Prevalence of various mutations in beta thalassaemia and its association with haematological parameters

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Abstract

Objective: To determine the prevalence of various mutations in beta (β) thalassaemia and its association with haematological parameters.

Methods: A descriptive cross sectional study was carried out in the Department of Haematology, Armed Forced Institute of Pathology (AFIP) from February 2009 to January 2010. A total of 515 carriers having β thalassaemia mutations characterized by Multiplex amplification refractory mutation system (ARMS) were included in the study. Frequencies of different β thalassaemia mutations were calculated. Mutations were analyzed for their haematological parameters which include total red blood cell count (TRBC), haemoglobin (Hb), mean cell volume (MCV), mean cell haemoglobin (MCH) and red cell distribution width (RDW).

Results: Frame shift (Fr) 8-9 was the most common mutation found in 183 (35.5%) of patients followed by intervening sequence I-5 (IVSI-5) in 126 (24.5%) and Fr 41-42 in 76 (14.8%) while IVSII-1 was the least common mutation found in 1 patient. Fr 8-9 was also the commonest mutation in Punjabis and Pathans. Predominant mutation in other ethnic carriers was IVSI-5. Patients with Fr 8-9 mutation had the lowest mean MCV and MCH of 63.7fl and 19.1pg, of all the mutations. Patients with CAP+1 mutation had mean TRBC, Hb, MCV, MCH and RDW of 5.5 x 1012/L,13.5g/dl, 78.0fl, 24.7pg and 41.9fl respectively.

Conclusion: Fr 8-9 is the most common β thalassaemia mutation with lowest red cell indices while CAP+1 mutation can present with normal red cell values therefore, a potential carrier should be screened for CAP+1 mutation by DNA analysis.

Keywords: Beta thalassaemia, Mutation and Haematological values (JPMA 62: 40; 2012).

Introduction

β-thalassaemia is a syndrome of inherited haemoglobin disorders characterized by a quantitative deficiency of functional β-globin chains.[1,2] Based on β globin chain synthesis β thalassaemia are either β^0 or β^+. In β^0 thalassaemia the gene is unable to encode for any functional mRNA and therefore, no β chain is synthesized. In β^+ thalassaemias, the mutated gene encodes from a small amount of normal mRNA and, thus, some amount of chain is still

synthesized.[1-3] Routine diagnostic modalities for identification of β thalassaemia include low red cell values (MCV, MCH, RDW) on complete blood picture (CP), altered erythrocyte morphology and increased Hb A2 levels on high performance liquid chromatography (HPLC) or Hb electrophoresis.[4,5] On blood CP such patients present with variable increase in TRBC and low MCV, MCH and RDW depending upon the extent of globin chain imbalance between α and non-α globin chains and size of free α chain pool.[4-6]

Silent β thalassaemias are β^+ thalassaemias in which the deficit in β globin chains production is minimal. Such carriers present with minimally reduced or normal red cells indices and with normal HbA2 levels on haemoglobin electrophoresis, making their identification difficult with conventional methods.[7,8] These carriers need to be evaluated by molecular studies e.g. -101 (C-T) mutation in Mediterranean region[9] and CAP+1(A-C) mutation in Pakistan and South Asia.[10,11]

β thalassaemia exists in 5% of our population as heterozygous state.[10,12] Over 5000 thalassaemia major children are born in Pakistan each year.[13,14] Present health system is unable to deal adequately with such a number of ailing children. Better approach to this problem is therefore, a community based preventive programme including identification of β thalassaemia carriers, genetic counseling and prenatal diagnosis (PND).[13-15] Blood CP is the first and an important laboratory investigation in the identification of thalassaemia carriers.[5] Therefore red cell parameters are usually assessed before going for further investigations. PND depends upon the comprehensive knowledge of the prevalent mutations.[10] This study was conducted to see the spectrum of β thalassaemia mutation and their association with haematological findings.

Subjects and Methods

A total of 515 carriers of β thalassaemia were included in the study who reported during the study period. Among them 259 were males and 256 were females. The red blood cell (RBC) values were obtained with an automated cell counter (Sysmex Kx-21). It was a descriptive cross sectional study carried out in the Department of Haematology, Armed Forces Institute of Pathology (AFIP) from February 2009 to January 2010.

Inclusion criteria were known carriers of βthalassaemia whose mutations had been characterized by polymerase chain reaction (PCR) using Multiplex ARMS.

While exclusion criteria were carriers of β thalassaemia with iron, vitamin B12 or folic acid deficiency.

