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Genotype-Phenotype Profile of Beta-thalassemia

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ABSTRACT

Background: Beta thalassemias are extremely heterogenous hereditary monogenic blood disorders and preventable genetic hemolytic anemia caused by >200 mutations in HBB gene. In Nepal, it is more prevalent in Tharu tribe but it seen in other communities as well. Out of more than 200 mutations of beta globin gene, approximate 20 different alleles are responsible for >80% of the mutations. Mutations vary in different geographic population and are responsible for manifestation of different phenotypes. This study was done to find common mutations of HBB gene in Nepal which were responsible for different phenotypic manifestations and to know clinical severity according to the mutations.

Methods: This was a descriptive, cross sectional study conducted in the pediatric and medicine department of Nepalgunj Medical College and Bheri Zonal Hospital, Nepalgunj from January 2020 to December 2020. The genotype and phenotype profiles of thalassemia cases were reported. The data was analyzed by SPSS 20.

Results: The results obtained showed that clinical presentation differed with different β-globin gene mutations present. Individuals with HBB:c.47G>A and HBB:c.20A>T/ c.79G>A mutations showed milder presentation than those with HBB:c.47G>A/-619del and HBB:c.20A>T/c.47G>A.

Conclusions: Therefore, these findings can be used to predict clinical severity so that we can take appropriate measures by early genotype identification for prenatal diagnosis of beta thalassemia.

Keywords: Genotype phenotype; prenatal diagnosis; thalassemia

INTRODUCTION

Beta thalassemias are the most common heterogeneous monogenic hematological disorder, preventable genetic hemolytic anemia, caused by >200 mutations in HBB gene and approximately 20 different alleles are responsible for >80% of the mutations.[1-3] Clinically, this is classified into 3 forms i.e. thalassemia major, thalassemia intermedia and thalassemia minor.[4] Carrier frequency in world population is 5.2% with Maldives (16%) having highest carrier frequency.[5] In Nepal, it is more prevalent in Tharu community of South Western part but no community is spared. Accurate prevalence of carriers is not reported in Nepal.

Mutations vary with different geographic population and is responsible for different phenotypes of this disease; varying from severe transfusion dependent thalassemia major to mild form of thalassemia intermedia.[6-8] Thus this study was conducted to find common mutations responsible for different phenotypic manifestations like age at presentation, transfusion requirement and mutation related clinical severity guiding for development of primer for prenatal diagnosis of beta thalassemia.

METHODS

The present study was a descriptive, cross-sectional study. The study was conducted in the pediatric and medicine outpatient department and pediatric and medicine ward of Nepalgunj Medical College and Bheri Hospital, Nepalgunj from January 2020 to December 2020. The sample size was 30. So far, no genotype study has been conducted by Sanger sequencing in Nepal to find common mutation so we are not able to calculate sample size and took sample size of 30 patients. Interview questionnaire schedule was used for data collection. All the cases already diagnosed as beta thalassemia (heterozygous, homozygous, sickle beta

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thalassemia) by Hb electrophoresis were included in the study. Written and informed consent were obtained from patients (guardians in case of children). Patients who refused to give consent were excluded from the study.

Four milliliters of venous blood sample were collected in ethylenediaminetetraacetic acid (EDTA) - containing tube for Sanger sequencing. Blood samples were stored in refrigerator at four degrees centigrade and send to laboratory, located in Kathmandu for DNA extraction and extracted sample was maintained in cold chain and were transported to Sanjay Gandhi Post Graduate Institute, Lucknow, India. The finding of the research was validated with reference to the already published articles of the same objective and by consulting the expert of subject matter. The data was analyzed by SPSS version 20. Annova was used to compare more than 2 groups and student's t test was used to compare 2 groups.

RESULTS

A total of 30 patients aged 2 years to 44 years were enrolled in the present study including homozygous or compound heterozygous beta-thalassemia, heterozygous beta-thalassemia and sickle beta thalassemia. HBB -619 del mutation was the most common mutation detected in 20 (50%) alleles; 16 (80%) of these were heterozygous mutant and 4 (20%) were homozygous mutant alleles. Distribution of other mutations can be seen in Figure 1.

