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Original Article

Co-Inheritance of Sickle Cell Trait and Thalassemia Mutations in South Central Iran

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Abstract

Background: We aimed to determine the incidence of co-inheritance as well as interaction of sickle cell trait (SCT) and $\alpha^{thal}/\beta^{thal}$ mutations in south and south central of Iran.

Method: We employed a PCR and restriction fragment length polymorphism techniques to confirm diagnosis of sickle cell trait. All subjects were screened for any α/β –thalassemia mutations using a gap-polymerase chain reaction and amplification refractory mutations system.

Results: Our results showed combination of sickle cell trait and β-globin mutation results in a severe clinical course of similar to sickle cell disease, while coinheritance of α-globin gene defects usually modulates the clinical course. A coexistence of sickle cell trait and α-globin gene mutation was the frequent genotype in overall samples (57. 5%).

Conclusion: Sickle cell trait mainly co-inherits with α-globin gene mutation in the south and south central region of Iran. This combination modulates hematological indices and interferes with the SCT diagnosis.

Keywords: Sickle cell trait, β-thalassemia, α-thalassemia**,** Iran

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 β^S-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 β^S 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^S gene (11) .It has been reported that co-inheritance of SCT and HbD results moderate to severe anemia (12). Coexistence of α-thalassemia (α^{thal}) 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-oclosive 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 (β^0) 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 (β^{thal}) 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 $\alpha^{thal}/\beta^{thal}$ 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 $\alpha^{thal}/\beta^{thal}$ 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).

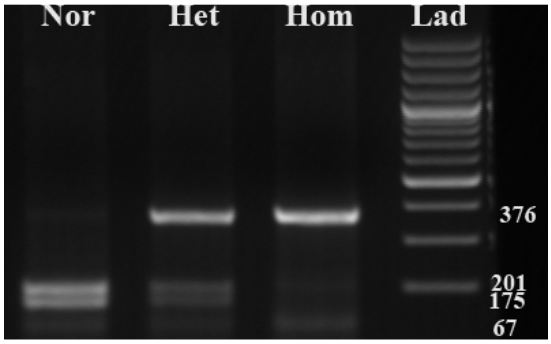

Fig. 1: Digestion a 443bp PCR product of β-globin gene by DdeI enzyme. A healthy subject (Nor) gives three bands (a 201bp, a 175bp and a 67bp). A heterozygote β^s (Het) individual results in four bands (a 376bp, a 201bp, a 175bp and a 67bp). A homozygote β^s (Hom) patient produce two bands (a 376bp and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

All subjects were screened for any $\alpha^{thal}/\beta^{thal}$ mutations. In β^{thal} 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 α^{thal} carriers were screened for the common deletion defects $-\alpha^{3.7}$, $-\alpha^{4.2}$, – –MED and $\alpha\alpha\alpha^{anti\text{-}3.7}$ triplication using the gap-polymerase chain reaction (gap-PCR) method (17). The Vienna-lab β-globin strip assay kit (viennalab labor-diagnostika 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 $\alpha^{thal}/\beta^{thal}$ 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 $\alpha^{thal}/\beta^{thal}$ mutation (HbS/Normal). Three subjects inherit sickle cell, α^{thal} and β^{thal} together (Table 1). Two patients with sickle cell trait, HbD and α^{thal} 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 β^{thal} mutation (β^s/β^{thal}) 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/–α3.7	15.6	28
HbS/Normal, IVSI-5/Normal	4.4	8
HbS/HbS, –α3.7/αα	1.6	3
HbS/Normal, IVSII-I/Normal	1.1	2
HbS/Normal,–α3.7/–α3.7,Fr8-9/Normal	0.5	1
HbS/Normal,–α3.7/–α3.7,IVSI5/Normal	0.5	1
HbS/Normal, –α3.7/αα, IVSI-5/Normal	0.5	1
HbS/Normal, -88(C>T)/Normal)	0.5	1
HbS/Normal, –α3.7/–α4.2	0.5	1
HbS/HbS, –α3.7/–α3.7	0.5	1
HbS/HbS, –α4.2/–α4.2	0.5	1
HbS/HbD, –α3.7/–α3.7	0.5	1
HbS/HbS	0.5	1
HbS/Normal, Fr 8-9/Normal	0.5	1
HbS/HbD, –α3.7/αα	0.5	1
HbS/Normal, α2 IVSI–5nt/Normal	0.5	1
HbS/Normal, $\alpha\alpha\alpha^{anti3.7}$	0.5	1
Total	100	179