Mutation analysis for β-thalassaemia:

The β thalassaemia mutations of the carriers were characterized by Multiplex ARMS PCR for the previously reported common mutations in Pakistani populations. The mutations include: (IVSI-5 (G-C), Fr 8-9 (+G), IVSI-1 (G-T), Fr 41-42 (-TTCT), Del 619 bp, Cd 15 (G-A), Cd 5 (-CT), Cd30 (G-C), Cd 30 (G-A), Fr 16(-C), IVSII-1 (G-A), Cap +1 (A-C), Fr 47-48 (+ATCT) and IVSI-25 (25b del)).[10]

PCR was carried out in a 25 µl reaction mixture containing 5 pmol of each primer, 0.3 units of Taq polymerase (Advanced Biotechnologies. U.K.), 30 µM of each dNTP, (Boehringer Manheim), 10 mmol Tris HCl (pH 8.3), 50 mmol KCl, 1.5 mmol MgCl2, 100 µg/ml gelatin and 0.3 - 0.5 µg of genomic DNA. The thermal cycling consisted of 25 cycles of denaturation at 94°C for 1 minute, primer annealing at 65°C for 1 minute and DNA extension reaction at 72°C for 1.5 minute. In the final cycle, the extension reaction was prolonged to 3 minutes. PCR products were detected by 6% polyacrylamide gel electrophoresis.

Results

The carriers DNA were screened for the presence of β thalassaemia mutations. Median age of the female carriers

Table-1: Frequencies of β thalassaemia mutations and their distribution in various ethnic groups.

Mutations	Frequency n (%)	95% Confidence interval	Punjabi	Pathan	Others
FR 8-9	183 (35.5)	31.4-39.6	111(31.9%)	71(48.3%)	1
IVSI-5	126(24.5)	20.8-28.2	97(27.9%)	23(15.6%)	6(30%)
Fr 41-42	76(14.8)	11.7-17.9	61(17.5%)	14(9.5%)	1
Cd 15	36(7.0)	4.8-9.2	25(7.2%)	11(7.5%)	-
Cd 5	34(6.6)	4.5-8.7	13(3.7%)	18(12.2%)	3
Cd 30 (G-C)	16(3.1)	1.6-4.6	13(3.7%)	2	1
IVSI-1	10(1.9)	0.7-3.1	6(1.7%)	2	2
CAP+1	9(1.7)	1.1-2.3	4	4	1
Del 619	8(1.6)	1.1-2.1	6(1.7%)	-	2
Fr 16	8(1.6)	1.1-2.1	6(1.7%)	-	2
Cd 30 (G-A)	4(0.8)	0.4-1.2	2	2	-
IVSII-1	1(0.2)		1	-	-
Uncharacterized	4(0.8)	0.4-1.2	3	-	1
			348	147	20

Table-2: β thalassaemia mutations and their haematological values (n=515).

Mutation	TRBCx10^{12}/L (4.5-5.5)	Hb g/dl (13-17)	MCV fl (76-96)	MCH pg (27-31)	RDW fl (39-45)
Fr 8-9	5.7 (±1.0)	11.0 (±1.7)	63.7 (±4.0)	19.1 (±1.8)	39.2 (±3.5)
IVSI-5	5.6 (±0.86)	11.1 (±1.6)	64.9 (±4.0)	19.8 (±1.7)	39.5 (±2.9)
Fr 41-42	5.7 (±1.0)	11.2 (±1.7)	65.4 (±4.6)	19.7 (±1.7)	40 (±3.5)
Cd 15	5.7 (±1.0)	11.0 (±2.2)	65.6 (±7.7)	19.9 (±2.0)	40.7 (±3.9)
Cd 5	5.7 (±1.0)	11.1 (±1.7)	65.4 (±4.0)	19.8 (±2.0)	40.0 (±3.3)
Cd 30 (G-C)	6.0 (±0.8)	11.0 (±2.8)	64.3 (±1.7)	19.3 (±0.9)	39.6 (±2.5)
IVSI-1	5.73 (±0.8)	10.9 (±2.3)	64.7 (±3.2)	20.3 (±1.2)	40.7 (±1.8)
CAP+1	5.5 (±0.6)	13.5 (±1.1)	78.0 (±6.0)	24.7 (±2.6)	41.9 (±2.7)
Del 619bp	6.1 (±0.8)	11.8 (±1.9)	65.8 (±4.0)	19.3 (±0.9)	39.4 (±2.3)
Fr 16	5.7 (±1.0)	10.8 (±1.4)	64.0 (±2.5)	19.3 (±1.2)	39.4(±10.7)
Cd 30 (G-A)	5.2 (±0.5)	10.0 (±1.6)	63.9 (±6.9)	19.2 (±3.3)	40.0 (±1.6)
IVSII-1 (n=1)	5.0	9.6	63.3	19.4	40.0
Uncharacterized	5.9 (±1.1)	12.0 (±1.2)	69.8 (±8.6)	20.5 (±2.6)	39.1 (±5.5)