The average age at diagnosis was 6.3± 4.94 years for homozygous or compound heterozygous beta-thalassemia, 30.11± 9.74 for heterozygous beta-thalassemia and 23.72± 16.16 years for sickle beta thalassemia. There was statistically significant difference between three groups (p-value << 0.01). Mean hemoglobin was 3.99± 0.81 for homozygous or compound heterozygous beta-thalassemia, 10.94± 1.12 for heterozygous beta-thalassemia and was 8.62± 0.86 for sickle beta thalassemia respectively. The regular blood transfusion was approximately 10.9± 1.9 times/ year in beta-thalassemia disease and 1.1± 1.2 times/ year in sickle beta thalassemia. There was statistically significant difference between two groups (p-value << 0.01). All the patients had either hepatosplenomegaly or splenomegaly in homozygous or compound heterozygous beta-thalassemia and sickle beta thalassemia group.

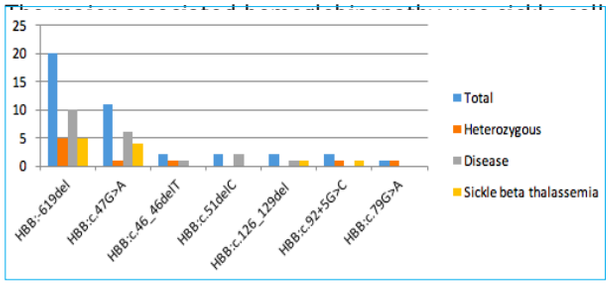

Figure 1. Distribution of beta- thalassemia mutant alleles in percent.

Table 1. Clinical, hematologic and molecular data of heterozygous beta- thalassemia patients.

N	Age/Sex	Hb	HbA2	HbF	Mutation
1	28/F	11.0	4.8	3.9	HBB:-619del
2	34/M	11.8	6	0.4	HBB:-619del
3	14/M	12.1	4.5	2.8	HBB:-619del
4	43/M	11.3	6.1	1.6	HBB:-619del
5	43/F	10.4	4	2.7	HBB:-619del
6	35/F	9.6	6.9	0.4	HBB:c.47G>A
7	26/M	11.2	5.3	0.2	HBB:c.47G>A
8	20/M	12.2	21	0.9	HBB:c.79G>A
9	28/F	8.9	5.3	1.3	HBB:c.46_46delT

Table 2. Clinical, hematologic and molecular data of homozygous and compound heterozygous beta- thalassemia patients.

N	Age/Sex	Hb	HbA2	HbF	Organomegaly	Mutation	Blood transfusion frequency per year	Phenotype	Severity
1	3/F	3.4	1.9	95.1	Hepatosplenomegaly	HBB: c.47G>A/-619del	10	CHT	Severe
2	6/M	4.7	1.3	82.2	Hepatosplenomegaly	HBB: c.47G>A/-619del	12	CHT	Severe
3	11/M	2.9	3.3	96.7	Splenomegaly	HBB: c.47G>A/-619del	11	CHT	Moderate

4	5/M	3.9	2.5	96.2	Hepatosplenomegaly	HBB: c.47G>A/-619del	12	CHT	Severe
5	2/M	5.2	2.9	40.7	Splenomegaly	HBB: c.51delC Homozygous	9	HM	Moderate
6	2/F	4.6	3.0	96.8	Hepatosplenomegaly	HBB: c.46_46delT/-619del	10	CHT	Severe
7	3/F	3.9	2.3	99.7	Hepatosplenomegaly	HBB: c.126_129del CTTT/-619del	12	CHT	Severe
8	6/M	2.8	2.9	85.5	Hepatosplenomegaly	HBB: -619del	11	HM	Severe
9	7/M	4.7	2.2	76.3	Hepatosplenomegaly	HBB: -619del	12	HM	Severe
10	18/F	3.8	2.8	97.2	Splenomegaly	HBB: c.47G>A	10	HM	Moderate

CHT- Compound heterozygous, HM- homozygous

Table 3. Clinical, hematologic and molecular data of sickle beta- thalassemia patients with codon 8 (-AA) mutation.

