EFFECT OF SNPS IN BCL11A GENE ON THE CLINICAL HETEROGENEITY OF THE PATIENTS WITH HBE/BETA-THALASSEMIA IN BANGLADESH

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Abstract

Hemoglobin E/ β -thalassemia is a blood disorder with highly variable clinical phenotypes. Increased production of fetal hemoglobin (HbF) can influence the degree of severity in β -thalassemia. This study aimed to unravel the disease modifying effects of two SNPs, *rs4671393* & *rs11886868* in *BCL11A* gene among HbE/ β -thalassemia patients. A total of 133 HbE/ β -thalassemia patients with mean of age 19.66 ± 10.22 years and 50 healthy controls with completely normal hematological parameters were recruited in this study and patients were classified as NTD/mild, moderate and severe, according to previously reported clinical scoring system. Laboratory investigation included hematological tests using Complete Blood Count analysis and Hemoglobin electrophoresis; SNPs were detected by High Resolution Melt-Curve (HRM) analysis based on Real-time PCR and Sanger Sequencing. The allelic distribution of *rs4671393* was 78.2% GG and 21.8% AG; and for *rs11886868*, 51.12% CC and 48.9% CT+TT among the patients. Both SNPs showed significant association with induction of HbF in HbE/ β -thalassemia patients, while *rs4671393* showed no significant effect on severity score. HbF level was significantly correlated with transfusion interval and clinical score while showed comparatively less correlation with the age of first blood transfusion. However, any of the two SNPs showed no HbF inductive effect in the absence of anemic condition.

Research Highlights

Highlight-1: Among the HbE/ β -thalassemia patients in Bangladesh, the minor allele frequency of *rs-4671393* (A) and *rs-11886868* (T) in the *BCL11A* gene was found as 0.11 and 0.25 respectively.

Highlight-2: Minor alleles of both SNPs showed significant association with induction of HbF ($p= 0.02$, $p= 0.03$ respectively) in HbE/ β -thalassemia patients. On the other, only the *rs-11886868* (T) allele was found to have statistically significant lowering effect on the disease severity of the patients.

Highlight-3: HbF level was positively correlated ($r= 0.5$) with transfusion interval and negatively correlated to clinical score ($r= -0.45$) implying that increased production of HbF decreases the rate of blood transfusion as well as disease severity in HbE/ β -thalassemia patients.

Highlight-4: In spite of having both SNPs (17% minor allele for *rs-4671393* and 25% minor allele for *rs-11886868*), no induction of HbF was found among SNPs positive healthy individuals.

Research Objectives

HbE/ β -thalassemia is an inherited hemolytic disease highly prevalent in Bangladesh as the carrier frequency of HbE and β -thalassemia traits are very high (8.68% and 2.24% respectively) in the population [1]. Increased level of HbF has been reported as an influencing phenotypic factor of HbE/ β -thalassemia and genom-wide studies have identified a number of single nucleotide polymorphisms (SNPs) within the gene of repressor protein *BCL11A* associated with elevated HbF levels [2,3]. This study aimed to evaluate whether genetic variability at *BCL11A* locus influences HbF levels in HbE/beta-thalassemia patients in Bangladesh. In particular, to determine the frequency of two SNPs, *rs11886868* & *rs4671393* at *BCL11A* gene and their association with increased production of HbF, thus the effect on the clinical heterogeneity among the patients with HbE/beta-thalassemia in Bangladesh. So far, it is the first study on *BCL11A* gene polymorphisms done in Bangladesh. In the former decades, the main inclination of research was HbF induction since ascending HbF levels uplift the severity of β -thalassemia and HbE/ β -thalassemia in adults [5,6]. Targeting *BCL11A* gene for gene editing to reactivate fetal hemoglobin gene expression in the HbE/ β -thalassemia patients, our study confers indispensable knowledge for developing targeted potential therapies hereafter.

Methodology

From Bangladesh Thalassemia Samity Hospital in Dhaka, a total of 133 HbE/ β -thalassemia patient with a mean of age 19.66 ± 10.22 years were enrolled in this study (56.4% male and 43.6% female). All laboratory analysis were performed at “Institute for Developing Science and Health Initiatives (ideSHi)” laboratory providing BSL-2 facilities. Ethical approval was provided by the ‘Bangladesh Medical Research Council (BMRC)’ for this study. Blood (~5mL) of patients were collected from venous just before blood transfusion and subjected to CBC analysis by ‘automated hematology analyzer Sysmex kx-21’ (Sysmex Corporation, Kobe, Japan) and hemoglobin electrophoresis by ‘CAPILLARYS 2 instrument (Sebia, France)’. For molecular analysis, DNA isolation from patients whole blood cells using ‘FlexiGene DNA kit’ (QIAGEN, Hilden, Germany) and Polymerase Chain Reaction using ‘Qiagen HotStart Taq DNA Polymerase’ (Qiagen, USA) were done carefully. Sanger Nucleotide Sequencing was performed to detect the SNPs for the first time and to use those as reference samples. Then for SNPs detection, HRM analysis based on Real-time PCR were accomplished on ‘BioRad CFX96 Touch Real-Time System’ by using ‘Precision Melt Analysis™ Software (BioRad)’ according to manufacturer’s instruction and statistical analysis including two-tail unpaired T-test, one-way ANOVA test and Pearson Correlation were done using ‘Graphpad Prism-version 7’.

Results

The patients were divided into three groups- Non Transfusion Dependent (NTD)/mild, moderate and severe according to the previously reported scoring system with little modification [4]. HbF level was significantly higher ($p < 0.0001$) in NTD/mildly severe group compared to other two groups. Extracted genomic DNA from 133 patients and 50 healthy controls were analyzed for detection of two SNPs at the *BCL11A* gene, namely *rs4671393* and *rs11886868* which have been reported to have significant association with elevated level of HbF in adults in several studies [2,3]. Among the patients, the allelic distribution of *rs4671393* was 78.2% GG, 21.8% AG and for *rs11886868*, there were 51.12% CC and 48.9% CT+TT. Both SNPs showed significant association with induction of HbF (g/dl) ($p = 0.03$ and $p = 0.02$) in the HbE/beta-thalassemia patients. However, clinical severity score was significantly lower in the patients only in *rs11886868* ($p = 0.03$) carrying the minor allele T. Pearson Correlation analysis revealed significant correlation of HbF with transfusion interval ($r = 0.5$) and clinical score ($r = -0.45$), and comparatively less correlation with the age of first blood transfusion ($r = 0.32$). In spite of having both SNPs (17% minor allele for *rs4671393* and 25% minor allele for *rs11886868*), no induction of HbF was found among SNPs positive healthy individuals.

Findings

The two SNPs of *BCL11A* *rs11886868* and *rs4671393* have significant effect on increased fetal hemoglobin level in the adult patients with HbE/beta-thalassemia in Bangladesh and thus shows similarity with the previous studies done on other populations. Though *rs4671393* showed no significant association with clinical severity of the patients, the mean value of the clinical score was much lower in the patients groups carrying the minor alleles. Moreover, pearson correlation test showed that high level of HbF decreases blood transfusion rates and clinical scores in the patients, thus both SNPs can be correlated with less severity of the disease.

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Author's Biography

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