Cap +1 mutation; an unsuspected cause of beta thalassaemia transmission in Pakistan

Cap +1 mutasyon; Pakistan'da beta talasemi taşıyıcılığının şüphe götürmez bir nedeni

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Abstract

Objective: Thalassemia is one of the most common genetic disorders worldwide. Cap +1 mutation which causes 'silent beta thalassemia' is present around all ethnic groups of Pakistan. This study was designed to detect the frequency of Cap+1 mutation in Pakistani Population.

Materials and Methods: Molecular genetic for Cap+1 beta thalassemic mutation was done by extracting DNA from whole blood by using Genomic DNA Purification Kit (Gentra system USA). Amplification Refractory Mutation System (ARMS) primers were designed for detection of normal and mutant DNA.

Basic hematological parameters were performed by using automated analyzer (Sysmex KX-21). Cellulose acetate hemoglobin electrophoresis was done by using semi-automated technique (INTERLAB Roma Microtech Series Electrophoresis system 4.23).

Results: The frequency of Cap+1 mutation was observed 5% (10/200) in targeted thalassemic families (having patients with beta-thalassemia intermedia) while its frequency was observed 2% (12/600) in total thalassemic genes in Pakistani population.

Conclusion: Cap+1 (A-C) is a silent mutation and it has very minimum effect on beta globin synthesis because of which it produces very less clinical severity and certain important laboratory diagnostic tests like basic hematological parameters and Hb A2 levels are also remain in normal range. Therefore individuals with Cap+1 mutation may produce children with beta-thalassemia intermedia if they marry an individual with beta-thalassemia minor. Cap+1 (A-C) mutation is an unsuspected cause of beta thalassemia transmission in Pakistani population. This mutation can identify at molecular level. As this molecular defect is difficult to diagnose in Laboratory with routine laboratory tests because of that it has become a serious hindrance for thalassemia prevention program in Pakistan. *(Turk J Hematol 2009; 26: 167-70)*

Key words: Cap+1(A-C) mutation, Silent beta thalassaemia, Polymerase chain reaction (PCR)

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Özet

Amaç: Talasemi dünya genelinde en yaygın genetik bozukluklardan biridir. 'Sessiz beta talasemi'ye neden olan Cap +1 mutasyonu Pakistan'daki tüm etnik gruplarda görülmektedir. Bu çalışma Pakistan nüfusu içinde Cap +1 mutasyonun sıklığını belirlemek için tasarlanmıştır.

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Yöntem ve Gereçler: Cap +1 beta talasemik mutasyon tayini Genomik DNA Purifikasyon Kiti (Gentra system ABD) kullanılarak kandan ekstre edildi. Normal ve mutant DNA tespiti için Amplifikasyon Refrakter Mutasyon Sistemi (ARMS) primerleri tasarlandı.Temel hematolojik parametreler otomatik analizör (Sysmex KX-21) kullanılarak çalışıldı. Selüloz asetat hemoglobin elektroforezi yarı-otomatik teknik (INTERLAB Roma Microtech Series Electrophoresis system 4.23) kullanılarak yapıldı.
Bulgular: Cap+1 mutasyon sıklığı, hedef talasemik ailelerde (beta-talasemi intermedia hastaları) %5 (10/200) olarak gözlemlenirken bu sıklık Pakistan popülasyonu içindeki total talasemik genlerde %2 (12/600) olarak bulundu.
Sonuç: Cap+1 (A-C) sessiz bir mutasyondur ve beta globin sentezi üzerinde minimum bir etkiye sahiptir. Bu yüzden klinik önemi azdır. Hematolojik parametreler ve Hb A2 seviyeleri gibi önemli laboratuvar tanı testleri de normal düzeyde kalmaktadır. Bu nedenle Cap+1 mutasyonu taşıyan bireyler eğer beta-talasemi minör bulunan bir bireyle evlenirlerse beta-talasemi intermediyalı çocuğa sahip olabilir. Pakistan popülasyonunda Cap+1 (A-C) mutasyon taşıyıcılığı, beta talasemi taşınmasının nadir ve şüphe duyulmayan bir nedenidir. Bu mutasyon moleküler düzeyde belirlenebilir. Bu moleküler bozukluk Polimeraz Zincir Reaksiyon (PCR) yardımı olmadan laboratuvarda zor teşhis edilebileceği için Pakistan'daki talasemi önleme programı için ciddi bir engel haline gelmiştir. *(Turk J Hematol 2009; 26: 167-70)*
Anahtar kelimeler: Cap+1(A-C) mutasyon, sessiz beta talasemi, polimeraz zincir reaksiyonu (PCR)

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Introduction

Thalassemia is the most common genetic disorder across the globe [1,2]. The term thalassemia comes from a Greek word "thalas" which means the sea, and "emia" that stands for blood [3,4]. The composite word came into use because this type of anemia was originally described only in countries bordering the Mediterranean Sea. Thalassemia was not recognized as a clinical entity until 1925, when Cooley and Lee, described a syndrome occurring early in life that was associated with splenomegaly and bony deformities [4].

