



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

Khoda Morad Zandian^{1,*}, Manizheh Kadkhodai^{1,2}, Hamid Galehdari¹, Mohamad Pedram¹, Bijan Kiekhaie¹, Kaveh Jaseb¹, Helen Zandian¹, Mozhgan Norbehbahani², Hedayatollah Hosseini², Bahaaldin Salehi¹, Roa Salehi¹, Elham Yousefi¹, Shekofeh Josheghani¹

1. Research Center of Thalassemia And Hemoglobinopathy, Ahvaz Jundishapur University of Medical sciences, Ahvaz, Iran
2. Department of Biochemistry, Ahvaz Jundishapur University of Medical sciences Ahvaz, Iran

Received 20 July 2012; received in revised form 12 November 2012; accepted 8 December 2012

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^0 , S/β^+ , Thalassemia) is the second most common hereditary

Hemoglobinopathy disorder in Khuzestan¹⁻⁷. 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

* **Corresponding Author;** Khoda Morad Zandian Professor in pediatric Hematology/Oncology Ahvaz Jundishapur University of Medical Sciences, Shafa hospital, Ahvaz, Iran. Telephone 09161187034. E-mail: Drzandian@yahoo.com

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 region¹¹⁻¹³.

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. EDTA 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 agaorse 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 II, 5' to the G gene by XmnI, within IVS 2 of the G and A genes by Hind III, 3' to $\psi\beta$ by HindII, 1VS 2 and others β^s gene by AvaI . Agaorse gel electrophoreses of PCR and enzyme digestive products were also performed¹¹⁻¹³ (Figures 1-2).

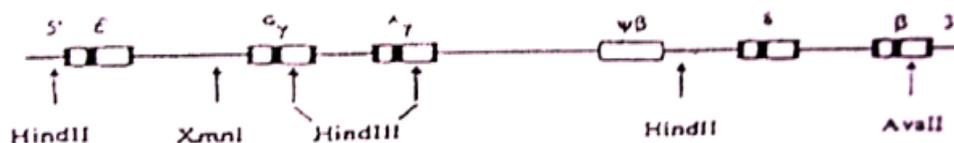


Fig: 1- Sites affects of six restriction enzyme on β^s -globin gene cluster.

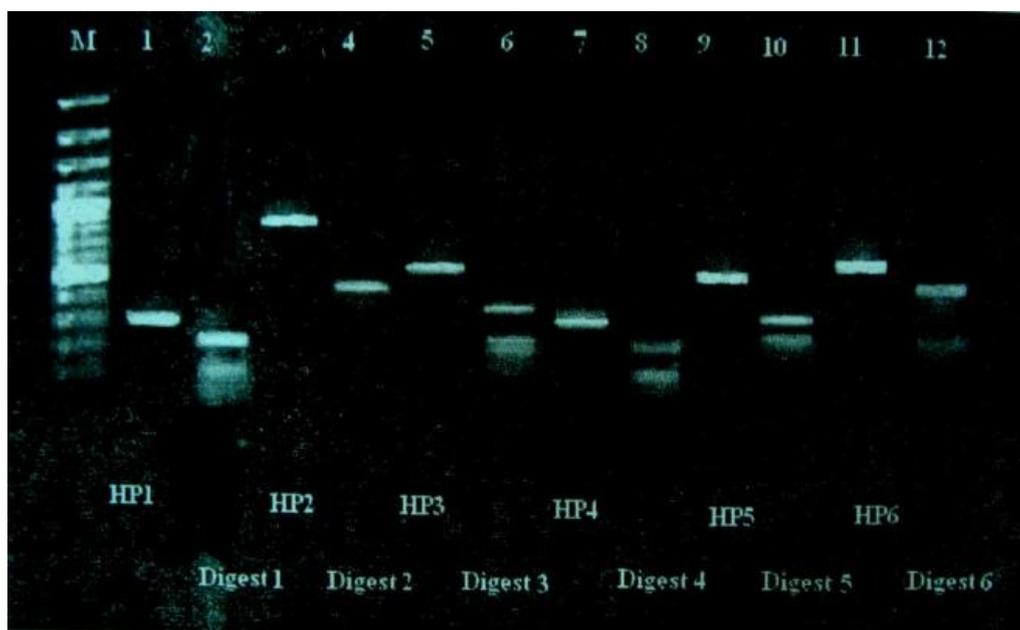


Fig: 2-PCR, RFLP, agaorse gel electrophoresis pattern products of β -globin gene cluster.

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 β^s globin 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 β^s cluster haplotypes is a useful tool for the detection of high risk patients with SCA¹¹⁻¹³. 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 severity¹²⁻¹³, 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 disorders¹⁷⁻¹⁸. The last point double heterozygote subtype of Benin, Bantu, Cameron, with Arabia-Indian or Senegal haplotype makes admixture haplotypes with intermediate to worst prognostic diseases¹⁹.

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 .

