

Haemoglobin Variants among the Bengali Muslims and the Meiteis in two Villages of Cachar District of Assam, India

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Abstract: *Present paper documents the presence of haemoglobin variants along with haematological parameters among the Bengali Muslims of Bhourikandi Part-II and the Meiteis of Dakshin Mohanpur Part-V village of Cachar District of Assam. Capillary electrophoresis has been performed to detect the haemoglobin variants on 27 Bengali Muslims and 26 Meiteis from whom 2 ml intravenous blood was collected in EDTA vials. Haematological parameters has been analysed by using haematology analyzer. The results reveal the presence of haemoglobin variants like HbE and β -thalassemia among the Muslim population with a gene frequency of 0.0556 each. On other hand, only HbE trait is found to be present among the Meitei population with a gene frequency of 0.0962. Haemoglobin concentration level (Hb in g/dl) is found to be lower in haemoglobin variants in contrast to normal haemoglobins (HbAA) in both the communities.*

Keywords: *HbE, β -thalassemia, Haematology, Bengali Muslim, Meitei, Cachar, etc.*

1. INTRODUCTION

Haemoglobin (Hb), the red respiratory protein found in mammalian erythrocytes is one of the most informative molecules in primate blood [1]. In adult humans, the most common haemoglobin type is a tetramer known as haemoglobin-A consists of four separate polypeptide chains of amino acids and each subunit is composed of a protein chain tightly associated with a non-protein haeme group that fix oxygen. Mutations in the genes for the haemoglobin result in haemoglobin variants, some of which cause a group of hereditary disorders in humans termed as haemoglobinopathies. Haemoglobin variants are a part of the normal embryonic and fetal development which can also be pathologic mutant forms caused by variations in genetics of haemoglobin in a population. Haemoglobinopathies covers a group of hereditary disorders in which the structure (qualitative change) or the rate of synthesis (quantitative change) of one of the normal haemoglobin chain is altered [1]. Haemoglobinopathies causes varying degrees of microcytic anemia that can range from insignificant to life threatening [2]. Although haemoglobinopathy is a hereditary disorder but environment potentially can determine the genotypes and it does it through natural selection.

Haemoglobinopathies are the most commonly encountered hereditary abnormalities of blood posing a major genetic burden and public health problem in Southeast Asia and the Indian subcontinent [3]. HbS, HbE and HbD as well as thalassemia are the most widely distributed haemoglobin disorders in India out of which HbE is widely distributed in north-eastern states of India [4, 5, 6]. Thalassemia results from the reduced rate of synthesis one of the globin chains can cause the formation of abnormal haemoglobin molecules which in turn causes the anemia. The sickle cell haemoglobin (HbS) is structurally abnormal variant results in the substitution of the amino acid valine for glutamic acid at 6th position of β -globin polypeptide chain of the molecule. Haemoglobin E is another structurally abnormal variant with glutamic acid residue replaced by lysine at the 26th position of the β -globin polypeptide chain [7].

India is a land of different endogamous communities and non random (consanguinity) mating pattern leads to the co-heritance of β -thalassemia and other structural variants (D, E, S). Interaction of these structural variants of haemoglobin along with the reduced synthesis of globin chains result to the combination of two abnormalities, resulting in double heterozygosity of the disease in India [8]. Carriers of haemoglobinopathies are partially protected against morbidity and mortality of falciparum malaria. The resistance of HbAE red cells to Plasmodium falciparum is most likely the cause for its

high prevalence throughout the world [9]. Although no clear evidence is established regarding the selection of haemoglobin genotypes in differential fertility but their role cannot be ruled out in endemic malarial areas of Eastern India where frequency of HbE gene is very high [10, 11].

2. AIMS AND OBJECTIVE

The main objective of the present research is to study the frequency and distribution of haemoglobin variants as well as haematological profile of the Bengali Muslims and the Meiteis.

3. MATERIALS AND METHODS

The present study has been carried out among the Bengali Muslims of Bhaurikandi Part-II village and the Meiteis of DakshinMohanpur Part-V village of Sonai Block of Cachar District of Assam, India.