was 27 years while median age of their male counterparts was 32 years. The three most common mutations; IVS-I-5 (G-C), Fr 8-9(+G) and Fr 41-42 (-TTCT) constitute 74.8% of all the mutation characterized. Four mutations remained uncharacterized. Fr 8-9 (+G) was the most common mutation in Punjabis and Pathans followed by IVS-I-5 (G-C). Predominant mutation in others ethnic group was IVS-I-5 (G-C). CAP+1 mutation, the only silent mutation in the study was present in 4 Punjabis, 4 Pathans and 1 other carrier (Table-1). Fr 8-9 (+G) presented with the lowest red cell values with mean MCV of 63.7(±4.0) fl, MCH of 19.1(±1.8) pg and RDW of 39.2(±3.5) fl while red cell parameters for CAP+1 mutation are close to normal with mean MCV of 78.0(±6.0) fl, MCH of 24.7(±2.6) pg and RDW of 41.9(±2.7) fl (Table-2).

Discussion

β globin gene is present on the short arm of chromosome 11 in a cluster with the other β like genes.[1,2] There are more than 200 mutations causing β thalassaemias.[4] With exception of a few deletions, vast majority of β thalassaemias are caused by point mutations within the gene or its immediate flanking sequences.[3,4] The degree of globin chain imbalance is responsible for the pathophysiologic features of thalassaemia syndromes. Globin chain imbalance is maximum in case of β^0 thalassaemias and minimal in case of silent β thalassaemias.[2,3] The heterozygous state of β thalassaemias show a tremendous phenotypic diversity.[3,4] In typical β^0 or severe β^+ thalassaemias, the alleles demonstrate lower Hb, MCV and MCH values while in some carriers the β thalassaemias is so mild that it is phenotypically silent with no anaemia or haematological abnormality.[5,7]

Assessment of red cell parameters on blood CP is the first and an important laboratory investigation in the diagnostic workup of thalassaemias.[5,6] Most of the mutations characterized in our study are either β^0 or severe β^+. So the red cell parameters for all these mutations characteristically showed a reduced Hb concentration, elevated TRBCs, low MCV and MCH and normal or just below normal RDW, a measurement which reflects red cell anisocytosis.

An exception was observed for red cell values of CAP+1, a silent mutation, which are either normal or nearly normal. These values speak for the silence of this mutation. Such mutations cause minor or no haematological abnormalities in heterozygotes but in the compound heterozygous state with a severe β thalassaemia allele, they are associated with clinically significant disease like thalassaemia intermedia. In homozygous state, patient can have a typical phenotype of β thalassaemia trait.[7,16] CAP+1 β thalassaemia mutation can be completely silent in some cases while in others it can be suspected on the basis of a slight reduction in MCV and MCH values or minimal changes in Hb A2 level which is either normal or slightly increased to border line.[8,11] Molecular techniques for DNA analysis like PCR become necessary for identification of such mutations.[9]

Our results for spectrum of β thalassaemia mutations are in agreement with other studies across the country[10,17] which show the predominance of FR 8-9 and IVSI-5 mutation in Punjabis and Pathans and CAP+1 as the commonest silent mutation. Same results have been observed for the Indian population as shown by the studies of Garewal G et al[11] and Varawalla NY et al[18] but this is in contrast to the Mediterranean region where IVSI-1 (G-A), IVSI-110 (G-A), IVSI-6 (T-C) are the predominant β thalassaemia mutations[19] and-101 (C-T) mutation is one of the most prevalent silent β thalassaemia mutation.[9]

CAP+1 mutation can cause serious difficulties in screening and counseling programmes in populations in which it occurs at a significant frequency.[11] Such silent mutations become significant in PND for thalassaemia.[20] At

risk couples don't seek for PND if one partner is a known β thalassaemia carrier and other is apparently normal (a silent carrier), as silent carriers remain undiagnosed by routine diagnostic modalities (Blood CP, HPLC and Hb electrophoresis). Coinheritance of a severe β thalassaemia allele and CAP+1 allele in a foetus may lead to thalassaemia intermedia after birth, making PND essential for such couples. Therefore in couples, where one of the partner is β thalassaemia trait, the other partner should be investigated by all possible diagnostic modalities including PCR for common silent mutations.[7]

Conclusion

Fr 8-9 (+G) is the most common β thalassaemia mutation with lowest red cell indices. CAP+1 (A-C) mutation can present with normal red cell values, therefore a potential carrier should be screened for CAP+1 mutation by DNA analysis if there is enough clinical suspicion or other partner is a known thalassaemia carrier.

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