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Hemoglobin E/β⁰ Thalassemia in south west Iran - A case series

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Abstract

Background: Beta thalassemia gene mutations are among common mutations in southwest Iran. However, Hemoglobin E (Hb E) and Hb E/β⁰ thalassemia account for a small number of hemoglobinopathies in Iran. This is the first study to directly address the existence of Hb E and consequently Hb E/β⁰ thalassemia in southwest Iran.

Methods: This retrospective study discovered seven cases of Hb E/β⁰ thalassemia among 700 patients with hemoglobinopathies referring to Health Institute and Research Center for Thalassemia and Hemoglobinopathy in southwest Iran. EDTA and clot blood samples were obtained and analyzed for complete blood counts, hemoglobin electrophoresis, LDH, bilirubin, ferritin and amplification refractory mutation system (ARMS) technique by polymerase chain reaction (PCR) and DNA sequencing.

Results: Out of 700 cases, seven patients with Hb E/β⁰ thalassemia were detected (1%). Four patients were classified into non-transfused dependent Hb E/β⁰ thalassemia and three cases were classified into transfusion dependent Hb E/β⁰ thalassemia group. Alpha thalassemia (deletional and non-deletional) and XmnI gene polymorphism were not found in either of cases.

Conclusion: Hb E/β Thalassemia is not a common hemoglobin disorder in southwest Iran. Phenotype heterogeneity is common in Iranian patients from a mild asymptomatic anemia to severe anemia that can be presented in the early years of life. This was the first report of Hb E/β⁰ thalassemia from Iran.

Keywords: Hb E/β⁰ Thalassemia, Southwest Iran, Transfusion dependent, Non-transfusion dependent, Hb E mutation.

Introduction

Iran is located on the thalassemia belt. The prevalence of thalassemia minor is about 4-10% in different parts of Iran with more than twenty thousand patients affected with thalassemia major [1]. There is no data on the number of Hb E/β⁰ thalassemia patients in Iran.

Hb E is caused by a single nucleotide base replacement at codon 26 of the β-globin gene, GAG-AAG, which results in the substitution of lysine for glutamic acid. This mutation activates a hidden splice site that produces an abnormal messenger RNA [2]. Since the normal donor site has to compete with this new site, the level of β Globin messenger RNA is reduced, resulting in a mild clinical phenotype of β thalassemia syndrome [3].

Hb E is a common hemoglobin variant in southeast Asia, especially in Thailand, Burma, Laos, Cambodia, Malaysia, Indonesia, and some areas of India [4, 5]. It accounts for nearly half of all the cases of severe thalassemia syndrome in the world population [4, 6].

The first identification and clinical descriptions of Hb E were reported during 1954-1956. However, there are still many gaps in understanding its pathophysiology [7]. Although Hb E heterozygous or even homozygous state do not cause any clinical problems, the interaction of Hb E with thalassemia produces a variable phenotype ranging from an asymptomatic syndrome to a severe form of anemia similar to thalassemia major [8]. The cause

of this diversity is not known yet but factors such as the kind of β thalassemia mutation, coexistence of alpha thalassemia, presence or absence of XmnI polymorphism, and other unknown factors may be involved [7].

The common clinical manifestation of symptomatic Hb E/β^o thalassemia consists of pallor, hepatosplenomegaly and jaundice. Delayed puberty and stunted growth may also be exhibited in children [6, 8].

Because of the extremely low frequency of Hb E and consequently Hb E/ β^o thalassemia in Iran, we intended to search for this hemoglobinopathy among patients who refer ro research center for thalassemia and hemoglobinopathies. This is the first study to address directly the existence of Hb E and consequently Hb E/β^o thalassemia in southwest Iran.

