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Research Paper

Prevalence of Hb E among adolescents in Basic Education High School, Anisakhan, Pyin Oo Lwin Township

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ABSTRACT
*Haemoglobin (Hb) E is the most common Hb variant found among Southeast Asian populations. Myanmar also has high prevalence of important haemoglobinopathies: Hb E - 1 to 28.3% accounting for 1 to 4.9 births per 1000 infants with a major haemoglobinopathy. This study was conducted to determine the prevalence of Hb E among adolescents in Basic Education High School, Anisakhan, Pyin Oo Lwin Township. This community-based cross-sectional descriptive study was done in Myanmar adolescents in 2017. A total 290 apparently healthy high school students in which male 140 (48.3%) and female 150 (51.7%) studying in Basic Education High School, Anisakhan, Pyin Oo Lwin Township, Mandalay District, Myanmar were involved. Then, three mililitres of venous blood samples were taken andHb E by NESTROFT (Naked Eye Single Tube Red Cell Osmotic fragility test) and DCIP (Di Chlorophenol Indol Phenol precipitation) test were done for screening and then Isoelectric focusing (IEF) method was done for confirmation of Hb E to above either of the two positive screening samples. Data entry and analysis was done by SPSS software 20.0 version. The overall prevalence of Hb E trait and Hb E disease was 15.5% (45/290) and, 19 cases observed in male (42.2%) and 26 cases were found in female (57.8%) in this study. Among 290 participants, 53 (18.3%) were positive with NESTROFT but not with the DCIP; 17 (5.9%) were negative with the NESTROFT but positive with DCIP; 61 (21.0%) were positive with both tests and 159 participants (54.8%) were negative with both tests. Among 131 either of the two positive cases, both positive (Positive/ Positive**)** 61 cases gave 23 cases (37.7%), (Positive/Negative) 53 cases gave 16 cases (30.2%) and (Negative/Positive) 17 cases gave 6 cases (35.3%) as identified as Hb E of total 45 cases (45/131, 34.3%) in which 41 samples (31.3%) were Hb E trait and 4 samples (3.1%) were Hb E disease. Therefore, this study highlighted that one of the main causes of anaemia was haemoglobinopathy and remains a common health problem among adolescents.*

KEY WORDS: *Hb E, high school students, NESTROFT, DCIP*

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I. INTRODUCTION

Mutations in human beta globin gene cause genetic disorders such as β-thalassaemia as well as sickle cell disease, Haemoglobin C (Hb C) and Haemoglobin E (Hb E).[1]Hemoglobin (Hb) E is one of the world's most common, important mutations and the second most prevalent haemoglobinopathy after Hb S showing the highest prevalence in South East Asia.[2]

Hb E is defined by the heterozygous condition associated with one, normal adult haemoglobin (HbA) β gene and one variant (Hb E) β gene. Hb E is common in South East Asia. Thailand and Myanmar have an overall prevalence of around 14-15 percent.[3] The most common combination of beta-thalassaemia with abnormal Hb or structural Hb variant with thalassemic properties is Hb E/β-thalassaemia which is most prevalent in South East Asia where the carrier frequency is around 50 percent.[4]

Haemoglobin E has the clinical phenotype of a mild form of β-thalassaemia, and is most frequent in south-east Asia, particularly eastern Thailand and Laos. The combination of Hb E with β-thalassaemia spans thalassaemia phenotypes, from a condition indistinguishable from thalassaemia major to a mild form of thalassaemia intermedia.[5] Hb E/β thalassaemia is the commonest severe form of thalassaemia in South East Asia and parts of the Indian subcontinent. Hb E is inefficiently synthesized and hence, when it is inherited together with β^0-thalassaemia, there is a marked deficiency of β-chain production.[6]

Many patients with HbE/ β-thalassemia disease seen in Myanmar, unlike thalassaemia major, may survive till adult life and full physical and sexual development is possible in a significant number.[7] Although not always transfusion dependent, haemoglobin values are in the 4-9g/dL range, with an average of 6-7 g/dL. Although very little is known about the natural history of this disorder, it is clear that in many part of South East Asia and India it causes a very high mortality in early life.[6]