Two (2) ml blood sample was collected in EDTA vials from vein of 27 Bengali Muslims and 26 Meitei. All these individuals were above 20 years of age. After collection blood samples were kept at 4°C until the sample arrived at laboratory. Prior consultation and written consent was taken from all the individuals before collection of the blood sample. Capillary electrophoresis was performed within 24 hours of blood sample collection to detect the presence of HbE and other haemoglobin variants. Haematological parameters like WBC (White Blood Corpuscles), RBC (Red Blood Corpuscles), MCV (Mean Cell Volume), MCH (Mean Cell Haemoglobin), MCHC (Mean Cell Haemoglobin Concentration), Hct (Haematocrit), RDW (Red Cell Distribution Width), Hb (Haemoglobin), etc. were also analysed for these 53 samples by using haematology analyzer.

After collection of data, these were treated in different statistical tools like number, percentage, mean, etc. Calculation of gene frequency of haemoglobin variants was done by following Das [12].

4. THE PEOPLE

The present study has been done among the Bengali Muslims and the Meiteis living in two neighbouring villages like Bhaurikandi Part-II and DakshinMohanpur Part-V respectively of Cachar District of Assam, India. Geographically Cachar district is located in the southernmost part of Assam. There are different endogamous ethnic communities inhabiting the district such as Bengali Hindu, Bengali Muslim, Meitei, Brishnupriya, Dimasa Kachari, Hmar, Khasi, etc. The people inhabiting in Barak valley (which includes Cachar, Karimganj and Hailakandi district) are primarily known as *Sylheti Bengali* (*sylheti*, a dialect) who mainly follow either Islam or Hinduism. The Bengali Muslims and the Meiteis of the area belong to two different religions such as Islam and Hinduism. The valley has a long history of Islam as half of the valley came under the rule of the Turk-Afghan dynasties of Bengal from the early 14th century and continued with the establishment of the Mughal Empire [13, 14]. Meitei people got its introduction to the valley from the early part of nineteenth century when its princes made Cachar a springboard for the reinvasion of the territory after being displaced from Meitribak, modern Manipur [15]. Linguistically the Bengali Muslims belong to the Indo-European ethnic group of Caucasoid racial stock while the Meiteis belong to the Tibeto-Burman ethnic group of Mongoloid racial stock [16]. Both the communities follow community endogamy. Marriage by negotiation is the prevailing practice among them. Consanguineous marriage is also present among the Bengali Muslims but it is prohibited among the Meiteis. They follow the patriarchal system of family structure. Rice is their staple food and agriculture is their mainstay of livelihood. The Muslims are generally non-vegetarian while most of the Meiteis are vegetarian.

5. RESULTS

Prevalence of haemoglobin variants has been observed among the Bengali Muslims (Table 1a). The percentage (%) of HbE and β -thalassemia among the Muslim population is found to be 3.7% each. Only one female (Figure 1a) is found to be heterozygous for HbE gene i.e. HbE trait (HbAE). One female (Figure 1b) is also found to have β -thalassemia trait (HbA β -thall). Apart from that other individuals are found to have a normal Hb pattern (HbAA). The gene frequency (each) for HbE and β -thalassemia among the Muslim population is found to be 0.0556 each (male-0.0000, female: 0.1154). The female who has HbE trait is found to have 23.8% and 3.5% of HbE and HbA₂ respectively (Table 2a). The female who has β -thalassemia trait is found to have HbA₂ of 5.5%.

Prevalence of only haemoglobin variant HbE has been observed among the Meitei population (Table 1b). The presence of HbE among the Meitei population is found to be 19.2% (Figure 1c). It is revealed that two males and three females are having heterozygous for HbE gene i.e. HbE trait

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(HbAE). The gene frequency for HbE among the Meitei population is found to be 0.0962 (male-0.08333, female: 0.10714). Average percentage of HbE and HbA₂ are found to be 24.5 and 4.1 respectively among the Meitei males. Average percentage of HbE and HbA₂ are 16.5 and 3.4 respectively among the Meitei females (Table 2b).