Beta thalassemia is the most common genetic disorder in Pakistan [5]. Pakistan has a population of 160 million people [6]. The annual rate of population growth is 3% and almost 40% of the population is below 15 years of age [7,8]. There are five major ethnic groups Punjabi, Pathan, Sindhi, Baluchi and Urdu speaking. Each ethnic group is subdivided into Casts or 'Biradris' of people ranging from a few thousands to a few millions. There is a very strong tradition for people to marry within their Biradris. Among very common custom is marriage between close relatives, especially the first cousins [8].

A number of studies have been done worldwide at molecular level to describe mutations, deletions and substitutions in beta globin gene causing beta thalassemia with different clinical severity. More than 200 causative molecular defects have so far been described in the ß-globin gene causing beta thalassemia [9]. Thirteen mutations are commonly reported in Pakistani population in which five are the most common mutations; these include IVS-1-5 (G-C), Frameshift (Fs) 8/9 (+G), Fs 41/42 (-TTCT), IVS-1-1 (G-T) and Del 619. Other mutations that were reported in Pakistani population are a few uncommon and some rare mutations [8,10]. Cap +1 (A-C) mutation is a silent mutation causes 'silent beta thalassemia' [10,11]. This mutation is present in all ethnic groups of Pakistan.

This study was designed to detect the prevalence of Cap+1 (A-C) mutation in Pakistan, to determine its effect on basic hematological parameters and Hb-A2 levels and to reveal the role of this mutation in propagation of beta thalassemia gene in Pakistani population.

Material and Methods

This molecular study was performed at Baqai Institute of hematology, Baqai Medical University, Karachi from March 2004 to April 2009. Venous blood samples from 610 patients with beta thalassemia major, beta thalassemia intermedia and beta thalassemia minor were collected in EDTA. Test population was divided into two groups; one group comprised of targeted families (having children with thalassemia intermedia) while the second group included random samples from patients with beta thalassemia major and minor.

All samples were tested by the modified method of Amplification of Refractory Mutation System (ARMS) for Cap+1 (A-C) mutation. Basic hematological parameters and Hb-A2 levels were also done on blood samples that had Cap+1 mutation.

Basic hematological parameters were determined by automated analyzer (Sysmex KX-21). Cellulose acetate hemoglobin electrophoresis was done by semi-automated technique (INTERLAB Roma Microtech Series Electrophoresis system 4.23).

DNA was extracted from whole blood by using Genomic DNA Purification Kit (Gentra system USA). Amplification refractory mutation system (ARMS) primers were designed for detection of normal and mutant DNA. A control pair of primers was included in each assay. Control primers A, B and C were amplified at 861 bp fragments from 3' end of the ß globin gene [12,13].

PCR was conducted with modified method in a mixture of 10 mmol/l tris (pH 8.3), 50 mmol/l KCl, 1.5 mmol/l MgCl2. 500 µM each dNTP's, 0.2 µmol/l of each primer, 0.5 units of Thermus aqaticus (Taq) 0.5 to 1 pg of genomic DNA was added to the PCR mixture in a total volume of 20 µl. The modified cycling reaction (DNA thermal cycle; Perkin-Elmer/Cetus) was programmed at 94°C for 1 minute (denature), 65°C for 1 minute (anneal) and 72°C for 1.5 minutes (extend). After 25 cycles, the samples were incubated for an additional 3 minutes at 66°C [12,13].

The amplified PCR products were observed using agarose gel electrophoresis and mutation was characterized with 100 bp or 50 bp ladder.

Table 1. Basic hematological parameters and Hb-A2 levels of beta thalassemia minor with Cap+1 mutation

Group	No. of samples	Hb (gm/dl)	RBC (millions/ul)	MCV (fl)	MCH (pg)	MCHC (gm %)	Hb-A2 (%)
Male	12	13.6 (±0.37)	4.8 (±0.34)	84.8 (±5.2)	29 (±1.37)	32.7 (±1.4)	3.0 (±0.12)
Female	10	12.0 (±0.71)	4.0 (±0.7)	80.4 (±3.1)	28.4 (±0.78)	31.4 (±1.8)	3 (±0.19)
Total no. of samples	22						

