Haemoglobinopathies – major associating determinants in prevalence of anaemia among adolescent girl students of Assam, India
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Introduction
Anaemia is a widespread public health problem associated with an increased risk of morbidity and mortality, especially in pregnant women and young children. As in other developing countries, anaemia is a major public health problem in India. Assam, a north-eastern state of India, also reflects a similar scenario of the national average in prevalence of anaemia. Studies conducted reflect high prevalence of anaemia among tea garden workers of Assam.

Prevalence of anaemia among adolescent girl students of Assam, a north-eastern state of India, was evaluated along with its associating determinants. The present study revealed that anaemia is a major public health problem among adolescent girl students of Assam. The overall prevalence of anaemia among adolescent girl students of Assam is as high as 71.5%. Non-nutritional factors such as infection due to helminths was substantially low (24.71%). Ascaris lumbricoides was the most frequent infection (10.6%), followed by Trichuris trichiura (6.2%), and hookworm infestations (3.9%). Polyparasitic infection (A. lumbricoides, T. trichiura and hookworm) was observed in 0.5% of the study subjects. While coinfection due to A. lumbricoides and T. trichiura was 2.3%, A. lumbricoides and hookworm was 1.1% and T. trichiura and hookworm was 0.9%. Serum ferritin level in a subgroup of samples was in the lower normal range. Malaria parasite was not detected in any of the slides. We have observed a gene frequency of 0.188 for βE-globin gene among the adolescent girl students of Assam. The gene frequency for βE-globin gene ranged from 0.071 to 0.266. Statistically significant difference (F=3.471; P=0.001) of mean haemoglobin level was observed in different types of haemoglobin variants. Multiple regression analysis, in a subset of samples having information on Hb levels (g/dl), helminthic infestation (A. lumbricoides, T. trichuria and hookworm), haemoglobin type, revealed haemoglobin type (Hb E) was the important determinant of anaemia among adolescent girl students in the present study.

Non-nutritional factors associated with anaemia, such as geohelminthic infestations, malaria, haemoglobinopathies etc. are widely prevalent in this part of the country due to the geoclimatic conditions and ethnic affiliation of the people of this region. Earlier studies indicate high prevalence of helminthic infestations in this part of the country. The north-eastern region of India exhibits unique topography with valleys and
hills with tropical evergreen rain forests and vivid isothermic zones. This geoclimatic condition of the region facilitates transmission of malaria parasites, especially *Plasmodium falciparum*, with widespread distribution of chloroquine-resistant strains. The north-eastern region of India is considered as a mosaic of people with diverse ethnicity and cultural entity. Streams of human waves had migrated to this part of the country centuries ago and settled in different parts of the region maintaining their sociocultural solidarity. As in other South-East Asian countries, the autochthonous inhabitants of this part of India are predominantly of Mon-Khamer, Tibeto-Burman, Astro-Asiatic origin. Thus, with the linguistic and cultural affiliation of the autochthonous inhabitants of north-east India with the south-east Asian population, the region exhibits high gene frequency for Hb E. Sickle cell haemoglobin is mostly confined to the tea garden labour communities of Assam. Prevalence of thalassemia carrier state among college students and expectant mothers was 3.24% and 3.2%, respectively. Further, a positive correlation of *P. falciparum* percentage and high gene frequency for βE was documented in malaria-endemic zones of north-east India.

Therefore, the present study is an attempt to find out the prevalence of anaemia among adolescent girl students of selected districts of Assam with an attempt to evaluate the associating determinants of anaemia.

**Materials and methods**

**Study area, sample size and collection of samples**

In the present study four districts, viz. Barpeta, Bongaigaon, Kamrup and Dibrugarh, were included purposively. Considering 50% prevalence of anaemia, to get maximum sample size, with confidence coefficient of 95%, confidence interval of ±5%, design effect of 1.3% and rate of homogeneity 2%, the estimated total sample required is of 510 individuals from 30 clusters from each districts. However, considering the refusal rate, a cluster size of 40 was maintained. The selection of the schools in the respective districts was based on probability proportional to size (PPS) sampling approach and students enrolled in VIII and IX standards were included in the study. In case of refusal by the school authority, an adjacent school under the same block was included in the study.

