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Original Research Article

Haemoglobinopathy: A Major Concern for Adolescents of Kokrajhar District of Assam

Dipankar Baruah[1], Miss. Noymi Basumatary[2], Abhinanda Barua[3], Jatin Sarmah[4]

[1]Associate Professor, Department of Pathology and in Charge Blood Bank, Gauhati Medical College, Guwahati, Assam, India.
[2]Ph. D. Scholar, Department of Biotechnology, Bodoland University, Kokrajhar, Assam, India.
[3]Assistant Professor, Department of Pathology, Tezpur Medical College and Hospital, Sonitpur, Assam, India.
[4]Professor, Department of Biotechnology, Bodoland University, Kokrajhar, Assam, India.

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Corresponding author: Dr. Jatin Sarmah
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Abstract

Background: Anaemia is prevalent among the adolescents of Assam, India. Different causes are responsible for this global health concern. Vector borne disease like malaria, helminthic infestations, nutritional deficiencies are some of the common causal factors. Similarly; haemoglobinopathy is one of the determinants for this public health problem.

Materials and Methods: The present study was conducted among 600 adolescents of Kokrajhar district of Assam to find out haemoglobin level, haemoglobinopathies, stool for helminthic infestations and peripheral blood smear for malarial infection.

Result and Observations: The present study revealed that Haemoglobinopathy associated anaemia is a major concern among adolescents of Assam. Out of 600 study samples adolescent boy was 305 (50.8%) and adolescent girl was 295 (49.2%) with a adolescent boy girl ratio of 1.03:1. The overall prevalence of anaemia among the studied subjects is as high as 67.5% (n=405). Out of 305 adolescent boy 140 was anaemic (45.9 % of total 305 adolescent boy and 23.3% of total 600 study cases) and out of 295 adolescent girl 265 was anaemic (89.8 % of total girls and 44.2% of total 600 study case). Non-nutritional factors like helminthic infestation is substantially low 21.5% (n=129). Ascaris is the most frequent infestation (14.2%, n=85, followed by Trichuris (5.1%, n=31), and hookworm (2.2%, n=13). Malaria parasite was not found in any of the cases. We found the gene frequency of 0.201 for globin gene among the subjects.

Conclusion: Regression analysis provides information on Hb levels (g/dl), helminthic infestation and haemoglobin type. Revealed haemoglobin type (Hb E) was the important factor of anaemia among adolescents in the present study.

Keywords: Haemoglobinopathies, Helminthic, Thalassaemias, Anaemia, Malaria.

Introduction

Anaemia is a public health concern associated with morbidity and mortality,

especially in young children and pregnant women.[1] Like other developing countries, India is also facing similar public health problem.[2] Assam reflects similar scenario of national average in prevalence of the problem.[3] Earlier studies reflect high occurrence of anemia among tribals of the state due to different forms of haemoglobinopathies.[4]

Non-nutritional factors such as malaria, helminthic infestations, haemoglobinopathies, etc. are widely prevalent in this part of the country due to the climatic conditions and ethnicity.[5-16] Prior studies indicate high prevalence of anaemia due to helminthic infestations in NE India.[5,6,7] The NE India is considered as a mosaic of people with diverse ethnicities.[17,18] Similar to other SE Asian countries, the inhabitants of this part of India are predominantly of Mongoloidorigin.[12,19] Thus, the region exhibits high gene frequency for Hb E.[12,13] Sickle cell disease is mostly confined to the tea garden areas of Assam. [14,15] Prevalence of α- and β-thalassemia traits among college going adolescents was 3.24%.[20] The pregnancy outcome in anaemic mothers is also a cause of concern in this part of the country [21]. Further, *Plasmodium falciparum malaria* percentages and high gene frequency of HbE was documented in malaria-endemic regions of NE India.[13]

Aims and Objectives

This study is designed to find out the prevalence of types of anaemia among adolescents of Kokrajhar districts of Assam with an attempt to evaluate the associating determinants of anaemia.

Materials and Methods

Study area, sample size and collection of samples

The present study was conducted among the adolescents of Kokrajhar district, Assam. Considering 50% prevalence of anaemia, to get maximum sample size, with confidence coefficient of 95%, confidence interval of ±5%, design effect of 1.5% and rate of homogeneity 2%, the estimated total sample required is of 570 individuals from 50 clusters from the district. However, considering the refusal rate, a cluster size of 60 was maintained. The selection of the schools and colleges in the district was based on probability proportional to size (PPS) sampling approach and students enrolled in 8^{th}-10^{th} and 11^{th} - 12^{th} standards were included in the study. In case of refusal by the authority, an adjacent school/college under the same block was included in the study.