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

Mutation	n	%
β^s	59	33
β^s & β^{thal}	12	6.7
β^s & α^{thal}	103	57.5
β^s & α^{thal}& β^d	2	1.1
β^s & α^{thal} & β^{thal}	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^s	13.194	39.926	75.964	26.011	60.835	3.071	0.835	35.504
β^s & β^{thal} (het)[a]	9.17	28.856	68.82	22.19	5.2125	4.58	18.54	72.76
β^s & α^{thal} (het)	13.096	39.475	73.659	24.564	63.140	3.097	0.894	34.163
β^s & α^{thal} (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 β^{thal} mutation.

In the present study, four different types of β^s/β^{thal} mutation were detected (Table 1). Among these, IVSI-5 was found to be the commonest mutation, followed by IVSII-I, Fr 8-9 and -88nt. The incidence of β^{thal} mutations in Kerman province was previously reported (24) the same as what was found in the present study. Therefore, it was not surprising to find the incidence pattern of β^s/β^{thal} similar to that previously detected. Coexistence of β^{thal} mutations is a modulating factor that may worsen the clinical and hematologic features of sickle cell patients (25). Our data shows that the combination of β^s/β^{thal} decreases levels of Hb, Hct, MCV, MCH, Hb and increases levels of HbA2, HbF and HbS more than the other single or multiple globin genes mutations (Table 3). Earlier studies showed that co-inheritance of β^s/β^{thal} studies decrease MCV, MCH and Hb indices as compared with SCT patients (26, 27). These findings are in agreement with the fact that coinheritance of β^s/β^{thal} will worsen the clinical course of the sickle cell from mild to sickle cell disease.

Among the detected β^{thal} mutation, IVSII-I and Fr 8-9 are classified as β^0, while -88 and IVSI-5 considered as β^+. The severity of sickle cell depends on the nature of the co-inherited β^{thal} mutations, hence β^0 thalassemia results in a more severe clinical course similar to SCD, while HbS-β^+ thalassemia is usually associated with a milder clinical course (28). Although inheritance of SCT and any of the β^{thal} mutations may result the birth of SCD children, combination of α^{thal} with them may modulate the severity of the disease. Interaction of SCT, β^{thal}, α^{thal} 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 α^{thal} and β^{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 β^{thal} was virtually reported zero for this area (24).

Conclusion

Co-inheritance of SCT with β^{s}/β^{thal} is mainly frequent in the south parts of Kerman province. Since the clinical manifestations of SCT are influenced by associated of β^{thal} and/or α^{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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References

1. Steinberg MH (2001). Sickle cell anemia, the first molecular disease: overview of molecular etiology, pathophysiology, and therapeutic approaches. *Scientific World Journal*, 8: 1295–1324.
2. Schechter AN (2008). Hemoglobin research and the origins of molecular medicine. *Blood*, 112(10): 3927-38.
3. Sebastiani P, Wang L, Nolan VG, Melista E, Ma Q, Baldwin CT, et al. (2008). Fetal hemoglobin in sickle cell anemia: Bayesian modeling of genetic associations. *Am J Hematol*, 83(3): 189-95.
4. Mukherjee MB, Nadkarni AH, Gorakshakar AC, Ghosh K, Mohanty D, Colah RB (2010). Clinical, hematologic and molecular variability of sickle cell-β thalassemia in western India. *Indian J Hum Genet*, 16: 154-8.
5. Schneider RG, Hightower B, Hosty TS, et al (1976). Abnormal hemoglobins in a quarter million people. *Blood*, 48(5): 629-37.
6. Jastaniah W (2011). Epidemiology of sickle cell disease in Saudi Arabia. *Annals of Saudi Medicine*, 31(3): 289-293.
7. Heller P, Best WR, Nelson RB, Becktel J (1979). Clinical implications of sickle-cell trait and glucose-6-phosphate dehydrogenase deficiency in hospitalized black male patients. *N Engl J Med*, 300(18): 1001-5.
8. Basak AN, Tuzman S (2011). Genetic predisposition to β-thalassemia and sickle cell anaemia in Turkey: a molecular diagnostic approach. *Methods Mol Biol*, 700: 291-307.
9. Habibzadeh F (2000). The sickle cell gene frequency in southern Iran. *Journal of tropical pediatrics* 46(3): 181.
10. Habibzadeh EM, Yadollahie M, Ayatollahie F (1999). The prevalence of sickle cell syndrome in south of Iran. *Iran J Med Sd*, 24: 32-34.
11. Noguchi CT, Schechter AN, Rodgers GP (1993). Sickle cell disease pathophysiology. *Baillieres Clin Haematol*, 6(1): 57-91.
12. Zakerinia M, Ayetollahi M, Rastegar M, Amanat SH, Askarinejad AR, Amirghofran S, et al. (2011). Hemoglobin D (Hb D Punjab/Los angles and HbD Iran) and co-inheritance with alpha and beta thalassemia in Iran. *Iran Red Crescent Med J*, 13(7): 493-498.

