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آموزش مهارت های کاربردی در تدوین و چاپ مقاله
Co-Inheritance of Sickle Cell Trait and Thalassemia Mutations in South Central Iran

*N Saleh-gohari 1, M Mohammadi-Anaie 2

1. Dept. of Genetic, Medical School, Kerman University of Medical Sciences, Kerman, Iran
2. Genetic laboratory, Afzalipour Hospital, Kerman, Iran

*Corresponding Author: Tel: +98-341-3222246 Email: n_salehgohari@kmu.ac.ir

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Introduction

Sickle cell anemia inherits as an autosomal recessive disorder that was first described by Herrick in 1910 and its alleles are frequent in regions where malaria is endemic. A homozygote A to T transversion results in the substitution of glutamic acid by valine in the sixth amino acid of the human βS-globin chain. Inheritance of a single mutated allele results in sickle cell trait (SCT), while homozygous mutations, one from each parent, cause clinical severity of sickle cell disease (SCD) (1). The disease is a multi-organ illness characterized by the production of abnormal hemoglobin S (HbS). The interaction of HbS tetramers results in the formation of polymers that cause red blood cells to become rigid and form sickle in deoxygenate condition (1, 2). Repetitions of the situation cause the cells to become fragile and subject to easy lysis, which produces chronic anemia (2, 3). Sickle cell is a major health problem that occurs commonly in people of African, Middle Eastern, Indian and Mediterranean backgrounds (4). The prevalence of the βS gene has been reported in about 40% of tribal groups of India (5). It occurs at similar gene frequencies across equatorial Africa, while reaching 1% - 2% on the North African coast and South Africa (6). About 7 to 9% of African Americans (5, 7), and 4.6% of Turkish (8) are carriers of this mutation. Habibzadeh and colleagues (9) announced the sickle cell gene frequency of 1.5 % in the south of Iran, while this value was reported about 0.01 in Pars province of the country (10).
Genetic factors such as α or β-globin gene mutations can modulate the hematological diagnostic data and clinical expression of the sickle cell, when co-inherited with the β gene (11). It has been reported that co-inheritance of SCT and HbD results moderate to severe anemia (12). Coexistence of α-thalassemia (αthal) lowers the mean cell volume (MCV) and the mean corpuscular hemoglobin (MCH) that results in milder anemia. But this condition causes a reduction in hemolysis and an increase in total hemoglobin which makes patients more prone to vaso-occlusive and painful crises of the disease (13). Phenotype of the β-globin gene defect determines the severity of the co-inherited sickle cell mutation (4). The absence synthesis of β-globin chain (β0) results in a severe disease, while the reduced one (β+) cause a milder clinical picture of the disease.

Therefore, HbS and thalassemia may interact to produce specific effects on haematological parameters. Understanding the influence of α and β-thalassemia (βthal) mutations on hematological characteristics of the SCT people has been helpful to diagnosis of the disease.

In the present study, we aim to determine the incidence of co-inheritance as well as interaction of SCT and αthal/βthal mutations in south and south central of Islamic Republic of Iran.

Materials and Methods

In a national premarital screening program red cell indices were measured to identify risk for specific hemoglobinopathies (sickle cell and thalassemia). Hemoglobin electrophoresis was performed in subjects with potential for the diseases. A group of 179 individuals found microcytic hypochromic (MCV< 82 and MCH < 26 pg) and positive HbS were examined to confirm the primary diagnosis between May 2006 and February 2011. A genetic counselor reassured the individuals regarding the objectives and explained the aims. After obtaining a consent sheet, we collected 8 ml whole blood from their brachial vein in tubes containing 200 µl EDTA (Ethylene Diamine Tetra-acetic Acid). Genomic DNA was isolated from leukocytes of the whole blood using the salt-saturation method as previously prescribed (14). The isolated DNA was applied to verify the sickle cell gene defect and to screen for any coexisting αthal/βthal thalassemia mutations.

The sickle cell hemoglobinopathy was confirmed using the PCR followed by restriction fragment length polymorphism (RFLP) method. To accomplish this, a 443bp DNA fragment of β-globin gene was amplified using a forward primer (ACCTCACCCTGTGGAGCCAC) and a reverse primer (GAGTGGACAGATCCCCAAAGGACTCAAGGA) (15).