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

Finger-pricked blood samples (20µl) were collected using micropipettes and transferred to individually labeled No:1 Whatman filter paper. The samples were air dried and packed individually after marking for estimation of hemoglobin. Collected samples were analyzed within 2 days of collection.[22]

To detect the prevalence of haemoglobinopathies, venous blood (3 ml) was collected from every 10th individual in K_3EDT Avials (Acuvet disposible). In case of refusal by the selected 10^{th} 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 labeled vials, one containing 10% formal-saline and the other having 2.5% potassium dichromate solution to study the prevalence of helminths. The vials were distributed to the selected adolescents on the day of collection of the blood samples. The students were advised to dispense about 2–3 gm of stool samples in each vial separately and hand over the samples to the field worker the next day. In case of defaulters, extra two attempts were made for collection of the samples. The samples were then transported to the laboratory.

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

Analysis of Samples

Haemoglobin levels of the individual dry blood samples were estimated by using Hemo Cue^{201+}. Prevalence of haemoglobinopathies and thalassaemias was determined by BioRad D10 HPLC based Variant Hemoglobin Testing System using β-thalassaemias short program as per protocol of the manufacturer. Detection of malaria parasite was based on examination of both thick and thin smears of the blood slides stained with 3% Giemsastain. Similarly, stool samples collected from the field were analysed microscopically for presence of helminthes by adopting the formalin-ether method of WHO, 1994.

The data generated were computed and analysed using SPSS (26.0 v). Gene frequency of Globin gene was calculated by following the Hardy–Weinberg Equilibrium principle. Further, to understand the correlation of Hb levels, multiple regression analysis was done in a sub-set of samples where information on hemoglobin type and helminthic infestation was available. The mean haemoglobin level across three different groups, based on haemoglobin types (HbAA, Hb AE, Hb EE) was compared by Analysis of Variance (ANOVA). 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 hemoglobin level (dependent variable) was entered as continuous variable.

Results

Dry blood samples numbering 600 were collected from, 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 6.1% (n=37). The mean (± SD) age group of the study subjects was 15.62 ±1.1 years. Out of 600 study samples adolescent boy was 305 (50.8%) and adolescent girl was 295 (49.2%) with a adolescent boy girls ratio of 1.03:1 (Figure-1).

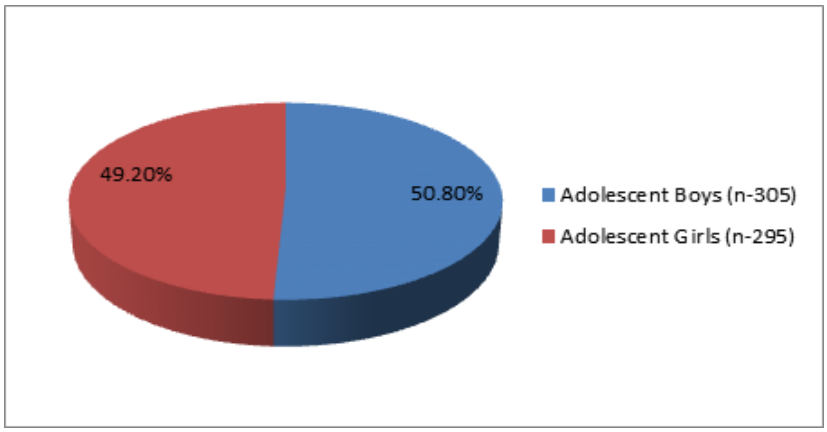

Figure 1: Showing adolescent boy and adolescent girl ratio in the study group

The mean (± SD) hemoglobin level of the adolescents in the study was 11.02 ± 1.09 g/dl. Hb level was <12.0 g/dl in 67.5% (n=405) of the adolescent students, indicating high prevalence of anemia. Table 1 shows that out of 305 adolescent boys 140 was anaemic (45.9 % of total 305 adolescent boy and 23.3% of total 600 study cases) and out of 295 adolescent girls 265 was anaemic (89.8 % of total girls and 44.2% of total 600 study case). No significant difference was observed in hemoglobin level in duplicate samples, obtained from every 10th individual in filter paper, for quality control for estimation of hemoglobin from dry blood samples.