N	Age/ Sex	Hb	HbA2	HbF	HbS	Organomegaly	Mutation	Blood Transfusion frequency per year	Severity
1	4/M	10.2	5.7	4.2	64.9	Hepatosplenomegaly	HBB: c.20A>T/ c.47G>A	3	Severe
2	44/F	7.3	7.1	21.1	71.8	Splenomegaly	HBB: c.20A>T/ c.47G>A	2	Severe
3	38/M	7.6	6.7	20.4	51.2	Splenomegaly	HBB: c.20A>T/ c.47G>A	1	Moderate
4	14/F	8.3	5.7	17.5	48.5	Splenomegaly	HBB: c.20A>T/ c.47G>A	1	Severe
5	10/M	9.2	4.9	31.3	49	Splenomegaly	HBB: c.20A>T/619del	5	Moderate
6	23/F	9.4	3.9	16.4	51.6	Splenomegaly	HBB: c.20A>T/619del	2	Moderate
7	44/F	8.8	8.3	18.5	55	Splenomegaly	HBB: c.20A>T/619del	1	Severe
8	6/M	7.9	3.8	20.2	73.2	Splenomegaly	HBB: c.20A>T/c.126_129 del	2	Mild
9	42/M	8.5	5.3	15.4	28.4	Hepatosplenomegaly	HBB: c.20A>T/ c.79G>A	2	Mild
10	8/M	9.3	3.1	14.8	32	Hepatosplenomegaly	HBB: c.20A>T/619del	1	Severe
11	28/M	8.4	18.2	5.9	0.8	Splenomegaly	HBB: c.20A>T/619del	1	Mild

DISCUSSION

The present study brought to light the distribution of the HBB gene mutations responsible for beta-thalassemia and also showed genotype-phenotype correlation in a Nepalese population of Western Terai of Nepal. Beta-thalassemia is mainly seen in Tharu community of Western Terai of Nepal but cases are seen in other communities as well. It can manifest with various conditions like asymptomatic thalassemia minor to clinical phenotypes ranging from thalassemia intermedia to thalassemia major. Knowledge of the molecular basis of the HBB gene mutations explicates the diversity of its clinical manifestations. Beta (zero)-thalassemia presents with a more severe clinical presentation, causing thalassemia major in homozygous and compound heterozygous states, whereas beta(+)-thalassemia manifests as milder cases of thalassemia intermedia.[9] Our study showed most

common mutation to be HBB -619 del (50%) mutation followed by HBB:c.47G>A (27.5%) whereas another Nepalese study done in 2017 by ARMS PCR showed most common mutation to be IVS 1-5 GC(50%) followed by HBB -619 del (30.76%) mutation.[10] Mishra et al. also reported 5 mutations namely F.S 41/42 (--TCTT), IVS1 nt5 (G-->C), IVS1 nt1 (G-->T), 619 bp deletion and F.S 8/9 (+G) constituting 87.82% mutations in their study F.S 41/42 (--TCTT) being the commonest one.[11] Our study showed sickle beta-thalassemia to be a common associated hemoglobinopathy in high malaria incidence population of past. Out of 30 patients, 11 (36.7%) showed mutation for Sickle cell anemia which is in concordance to a study done by Kumar R et al. showing 48% sickle mutation.[12]

This is the first study done in Nepal by Sanger sequencing of HBB gene to find common mutations in Thalassemic patients. We are keen to do more studies by Sanger sequencing to find common mutations in Thalassemic patients of other parts of Nepal from different ethnic population on larger scale and utilize our result to develop a primer incorporating common mutations of our country for prenatal diagnosis and genetic counseling to reduce burden of thalassemia in our country.

CONCLUSIONS

Clinical presentation differs with different ß-globin gene mutations present. This information can be used to predict severity of beta thalassemia patients and prenatal diagnosis of beta thalessemia in fetus.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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