Forty girl students were selected randomly after serially listing the total girl students of each selected school using random number table. In case of refusal or non-availability, additional students were selected on the basis of the random table. Identified schools were visited prior to the collection of the samples and briefed about the programme. On the day of sample collection, details about the students pertaining to age, class in which studying, name of the head of the family, information on residential address etc. were recorded in a predesigned proforma. Written consent from the parents was obtained before collection of samples.

Finger-pricked blood samples (20 µl) were collected using fixed volume micropipette and transferred to individually labelled 2.5 multi 2.5 inch Whatman filter paper (No: 1). The samples were air dried and packed individually in low-density polyethylene (LDPE) autoseal pouches after proper marking for estimation of haemoglobin. Collected samples were analysed within 10 days of collection.

Prevalence of haemoglobinopathies, thalassaemia and serum ferritin level, venous blood (3 ml) was collected from every 10th individual in K3EDTA vials (AcCuvet Disposible) as well as in plain Clot activator vials (AcCuvet Premium). In case of refusal by the selected...
10th individual, the next subject was included in the study. Necessary precautions were taken in storage and transportation of the samples.

Stool samples were collected in two labelled vials, one containing 10% formal-saline and the other having 2.5% potassium dichromate solution to study the prevalence of helminthic infestations. The vials were distributed to the selected individuals on the day of collection of the blood samples. The students were advised to dispense about 2–3 g of stool samples in each vial separately and hand over the samples to the field team the next day. In case of defaulters, an additional two attempts were made for collection of the stool samples. The samples were finally transported to the laboratory.

Thick and thin smears of fresh blood samples were prepared for individual students to study malarial infections. The slides were labelled and air dried and transported to the laboratory for screening.

Analysis of samples

Haemoglobin levels of the individual dry blood samples were estimated by standard cyanmethaemoglobin method after elution in Drabkin’s solution.21 Prevalence of haemoglobinopathies and thalassaemias was determined by cation exchanger HPLC based Variant Hemoglobin Testing System (BioRad) using β-thalassaemia short programme as per protocol of the manufacturer (BioRad) provided along with the kit. Serum ferritin level of individual serum samples were determined by commercially available ferritin ELISA kit (Calbiotech Inc.). Protocol provided by the manufacturer was followed for estimation of serum ferritin. Detection of malaria parasite was based on examination of both thick and thin smears of the blood slides stained with 3% Giemsa stain. Similarly, stool samples collected from the field were analysed microscopically for presence of helminthic infestation by adopting the formalin-ether method of World Health Organization (WHO), 1994.

The data generated were computed and analysed using suitable statistical software packages such as Epi Info, SPSS etc. Gene frequency of βE-globin gene was calculated based on the Hardy–Weinberg Equilibrium. Further, in order to understand the correlation of Hb levels multiple regression analysis was performed in a sub set of samples where information on haemoglobin type and helminthic infestation was available. The mean haemoglobin level across three different groups, based on haemoglobin types (Hb AA, Hb AE, Hb EE) was compared by Analysis of Variance (ANOVA). Multiple regression analysis was used to determine factors influencing haemoglobin level in a sub set of adolescent girls. The factors (independent variables), haemoglobin type, helminthic infestations, due to Ascaris, Trichuris and hookworm, were entered as categorical variables (0=absence and 1=presence) and the haemoglobin level (dependent variable) was entered as continuous variable. We used “Enter” option for regression analysis. In this type of analysis all independent variables are entered in a block in a single step.

Results

Dry blood samples numbering 4457 were collected from Barpeta (n=1116), Bongaigaon (n=1150), Dibrugarh (n=1069) and Kamrup (n=1122) districts were screened to study the prevalence of anaemia by estimating haemoglobin (Hb) levels. The overall refusal rate, i.e. total number of individual samples who refused to participate in the study divided by numbers of individual approached for analysis of blood samples was 5.8%. However, the district-wise dropout rate in Barpeta,
Bongaigaon, Dibrugarh and Kamrup district was 7%, 4.3%, 10.9% and 6%, respectively. The mean (± SD) age group of the study subjects was 13.62 ± 1.1 years.