Complications include a marked proneness to infection, secondary hypersplenism, progressive iron loading, neurological lesions due to masses of extramedullary erythropoietic tissues extending in from the inner tables of the skull or vertebrae, folate deficiency, leg ulcers and recurrent pathological fractures. On the other hand, some patients grow and develop normally with few complications.[6]

In Myanmar 2000, peripheral bloods of 132 children were examined for Hb E by using anion-exchange high performance liquid chromatography (DEAE-HPLC_) and iso-electric focusing (IEF). But Hb E and Hb A_2 bands were not separated by these two methods. The prevalence of Hb E was confirmed by β^E globin gene detection of a restriction enzyme (MnL I) assay of PCR products.[8] More sophisticated techniques such as real-time PCR and oligonucleotide microarray analysis have been described for the rapid analysis of thalassemia and hemoglobinopathies; however, these techniques are more sophisticated and expensive than current screening tests.[9]Therefore, accurate determination of Hb E was important by using effective diagnosis protocol for the Hb E carrier screening in Myanmar. The purpose of this study aimed to assess distribution of Hb E in which it could be detected firstly by screening methods. As the combined NESTROFT/DCIP was simple, reliable, economically appropriate and practical methods for screening of Hb E, it could be used as preliminary screening test for identifying the carriers of Hb E. Then, Isoelectric Focusing will be performed in this study to confirm Hb E carrier.

The management of Hb E trait and disease includes only folic acid but in Hb E/β thalassaemia, periodic blood transfusions may be required to maintain the haemoglobin level.The purpose of this study is to assess distribution of Hb E among these populations in which it highlights the increasing prevalence of Hb E variants in upper Myanmar. The health and well-being of these high school students has a major impact on the overall social, economic development of the country as they are tomorrow's workforce, parents and leaders. Moreover, this study may be useful for early diagnosis and important to avoid unnecessary prescription of iron salts and then, the results of this study may provide the data for further research.

II. MATERIALS AND METHODS

This community-based cross-sectional descriptive study was done at Pathology Research Division, Department of Medical Research (Pyin Oo Lwin Branch) and Basic Education High School, Anisakhan, Pyin Oo Lwin Township**.** A total of 290 apparently healthy Grade IX students, totally unrelated whose parents/guardian had given the written informed consent were recruited by using systematic sampling procedure during January to December 2017**.** Then, three mililitres of venous blood samples were taken andHb E by NESTROFT (Naked Eye Single Tube Red Cell Osmotic fragility test) and DCIP (Di Chlorophenol Indol Phenol precipitation) test were done for screening and then Isoelectric focusing (IEF) method was done for confirmation of Hb E to above either of the two positive screening samples. Data entry and analysis was done by SPSS software 20.0 version. Frequency charts were defined and continuous variables' averages and standard errors (SE) were calculated. The prevalence rates were calculated with 95% Confidence Interval (95% CI). Regarding the Ethical consideration, the approval was obtained from the Ethical Review Committee of the Department of Medical Research before the study was conducted.

III. RESULTS

Background subjects' characteristics revealed that total 290 Grade IX high school students aged between (14-15yrs) who were studying at Basic Education High School, Anisakhan, Pyin Oo Lwin Township were randomly selected within the period of January to June, 2017.

They were apparently healthy and after getting both informed consent and assent forms by their parents/guardians and themselves, their personal identifications were noted down in the pro-forma. In this study, these were 140 male (48.3%) and 150 female (51.7%) participants of high school students. Female was more preponderant than male with male to female ratio of approximately 1: 1.1.