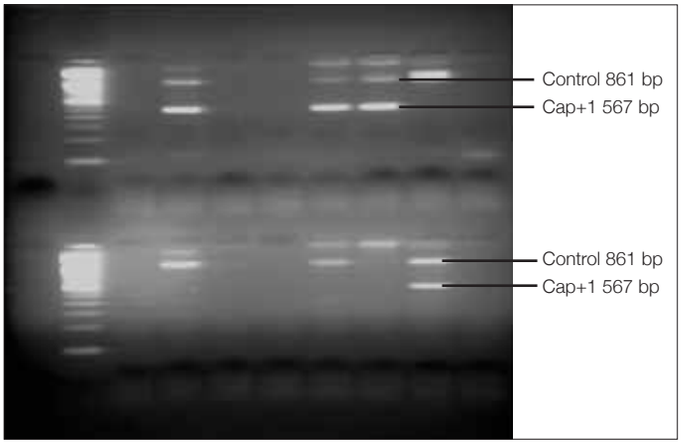

Figure 1. Analysis of DNA sample with mutation primers Cap+1 (A-C) using 100 bp ladder

Statistical analysis

Results were analyzed using SPSS statistical software version 13.

Results

Result of the first group which included 160 individuals from targeted families (having children with thalassemia intermedia) identified 200 beta thalassemia genes in which frequency of Cap+1(A-C) mutation was observed at 5% (10/200). While result of the second group which included 450 random samples from patients with beta thalassemia major and minor revealed 600 beta thalassemia genes in which frequency of Cap+1(A-C) mutation was 2% (12/600) in Pakistani population. Prevalence of this mutation in five major ethnic groups was observed with a frequency of 2.1% in Punjabi's, 1.7% in Pathan's, 1.7% in Sindhis, 2% in Baluchi's and 2.5% in Urdu speaking population of Pakistan.

Discussion

Result of this study revealed that Cap+1(A-C) is one of the common mutation which is present in all major ethnic groups of Pakistan. Frequency of Cap+1 (A-C) mutation was observed at 5% (10/200) in targeted beta thalassemic families (having patients with beta thalassemia intermedia) while its frequency was 2% (12/600) in total beta thalassemic genes in Pakistani population. Prevalence of this mutation in five major ethnic groups was observed with a frequency of 2.1% in Punjabi's, 1.7% in Pathan's, 1.7% in Sindhis, 2% in Baluchi's and 2.5% in Urdu speaking population of Pakistan.

Cap+1(A-C) mutation is also reported in Indian population and U.A.E (United Arab Emirates) nationals [14-18]. This mutation is also identified in Arab population (Jordan, Egypt, Syria, Lebanon, Yemen and Saudi Arabia) [19]. Cap+1 mutation is also reported in Malaysian population [20].

This study revealed that Cap+1 (A-C) is a silent mutation and it has only minimal effect on beta globin synthesis. Because of this it produces very few clinical manifestations. Also various basic hematological parameters (Hb, RBC, MCV, MCH, MCHC) and Hb A2 levels remain in normal range (Table 1). Commonly prevalent beta thalassemia mutations other than Cap+1(A-C) mutation present in Pakistani population show characteristic hematological variation in the form of low Hb, increased RBC count, decreased MCV, decreased MCH, normal MCHC and increased level of Hb-A2. Because of the negligible effect of Cap+1 mutation on these hematological parameters and Hb-A2 levels it is difficult to diagnose beta thalassemia minor caused by Cap+1(A-C) mutation. Molecular techniques like PCR are essential for the diagnosis of beta thalassemia minor associated with Cap+1 mutation.

It is therefore important to accurately diagnose Cap+1 mutation lest it propagates beta thalassemia major as a result of marriage between a known case of beta thalassemia minor and a "normal" individual for beta thalassemia minor who indeed is a carrier of Cap+1 mutation which remain undetected on routine hematological parameters including Hb-electrophoresis for Hb-A2 level.

This mutation was not observed in homozygous state during this study and no case of beta thalassemia major was identified having Cap+1 (A-C) mutation. This mutation was observed as one of the most important molecular defects causing beta thalassemia intermedia if inherited with other major beta thalassemic genes reported in Pakistani population. Therefore individuals with Cap+1 mutation may produce children with beta thalassemia intermedia if they marry an individual with beta-thalassemia minor. Cap+1 (A-C) mutation is an uncommon and unsuspected cause of beta thalassemia transmission in Pakistani population. Population study at a larger scale should be conducted to determine the frequency of CAP+1 mutation in Pakistani population; this will stop the propagation of thalassemia genes in homozygous state and prevent the birth of children with beta thalassemia intermedia.

