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Original Article

Elucidation of β^s/globin gene cluster haplotypes related to sickle cell anemia in Khuzestan province, southwest of Iran

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Abstract

Background & objectives: The researcher clarified that β/Globin gene cluster haplotypes in patients with sickle cell anemia provide useful population data as predictors of the disease severity, gene flow, and the origins of sickle cell mutation in this region.

Materials and methods: A total of 150 subjects was investigated in two different groups for five polymorphism restriction sites of the beta Globin gene cluster. The first groups 100 cases were examined in March 2008 and the second groups 50 cases in May 2010. ETDA blood was taken and DNA was prepared from subjected leukocytes and the β^s gene cluster were amplified via polymerase chain reaction (PCR) using a DNA TM KIT. The sickle mutation eliminates the recognition sequence of this enzyme.

Results: Both studies showed five haplotypes exist in a population of one hundred and fifty sickle cell anemia patients (300 chromosomes). The Arab-Indian haplotype was found to be the highest ranking in prevalence (43.66). The second most common haplotype was the Benin haplotype (24.66%) followed by the Bantu haplotype (18%) and the Cameron haplotype which was ranked fourth (7.66%). The Senegal haplotype was ranked fifth in terms of prevalence (5.33%).

Conclusions: The overall conclusion from the SCA gene analysis in Khuzestan showed a spectrum of all 5 haplotype types of the African, Arab-Indian sickle cell haplotype which produced various types of homozygote and heterozygote admixture SCAs with various mild to very severe clinical course and clinical manifestation in the region. It is best recommended that the history, patient's exam, CBC, RBC indices, hemoglobin electrophoresis, sickle preparation, DNA mutation analysis, haplotype determination and family pedigree be taken into account.

Keywords: Sickle cell; gene cluster haplotype; South west of Iran

1. Introduction

Practical experience on the basis of daily referral cases to the Thalassemia and Sickle Cell Center of Shafa hospital in Ahvaz, the capital of the Khuzestan Province in the southwest of Iran,showed sickle cell anemia (SCA), and its heterozygote-compounds (S/β^o ,S/β^+, Thalassemia) is the second most common hereditary Hemoglobinopathy disorder in Khuzestan[1-7]. The previous reports of SCA in the southwestern of Iran showed that there is a mild disease with high HbF level[4,5,8-10], however, medical experience has shown that some sickle cell patients, whom were referred to Shafa hospital in a serious ill condition and even dead on arrival in the emergency room or in the ICU after a few days after post admission, showed acute chest

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syndrome, suffered strokes and others vaso-occlusive events without any response to medications[3,4,7]. Factors that predispose patients to this complication have not been well established. It was on the basis of such an assumption that the researchers tried to elucidate β/Globin genes cluster haplotype in patients with sickle cell disease in the Khuzestan Province because β^s globins gene cluster haplotype provide useful population data as predictors of disease severity, gene flow and the origin of sickle cell mutation in this region[11-13].

2. Materials and Methods

Molecular genetic studies were undertaken to determine the haplotype of the chromosome carrying capacity of the sickle cell allele. A total of 150 subjects was investigated in two different groups for five polymorphism restriction sites of the beta Globin gene cluster. In the first group 100 cases were examined in March 2008 and in the second group 50 cases in May 2010. ETDA blood was taken and DNA was prepared from subjected leukocytes using a phenol-chloroform extraction method and kept –on 20°C until analyzed. The Six surrounding regions within the β^s gene cluster were amplified via polymerase chain reaction (PCR) using a DNA TM KIT. The PCR from each patient was treated with Restriction Fragmented Length Polymorphism (RFLP) method by appropriate enzymes which resulted in fragmentation and separation on a 3% multipurpose agaorose gel containing ethidium bromide. The bands were visualized using a UV light box and photographed using a U Vitec**.** The sickle mutation eliminates the recognition sequence of this enzyme. Therefore, the HbS allele is visualized as a 340-bp band and the HbA allele as 200- and 140-bp bands. A constant band of 100 base pairs is present in all individuals' gel docu-mentation system. The polymorphic restriction sites studies were done 5′ to ε , gene by Hind 11, 5′ to the G gene by Xmn1, within IVS 2 of the G and A genes by Hind 111, 3′ to ψβ by Hind11, 1VS 2 and others β^s gene by Ava11 . Agaorose gel electrophoreses of PCR and enzyme digestive products were also performed[11-13] (Figures 1-2).

Fig: 1- Sites affects of six restriction enzyme on β^s-globin gene cluster.