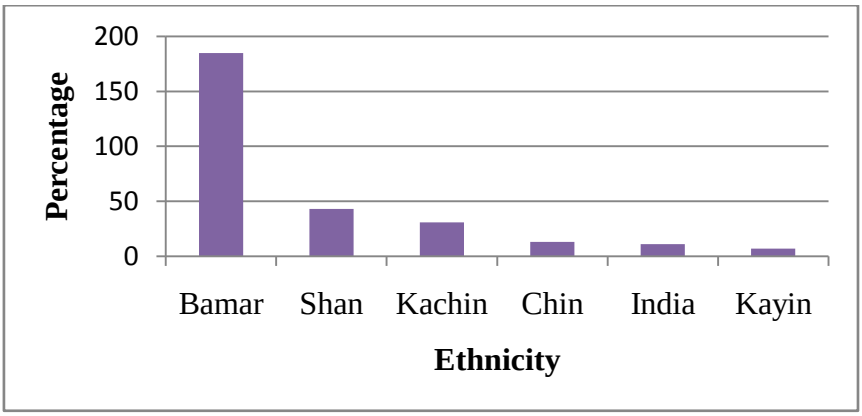

Figure 1. Distribution of participants by Ethnicity (n=290)

Overwhelming majority of the participants in the study was Bamar (63.8%). Others include Shan (14.8%), Kachin (10.7%), Chin (4.5%), India (3.8%) and Kayin (2.4%). In this study, 185samples were Bamar and105were otherraces such as Shan, Kachin, Chin, India and Kayin. BamardominatedthestudypopulationsfollowedbyShan.

In this study, 131 cases were detected as possible carrier of heterozygous β-thalassaemia. Contribution of races among β-thalassaemia cases were 92 cases (49.7%) of Bamar, 15 cases (48.4%) of Kachin, 3 cases (42.9%) of Kayin, 3 cases (23.1%) of Chin, 14 cases (32.6%) of Shan, and 4 cases (36.3%) of Indian respectively.

The 290 participants were screened for possible carrier of heterozygous β-thalassaemia including Hb E by doing two separate thalassaemia screening tests. Among 290 participants, 114 participants (39.3%) were positive in NESTROFT screening test and the rest 176 (60.7%) were negative screening test. In this study, positive DCIP screening was observed in 78 participants (26.9%) and negative in 212 participants (73.1%).

Table 1. Determination of results of both NESTROFT/DCIP tests in participants

Combined (NESTROFT/DCIP) Test	Number (n=290)	Percentage (%)
(NESTROFT +/DCIP +)	61	21.0
(NESTROFT +/DCIP -)	53	18.3
(NESTROFT -/DCIP +)	17	5.9
(NESTROFT -/DCIP -)	159	54.8
Total	290	100.0

Among 290 participants, 53 (18.3%) were positive with NESTROFT but not with the DCIP; 17 (5.9%) were negative with the NESTROFT but positive with DCIP; 61 (21.0%) were positive with both tests and 159 participants (54.8%) were negative with both tests. Those positives with either of the two screening tests (131 samples) were considered as heterozygous beta thalassemia including Hb E.

Among 131 either of the two positive cases, both positive (Positive/ Positive**)** 61 cases gave 23 cases (37.7%), (Positive/Negative) 53 cases gave 16 cases (30.2%) and (Negative/Positive) 17 cases gave 6 cases (35.3%) as identified as Hb E of total 45 cases(45/131, 34.3%).

Table 2. Screening and confirmatory cases of combined (NESTROFT/DCIP) results on Hb E

Combined (NESTROFT/DCIP)	Hb E	
	Number	Percentage
(Positive/ Positive) 61	23	37.7
(Positive/Negative) 53	16	30.2
(Negative/Positive) 17	6	35.3
Total = 131	45	34.3

After using combined NESTROFT and DCIP screening tests, out of 290 participants, 131 samples with positive in either one of the two screening tests or both were determined for heterozygous beta thalassemia including haemoglobin E by using IEF method; 45/131 (34.4%) showed Hb E carrier status such as 41 samples (31.3%) were Hb E trait and 4 samples (3.1%) were Hb E disease.

Table 3. Distribution of combined (NESTROFT/DCIP) results in Hb electrophoresis results by IEF

(NESTROFT/DCIP)	Hb AE	Hb EE	Hb A
(NESTROFT+/ DCIP+) n=61	22 (36.1%)	2 (3.3%)	37 (60.6%)
(NESTROFT+/ DCIP-) n=53	14 (26.4%)	2 (3.8%)	37 (69.8%)
(NESTROFT-/ DCIP+) n=17	5 (29.4%)	0 (0.0%)	12 (70.6%)
Total n=131	41	4	86

It showed the distribution of combined NESTROFT/ DCIP screening results in different Hb types. Hb AE of 22 cases (36.1%), Hb EE of 2 cases (3.3%), and Hb A of 37 cases (60.6%) were both positives screening (+,+). The positive NESTROFT and negative DCIP revealed that Hb AE of 14 cases (26.4%), Hb EE of 2 cases (3.8%), and Hb A of 37 cases (69.8%) respectively. The (-,+) screening result gave 5 cases of Hb AE and 12 cases of Hb A.