Patients and Methods

This study was conducted in Ahvaz Research Center for thalassemia and hemoglobinopathy on patients with hemoglobinopathy who were referred for genetic studies. The study was also approved by Ahwaz university. All patients and/or their parents gave informed consent. From each patient and their parents 3 milliliters of blood were obtained and collected into EDTA, and all blood tests were performed as follows: Cell blood count and red blood indices by automated cell counter Sysmex K1000, hemoglobin A_2 by column chromatography, hemoglobin electrophoresis on cellulose acetate at PH 8.5 and high performance liquid chromatography (HPLC) (Bio-rad Variant, Hercules, CA) was performed on anion exchange columns with Gynkotek-High precision pump, model 300C to separate Hb A_2 from Hb E. Bilirubin and ferritin were measured using enzyme-linked immunosorbent assays (ELISAs; R&D Systems, Abingdon, UK).

DNA was extracted from peripheral blood leukocytes. The β thalassemia mutations were identified using amplification refractory mutation system (ARMS) technique by the polymerase chain reaction (PCR). Alpha thalassemia was screened for using Southern blotting. The XmnI gene polymorphism was detected by PCR amplification and restriction enzyme digestion. DNA sequencing was performed for all samples.

Results

Out of 700 patients with hemoglobin disorders in Southwest Iran, 7 cases of Hb E/β^o thalassemia were detected. Four patients (cases 1-4) who had not received any or only two to four occasional blood transfusions were classified into non transfused dependent Hb E/β^o thalassemia and the remainder (cases 5-7,Table-1) who were receiving regular transfusions every four weeks were classified into transfusion dependent Hb E/ β^o thalassemia group. The mean pre-transfusion Hb in transfusion dependent and non transfusion dependent groups were 7.3 and 8.1 g/dl, respectively. The mean Hb E in non transfusion

Table 1: characteristics of the patients

	Age,sex	Severity of anemia	hepatosplenomegaly	Thalassemic face	History of blood transfusion
1	9 yr,male	moderate	+	mild	2 times
2	37 yr,male	mild	+	_	_
3	40 yr, female	Moderate	+	+	2 times
4	4.5 yr, female	mild	_	_	_
5	32 yr, male	severe	splenectomized	severe	Regular transfusion
6	32 yr, male	severe	splenectomized	severe	Regular transfusion
7	35 yr, male	severe	splenectomized	severe	Regular transfusion

dependent and transfusion dependent groups was nearly the same (78.7% vs. 79.6%). The mean Hb F was slightly higher in the non transfusion dependent group compared with the transfusion dependent patients (18.9 % vs 16 %). Due to small number of patients in both groups statistical comparison was not applied for them.

Patients in transfusion dependent group had higher mean serum ferritin levels compared with the other group (2586 vs 855 ng/ml). Alpha thalassemia (deletional and non-deletional) and XmnI polymorphism were not found in either of the cases. The mean MCV and MCH were 59.1 fl (range, 51 to 71.2) and 19.2 pg (range, 16.1 to 23.2), respectively. Two patients in each group were receiving hydroxyurea along with iron chelators. Splenectomy and cholecystectomy were done in all patients who were transfusion dependent and one from the other group.

Some characteristics of the patients are summarized in Table 1. In three patients belonging to one family the mutation was Cd 36/37 – T mutation. In three other siblings from another family the detected mutation was IVS I-5. One patient was positive for Cd 8 –AA mutation.

