RESULT AND DISCUSSION

Results: Patients of group I & II included 60% males and 40% females. Positive consanguinity was found in 63.4% and positive family history in 34.4% of the studied patients. While patients of group III were 56% males & 44% females with 68% positive consanguinity and 50% positive family history. Among the 93 studied patients with high HBF level, 36 were classified as thalassemia intermedia (TI) and 57 as thalassemia major (TM). The mean age at the time of the study was 9±4.5 years for the TI patients, 5±1.2 years for the TM patients and 7±3.2 years in group III. The age of disease onset ranged from 6 to 72 months in the TI patients and 2 to 42 in the TM patients and to 2 to 48 months in group III.

Correlation of the high HBF level cases and studied molecular defects revealed the association with 80 β+ alleles, 56 β− alleles and 39 β0 alleles. 11 alleles were uncharacterized. Among the TI patients the underlying molecular defect in 36 (50%) alleles was of β− type while in 5 (6.9%) alleles it was β0.

In the TM group the β+ (68.7%) were the most commonly associated alleles and β0 alleles were more prevalent (29.8%) than in group I. XmnI Ggamma polymorphism was detected in only one TI case (4%) among 23 TI and 14 TM previously characterized cases.

Table (1) compares the clinical data of the three studied groups. The height of the patients was the mostly affected growth parameter among patients in the three groups, the percentages include those with more than -2SD from normal. As shown in Table (1), some patients with high HBF level had a milder phenotype taking the parameters of the scoring system in consideration[6,16,17]. A considerable number of the cases still presented as TM in spite of the almost equal mean HBF levels in the TM and TI groups. Many parameters showed almost the same clinical presentation (rate of blood transfusion, mean Hb level, the incidence of hepatosplenomegaly and growth affection) in both groups II & III.

In Table (2) about 60% of the patients in group I, were among the mild and moderate cases. However, the number of cases presenting as mild within the TM group (7.5%) was found to be less than within group III (14.6%).

Discussion: In Egypt, β-thalassemia mutations occur at high frequency. As calculated in previous studies the cumulative frequency of 5% corresponding to a carrier frequency of 10% would be expected[21]. Despite of showing a marked genetic homogeneity, patients display an extensive phenotypic variation only partially explained by coinherited α-thalassemia or hereditary persistence of HbF mapping within the β-cluster [(−158 C−T Gγ) polymorphism] [21]. Some studies reported variation in foetal haemoglobin (HbF) expression as inheritable disease modifier and correlated it with reduced morbidity and mortality in beta thalassemia and sickle cell disease (SCD) in different populations as India, Hong Kong, Iran and European Caucasians[11,22-24]. The ameliorating effect of persistent HbF has prompted different approaches – pharmacological and gene transfer – for the reactivation of HbF synthesis[24-27]. No exclusive studies according
to review of the literature discussed the detailed correlation of high level of HbF as a factor of clinical heterogeneity among Egyptian beta-thalassemia patients.

Our motivation in this study was the need for clarification of the clinical heterogeneity among beta thalassemia patients, which poses a problem for proper genetic counseling, especially with the lack of a persistent genotype/phenotype correlation\(^4\). Another motivation was the trends in management of beta thalassemia by the induction of \(\gamma\) chain production\(^{[24-27]}\) that necessitates the study of the clinical outcome of high HbF coinheritance in our Egyptian beta thalassemia patients. While persistence of high levels of HbF production has no clinical consequences on healthy individuals, previous studies indicated that high HbF levels confer clinical benefits associated with milder disease progression and fewer complications in patients with SCD and \(\beta\) thalassaemia\(^{[3,11]}\).

The co-existence of high HbF was associated with mild clinical phenotype in some of our studied patients with resulting TI phenotype in 36 patients and delayed mean age of disease onset to 38.3±21.7 and 15.4±17.5 months in the TI and TM groups compared to 9±8.6 months in group III with normal adult Hb F level. Still 50% of the TI patients have \(\beta^\text{+}^+\) mild \(\beta\)-globin mutations. One extreme of increased HbF response can be observed in \(\beta^\text{+}\) thalassaemia intermedia patients who are transfusion-independent with a mild disease despite the absence of HbA\(^{[28-31]}\). This could be an example of the clinical amelioration in some of our studied cases as the \(\beta^\text{+}\) mutations associated with TI in group I and mild &/or moderate phenotype in group II. However among our studied patients the high Hb F level did not always confer milder clinical disease. As seen in Table (1) the 57 cases included in group II presented as TM in spite of associated almost equal mean Hb F levels in the TM and TI groups.

Many parameters showed almost the same clinical presentation (rate of blood transfusion, mean Hb level, the incidence of hepatosplenomegaly and growth affection) in both groups II with mean HbF 50.9±22.4 & group III with normal HbF adult level. This could be explained by ethnic variability in addition to possibly undiscovered role in the variation of HbF production in response to erythropoietic reactivity &/or capacity to respond to increased HbF synthesis\(^{[32,33,34]}\). In addition as shown in Table (2) 49.1% of the TI cases in group I presented with severe phenotype and the number of cases presenting as mild within the TM group (7.5%) was found to be less than within group III (14.6%). Again questioning the establishment of the co-inheritance of high HbF as persistently clinical ameliorating factor.
Weatherall & Clegg\(^{(1)}\) stated that all β thalassaemias, heterozygotes or homozygotes, have variable increases in Hbf. However in our study the 50 patients included in group III had Hbf level ranging with less than 10% from 0-5% which is within normal adult values\(^{(3)}\) indicating the possible ethnic variability both regarding the coinheritance and response to Hbf levels. Many genetic variants have been associated with elevated F cells levels in healthy adults, including several in the β-globin gene complex Xmn1-\(\gamma\) site\(^{(11)}\). Xmn1-HBG2 single nucleotide polymorphism was estimated as one major locus. However the Xmn1 \(\gamma\) globin polymorphism was detected in only one TI case (4%) among 23 TI and 14 TM of our studied cases previously characterized\(^{(21)}\). Linkage studies have suggested that loci within the β-globin gene cluster and quantitative trait loci (QTL) mapping elsewhere in the genome may play a role in the regulation of Hbf levels\(^{(31)}\).

In the majority of β thalassaemia and SCD-SS, the mutation itself does not directly result in increased Hbf production, a large part of the Hbf response is related to the erythropoietic stress and expanded erythropoiesis mass secondary to the ineffective erythropoiesis or haemolytic process, and preferential survival of the red cell precursors that contain Hbf (i.e., FC). Acting against this background is the innate ability for Hbf synthesis related to inheritance of different quantitative trait loci (QTLs) for Hbf. These Hbf QTLs and others yet to be discovered, presumably play an important role in the variation of Hbf production in response to erythropoietic stress, and possibly, in the capacity to respond to pharmacological inducers of Hbf synthesis\(^{(32-34)}\). This complex background of genetics of Hbf production and its correlation to ineffective erythropoiesis which is not yet established till date\(^{(18)}\) can explain the lack of persistent clinical amelioration in response to high Hbf in our patients.

We conclude that the persistence of Hbf can not be constantly considered as a clinical modifier during genetic counseling. We also, cannot predict the capacity of the patient to respond to γ chain inducers nor their effect on disease progression.

It is likely that the remaining trait variance is due to numerous other loci. Identification and translation into new therapeutic approaches for Hbf reactivation would be an improved prediction of one's ability to produce Hbf, which in turn, may improve prediction of disease severity.

REFERENCES