IV. DISCUSSION

The prevalence of β-thalassaemia varies with gender and race. Among 131 possible carrier of heterozygous β-thalassaemia, female was predominant than male (52.6% vs 37.1%) and Hb E trait and Hb E disease (17.3% vs 13.6% vs). The overall prevalence of Hb E trait and Hb E disease was 15.5% (45/290) and, 19 cases observed in male (42.2%) and 26 cases were found in female (57.8%) in this study.

In 2004, Yi Yi Tin studied that female preponderance with male to female ratio being 1: 1.81 in out of 90 family members of transfusion dependent β-thalassaemia major cases and clinically suspected of thalassaemia intermedia cases.[10] In the study of Genc et al. (2012), they applied for a premarital screening program in Turkey in which there was a female preponderance among 1616 patients, 813 individuals (50.3%) were male and 803 (49.7%) were female. [11] In the United States (US) study done by Giardina et al. (1997), 35 males and 36 females were included (male: female 1:1.03). [12] In the study of Indian thalassaemia intermedia, 60 males and 33 females were included (male: female 1.82:1) (Seema et al., 2003). [13] In another Indian study, 35 males and 19 females were included (male: female 1.84:1) (Phadke & Agarwal, 2003).[14] Therefore, the sex distribution in this study was not much different from foreign studies and local studies.

Mostoftheβ-thalassemia mutationsarecausedbypointmutations,smalldeletions or insertions within the coding regions and the exon-intron junctions. Thetypesofthemutationaretypicallyethnic specific. [15,16,17] Betathalassaemiaisextremelyheterogeneousatthemolecularlevelwithmorethan200differentmutations identified.Thevastmajorityofthemutationsarecausedbypointmutationswiththeexceptionofafewdeletions.However, eachethnicgrouphasitsowncharacteristicsetof β-thalassaemiamutationswithcommonandsome rare mutations. [18]

Among the 290 participants, 6 different races were identified. Different ethnic groups from Mandalay region, from most areas of Northen Shan State and Kachin state had migrated to this Pyin Oo Lwin. Among 131 possible carrier of heterozygous β-thalassaemia, Bamar was (49.7%). Others include Kachin (48.4%), Kayin (42.9%), Chin (23.1%), Shan (32.6%) and India (36.3%).

Overall prevalence of Hb E trait and Hb E disease was 15.5% (45/290) in which 32 (71.1%) were Bamar and 13 (28.9%) were other ethnics respectively. In the study done by Yi Yi Tin (2004) [10], 81.8% were Myanmar races (Bamar, Kayin, Yakhine) and 18.2% were other races such as Chinese and Indians. In the study done by Sein Win (2010) [19], 79.61% were Myanmar races which included Bamar, Kayin, Shan, Yakhine, and Mon and 20.39% were either Chinese, or Indian, or Myanmar races with Chinese and Indian blood. Thus, racial distributions in these two local studies are quite similar. But in this study, Myanmar races are highly dominated (96.2%) and followed by Indian (3.8%).

A combined NESTROFT and DCIP tests for Hb E had been proposed to screening in rural communities of South East Asia. [20] In this study, 114 out of 290 samples (39. 3%) were positive NESTROFT and that of 176 samples (60.7%) were negative.This finding was consistent with the other studies which were done in Myanmar. One of the studies stated that 155 High School Students were determined for haemoglobinopathies by NESTROFT screening test, 60/155 (38.7%) were positive and also 21/60 cases were positive with confirmatory IEF method. [21]

Among 290 participants, single DCIP precipitation test alone was positive in 78 samples (26.9%) and negative in 212 samples (73.1%) in this study. The results were more or less similar to the results of previous studies in Myanmar. Aye Aye Win (2015) [22] stated that DCIP screening test in 140 cases were done in which 83 cases (59.3%) were DCIP positive and remaining 57 cases were DCIP negative. In another study, 27 out of 155 participants (17.4%) showed positive DCIP test. [21]If this method was used together with the NESTROFT, the results of the Hb E screening will be easier to interpret.