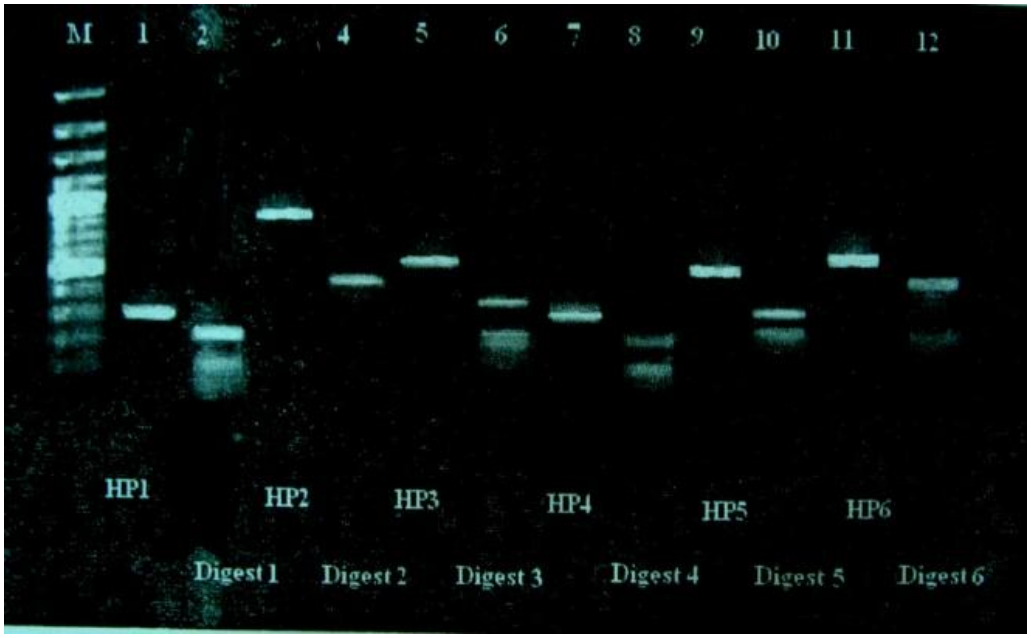

Fig: 2-PCR, RFLP, agaorose gel electrophoresis pattern products of β-globin gene cluster.

3. Results

Both studies showed five haplotypes to exist in a population of one hundred and fifty sickle cell anemia patients (300 chromosomes). This is shown in Figure 3. The Arab-Indian haplotype was found to be the highest ranking in prevalence (43.66). The second most common haplotype was the Benin haplotype (24.66%) followed by the Bantu haplotype (18%), and the Cameron haplotype which was ranked fourth (7.66%). The Senegal haplotype was ranked fifth in terms of prevalence (5.33%).

Table1. Haplotype distribution from β ^{s}-globin gene cluster in 300 Chromosome in Khuzestan, Iran

SCA Haplotype*	First R.H. D**	Second R.H.D***	Total Gene summations	Total $^{\beta s}$ goblin Haplotype%
Arab-Indian	95	38	133	44.33 %
Benin	56	18	74	24.66 %
Bantu	27	27	56	18 %
CAR	11	12	23	7.66 %
Senegal	11	5	16	5.33 %
Total	200	100	300	99.98%

**Sickle cell Anemia Haplotype*
***Fires Research Haplotype distribution*
**** Second Research Haplotype Distribution*

4. Discussion:

The identification of βs cluster haplotypes is a useful tool for the detection of high risk patients with SCA[11-13]. The Analysis of β^{s} /Globin genes cluster haplotypes in the aforementioned studies show that:

First: In the first group two benign haplotypes of the Arab-Indian haplotype with an occurrence rate of 43.66% was the highest and most common haplotype in prevalence and Senegal haplotype with an occurrence rate of 5.33% was the lowest and least common haplotype. The total sum of these two mild parameters (Arab-Indian +Senegal haplotype) = 49% was approximately half of the total haplotypes in both studies. The Relationship between long–term clinical course and the aforementioned parameters shows that the haplotypes designated as Arabian- Indian and Senegal have a decreased severity[12-13], moreover, they were reported as being mild and benign haplotypes with a high level of fetal hemoglobin (HbF), and packed cell volume (PVC), having a less acute recurrent clinical event, the lowest clinical course, including hospitalization, infection, sickle cell crisis, bone infarction, splenic sequestration, acute chest syndrome, cerebral thrombosis)[13-15].

Second: In the second group Benin haplotype was the highest haplotype in prevalence with an occurrence frequency of 24.66%, and was thus the second most common haplotype in both studies. The Bantu haplotype with an occurrence frequency of 18% was the third most common haplotype in both studies. The heterogeneously in the haplotypes (Bantu 1 to 6) makes it a worldwide difference. The Cameron haplotype with an occurrence frequency of 7.66% was the fourth most common haplotype in prevalence in both studies. The first three aforementioned parameters (Benin,Bantu, Cameron haplotypes) encompassed 51% of the total population in the second part of the two studies. The relationship between long–term clinical course reveals that those with the haplotype designated (Benin, Bantu, Cameron) have an increased severity in clinical course whereas in the low to lowest of HbF, (PVC)[15-16], shows a poor to worst clinical course with the highest recurrent clinical events, including more recurrent hospitalization, sickle cell crisis, bone infarction, infection, splenic sequestration, acute chest syndrome, cerebral thrombosis , toxemia pregnancy, and others critical painful crisis events and psychological disorders[17-18]. The last point double heterozygote subtype of Benin, Bantu, Cameron, with Arabia-Indian or Senegal haplotype makes admixture haplotypes with intermediate to worst prognostic diseases[19].

5. Conclusion

The overall conclusion from SCA gene analysis in the Khuzestan Province showed a spectrum of all 5 haplotypes of African, Arab-Indian sickle cell haplotypes which produce various types of homozygote and heterozygote admixtures of SCA with varying degrees of clinical courses ranging from mild to very severe and serious clinical course and clinical manifestation in the Khuzestan Province. The

researchers recommend that for a deeper understanding for each individual sickle cell patients' sickle cell syndrome, diagnosis, prognosis, treatment, legalized problems etc., it is necessary to take the history, Patients exam, CBC, RBC indices, Hemoglobin electrophoresis, sickle cell preparation, DNA mutation analysis, haplotype determination and family pedigree into account .

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