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Beta-Globin Gene Cluster Haplotypes in Iranian Sickle Cell Patients: Relation to Some Hematologic Parameters

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Abstract

Background: Sickle cell anemia is relatively common in Khuzestan province located in Southwest Iran. The characteristics of sickle cell disease in Iran are apparently different from other regions; some of these characteristics might be related to β-chain haplotypes. The purpose of this study was to determine the frequency of β-chain haplotypes in 50 patients with homozygous sickle cell anemia in Khuzestan Province.

Materials and Methods: The haplotypes were explored around and within the ε–Gγ–Aγ–ψβ–δ–β globin gene complex by analysing seven polymorphic restriction sites [(1) HincII 5′ to ε (2) XmnI-5′γG, (3) Hind III-γG, (4) Hind III-γA, (5) HincII- ψβ, (6) HincII- 3′to Ψβ, and (7) HinfI and RsaI - 5′to β], followed by restriction digestion and agarose gel electrophoresis. The effect of beta globin haplotypes upon hematologic parameters such as hemoglobin, hemoglobin F, mean corpuscular hemoglobin concentration, reticulocyte count, serum bilirubin and Lactate Dehydrogenase was also studied.

Results: The Arab/Indian was the most frequent haplotype, present in 38 percent of chromosomes, followed by Benin haplotype (18%), Senegal haplotype (16%), Bantu haplotype (16%) and Cameroon haplotype (12%). The mean percentage of hemoglobin F in sickle cell anemia patients was 17.18±8.81%, and in the homozygous Arab/Indian haplotype it was higher (20.90%), but the difference was not significant. The hemoglobin F was significantly higher in females compared to males (19.10 versus 14.50, P<0.005).

Conclusion: There was no significant correlation between haplotypes and hematological characteristics like fetal hemoglobin level among our patients.

Key words: Sickle cell anemia, haplotypes, hemoglobin, fetal, Iran.

Introduction

Sickle cell disease (SCD) occurs commonly in Africa and less commonly in America, the Mediterranean region and in some Arabic and Indian groups [1]. SCD is not a common disease in Iran and there is no comprehensive data on epidemiology of the sickle cell trait, however SCD is more common in south and south west Iran.

The clinical manifestations of SCD are characteristically different ranging from death in early infancy period to a normal life expectancy with few complications [2,3]. The SCD in India, Eastern province of Saudi Arabia, Qatar, Oman, Azerbaijan, Baluchistan, Iraq, Afghanistan and Iran is clinically milder than SCD of African origin [4-9]. In Khuzestan, the clinical presentation of SCD patients is usually mild to moderate; however, cases with severe manifestations do exist. The causes of this diversity are not totally understood. The behaviour of SCD appears to be affected by some acknowledged modulators such as haplotype, α-thalassemia, hemoglobin F (HbF) level, hematological parameters, environmental factors, and some other unknown factors [10]. A large part of this dissimilarity is attributed to the existence of linked β-globin gene cluster haplotypes, which modify the expression of the disease. Based of geographic regions, four major haplotypes have been recognized in Africa which are designated Senegal, Benin, Banto (Central African Republic), Cameroon; and one in Asia called Arab/Indian haplotype [11,12]. A β-globin gene haplotype is defined as different combinations of polymorphic sites by the non-random link of restriction endonuclease cleavage points located in the β-globin gene complex. The pattern of

restriction sites for the β-sickle haplotypes has been demonstrated (a "plus" indicates susceptible to restriction enzyme digestion, while a "minus" indicates resistance to restriction endonuclease digestion) [13].

The Study of beta chain haplotypes among SCD patients is crucial for detection of origin, distribution and the clinical expression [10]. Several studies of SCD patients with different hematologic features have suggested that the β-globin gene cluster haplotype might be a useful predictor of disease severity and HbF levels [10,11]. Some reports have suggested that patients with homozygous form of Senegal and Arab/Indian haplotypes appear as mild disease with high hemoglobin F levels but homozygous form of Bantu, Benin and Cameroon haplotype manifest as intermediate severity [14,15].