Table1a. Prevalence of Haemoglobin Variants among the Bengali Muslims

Sex		HbAA	HbAE	HbAβ-thal	Total	Gene Frequency		
						HbA	HbE	β-Thal
Male	No.	14	0	0	14	1.0000	0.0000	0.0000
	%	100.0	0.0	0.0	100.0			
Female	No.	11	1	1	13	0.7692	0.1154	0.1154
	%	84.6	7.7	7.7	100.0			
Total	No.	25	1	1	27	0.8888	0.0556	0.0556
	%	92.6	3.7	3.7	100.0			

Table1b. Prevalence of Haemoglobin Variants among the Meiteis

Sex		HbAA	HbAE	Total	Gene Frequency	
					HbA	HbE
Male	No.	10	2	12	0.91667	0.08333
	%	83.3	16.7	100.0		
Female	No.	11	3	14	0.89286	0.10714
	%	78.6	21.4	100.0		
Total	No.	21	5	26	0.90385	0.09615
	%	80.8	19.2	100.0		

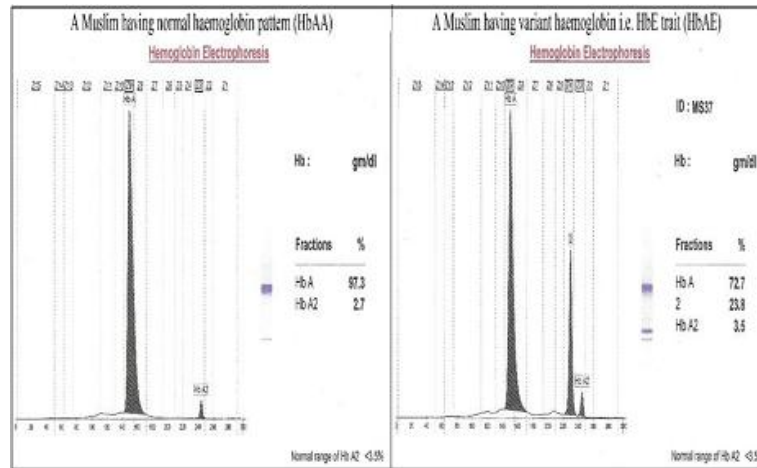


Figure1a. Haemoglobin Electrophoresis of Two Bengali Muslim Individuals, One Having Normal Haemoglobin Pattern and Another Having Haemoglobin E Trait

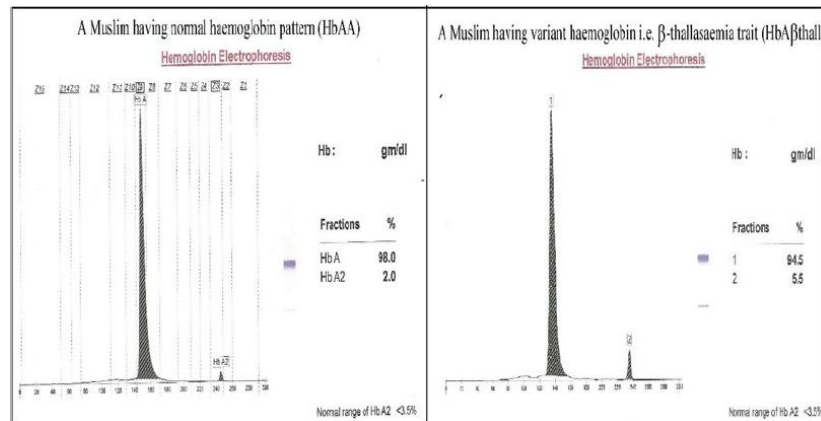


Figure1b. Haemoglobin Electrophoresis of Two Bengali Muslim Individuals, One Having Normal Haemoglobin Pattern and Another Having B-Thalassaemia Trait

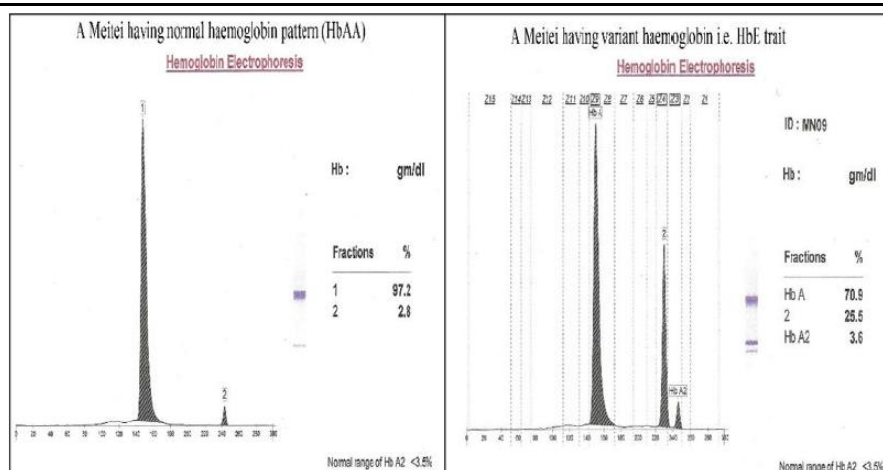


Figure1c. Haemoglobin Electrophoresis of Two Meitei Individuals, One Having Normal Haemoglobin Pattern and Another Having Haemoglobin E Trait.

