INCIDENCE AND TYPES OF HAEMOGLOBINOPATHIES IN TEA GARDEN COMMUNITY AROUND DIBRUGARH

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ABSTRACT

BACKGROUND

The term haemoglobinopathy is used to designate a group of inherited abnormalities of haemoglobin synthesis characterized by structurally abnormal haemoglobin. By far haemoglobinopathies are largest group of congenital anaemia. Their prevalence varies considerably with geographic location and racial group.

AIM

The present study was carried out to determine the frequency and types of haemoglobinopathies in the tea garden community near Dibrugarh town.

MATERIALS AND METHODS

Total number of 250 cases from two different tea gardens around Dibrugarh town, irrespective of age and sex, were studied. Laboratory investigation including Blood Examination, Peripheral Blood Smear Examination and Special Investigations: Agarose gel electrophoresis and Sickling test were done.

RESULT

In the present study, the age of the subjects with haemoglobinopathies ranged from the first decade to the sixth decade. Maximum cases were seen from first to second decade. In the present study, frequency of abnormal haemoglobin in males was 16.13%, while in females, frequencies were 14.74%. In the present study, abnormal haemoglobins found were HbE trait (1.6%), HbS trait (12.0%) and HbS disease (2.0%). Altogether, 39 subjects (15.6%) have the disorders. The haematological parameters of different haemoglobin types found in the present study were analysed. In comparison to adult haemoglobin (HbAA), low mean values of complete haemogram were found in haemoglobinopathies, only exceptional was in case of MCV and MCHC values of HbS disease, where normocytic normochromic anaemia was found to be more pronounced.

CONCLUSION

From the present study, it has been observed that haemoglobinopathic disorder is prevalent in all age group of individuals and among the haemoglobinopathic disorder high frequency of HbS disorders has been observed.

KEYWORDS

Haemoglobinopathy, Tea Garden Community, Complete Haemogram.

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INTRODUCTION

Haemoglobinopathies and Thalassemias are wide spread, inherited genetic disorders that affect millions of people worldwide.

Hereditary Disorders of Haemoglobin Structure and Synthesis

John N. Lukens and G. Richard Lee, Wintrobe, 2004, have classified inherited abnormalities of haemoglobin into three main groups.

Haemoglobinopathy	: Characterized by structurally abnormal haemoglobin.
Thalassaemia	: Those in which one or more of the normal polypeptide chain of haemoglobins are produced at a reduced rate.
Thalassaemic	: Characterized by not only structurally abnormal.
Haemoglobinopathy	: Haemoglobin, but also associated with reduced synthesis of the globin chain.

Some Important Haemoglobinopathies: [Table-1] The Abnormal Haemoglobin results from

- Point Mutation.
- Frame Shift Mutation.
- Crossovers.

The abnormal haemoglobin diseases are inherited in a fashion referred to as autosomal co-dominant (John N. Lukens and G. Richard Lee, 1998).

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Abnormal Hbs and Thalassaemias are a major health problem in many countries in South and South East Asia.

Haemoglobinopathies are one of the commonest massive disorder in the Indian subcontinent (A. Kundu, S. Ganguli, A. Gupta, P. Pal and S. Majumdar, 1997.¹). In Assam, two most common haemoglobinopathic disorders found are HbE and HbS. Study carried out in ICMR revealed sickle cell disease is prevalent among the tea garden population of Assam and frequency ranges from 15-29% among the different tea garden labour population.

Assam is the largest tea producing state in India about 16.5% of total population of upper Assam is made of the Tea tribal community. In Dibrugarh District, there are 258 tea gardens with 1,70,000 employed labourers and 2.5 lakhs registered dependants.

Hence the Present Study has been Aimed to

- To find out incidence and types of haemoglobinopathies in tea garden community around Dibrugarh.

MATERIALS AND METHODS

Duration of Study	One Year.
Place of Study	Department of Pathology Assam Medical College and Hospital Dibrugarh.

Sample Size

A total number of 250 subjects were studied for haemoglobinopathies. Two Tea gardens were selected randomly among the tea gardens around Dibrugarh Town. So, a total number of 125 subjects were studied from each garden.

Selection of Cases

Cases were selected randomly after knowing the total population of the tea garden community in a selected area around Dibrugarh irrespective of age and sex. Person uncooperative, person with fever, diabetes mellitus or person undergoing any drug treatment which might hamper the study was excluded.

