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Ava II Site as a Marker of β-Globin Gene Polymorphism, among Normal and Sickle Cell Patients in Iran

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ABSTRACT

The restriction enzyme Ava II detects the base change of the intervening sequence II (IVS II) which is used as one of the markers of β-globin gene polymorphism. This study was conducted to determine the frequency of the Ava II site on the β-globin gene among normal people and patients with sickle cell syndrome (SCS) in Iran. DNA fragments containing the IVS II region of the β-globin gene from 30 patients with sickle cell anemia and 30 normal individuals were amplified using PCR technique. The amplified DNA of various subjects was digested with the Ava II enzyme and the products were examined by electrophoresis on agarose gel. The Ava II site was present in all 60 chromosomes of the patients while it was present with a frequency of 78% in the chromosomes of the normal individuals. The results were compared with those of Afro-American blacks, Italian and some Indian populations. Our results demonstrate the association of the Ava II site in the β-globin gene with sickle cell mutation in the Iranian population. *Iran. Biomed. J. 5 (4): 117-119, 2001*

Keywords: Sickle cell syndrome (SCS), Beta globin gene, Polymorphism, Iran

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INTRODUCTION

The sickle cell anemia can cause numerous disorders that vary with respect to degree of anemia, frequency of crises, extent of organ injury and duration of survival [1]. This disease affects over 2 million people in Nigeria with a generally severe clinical course [1]. Several hemoglobinopathies are prevalent in the Middle East. Like other countries in this region, Iran has also a large number of patients with hemoglobinophaties including thalassemia [2]. The frequency of sickle cell gene, though with less serious than thalassemia, has also been reported [3]. On account of their level of fetal hemoglobin (HbF), Iranian patients, compared to Afro-American patients, have a less severe clinical picture [4]. Advances in methodology in recombinant DNA technology have allowed the elucidation of the molecular basis of some genetic factors, which may affect the clinical severity of sickle cell disease [5]. The Ava II marker, a restriction endonuclease, detects a site present in the IVS-II region of β-globin gene. This site in association with other genetic determinants within the β-gene cluster, affects clinical conditions of the sickle cell patients [6]. The present investigation was conducted to determine the frequency of this marker among normal people and patients with sickle cell syndrome (SCS) of Iran.

MATERIALS AND METHODS

Patients. Blood samples from 30 sickle cell patients were obtained from Hematology Research Laboratory of Shiraz University of Medical Sciences. Sickle cell anemia was identified by a positive sickling test and confirmed by hemoglobin electrophoresis at pH 9.2 on cellulose acetate by application of a 300-volt current for 35 minutes. Thirty normal individuals with no signs and symptoms of SCS were selected from the laboratory personnel. Patients and normal controls were randomly selected from both males and females.

Hematological analysis. Complete blood count was carried out using a Counter Model (Sysmex, England). Sickle cell hemoglobin was quantified after elution from a microcolumn of diethyl-aminoethyl cellulose (DE 52) resin as described earlier [3]. Fetal hemoglobin quantity was performed by alkaline denaturation procedure [7].

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DNA extraction. One ml of cold lysis buffer, containing 0.2 g Tris, 21 g sucrose, 0.2 g $MgCl_2$, 2 ml Triton 100X in 200 ml of distilled water, was added to 500 µl of EDTA-treated peripheral blood and centrifuged. The precipitate was washed three times and genomic DNA was extracted by adding 100 ml of 50 mM NaOH. Subsequently, the tube was heated in a boiling water bath to solublize the DNA.

Polymerase chain reaction (PCR). DNA fragments containing Ava II site (16 bp 3′ to 5′ splice junction of IVS II region of β-globin gene) from 30 sickle cell patients and 30 normal individuals were amplified with the standard PCR condition [8], using the following pair of primers: 5′-GTGCTCGGTGCCTTTAG-3′ and 5′-CGAT CC TGAGACTTCCACAC-3′ (from TIB, Molbiol, Berlin, Germany).

Restriction endonuclease analysis of the amplicon DNA. The amplified product (30 µl) containing Ava II site (237 bp) was treated with 1.5 µl of 10U/ml of Ava II restriction enzyme solution (Boehringer, Mannheim Germany), and digested at 37ºC for 1 hour in SURE/cut buffer A in a total volume of 35 µl.

Subsequently, the digestion products were separated according to size on a 2% agarose gel (Pharmacia, Sweden), by application of a 70-volt current for 45 minutes and visualized by ethidium bromide staining under ultraviolet light.

RESULTS AND DISCUSSION

Sickle cell disease is a major health problem in many countries with a wide spectrum of clinical severity. Some genetic factors affect the clinical severity of sickle cell disease, including fetal hemoglobin level [9], and β-gene haplotypes associated with the βS-chromosomes [6]. The highest prevalence of hemoglobin S (HbS) has been found in Afro-American people [1]. The results of DNA polymorphisms linked to the βS- gene suggest that this gene arose from three independent mutations in tropical Africa [10]. The most common haplotype in βS-chromosome is found in Benin (neighboring Nigeria) and central West Africa. The second haplotype is prominent in Senegal and the African West Coast. The third one is seen in the Central African Republic (Bantu-speaking).