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References

1. Quek L, Thein SL. Molecular therapies in beta thalassemia. Br J Haematol 2007;136:353-65.
2. Pan HF, Long GF, Li Q, Feng YN, Lei ZY, Wei HW, huang YY, Lin N, Xu QQ, Ling SY, Chen XJ, Huang T. Current status of thalassemia in minority population in Guangxi, China. Clin Genet 2007;71:419-26.
3. Weatherall DJ, Clegg JB. The biology of the thalassemias. The thalassemia syndromes. 4th ed. Blackwell Science Ltd. Oxford; 2001:121-284.
4. Weatherall DJ. Molecular biology at the bedside. Br Med J 1986;292:1505-8.
5. Colah R, Nadkarni A, Gorakshakar A, Phanasgaonkar S, Surve R, Subramaniam PG, Bondge N, Pujari K, Ghosh K, Mohanty D. Impact of beta globin gene mutations on the clinical phenotype of beta thalassemia in India. Blood Cells Mol Dis 2004;33:153-7.
6. Hafeez M, Aslam M, Ali A, Rashid Y, Jafri H. Regional and ethnic distribution of Beta thalassemia mutations and effect of consanguinity in patients referred for prenatal diagnosis. J Coll Physicians Surg Pak 2007;17:144-7.
7. Baig SM, Azhar A, Hassan H, Baig JM, Aslam M, Ud Din MA, Qureshi JA, Zaman T. Prenatal diagnosis of beta-thalassemia in Southern Punjab, Pakistan. Prenat Diagn. 2006;10:903-5.
8. Ahmed S, Petrou M, Saleem M. Molecular genetics of beta thalassaemia in Pakistan: a basis for prenatal diagnosis. Br J Haematol 1996;94:476-82.
9. Baig SM, Azhar A, Hassan H, Baig JM, Kiyani A, Hameed U, Rabbi F, Bokhari H, Aslam M, Ud Din MA, Baig SA, Hassan K, Qureshi JA, Zaman T. Spectrum of beta-thalassemia mutations in various regions of Punjab and Islamabad, Pakistan: establishment of prenatal diagnosis. Haematologica 2006;91:ELT02-ELT02
10. Ghani R, Manji MA, Ahmed N. Hemoglobinopathies among five major ethnic groups in Karachi, Pakistan. Southeast Asian J Trop Med Public Health 2002;33:855-61.
11. Garewal G, Das R, Awasthi A, Ahluwalia J, Marwaha RK. The clinical significance of the spectrum of interactions of CAP+1 (A-->C), a silent beta-globin gene mutation, with other beta-thalassemia mutations and globin gene modifiers in north Indians. Eur J Haematol 2007;79:417-21.
12. El-Gawhary S, El-Shafie S, Niazi M, Aziz M, El-Beshlawy A. Study of beta thalassemia mutations using the polymerase chain reaction-amplification refractory mutation system (ARMS) and direct DNA sequencing techniques in a group of Egyptian thalassemia patients. Hemoglobin 2007;31:63-9.
13. Najmabadi H, Teimourian S, Khatibi T, Neishabury M, Pourfarzad F, Jalil-Nejad S, Azad M, Oberkanins C, Krugluger W. Amplification Refractory Mutation System (Arms) and Reverse Hybridization in the detection of Beta-thalassemia mutations. Archives of Iranian Medicine 2001;4:165-70.
14. Vaz FE Thakur CB, Banerjee MK, Gangal SG. Distribution of beta-thalassemia mutations in the Indian population referred to a diagnostic center. Hemoglobin 2000;24:181-94.
15. Gupta A, Hattori Y, Gupta UR, Sarwai S, Nigam N, Singhal P, Agarwal S. Molecular genetic testing of beta-thalassemia patients of Indian origin and a novel 8-bp deletion mutation at codons 36/37/38/39. Genet Test 2003;7:163-8.
16. Colah R, Nadkarni A, Gorakshakar A, Phanasgaonkar S, Surve R, Subramaniam PG, Bondge N, Pujari, K, Ghosh K, Mohanty D. Impact of beta globin gene mutations on the clinical phenotype of beta thalassemia in India. Blood Cells Mol Dis 2004;33:153-7.
17. Chakrabarti P, Gupta R, Mishra A, Rai M, Singh VP, Dash D. Spectrum of beta-thalassemia mutations in North Indian states: a beta-thalassemia trait with two mutations in cis. Clin Biochem 2005; 38:576-8.
18. Quaife R, al-Gazali L, Abbes S, Fitzgerald P, Fitches A, Valler D, Old JM. The spectrum of beta thalassaemia mutations in the UAE national population. J Med Genet 1994; 31:59-61.
19. El-Hazmi MA, Warsy AS, al-Swailem AR. The frequency of 14 beta-thalassemia mutations in the Arab populations. Hemoglobin 1995; 19:353-60.
20. Chan Yoke Fan, Tan Kim Lian, Wong Yean Ching, Wee Yong Chui, Yap Sook Fan, Tan Jin Ai Mary Anne. The use of the amplification refractory mutation system (arms) in the detection of rare beta-thalassemia mutations in the Malays and Chinese in Malaysia. Southeast Asian J Trop Med Public Health 2001;32:872-9.