Table 1: Aneamic and non-aneamic among male and female adolescent

Gender	Anemic	Non-anaemic	Total
Adolescent Boy	140 (23.3%)	165 (27.5%)	305 (50.8%)
Adolescent Girls	265 (44.2%)	30 (5.0%)	295 (49.2%)
Total	405 (67.5%)	195 (32.5%)	600 (11%)

Stool samples numbering 600 were analysed for the prevalence of helminthic infestations. Helminthic infestation was observed only in 21.5% (n=129) of the students (Table-1). Overall, *A. lumbricoides* was the most frequent helminthic infection (14.2%, n=85), followed by *T. trichiura* (5.1%, n=31), and hookworm infestations (2.2%, n=13). Polyparasitic infection was observed in 0.5% (n=3) of the study subjects. While co-infection due to *A. lumbricoides* and *T. trichiura* was 1.8% (n=11), *A. lumbricoides* and hookworm was 1.0% (n=6) and *T. trichiura* and hookworm was 0.7% (n=4).

Table 2: Different types of helminthic infestation among the study group

Types of helminthic infestation	Number of cases	Percentage
A. lumbricoides	85	14.2
T. trichiura	31	5.1
Hookworm	13	2.2
Total	129	21.5

Absence of malaria parasite in any of the collected blood samples (*n*=600) indicates lack of even asymptomatic malaria cases in the study population. Ten per cent of the slides were cross-checked for inter-observation variance and 100% concordance was noted.

Screening of blood samples by D10 HPLC based Variant Hemoglobin testing system (BioRad) indicates Hb E is a widely prevalent variant hemoglobin in the present study. The gene frequency for Globin gene among adolescents in the present study was 0.201.

In addition to Hb E, other variant haemoglobins like HbS was also observed in very low frequency. While heterozygous state of Hb S was observed in four subjects, ones ubject was detected with double heterozygous state of Hb ES.

Hb E is the major variant hemoglobin prevalent among the students of Assam. A statistically significant difference (*F*=3.471; *P*=0.001) of mean hemoglobin level was observed in different types of hemoglobin variants, particularly Hb E. Further, in a subset of 310 samples, information on all parameters i.e., Hb levels (g/dl), helminthic infestation (*A. lumbricoides*, *T. trichuria* and hookworm) and hemoglobin type were available. Multiple regression analysis revealed hemoglobin type (Hb E) is the important determinant of anemia among adolescent students of Assam in the present study.

Discussion

The present study revealed that anemiais a major public health problem among the adolescents of Assam with the overall prevalence as high as 67.5%. Anaemia is more among adolescent girls then adolescent boys. This corroborates the findings of the earlier National Family Health Survey-4(NFHS-4). Among the non-nutritional factors, infection due to helminths was substantially low in the present study. Prevalence of *A. lumbricoides* was observed in 14.2% of the students against 63% documented among school students of Assam earlier.[6] Similarly, prevalence of *T. trichiura* and hookworm was found to be low in the present study in comparison to earlier studies reported from Assam.[5,6,7] The de-worming program adopted by the government among the school students may have some impact in lowering the helminthic infestation in the present study. In the present study other infections, except malaria parasite and helminths, were not included.

Among the non-nutritive factors, as associating determinants of anemia, only haemoglobinopathies were included in the present study. A gene frequency of 0.201, was observed for Globin gene among the students. Prevalence of highgene frequency for Globin gene has been documented earlier among the autochthonous inhabitants of NE India.[12,13] Prevalence of other haemoglobinopathies was substantially low in the present study. Earlier studies also reflect the prevalence of sickle cell hemoglobin, both in heterozygous state (Hb AS) and double heterozygous state with Hb E (Hb ES) in Assam.[14,15] We have observed a statistically significant difference (F=3.471; P=0.001) of mean hemoglobin level in different types of hemoglobin variants, particularly with HbE. 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 hemoglobin level with different types of hemoglobinvariants.

Conclusion

One of the significant findings of the present study is the role of hemoglobin E (Hb E) as an important non-nutritional determinant of anemia among adolescent school children. Thus, the present study indicates the need of a further comprehensive study to determine the role of hemoglobin type as an associating determinant of anemia in this part of the country. Further, a significant decline in prevalence of helminths, as compared to earlier studies, 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 students of Assam and consequently improve their hemoglobin status.

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