RFLP was performed via digestion of the PCR products by DdeI enzyme. Normally, two restriction sites for DdeI exist in the DNA sequences of the amplified beta-globin gene fragment. Digestion of the PCR products by the enzyme results a 201bp, a 175bp and a 67bp DNA fragment in the wild type gene (15). The sickle cell mutation changes one of the restriction sites, giving a 376bp and a 67bp DNA fragment flowing DdeI digestion (Fig. 1).

![Fig. 1: Digestion a 443bp PCR product of β-globin gene by DdeI enzyme. A healthy subject (Nor) gives three bands (a 201bp, a 175bp and a 67bp). A heterozygote β (Het) individual results in four bands (a 376bp, a 201bp, a 175bp and a 67bp). A homozygote β (Hom) patient produce two bands (a 376bp and a 67bp)](image)

All subjects were screened for any αthal/βthal mutations. In βthal work-up, the 15 most common β-globin gene defects were examined by
amplification refractory mutations system-polymerase chain reaction (ARMS-PCR) technique (16). Suspicious αthal carriers were screened for the common deletion defects –α3.7, –α4.2, –αMED and αααanti3.7 triplication using the gap-polymerase chain reaction (gap-PCR) method (17). The Vienna-lab β-globin strip assay kit (viennalab labor-dagnostika GmbH, Vienna, Austria) was used to identify those thalassemia mutations that not covered by the above methods. Rare and unknown mutations were studied using DNA sequencing.

Results

A cohort of 179 subjects was screened for sickle cell and αthal/βthal mutation. All the subjects were married and some of them had children. They comprised 23 couples and 133 independent individuals who their spouse was not included in this study due to lack of HbS. Of the cohort, 56 patients came from Hormozgan as the molecular analyzing laboratory did not exist in their province. From the 23 studied couples 13 were consanguineous.

Among the studied group, 19 different single or compound genotypes were found in globin genes. Combination of sickle cell trait and single α-globin gene deletion (HbS/Normal, –α3.7/αα) was the most common genotype (37.4%), while the second most frequent gene defect was sickle cell trait (32.4%) without αthal/βthal mutation (HbS/Normal). Three subjects inherit sickle cell, αthal and βthal together (Table 1). Two patients with sickle cell trait, HbD and αthal deletion were detected in this survey (Table 1).

The frequency of α-globin and sickle cell mutations was 57.5% in overall samples (Table 2). Sickle cell trait and minor βthal mutation (β+/βd+) was found in 6.7% of the referred cases (Table 2). Interaction between these two genotypes reduced Hb, Hct, MCV, MCH, & Hb and increased HbA2, HbF, & HbS more than the other single or multiple mutations (Table 3).

The studied cases of Kerman province were referred commonly from southern cities of the territory where Kahnooj, Jiroft, Bam, and Baft are located (Table 4). A few subjects were from cities of Shahr Babak and Kerman as the capital of province.

Table 1: Alfa and beta-globin gene mutations found in Kerman and Hormozgan Provinces, Iran

| Genotype | %   | n   |
|----------|-----|-----|
| HbS/Normal, –α3.7/αα | 37.4 | 67  |
| HbS/Normal | 32.4 | 58  |
| HbS/Normal, –α3.7/αα | 15.6 | 28  |
| HbS/Normal, IVS1-5/Normal | 4.4 | 8   |
| HbS/HbS, –α3.7/αα | 1.6  | 3   |
| HbS/Normal, IVS1-5/Normal | 1.1  | 2   |
| HbS/Normal, –α3.7/–α3.7, Fr8-9/Normal | 0.5  | 1   |
| HbS/Normal, –α3.7/–α3.7, IVS1-5/Normal | 0.5  | 1   |
| HbS/Normal, –α3.7/–α3.7, IVS1-5/Normal | 0.5  | 1   |
| HbS/Normal, –α3.7/–α3.7, IVS1-5/Normal | 0.5  | 1   |
| HbS/Normal, –α3.7/–α3.7, IVS1-5/Normal | 0.5  | 1   |
| HbS/Normal, –α3.7/–α3.7, IVS1-5/Normal | 0.5  | 1   |
| HbS/Normal, βs & βthal | 1.1  | 3   |
| HbS/Normal, α2 IVS1-5nt/Normal | 0.5  | 1   |
| HbS/Normal, α2 IVS1-5nt/Normal | 0.5  | 1   |