Table2a. Haematological Comparison Between Normal Haemoglobins And Variant Haemoglobins Among The Bengali Muslims

Muslim			Average Values				
Haematology	Sex	Normal Range	Only HbAA ¹	Only Cases ²	Only HbAE ³	Only Hbβthal ⁴	Total (Cases also) ⁵
WBC	Male	(4.0-10.0) m/mm ³	5.21	-	-	-	5.21
	Female		7.25	6.00	4.70	7.30	7.06
RBC	Male	(4.0-5.9) m/mm ³	4.51	-	-	-	4.51
	Female	(3.8-6.0) m/mm ³	3.75	4.65	5.10	4.20	3.89
MCV	Male	(83.0-93.8) %	91.40	-	-	-	91.40
	Female	(80.0-100.0) %	84.78	71.10	72.90	69.30	82.68
Hct	Male	(35.0-54.0) %	40.93	-	-	-	40.93
	Female	(33.0-54.0) %	31.66	32.80	36.80	28.80	31.84
MCH	Male	(25.0-33.0) pg	30.67	-	-	-	30.67
	Female	(25.0-32.0) pg	28.15	23.70	24.90	22.50	27.46
MCHC	Male	(28.0-36.0) g/dl	33.66	-	-	-	33.66
	Female		33.05	33.40	34.20	32.60	33.10
RDW	Male	(8.0-12.0)	12.02	-	-	-	12.02
	Female		12.95	11.80	11.40	12.20	12.77
Hb	Male	(12.0-18.0) g/dl	13.76	-	-	-	13.76
	Female	(10.0-16.5) g/dl	10.60	11.00	12.60	9.40	10.66
HbA	Male	%	97.33	-	-	-	97.33
	Female		97.45	83.60	72.70	94.50	95.32
HbA2	Male	(<3.5) %	2.67	-	-	-	2.67
	Female		2.49	4.50	3.50	5.50	2.80
HbE	Male	%	-	-	-	-	0.00
	Female		-	11.90	23.80	-	1.83

No. of individuals: ¹Male-14, Female-11; ²Male-0, Female-2; ³Male-0, Female-2; ⁴Male-0, Female-2

Table2b. Haematological Comparison between Normal Haemoglobins and Variant Haemoglobins among the Meiteis

Meitei			Average Values		
Haematology	Sex	Normal Range	Only HbAA ¹	Only Cases (HbAE) ²	Total (Including HbAE) ³
WBC	Male	(4.0-10.0) m/mm ³	7.15	5.60	6.89
	Female		6.64	7.93	6.92
RBC	Male	(4.0-5.9) m/mm ³	4.18	4.60	4.25
	Female	(3.8-6.0) m/mm ³	3.72	4.03	3.79
MCV	Male	(83.0-93.8) %	90.60	80.85	88.98

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	Female	(80.0-100.0) %	87.65	76.27	85.21
Hct	Male	(35.0-54.0) %	37.37	36.95	37.30
	Female	(33.0-54.0) %	32.45	30.53	32.04
MCH	Male	(25.0-33.0) pg	32.43	25.60	31.29
	Female	(25.0-32.0) pg	31.28	27.30	30.43
MCHC	Male	(28.0-36.0) g/dl	35.88	31.80	35.20
	Female		35.70	35.83	35.73
RDW	Male	(8.0-12.0)	11.39	13.00	11.66
	Female		11.06	10.83	11.01
Hb	Male	(12.0-18.0) g/dl	13.48	11.75	13.19
	Female	(10.0-16.5) g/dl	11.66	10.97	11.51
HbA	Male	%	97.22	71.50	92.93
	Female		97.38	80.10	93.68
HbA2	Male	(<3.5) %	2.78	4.05	2.99
	Female		2.62	3.37	2.78
HbE	Male	%	-	24.45	4.08
	Female		-	16.53	3.54