The β-globin gene cluster haplotype among SCD patients from Khuzestanian, Iran has not been studied before. Thus, the study reported here was undertaken to identify the β-gene cluster haplotypes among SCD patients from Khuzestan and their influence on hematological parameters such as hemoglobin (Hb), HbF, mean corpuscular hemoglobin concentration (MCHC), reticulocyte count, serum bilirubin and Lactate Dehydrogenase (LDH).

Materials and Methods

The study population consisted of 50 (30 females, 20 males) non-related patients with homozygous SCD. The patients were attended at the Shafa hospital, affiliated with Ahvaz Jundishapur University of Medical Science. The age of the subjects was 5 through 60 (mean: 24.12 ± 11.50 years) and all participants were from Arab ethnic group and were randomly selected. Individuals with Sickle cell trait and compound heterozygote cases were excluded from the study. Written informed consent was obtained from all patients or their parents. All samples from these subjects were prepared and analyzed in accordance with the ethical recommendations of the Ethics committee of Jundishapur University of Medical Sciences. Neither of the patients was transfused for at least six months before blood collection and none of patients was under treatment with hydroxyurea. Blood sample (4ml) was obtained from each patient during a regular clinic visit and if the patient was in a steady state condition.

CBC was performed using automated cell counter model BC-3000 Plus (Mindray, Bio-Medical and Electronics. Co. Ltd, Shenzhen - China). Hemoglobin electrophoresis was performed and HbF was measured by high-performance liquid chromatography (HPLC) and alkali denaturation (HPLC model D10, Bio-Rad, France, using Elitech Kit, France). LDH and bilirubin were measured using automatic auto-analyzer, (BT 3000-Italy) (bilirubin measurement via Malloy-Evelyn, End point approach with Elitech kit and LDH via photo metric approach with Pars Azmoon Kit). Reticulocyte count was performed by supravital staining and microscopic eye observation counting. Solubility test was used to distinguish HbS and HbD in some suspicious cases. DNA was prepared from peripheral blood leukocytes and haplotype analysis was performed using a polymerase chain reaction based approach, studying the following restriction enzyme sites of the β-globin gene cluster: [(1) HincII 5′ to ε (2)XmnI-5′γG, (3) Hind III-γG, (4) Hind III-γA, (5) HincII- ψβ, (6) HincII- 3′to Ψβ, and (7) HinfI and RsaI - 5′to β] using the method described by the manufacturer protocol. After amplification and digestion with the respective restriction enzymes, the samples were run on an agarose gel electrophoresis and stained with ethidium bromide for visualizing in an ultraviolet trans illuminator. Plus and minus were used to symbolize the presence or absence of the restriction digestion sites respectively. The hematological parameters were compared among different haplotypes and genders. The Chi-square, ANOVA, and the Student's t-test were used for statistical comparisons. P values of <0.05 were considered statistically significant.

Results

The distribution of β-sickle haplotypes in our patients is shown in Table 1. Among the fifty SCD patients who were investigated 26 % had Arab/Indian haplotype in homozygous form followed by Arab Indian/Senegal (22%), Benin/Cameroon (20%), Benin/Benin (12%) and Bantu/Bantu in 10% of patients. The frequency of the other homozygous form haplotypes such as Arab Indian/Cameroon, Senegal/Cameroon and Senegal/Senegal were lower than 4%. Haplotypes had Asian origin and /or African origin. The African origin haplotypes in homozygous form were identified in 50% of patients and Asian origin haplotypes in homozygous