Laboratory Investigation

a. Blood Examination

Hb%	RBC Count
Total WBC Count	Reticulocyte Count
Haematocrit (Micro Method)	MCV
MCH	MCHC

b. Peripheral Blood Smear Examination (Under Leishman Stain)

Hypochromia	Target Cell
Microcytosis	Polychromasia
Anisocytosis	Normoblast
Poikilocytosis	Basophilic Stippling
Spherocytes	

c. Special Investigations

- Electrophoresis of haemoglobin.
 - Agarose gel electrophoresis.
 - Cellulose acetate electrophoresis.
- Sickling test.

METHODS

Collection of Blood

2 mL of blood will be transferred to one EDTA vial and blood will be processed for preparation of haemolysate.

Procedure of Special Investigation

- Preparation of Haemolysate.
- Electrophoresis of Haemolysate for detection of the haemoglobin type, agarose gel electrophoresis was applied. A known sample of HbE – trait was run as control with each sample.

Principle

When charge particles (Protein, haemoglobin, etc.) are placed in an electrical field, they migrated towards the cathode or anode depending upon the net charge of the protein and molecular weight as well as the pH, temperature and ionic strength of the buffer solution and the type of stabilizing media used.

Relative mobility of haemoglobins in paper, agarose gel, starch gel and cellulose acetate electrophoresis, pH 8.6 noted [Fig. – I].

Sickling Test

Principle

The unique solubility of HbS in its reduced form leads to production of sickled shaped red cells containing HbS. This phenomenon is demonstrated by sealing thin film of the patient's blood between slide and cover glass by means of Vaseline.

Interpretation

In sickle disease, the test is positive within 15 minutes and may be delayed in sickle cell trait.

RESULTS AND OBSERVATIONS

From present study, maximum number of subjects fall in the 10-19 years' age group. A total 155 number of males and 95 number of female subjects were examined.

Total number of individual examined was - 250.

HbE trait was found in 4 cases (1.60%).

HbS trait was found in 30 cases (12.0%).

HbS disease was found in 5 cases (2.0%).

In the present study, not a single case of HbE disease was found. The remaining 211 subjects had normal adult haemoglobin (HbAA-84.40%) [Table-2].

Maximum cases of haemoglobinopathies have been seen in the age group of 10-39 years. Total 31 cases were diagnosed (79.48%) [Table-3].

Total 155 male subjects were examined, out of which 25 were found to have haemoglobinopathic disorder (16.13%).

In female subjects, total 95 cases were examined out of which 14 cases (14.74%) were with haemoglobinopathic disorder. Mild anisocytosis and poikilocytosis were observed in case of HbE trait. In case of HbSA mild anisocytosis, mild poikilocytosis and few target cells were seen. Normoblast and basophilic stippling were seen occasionally.

In case with HbSS anisocytosis and poikilocytosis were more frequently seen compared to HbSA. Normoblast were few in numbers, but target cells were plenty. Other important

features were basophilic stippling, which were prominent in peripheral blood smear.

In PBS, few sickle cells were seen in case of HbSS. But it was absent in case of HbSA. The mean values of all the six parameters of HbAA, HbEA, HbSA and HbSS were studied [Table-4].

The mean values were lower in case of HbEA compared to normal adult haemoglobin (HbAA).

In case of HbS disease, mean value of Hb_{gm}% was lowest compared to others. But the mean values of MCV, MCHC were in normal range and MCH was in lower limit of normal range.

In case of HbSA compared to HbSS, the MCV mean value was low, mean value of Hb_{gm}% was high. But mean values of MCH and MCHC were near about the same.

Fig. 1: Relative Mobility of Haemoglobins in Paper, Agarose Gel, Starch Gel and Cellulose Acetate Electrophoresis (pH-8.6)

Common Name	Scientific Designation
1 HbE	: $\beta_{26}(\text{B}_8) \text{Glu} \rightarrow \text{Lys}$
2 HbS	: $\beta_6(\text{A}_3) \text{Glu} \rightarrow \text{Val}$
3 HbC	: $\beta_6(\text{A}_3) \text{Glu} \rightarrow \text{Lys}$
4 HbD Punjab	: $\beta_{121}(\text{GH}_4) \text{Glu} \rightarrow \text{Gln}$
5 HbH	: β_4
6 HbM Boston	: $\alpha_{58}(\text{E}_7) \text{His} \rightarrow \text{Tyr}$
7 HbO Arab	: $\beta_{121}(\text{GH}_4) \text{Glu} \rightarrow \text{Lys}$
8 HbC Harlem	: γ_4
9 Hb Boyle Height	: $\beta_6(\text{A}_4) \text{Glu} \rightarrow \text{Val}$ $\beta_{73}(\text{E}_{17}) \text{Asp} \rightarrow \text{Asn}$
10 HbTak	: $\beta+11\text{C}(147\text{Stop} \rightarrow \text{Thr})$
11 Hb Lepore Baltimore	: $\delta(1-50)\beta(83-146)$
12 Koya Dora	: $\alpha+16 \text{ or } 17\text{C} (142 \text{ Stop} \rightarrow \text{Ser})$
13 Hb Constant Spring	: $\alpha+31\text{C} (142 \text{ Stop} \rightarrow \text{Glu})$