¹No.-Male-10, Female-11; ²No.-Male-2, Female-3; ³No.-Male-12, Female-14

Haematological profile (Table 2.a) of the Muslim population has been observed to see the difference between individuals having normal haemoglobin pattern (HbAA) and cases (individuals having Hb variant). Average values of haematological parameters like WBC, RBC, MCV, Hct, MCH, MCHC, RDW and Hb are found to be within normal range among the males (no variant cases detected among the Muslim males). Average values of all the haematological parameters are also found to be in normal range among the females (excluding the cases of HbE and Hb β -thal) except Hct (31.66 %) and RDW (12.95). MCV and MCH values in HbE trait are found to be 72.9 8 (in %) and 24.9 5 (in pg) respectively which are lower than the normal range. Muslim female who is having β -thallasaemia trait is found to have lower than normal values of MCV (69.3 %), Hct (28.8 %), MCH (22.5 pg) and Hb (9.4 g/dl).

Haematological profile of the Meitei population shows that the average values of haematological parameters like WBC, RBC, MCV, Hct, MCH, MCHC, RDW and Hb are found to be within normal range among the normal (HbAA) males (Table 2.b). Average values of all the haematological parameters are also found to be in normal range among the normal females (HbAA) except RBC (3.7 m/mm³) and Hct (32.5 %). Average values of MCV (80.9 %) and Hb (11.8 g/dl) are found to be below normal range among the Meitei males who are having HbE trait. Average values of MCV (76.3 %) and Hct (30.5 %) are also found to be lower than normal range among the Meitei females who are heterozygous for HbE.

6. DISCUSSION

Haemoglobinopathies are the most commonly encountered hereditary abnormalities of blood posing a major genetic burden and public health problem in Southeast Asia and the Indian subcontinent [3]. HbS, HbE and HbD as well as thalassaemia are the most widely distributed haemoglobin disorders in India, out of which HbE is widely distributed in north-eastern states of India [4, 5, 6, 17, 18, 19]. The cumulative gene frequency of haemoglobinopathies in India is 4.2% [19]. The carrier frequency of haemoglobinopathies varies from 3.0% to 17.0% in different population groups of India [8].

Guha and Sharma [20] mentioned that HbE is the most prevalent variant haemoglobin in ethnic groups affiliated to Tibeto-Burman linguistic family. HbE gene frequency was found to be very high as reported by Das et al. [21] in some of the tribal populations of Assam like 0.549 among the Kacharis of lower Assam, 0.535 among the Rabhas 0.496 among the Garos and 0.436 among the Lalungs. Gene frequency of HbE was also found to be very high as much as 0.349 [22], 0.359 [23], 0.343 [24] among the Ahoms of Assam.

In the present study, presence of haemoglobin variants like of HbE (3.7%) and β -thalassaemia (3.7%) is observed among the Muslim population and all the cases are heterozygous. The gene frequency for

HbE and β -thalassemia among the Muslim population is found to be 0.0556 each (male-0.0000, female: 0.1154). On other hand, only HbE (19.2%) is found to be present among the Meitei population and all the cases are heterozygous for HbE gene i.e. HbE trait (HbAE). The gene frequency for HbE among the Meitei population is found to be 0.0962 (male-0.08333, female: 0.10714). So the presence of HbE, the most common haemoglobin variant in the whole Southeast Asia as well as Northeast India, is also observed among the two studied populations of Cachar District, Assam despite their genetic differences.

Earlier studies by Chakraborty and Roy [25] and Singh et al. [26] among the Meiteis of Manipur reported an allele frequency of 0.071 and 0.101 respectively. Singh and Singh [27] revealed the prevalence of HbE among the Meitei Muslims with an allele frequency of 0.059. Singh et al. [28] reported on HbE distribution in four endogamous populations from neighbouring Manipur state and their study reveals the allele frequency of HbE and β -thalassemia are 0.101 and 0.004 respectively among the Meitei population. They demonstrated the frequency of HbE in the remaining three populations namely, the Kabui (0.035), the Koirang (0.029) and the Simte (0.012) also. Indian Council of Medical Research (ICMR) conducted a study covering mostly city based populations amongst six cities which includes Kolkata in West Bengal in the east, Dibrugarh in Assam in the north-east reported that the prevalence of HbE trait as 3.92% in Kolkata and 23.90% in Dibrugarh [29]. Mandal et al. [30] studied on large samples from different rural areas of West Bengal and found the prevalence of HbE trait (2.78%).