Regarding with thalassaemia screening tests, NESTROFT is positive in 87% of Hb E carrier and DCIP is positive in 64% of Hb E carrier which was proved by IEF method. Both positive combined (NESTROFT/

DCIP) screening was more sensitive than either of the two positive test (77.0% vs 54.7% and 76.5%). Therefore, it can be used as preliminary screening test for identifying the carriers of heterozygous β-thalassaemia including Hb E in population screening. By doing these screening tests together, the diagnosis for β-thalassaemia was more accurate for asymptomatic cases.

Molecular diagnosis, however, seems to be better for definitive diagnosis of thalassaemia syndromes at birth, IEF can be applied in a resource-limiting setting with acceptable reliability (Teinthavorn, 2006). IEF detects both normal and abnormal Hb variants, only qualitatively. The bands from Hb E and Hb A_2 are not separated in IEF. The more the concentration and the thicker the IEF band, the more likely is Hb E.[23, 24]

In this study, the overall Hb E carrier status was detected in 45 out of 290 samples (15.5%). 131 out of 290 students were chosen for IEF method in which heterozygous A (Hb A) 86 /131 samples (65.6%), haemoglobin E trait (Hb AE) 41 samples (31.3%) and haemoglobin E disease (Hb EE) 4 cases (3.1%) were found among those 131 students. The findings were concordance with Yi Yi Tin (2004)[10] who have shown that different haemoglobin phenotypes such as heterozygous A (Hb A) 55/90 cases (61.1%), Hb E/ β-thalassaemia (Hb EF) 6/90 cases (6.6%), haemoglobin E trait (Hb AE) 23 cases (25.6%) and haemoglobin E disease (Hb EE) 2 cases (2.2%) respectively. In 2010, Sein Win[19] reportedthat both cellulose acetate paper electrophoresis and isoelectric focusing electrophoresis were used to identify the haemoglobin phenotype of 103 patients with β-thalassaemia intermedia and found out that 94 patients were Hb E/β-thalassaemia intermedia patients and 9 patients were β-thalassaemia intermedia patients with no associated Hb E and he concluded that Hb E/β-thalassaemia was the commonest thalassaemia in Myanmar.

Hb Eoccursatahighfrequency inpartsof North EastIndiaandthroughoutSouth EastAsia.In Thailand and other South East Asian countries, thalassaemia is very common with 20-30% of the population having the α-thalassaemia trait, 3-9% having β-thalassaemia trait whereas 20-30% having the Hb E trait.[25]Accordingtoaprevious studydoneinSri Lanka,HbE/β-thalassaemia(CD26G-A)mutationwasobservedatafrequencyof26.3%.[26]This study was aggrement with other study which was done on 152 participants with normal Hb level and normal MCV of healthy individuals, there was 24 Hb E traits (15.8%), no α-thalassaemia trait and 1 case of β-thalassaemia trait.[27]

Perceived to be proactive and receptive to various screening programs and awareness generation activities, schools serve as receptive area for adolescents. Catering a mix of various racial groups and ethnicities, schools represent the existent local community. Comprehensive data on β-thalassemia trait amongthe adolescents are still absent.Hence,thereis an urgentneed ofraisingpublicawarenessandgovernmental actionin educating theyounggenerationabouttheimportanceto alleviateandcontrolthediseaseinthefuture.

In this study, total of 290 Grade IX High School Students were studied to screen for β-thalassaemia by analyzing combined NESTROFT and DCIP precipitation tests and haemoglobin analysis for IEF method.As the screening tests used in this study were simple, reliable, economically appropriate and practical methods, they could be used as preliminary screening procedures for identifying carriers' status (α^0-thalassaemia, β-thalassaemia and Hb E). It would be very useful in screening survey with restricted human resources and laboratory capacity in areas of thalassaemia ethnic region like Myanmar.

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