Acknowledgements

Proposal of First study was supported by Management company: Education and research management of Khuzestan Province Research registration N0: 84I121 March 2008. Second Study was a thesis I pediatric hematology oncology, supported by Research center For Thalassemia And Hemoglobinopathy, Ahvaz Jundishapur University of Medical sciences Ahvaz Iran. May 2010. Thesis No: d/425.

References

- 1- Zandian KM, Pedram M, Kianpour Ghahfarokhi F. Pre-marriage sickle cell screening program in south region of Iran, a pilot study on 50 cases of sickle trait. *Iran J Blood Cancer* 2009; 1(2): 55-7.
- 2- Zandian KM, Keikhaie B, Pedram M, Kianpour Ghahfarokhi F. Prenatal diagnosis and frequency determination of alpha and beta thalassemia, SDC, and H hemoglobinopathies: globin mutational genes analysis among voluntary couples from Ahvaz. *Iran J Blood Cancer* 2009; 1(3): 95-8.
- 3- Molavi MA, Naderi A, Zandian KM, Pedram M, Saadati M. Myocardial ischemia in a patient with diagnosis of sickle β^0 Thalassemia and its response to Hydroxyurea. *Jundishapur Sci Med J* 2011; 19(3): 240-48.
- 4- Zandian KM, Nateghi J, Keikhaie B, Pedram M, Hafezi-Nejad N, Hadavi V. α -thalassemia mutations in Khuzestan Province, Southwest Iran. *Hemoglobin* 2008; 32(6): 546-52.
- 5- Keikhani B, Zandian K. A report on new findings in view of revising on how to prevent major thalassemia and sickle cell anemia in Iran. *Sci Med J Ahvaz Univ* 2004; 3(42): 77-85.
- 6- Zandian KM, Parsi M, Pedram M, Najafian M. Pregnancy and sickle cell hemoglobinopathy. *Sci Med J Ahvaz Univ* 2003; 35: 28-35.
- 7- Sharaf Aldinzadeh N, Majdinasab N, Zandian KM, Taherpour K, Moravej A, Pipelzadeh M. Determining of hydroxyurea influence on TCD (Transcranial Doppler) of sickle cell patients. *IUMS* 2009; 27(95): 268-74.
- 8- Haghshenass M, Beigi F, Clegg J, Weatherall D. Mild sickle-cell anaemia in Iran associated with high levels of fetal haemoglobin. *J Med Gene* 1977; 14(3): 168-71.
- 9- Rahimi Z, Karimi M, Haghshenass M, Merat A. β -Globin gene cluster haplotypes in sickle cell patients from southwest Iran. *Am J Hematol* 2003; 74(3): 156-60.
- 10- Rahgozar S, Poorfathollah AA, Moafi AR, Old JM. β S gene in Central Iran is in linkage disequilibrium with the Indian-Arab haplotype. *Am J Hematol* 2000; 65(3): 192-95.
- 11- El-Hazmi MA, Wary As, Bashir N, Beshiawi A, Hussin IR. Haplotypes of beta-globin gene as prognostic factors in sickle cell disease. *East Mediterr Health J* 1999; 5(6): 1154-58.
- 12- Powar DR, Chanl, Schroeder WA. β -gene cluster haplotype in sickle cell anemia clinical implications. *Am J Pediatric Hematol Oncol* 1990; 12(3): 367-74.
- 13- Powars D, Hiti A. Sickle cell anemia, β s gene cluster haplotypes as genetic marker for severe disease expression *Am J Dis Child* 1993; 147(11): 1197-202.
- 14- Steinberg MH. Genetic etiologies for phenotypic diversity in sickle cell anemia. *Sci World J* 2009; 9: 46-67.
- 15 - Nagel RL, Erlingsson S, Fabry M, Croizat H, Susuka S, Lachman H, et al. The Senegal DNA haplotype is associated with the amelioration of anemia in African-American sickle cell anemia patients. *Blood* 1991; 77 (6): 1371-75.
- 16- Kulozik A, Wainscoat J, Serjeant G, Kar B, Al-Awamy B, Essan G, et al. Geographical survey of β s-globin gene haplotypes: evidence for an independent Asian origin of the sickle-cell mutation. *Am J Hum Genet* 1986; 39(2): 239-44.
- 17- Nagel RL, Rao SK, Dunda-Belkhodja O, Connolly MM, Fabry ME, Georges A. The hematologic characteristics of sickle cell anemia bearing the Bantu haplotype: the relationship between G gamma and HbF level. *Blood* 1987; 69(4): 1026-30.
- 18- Sarnaik SA, Ballas SK. Molecular characteristics of pediatric patients with Sickle cell anemia and stroke. *Am J Hematol* 2001; 67(3): 179-182.

- 19- Ayatollahi M, Zakernia M, Haghseenas M. molecular analysis of Iranian with sickle cell disease. J Trop Pediatr 2005; 51(3): 136-40.