state were encountered in 26% of patients. The rest (24%) belonged to the mixed type of Asian/ African origin haplotypes. Arab/Indian haplotype was identified in 38 percent of chromosomes followed by Benin (18%), Senegal (16%), Bantu (16%) and Cameroon in 12% of chromosomes. The hematological data relating to the different haplotypes are depicted in Table 2. The mean levels for hematologic values in 50 SCD patients were as follow: Hb: 8.55±4.50 g/dl, HbF: 17.18±8.81%, MCHC: 31.80±1.80g/dl, LDH: 1369.42±666.38 IU/ml, serum total bilirubin: 4.85±4.58 mg/dl and reticulocyte count: 4.60±3.28%. HbF level had a variation between 1.5% and 38%. The majority of patients (about 65%) had HbF in the range of 10% to 20%. The mean HbF levels were significantly higher among females compared to males (19.10 versus 14.50, p <0.005). The hematological variables did not differ significantly among the homozygous and heterozygous haplotype groups.

Discussion

Khuzestan province with 4,277,998 inhabitants, according to the most recent census taken in 2004, represents 6% of the Iranian population. Khuzestan, unlike most other states in Iran, is inhabited by different ethnic groups: Persians in major cities, Arabs, Bakhtiaries, Lurs, Behbahanis, Laks, the Turkic-speaking Qashqai and Afshari tribes and the Khuzis of Shush/Susa, Dezful, Shushtar, Andimeshk as well as the inhabitants of the coastal regions of the Persian Gulf. It is estimated that Arab ethnicity constitutes about 40 to 45% of Khuzestan population. SCD as an important health problem exclusively exists among Arab population. Being on the border with Iraq, Khuzestan suffered the heaviest destruction during the Iraq vs. Iran war (1980–1988). This created a mass emigration of people to other Iran provinces. Many Arab people settled in different parts of Iran. It caused a new distribution of SCD in Iran. The high frequency of Arab/Indian haplotype in our study compared to other Iranian studies enhances this hypothesis[16,17]. Iranian Arab communities have also interacted with people in Bahrain, Kuwait, Iraq, Qatar, and the United Arab Emirates. The distribution pattern of haplotypes in the present study is similar to that observed among neighbouring Arab countries and some tribes from Indian subcontinent [4-6,18,19]. The Arab/Indian haplotype has been related with a more benign clinical course of SCD having less anemia and organ damage because of higher HbF levels [1,3,5,6,8,16,18,19]. In our patients with Arab/Indian haplotype in homozygous form, the level of HbF was 20.9% comparable with the other studies (4,6,8,18,19). The range of HbF in our SCD patients was comparable with the Bahrainis, Eastern Saudi Arabia and those of Orissa and India [4,5,18] and lower than some other studies [16,17].

The average of HbF for the female patients was significantly higher than males. This is in agreement with Steinberg et al. and Adams et al. studies [11,20], and in contrast to a study by Inati et al. [21]. Among our patients the HbF level revealed no significant correlation with the β-sickle haplotypes. This finding is in accordance with some other studies [22,23], but differs from Steinberg et al. and Figueiredo et al. studies [11,24].

The distribution of β sickle chromosomes with African origin haplotypes is demonstrated in

Table1: Distribution of Haplotypes in Homozygous form

Haplotype	Frequency	Percent
Arab Indian/Arab Indian	13	26
Arab Indian/Cameroon	1	2
Arab Indian/Senegal	11	22
Bantu/Bantu	5	10
Benin/Bantu	6	12
Benin/Benin	1	2
Benin/Cameroon	10	20
Senegal/Cameroon	1	2
Senegal/Senegal	2	4

Table1. According to this table the Benin haplotype is the most common African origin haplotype among our patients followed by Senegal, Bantu and Cameroon haplotypes. The Benin/Cameroon was the most common double heterozygous form of African origin haplotype. The Senegal haplotype was the most common African origin haplotype which co-exists with the Asian origin haplotype in double heterozygous form. Our data is in line with historical records indicating that most of the Sickle cell diseases in Khuzestan have originated from a multicentric African origin. The Benin haplotype is the predominant haplotype in the state of Aragua in Venezuela, the western provinces of the Saudi Arabia, Oman and Lebanon [21,25-27]. In our study, the frequency of Benin haplotype was higher than the other Iranian studies [9,16,17]. The African origin haplotype paired with Arab/Indian haplotype in double heterozygous form has been reported in Iran by Rahimi et al. [16]. Another study from Fars province, Iran, has reported Bantu haplotype[16]. The presence of African origin haplotypes in other Iranian studies supports the hypothesis of multicentric origin of Iranian SCD mutation [16,17].