Table 2: Frequency of HbS, α/β-thalassemia and HbD mutations found in Kerman and Hormozgan Provinces, Iran

| Mutation | n | % |
|----------|---|---|
| βs       | 59 | 33 |
| βs & βthal | 12 | 6.7 |
| βs & αthal | 103 | 57.5 |
| βs & αthal & βd | 2 | 1.1 |
| βs & αthal & βd | 3 | 1.7 |
| Total | 179 | 100 |
Table 3: Hematologic data of sickle cell and/or thalassemia cases from Kerman and Hormozgan Provinces, Iran

| Mutations          | Hb(g/dL) | HCT(%) | MCV(fL) | MCH(pg) | HbA(%) | HbA2(%) | Hbf(%) | HbS(%) |
|--------------------|----------|--------|---------|---------|--------|---------|--------|--------|
| β^c               | 13.194   | 39.926 | 75.964  | 26.011  | 60.835 | 3.071   | 0.835  | 35.504 |
| β^c & βthal (het)^a | 9.17     | 28.856 | 68.82   | 22.19   | 5.2125 | 4.58    | 18.54  | 72.76  |
| β^c & αthal (het) | 13.096   | 39.475 | 73.659  | 24.564  | 63.140 | 3.097   | 0.894  | 34.163 |
| β^c & αthal (homo)^b | 13.093  | 39.932 | 69.479  | 22.536  | 66.456 | 3.207   | 1.133  | 29.778 |

^a heterozygote; ^b homozygote

Table 4: Geographical distribution of the referred sickle cell and/or thalassemia cases from Hormozgan Province and Different Cities of Kerman, Iran

| City          | n  | %  |
|---------------|----|----|
| Hormozgan     | 56 | 31.3|
| Kahnooj       | 48 | 26.8|
| Jiroft        | 42 | 23.5|
| Baft          | 17 | 9.5 |
| Bam           | 7  | 3.9 |
| Kerman^a      | 7  | 3.9 |
| Shahr Babak   | 2  | 1.1 |
| Total         | 179| 100|

^a Capital of Kerman Province, Iran

Discussion

The high co-inheritance of sickle cell trait and α-globin gene deletions (HbS/Normal, –α3.7/αα) was predictable, since α-globin deletion was previously reported as the most common globin gene mutation in Kerman province (18), other regions of the country (19-21) and what has been reported in other studies around the world (22, 23). As can be seen in table 3, the interaction of α-globin gene deletions with sickle cell mutation did not significantly increase the level of HbS, compared to βthal mutation.

In the present study, four different types of β^c/βthal mutation were detected (Table 1). Among these, IVSII-1 and Fr 8-9 are classified as β^c, while -88 and IVSII-5 considered as β^c. The severity of sickle cell depends on the nature of the co-inherited βthal mutations, hence β^c thalassemia results in a more severe clinical course similar to SCD, while HbS-β^c thalassemia is usually associated with a milder clinical course (28). Although inheritance of SCT and any of the βthal mutations may result the birth of SCD children, combination of αthal with them may modulate the severity of the disease. Interaction of SCT, βthal, αthal and/or HbD mutations was not possible due to the small number of patients having all of the gene defects together (Table 2).

In Kerman Province, the SCT is limited to definite geographical areas. The highest cases were from Kahnooj and Jiroft (Table 4), at the border of Hormozgan province where 57 suspicious SCT subjects were referred to our laboratory. This implies that sickle cell mutation was spread by gene flow from Hormozgan province to these regions. Thalassemia and sickle cell mutations confer resistance to malaria, and high prevalence of the both gene defects are found in the same areas due to
the advantage of heterozygosity. It is surprising that Bam, with the highest incidence rate of $\alpha$thal and $\beta$thal carriers (24), was not among the high frequent sickle cell carrier regions. This agrees with the gene flow phenomenon, since Bam is not contiguous to Hormozgan province. No patients were referred to our laboratory from northern cities (Rafsanjan, Sirjan, Zarand, and Ravar) of Kerman territory, since the prevalence of $\beta$thal was virtually reported zero for this area (24).

Conclusion

Co-inheritance of SCT with $\beta / \beta$thal is mainly frequent in the south parts of Kerman province. Since the clinical manifestations of SCT are influenced by associated of $\beta$thal and/or $\alpha$thal mutations, the birth of affected people with SCD is inevitable. This disorder imposes a significant burden on health resources and furthermore has psychological effects on relatives. These authors suggest an educational and premarital screening program for the at risk population. Further investigations in other regions of Islamic republic of Iran will be helpful for the support of such a program.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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