The mean (± SD) haemoglobin level of the adolescent girl students enrolled in the study was 11.02 ± 1.79 g/dl. In Barpeta, Bongaigaon, Dibrugarh and Kamrup districts the mean (± SD) haemoglobin levels of adolescent girl students was recorded as 11.15 ± 1.72, 11.40 ± 1.69, 10.81 ± 1.95 and 10.69 ± 1.72 g/dl respectively. Hb level was <12.0 g/dl in 71.5% of the adolescent girl students, indicating high prevalence of anaemia. District prevalence of anaemia and severity of anaemia among the study population is depicted in Figure 1. No significant difference was observed in haemoglobin level in duplicate samples, obtained from every 10th individual in filter paper, for quality control for estimation of haemoglobin from dry blood samples (Figure 2).

Stool samples numbering 2499 were analysed for the prevalence of helminthic infestations. The number of stool samples received from Barpeta, Bongaigaon, Dibrugarh and Kamrup districts was 705, 707, 403 and 684, respectively. The refusal rate in collection of stool samples was 43.8%. The district-wise rate of refusal was 36.8%, 38.5%, 36.0% and 64.1% for Barpeta, Bongaigaon, Dibrugarh and Kamrup districts respectively. Helminthic infestation was observed only in 16.97% of the students. Dibrugarh district exhibits maximum prevalence of helminthic infestation at 24.71%. Helminthic infestation in Barpeta, Bongaigaon and Kamrup districts was 18.01%, 10.33% and 13.65%, respectively. Overall, *A. lumbricoides* was the most frequent helminthic infection (10.6%), followed by *T. trichiura* (6.2%), and hookworm infestations (3.9%). Polyparasitic infection (*A. lumbricoides, T. trichiura* and hookworm) was observed in 0.5% of the study subjects.

![Figure 1: Prevalence and severity of anaemia among adolescent girl students of Assam](image-url)
While coinfection due to \textit{A. lumbricoides} and \textit{T. trichiura} was 2.3%, \textit{A. lumbricoides} and hookworm was 1.1% and \textit{T. trichiura} and hookworm was 0.9%. Prevalence and distribution of different helminthic infestation among adolescent girl students of Assam is reflected in Figure 3.

Absence of malaria parasite in any of the collected blood samples \((n=4457)\) indicates lack of even asymptomatic malaria cases in the study population. Ten per cent of the slides were cross-checked for interobservation variance and 100% concordance was noted.

Screening of 419 blood samples by cation exchanger HPLC based Variant Hemoglobin testing system (BioRad) indicates Hb E is a widely prevalent variant haemoglobin in the present study. The gene frequency for \(\beta^E\)-globin gene among adolescent girl students of Assam in the present study was 0.188. \(\beta^E\)-globin gene frequency was highest in Dibrugarh district (0.266). Gene frequency for \(\beta^E\)-globin gene in Barpeta, Bongaigaon and Kamrup districts was recorded as 0.071, 0.239 and 0.173, respectively. Figure 4 represents the prevalence of Hb E and other variant haemoglobins along with suspected \(\beta\)-thalassaemia observed in the present study.

In addition to Hb E, other variant haemoglobins like HbS and HbD was also observed in very low frequency. Two cases of heterozygous state of sickle cell haemoglobin were observed in Dibrugarh district and both the cases were from the tea garden labour community. While heterozygous state of Hb D was observed in one subject of Bongaigaon district, one subject was detected with double heterozygous state of Hb ED in Kamrup district.
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Figure 3: Prevalence of helminthic infestation among adolescent girl students of Assam

Figure 4: Prevalence of haemoglobinopathies and thalassaemias among adolescent girl students of Assam
Serum ferritin levels of adolescent girl students were screened in the present study. Of 419 samples, 112 were from Barpeta, 95 from Bongaigaon, 111 from Dibrugarh and 101 from Kamrup districts, and were screened for serum ferritin level. Mean ± (SD) serum ferritin level among the adolescent girl students was 20.94±7.3 ng/dl. Considering <10 ng/dl of serum ferritin as the cut off for females, it was observed that only two samples (0.5%), one each with normal (HbAA) and heterozygous (HbAE), were with <10 ng/dl of serum ferritin. The remaining 99.5 % of samples were with normal level of serum ferritin.