Senegal haplotype like Arab/Indian haplotype has been associated with the most benign clinical course. The Arab/India and Senegal haplotypes in the homozygous form have been associated with higher levels of HbF [28]. Nagel et al. [28] showed that the presence of at least one chromosome background with Senegal haplotype is associated with higher hemoglobin compared to other haplotypes, however our findings do not support this finding. Steinberg et al. [11] found that the presence of Senegal haplotype in combination with female gender produces the highest level of HbF among SCD patients, however our data did not support this possibility. It has been revealed that the Senegal and Arab/Indian haplotypes are associated with the C > T mutation at position –158 of the Gγ promoter, which results in an increased number of Gγ chains leading to raised HbF level in some cases but, patients with either the Cameroon or Benin haplotype have significantly lower HbF concentrations [13,14]. These findings are not consistent with the results of the present study. Nagel et al. [28] showed that patients with Cameroon haplotype and low HbF should be associated with more severe clinical presentations. Steinberg et al. have suggested that the presence of Cameroon haplotype have a suppressive effect on the HbF response to hydroxyurea in patients with SCD [10].

Table 1 shows Hb concentration, HbS level, MCHC, LDH, bilirubin values and reticulocyte count did not differ among homozygous forms of haplotypes among our patients. It also shows that HbF and HbS levels as well as other hematologic parameters were not significantly different between different haplotypes. This suggests that factors other than haplotype might influence the HbF and HbS levels as well as hematologic parameters. Our data is in line with Rieder et al. Findings [23]. Rusanova et al. [29] showed that the predominant haplotype in Panama is Bantu haplotype and hemoglobin concentrations, hematocrit, and mean corpuscular

Table2: Haplotypes and Hematologic Parameters

Haplotype	HbF	Hb S	MCHC	LDH	Bilirubin	Reticulocyte	Hb
Arab Indian /Arab Indian	20.9	77.03	32.0	1256	6.4	4.0	9.1
Arab Indian/Cameroon	16.7	81.2	31.1	557	1.5	1.1	11
Arab Indian/Senegal	13.9	82.4	31.7	1324	4.1	4.9	8.3
Bantu/Bantu	11.9	85.8	32.4	1149	3.1	5.1	9.8
Benin/Bantu	17.6	81.0	31.6	1592	4.0	3.9	8
Benin/Benin	13.2	84.4	32.3	2300	6.7	1.9	6.7
Benin/Cameroon	18.5	79.1	31.4	1424	4.3	6.1	7.6
Senegal/Cameroon	22.6	74.7	32.2	569	1.8	1.7	13
Senegal/Senegal	14.8	78.1	30.8	2297	3.5	6.0	6.5

hemoglobin concentration values did not differ among homozygous forms of haplotypes. However, the percentage of reticulocytes was highest in Benin and Cameroon homozygous forms which was associated with the worst prognosis [29].

In conclusion, the present study in south west Iran indicates that Arab/Indian haplotype in homozygous form is the predominant type in this area. In addition, the Benin haplotype is the most frequent haplotype with African origin. In this study, different haplotypes did not show a significant influence on the level of HbF and other hematologic parameters, so the genotype–hematologic phenotype relationship was incompletely defined. The presence of Asian origin haplotype co-existence with the African origin haplotypes in Khuzestan province are in agreement with the multicentric origin of SCD in this province.

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