The presence of sickle cell gene with lower frequency has also been reported in southern India, Saudi Arabia, the Mediterranean basin and the Middle East [10]. In the previous study, we reported the frequency of the sickle cell gene in the south of Iran [3]. Iranian patients with sickle cell anemia, on account of their genetic factors, compared to Afro-American patients, have a less severe clinical picture [4]. In the present study, the frequency of the Ava II site (as a marker of β-globin gene polymorphism) among normal people and patients with SCS was studied. Figure 1 shows the agarose gel electrophoresis of the amplified DNA from eight subjects and Figure 2 shows the agarose gel electrophoresis of the amplified DNA along with the digested products with Ava II for five patients and a normal subject.

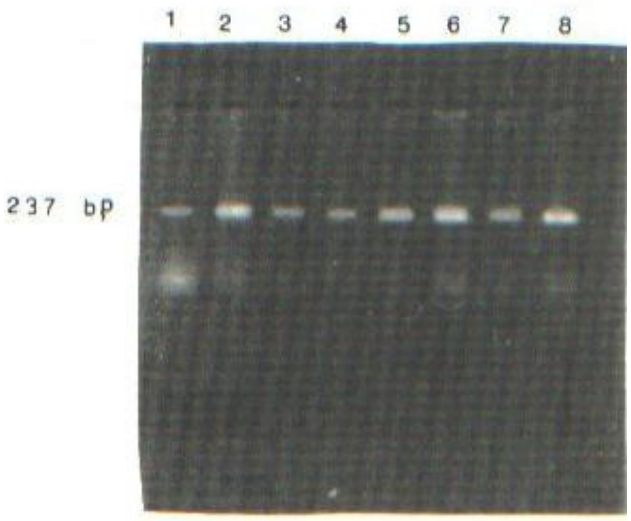

Fig. 1. The agarose gel electrophoresis of the amplified DNA from 8 subjects.

Lanes 3, 7, 9 and 11 in Figure 2 represent the presence of Ava II site in homozygous subjects. However, the single band with a molecular weight (MW) of about 120 bp observed in these four lines is actually composed of a mixture of two equal size products due to the fact that the Ava II site lies almost exactly in the middle of the amplified DNA. Lane 5 shows a heterozygous state with two different bands. One band with a MW of about 237 bp corresponding to the original amplified DNA with no Ava II site and another band which is a mixture of two equal size products as in lanes 3, 7, 9 and 11. While this site was present in all 60 choromosomes (100%) of the patients with SCS, it was found with an incidence of 78% in normal individuals. These data were comparable to those

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of Afro-American blacks (15.8%), Greeks (15.7%), Italian (19%), and some Indians (23%) [11].

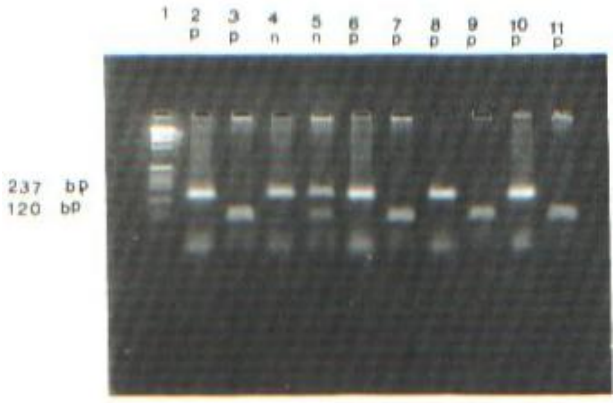

Fig. 2. Lane l, the molecular marker (0.07-12.2 kb); Lanes 2, 4, 6, 8 and 10 show the PCR products of the amplified DNA from 5 patients (p) and a normal (n) subject; Lanes 3, 5, 7, 9 and 11 are the enzyme digested products corresponding to the amplified DNA on its left line.

While the same three haplotypes are associated with the βS-gene in black Americans and Jamaicans, only the Benin and Senegal haplotypes are prevalent among North Africans, Greeks and Italians [10]. On the other hand, the HbS gene found among eastern Saudi Arabians, Iranians and some Indians had different DNA structure not encountered in Africa, and probably represents a fourth independent occurrence of the sickle cell mutation [12]. This haplotype is associated with much higher HbF level (15-40%), fewer vaso-occlusive sickle crisis, lower complication rate and mild clinical conditions [13]. Therefore, the geographical distribution of the Asian βS-haplotype, corresponds to a benign clinical presentation of sickle cell anemia, even in those homozygous for the disease [14]. Thus the geographical survey of βS-globin gene haplotype among Iranians, like the Saudi Arabians, is the most informative for the further studies.

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