Information on haemoglobinopathies was available for 419 girl students. Hb E is the major variant haemoglobin prevalent among the adolescent girl students of Assam. A statistically significant difference (F=3.471; P=0.001) of mean haemoglobin level was observed in different types of haemoglobin variants, particularly Hb E, among the adolescent girl students (Figure 5).

Further, in a sub set of 256 samples, information on all parameters i.e. Hb levels (g/dl), helminthic infestation (A. lumbricoides, T. trichuria and hookworm) and haemoglobin type were available. Multiple regression analysis revealed haemoglobin type (Hb E) is the important determinant of anaemia among adolescent girl students of Assam in the present study (Table 1 and Figure 6).

**Discussion**

The present study revealed that anaemia is a major public health problem among the adolescent girl students of Assam with the overall prevalence as high as 71.5%. This corroborates the findings of the earlier National Family Health Survey-3 (NFHS-3).

![Figure 5: Box and Whisker plot showing the mean, range and interquartile haemoglobin levels according to haemoglobin type](image-url)
NFHS-3 data revealed 69.6% prevalence of anaemia among ever-married women (in the age group 15–49 years) and 77.3% among children (in the age group of 6–35 months). Thus, the prevalence of anaemia remains unchanged in the State.

<table>
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*Dependent variable: Hb (g/dl) for all samples
Among the non-nutritional factors, infection due to helminths was substantially low (24.71%) in the present study. Prevalence of *A. lumbricoides* was observed in 10.6% of the adolescent girl students against 63% documented among school students of Assam earlier.\(^8\) Similarly, prevalence of *T. trichiura* (6.2%) and hookworm (3.9%) was found to be low in the present study in comparison to earlier studies reported from Assam.\(^5,8,9\) The de-worming programme adopted by the state government among the adolescent girl students may have some impact in lowering the helminthic infestation in the present study. Serum ferritin level in a sub group of samples was in the lower normal range. Probably due to the lower life span of the RBCs with Hb E, severe lower respiratory infection may be associated with lower normal level of serum ferritin.\(^22\) However, normal or elevated levels of serum ferritin do not indicate adequate iron stores necessarily, particularly in populations exposed to recurrent infections.\(^23,24\) Further, it was also observed in the study, that IFA supplementation programme for school girls was part of a routine programme in some of the districts of Assam included in the study districts. The supplementation programme was, however, not regular. In the present study other infections, except malaria parasite and helminths, were not included.

Among the non-nutritive factors, as associating determinants of anaemia, only haemoglobinopathies and thalassaemias were included in the present study. A gene frequency of 0.188, ranging from 0.071 to 0.266, was observed for β\(^E\)-globin gene among the adolescent girl students of Assam. Prevalence of high gene frequency for β\(^E\)-globin gene has been documented earlier (1987 and 2009) among the autochthonous inhabitants of north-eastern India.\(^14,15\) Prevalence of other haemoglobinopathies was substantially low in the present study. Earlier studies (1988, 1958 & 2003) also reflect the prevalence of sickle cell haemoglobin and Hb D, both in heterozygous state (Hb AD) and double heterozygous state with Hb E (Hb ED) in Assam.\(^16,17,25\)

We have observed a statistically significant difference \((F=3.471; P=0.001)\) of mean haemoglobin level in different types of haemoglobin variants, particularly with Hb E. Further, analysis of a subset of samples comprising all available parameters of the study also indicates statistically significant difference \((F=5.062; P= <0.001)\) of mean haemoglobin level with different types of haemoglobin variants.

One of the significant findings of the present study is the role of haemoglobin E (Hb E) as an important non-nutritional determinant of anaemia among adolescent school children. Thus, the present baseline study indicates the need of a further comprehensive study to determine the role of haemoglobin type as an associating determinant of anaemia in this part of the country. Further, a significant decline in prevalence of helminthic infestation, as compared to earlier studies (2004, 2006 & 2009), is also a striking observation of the present study. De-worming at regular intervals and creation of awareness on personal hygiene and sanitation may further reduce helminthic infestation among adolescent girl students of Assam and consequently improve their haemoglobin status.

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References