Agarwal [31] indicated that β -thalassemia is detectable in almost every Indian population, however, it is seen with the highest frequency in north-west and far-east. Two large scale studies on haemoglobinopathy reported by Madan et al. [32] and Mandal et al. [30] on the prevalence of β -thalassemia trait was 5.5% in northern India, 2.7% in western India and 6.6% in eastern India. Earlier study conducted by Indian Council of Medical Research (ICMR) from different cities of eastern India (Kolkata, West Bengal), north east India (Dibrugarh, Assam) and southern India (Bangalore, Karnataka) showed prevalence of β -thalassemia carriers was 3.64%, 1.48% and 2.16%, respectively [30]. Uddin et al. [33] reported on haemoglobinopathy from a hospital based study among the 210 peoples and revealed that the prevalence of thalassemia trait was 47.14%, HbE- β -thalassemia was 30.47%, HbE trait was 13.3%, HbE disease was 5.71% and thalassemia major were 3.33%.

In the present studied population, average values of haematological parameters are found to be within normal range among the Muslims who are having normal (HbAA) haemoglobin pattern except Hct (31.66 %) and RDW (12.95) among the females which is indicating the presence of iron deficiency anemia. MCV (72.9 %) and MCH (72.9 %) values are found to be lower than the normal value (MCV: 80.0-100.0 %; MCH: 25.0-32.0 pg) in HbE heterozygous cases among the Muslims. Muslim female who is having β -thalassemia trait is also found to have lower than normal values of MCV (69.3 %), Hct (28.8 %), MCH (22.5 pg) and Hb (9.4 g/dl). Average values of the haematological parameters are found to be within normal range among the Meiteis who are having normal haemoglobin (HbAA) pattern except RBC (3.7 m/mm^3) and Hct (32.5 %) among the females; but the average values of MCV (80.9 %) and Hb (11.8 g/dl) are found to be lower than normal range among the Meitei males who are having HbE trait. Average values of MCV (76.3 %) and Hct (30.5 %) are also found to be lower than normal range among the Meitei females who are heterozygous for HbE gene. 35. Das et al. [34] showed a trend of more anemic individuals among the HbE homozygotes in contrast to HbE heterozygotes and normal HbA individuals. Earlier research carried out by Balgir et al. [35] among the Bhuyan and Kharia tribes of Sundargarh district in Orissa showed low MCV and low MCH counts in HbE trait and β -thalassemia trait as compared to individuals having normal haemoglobin pattern.

HbE trait is an asymptomatic state with no clinical significance, except for the threat of compound heterozygotes with β -thalassemia. However such cases of compound heterozygotes have not been observed in the present studied populations. Most of the individuals with HbE trait have reduced mean corpuscular volume (MCV) and Mean cell haemoglobin (MCH) with or without mild anemia [36]. Fucharoen and Winichagoon [3] mentioned that HbE trait has no clinical significance and individuals may have mild microcytosis without anemia. The red cell morphology may show individuals having HbEE pattern are asymptomatic with very mild anemia and microcytosis. [37]. Das and Deka [38] mentioned that HbE homozygotes are mildly to moderately anemic. Swarup et al. [39] mentioned that severe iron deficiency anemia reduces the proportion of HbE in heterozygotes which indicated that haemoglobin production governed by the variant gene is more severely affected.

The presence of HbE is not restricted only to the populations of Mongoloid stock of North East India but also been reported in other genetically diverse populations of Southern, Northern and Western parts of India which is visible from various other studies mentioned earlier as well as from the present study. Mandal et al. [30] indicated that the occurrence of larger frequency of HbE in populations of Northeast India, Laos, Thailand and Cambodia forms 'haemoglobin E quadrangle'.

7. CONCLUSION

Presence of haemoglobin variants like of HbE (3.7%) and β -thalassemia (3.7%) is observed among the Muslim population and all the cases are heterozygous. The gene frequency for HbE and β -thalassemia among the Muslim population is found to be 0.0556 each (male-0.0000, female: 0.1154). On other hand, only HbE trait (19.2%) is found to be present among the Meitei population. The gene frequency for HbE among the Meitei population is found to be 0.0962 (male-0.08333, female: 0.10714). Mean Corpuscular Volume (MCV) and mean haemoglobin concentration level (Hb) are found to be lower in haemoglobin variants in contrast to normal haemoglobin pattern (HbAA) in both the communities.

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