Austin Hematology

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Molecular Screening of Hemoglobin E Variant in Anemia Patients of Eastern UP Population, India

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Received: October 22, 2021; **Accepted:** November 16, 2021; **Published:** November 23, 2021

Abstract

Hemoglobinopathy is one of the most common monogenic disorders. This is prevalent in South East Asia. More than 700 heboglobinopathies are reported worldwide, out of which HbS and HbE are also prevalent in India. The objective of the present study is to determine the frequency of HbE in Eastern UP population. After taking written consent, blood samples was collected from 350 individuals and genomic DNA was extracted from all the collected blood samples. PCR-RFP method was used to analyze the HbE mutation. Out of 350 samples analyzed, one individual was Heterozygous (HbE/N) and one individual was Homozygous (HbE/E) for HbE mutation. In conclusion, the β^E allele frequency was observed as 0.42% in Eastern UP population. Percentage of both heterozygous and homozygous genotypes were 0.28%.

Keywords: Hemoglobin; Hemoglobinopathy; HbE; Globin gene

Introduction

Hemoglobin E (HbE) is the most common hemoglobinopathy in Asia and the second most common hemoglobinopathy in the world. It has been estimated that approximately 50 million people in the South East Asia alone carry the gene for hemoglobin E. It occurs at up to 50% in Khmer people living on the borders of Laos, Thailand and Cambodia which have been known as "Hb E triangle". The hemoglobin E has a wide spread distribution, being most frequent in the Eastern and Far Eastern Region in the India [1]. However, sporadic reports are also available from other parts of the country. Average gene frequency has been found to be 10.9% for North Eastern states of India.

HbE is caused by a substitution of glutamic acid by lysine at 26th position of β-globin protein, and G-->A substitution in β-globin gene (β^{26} Gluà Lys; GAGàAAG). The mutation underlying HbE creates an abnormal splicing site and thereby drastically reduces the formation of functional β-globin mRNA, this gene is similar to β ± thalassemia gene [2,3]. HbE has a weekend a/b interface, leading to some instability during conditions of increased oxidant stress [4]. Heterozygotes for HbE are microcytic, minimally anemic, and asymptomatic, however, homozygotes are clinically anemic. No reports of HbE frequency was available from Uttar Pradesh, hence we have screened anemic patients for HbE mutation.

Methods

The present study was approved by the Institutional Ethics Committee of VBS Purvanchal University, Jaunpur, India and all participants gave their written informed consent. 2 ml blood sample was collected from 350 anemia patients. Subjects for the present study selected from the three districts of Eastern UP like Varanasi, Allahabad and Jaunpur. Genomic DNA was extracted using the standard method of Bartlett and White [5]. And HbE mutation analysis was done by PCR-RFLP method of Tachavanich et al. [6].

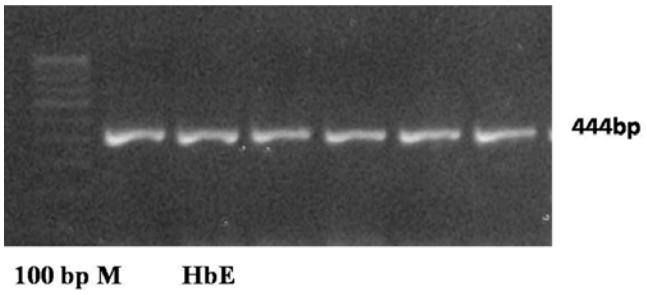

Figure 1: Gel picture showing 444bp of HbE amplicon.

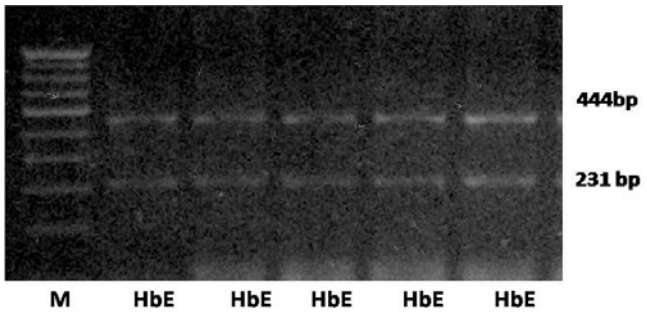

Figure 2: Gel picture showing MnII digested HbE amplicon.

Results and Discussion

Out of 350 samples, 50 samples were of Muslims, 100 from Scheduled cast, 100 from Brahmin and 100 samples from OBC. HbE specific primers amplified 444 long DNA amplicon (Figure 1). In case of normal β-globin allele, MnII enzyme cleave 444 bp long amplicon in to five fragments of 231/89/56/35/33bp long. HbE mutation (GAGàAAG) abolishes the MnII restriction site and 444p long amplicon remained uncut after restriction digestion (Figure 2). Out of 350 samples, one individuals was heterozygous (β^A / β^E) and one individual was homozygous (β^E / β^E). Both individuals

Citation: Rai V, Yadav U and Kumar P. Molecular Screening of Hemoglobin E Variant in Anemia Patients of Eastern UP Population, India. Austin Hematol. 2021; 6(3): 1042.

belong to Scheduled caste. The β^E allele frequency was observed as 0.42% in Eastern UP population. Percentage of both heterozygous and homzoygous genotypes were 0.28%. Heterozygous HbE are asymptomatic and it is silently propagated in the community. Hence mass screening and detection and counseling to prevent further spread of Hb E gene in this region is urgently required.

Acknowledgements

We wish to thank all of the patients who participated in this study. This work was supported by a grant from Council of Scientific and Industrial Research, Government of India to Vandana Rai (No 27(0204)/09/EMR-II).

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