13. Higgs DR, Aldridge BE, Lamb J, Clegg JB, Weatherall DJ, Hayes RJ, et al (1982). The interaction of alpha-thalassemia and homozygous sickle-cell disease. *N Engl J Med*, 17;306(24): 1441-6.
14. Miller SA, Dykes DD, Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res,* 16: 1215-1216.
15. Old J (1996). Haemoglobinopathies. *Prenat Diagn*, 16(13): 1181-6.
16. Newton CR, Graham A, Heptinstall LE, Powell SJ, Summeres C, Kalsheker N, et al. (1989). Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res*, 17 (7): 2503-16.
17. Oron-Karni V, Filon D, Oppenheim A, Rund D, et al (1998). Rapid detection of the common Mediterranean alpha-globin deletions/rearrangements using PCR. *Am J Hematol*, 58(4): 306-10.
18. Saleh-Gohari N, Khosravi-Mashizi A (2010). Spectrum of α-globin gene mutations in the Kerman Province of Iran. *Hemoglobin*, 34(5): 451-60.
19. Hadavi V, Taromachi AH, Malekpour M, Gholami B, Law HY, Almadani N, et al. (2007). Eluciding the spectrum of alpha-thalassemia mutations in Iran. *Haematologica*, 92(7): 992-3.
20. Neishabury M, Abbasi-Moheb L, Poorfathollah A, Kahrizi K, Keyhany E, Krugluger W, et al. (2001). Alpha-thalassemia: Deletion analysis in Iran. *Arch Irn Med*, 4 (4): 160-164.
21. Garshasbi M, Oberkanins C, Law HY, Neishabury M, Kariminejad R, Najmabadi H, et al. (2003). alpha-globin gene deletion and point mutation analysis among in Iranian patients with microcytic hypochromic anemia. *Haematologica*, 88(10): 1196-7.
22. Chong SS, Boehm CD, Higgs DR Cutting GR (2000). Single-tube multiplex-PCR screen for common deletional determinants of alpha-thalassemia. *Blood*, 95(1): 360-2.
23. Shaji RV, Srivastava A, Chandy M, Krishnamoorthy R (2000). A single tube multiplex PCR method to detect the common alpha+ thalassemia alleles. *Blood*, 95(5): 1879-80.
24. Saleh-Gohari N, bazrafshani MR (2010). Distribution of β-globin gene mutations in thalassemia minor population of Kerman/Iran province. *Iranian J Publ Health*, 39(2): 69-76.
25. Mohanty D, Mukherjee MB (2002). Sickle cell disease in India. *Curr Opin Hematol*, 9: 117-22.
26. Valavi E, Alemzadeh M, and Zandian K (2010). How to reach rapid diagnosis in sickle cell disease. *Iran J Pediatr*, 20(1); 69-74
27. Rahimi Z, Karimi M, Haghshenass M, Merat A (2003). B-globin gene cluster haplotypes in sickle cell patients from southwest Iran. *Am J Hematol*, 74; 156-160.
28. Weatherall DJ, Clegg JB (2001). *Thalassemia Syndromes*. 4th ed. Blackwell Scientific Publications, Oxford, pp.:1-846.