Discussion

Hemoglobinopathies are among common hematologic diseases in southwest Iran. The Hb C, Hb O Arab, and Hb E are occasionally found in this area [9, 10]. The most common hemoglobinopathies are Hb S, Hb D and other abnormal Hb variants such as Hb J Iran. The gene frequency of Hb E is very low in Iran compared with southeast Asia and India [4, 5]. The frequency of Hb E is 10.9% in North Eastern regions of India and up to 30% in Burma, Thailand, Laos, Cambodia, Malaysia and Indonesia[11,12]. Clinical manifestation of our patients was remarkably variable. Thalassemia mutation type is not a reliable indicator for severity of Hb E/β^o thalassemia syndrome. In our patients three beta gene mutations were detected; Cd 36/37 – T, IVS I-5 and Cd 8 –AA mutations. Patients with IVS I-5 beta mutation were dependent on regular blood transfusion. The predominant beta mutation in patients with Hb E/β^o thalassemia and thalassemia major in north India was IVS 1-5 G-> C [13]. The other dominant beta mutation in our patients was Cd 36/37 – T which is a frameshift mutation at codons 36/37 and was observed in 3 of them [14]. It might be expected that a patient with more Hb F to have a better clinical manifestation. Despite seemingly identical genotype and equal Hb F levels, the patients had remarkably variable phenotypes. The Hb E level in our patients was between 40-97.8% that is higher than other reports showing Hb E ranges between 30-70% of total hemoglobin 4. The range of Hb F in our patients was 0.3 to 56.7%. The mean level of Hb F in a study from Sri Lanka was approximately 28%, ranging from 10-50%, and even higher levels of Hb F have been reported from Thailand [15, 16]. Hemoglobin A_2 level in our patients was 1.6-7% nearly close to a previous report [16]. The mean Hb E level in the parents as carriers was 31.8% ranging from 20-35.8%, which was higher than the previous study [16]. There was no correlation between Hb F, Hb E/F ratio and beta thalassemia mutation in our study with the severity of the disease as well as another report [16]. The mean MCV and MCH in our patients was 59.5 fl and 19.2 pg, respectively. It was slightly lower than Pakistanis patients [17]. All adult patients were married which could indicate a normal developed sexual endocrine system.

In our study, the frequency of blood transfusions and splenectomy was consistent with a similar study [18]. Splenectomy was significantly more common in patients who had underwent regular transfusions compared with those who did not. We did not find any deletional and non-deletional alpha thalassemia and XmnI gene polymorphisms in our patients which was inconsistent with another study [19]. Pallor was a commonly observed sign in our patients. The mean pre-transfusion hemoglobin in patients with transfusion dependency was 7.3 g/dl and 8.1g/dl in patients who were not transfusion dependent. This finding was consistent with results obtained in Thai patients. Thai patients with Hb E/β0-thalassemia and a XmnI +/+ genotype had higher total hemoglobin and hemoglobin F concentrations than those with XmnI -/- genotype [20].

Chronic hyperbilirubinemia and gallstone may significantly worsen the phenotype of the patients with Hb E/β^o thalassemia. Hyperbilirubinemia was seen in all patients with transfusion dependency and at lower levels in some patients who were not transfusion dependent. The increased level of bilirubin and higher number of patients who underwent cholecystectomy in our series may

be related to a polymorphism of the promoter region of the enzyme responsible for hepatic glucuronidation of bilirubin [21]. We did not study the presence of UDP-glucuronosyltransferase 1 (UGT1A1) promoter polymorphism in our patients.

Iron overload is a serious problem in multiple transfused patients and even in some who do not receive regular blood transfusion because of enhanced gastrointestinal iron absorption. The mean ferritin level in patients who had received multiple transfusions and non transfusion dependent group was approximately 2500 and 855 ng/ml, respectively. Two patients in each group were receiving hydroxyurea with doses of 10-15 mg/kg/day. Hydroxyurea had been effective in increasing hemoglobin Hb F and RBC indices and decreasing Hb E and bilirubin levels. Although there had been reported no dramatic change in hemoglobin level in patients who were on transfusion, the interval of blood transfusions had been prolonged and they exhibited a decrease in serum bilirubin levels associated with a feeling of well being. Further studies will be required to evaluate the long-term efficacy of hydroxyurea among these patients.

Conclusion

Our study suggests that we should be alert of the presence of Hb E/beta thalassemia mainly in southern areas of Iran. Various phenotypes could be expected among our patients. This phenotype heterogeneity was not related to the known modifying factors such as alpha thalassemia and XmnI polymorphism in our patients. Further studies will be required to clarify these contributing factors.

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