Table 1: Some Important Haemoglobinopathies

Haemoglobin Pattern	Number of Cases	Percentage (%)
Hb AA (Adult Hb)	211	84.40
Hb E Trait (E+A)	4	1.60
HbE Disease (E+E)	–	–
HbS Trait (S+A)	30	12
HbS Disease (S+S)	5	2

Table 2: Showing Distribution of Cases with Haemoglobin Pattern

Age Group (In Years)	EA		EE		AS		SS	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
0 – 9	–	–	–	–	–	–	3	18.75
10 – 19	1	1.18	–	–	16	18.82	2	2.35
20 – 29	2	3.45	–	–	7	12.07	–	–
30 – 39	1	2.27	–	–	4	9.09	–	–
40 – 49	–	–	–	–	2	5.56	–	–
≥ 50	–	–	–	–	1	9.09	–	–

Table 3: Showing Age Distribution of Haemoglobinopathy Disorders

Parameters	HbAA	HbEA	HbSA	HbSS
Hb _{gm} %	10.2–13.4	8.2–12.8	7.4–12.0	4.5–6.25
Range Mean SD±	12.09 ± 0.8	10.62 ± 2.14	9.74 ± 1.89	5.53 ± 0.86
RBC Millions/cu mm	4.0–5.5	4.0–5.0	2.5–4.3	1.5–2.2
Range Mean SD±	4.63 ± 0.4	4.3 ± 0.48	3.1 ± 1.52	1.88 ± 0.31
PCV%	40–50	30–42	20–40	12–20.6
Range Mean SD±	44.83 ± 5.07	36.5 ± 5.50	28.5 ± 4.70	17.46 ± 3.50
MCV (fl)	80–100	71.42–100	70.96–97.13	80–100
Range Mean SD±	93.74 ± 4.8	85.0 ± 11.66	81.13 ± 9.04	92.48 ± 7.44
MCH (pg)	25.5–31	20.5–32	22.97–33.33	25–32.7
Range Mean SD±	27.0 ± 1.83	24.78 ± 5.46	28.84 ± 2.04	28.94 ± 2.8
MCHC (gm/dL)	25.77–32.5	24.12–32	27.0–39.0	26.31–37.5
Range Mean SD±	28.97 ± 1.93	29.0 ± 3.65	31.4 ± 4.0	31.45 ± 4.09

Table 4: Showing Mean and Standard Deviation of Complete Haemogram in HbAA, HbEA and HbEE

DISCUSSION

The State of Assam, situated in the North Eastern corner of India is comprised of population with high degree ethnic and racial heterogeneity, like Mediterranean (Dravidian), Mongoloid (Kirata), Nordic (Aryan/Indo-Caucasian), Proto-Australoid (Austrian) and Negrito (Dr. P. Bhattacharya, 1966).

Different studies have shown high prevalence of haemoglobinopathies in some of these ethnic groups.

The two most common haemoglobinopathic disorder found are HbE and HbS. HbS is prevalent among the tea garden population of Assam.

The ICMR annual Report, 1989-90 reveals high prevalence of HbE in Kacharis AE=50%, EE=31.6%, Ahoms AE=41.6%, EE=9.6% and in other population group AE=37.8%, EE=6.7% of Dibrugarh District.

The most of the tea garden labours, coal-mine workers in Assam which belong to various subtypes like Kol, Munda, Orao, Ghatio, Kharia, Chaotal etc. belong to Mediterranean or Dravidian stock.

Balgir RS and Sharma SK, in their article. "Distribution of sickle cell haemoglobin in India," 1988, reported the frequency of sickle cells haemoglobin to be highest in the Eastern Zone (Assam) followed by Central Zone (Madhya Pradesh and Orissa); Southern (Tamil Nadu, Andhra Pradesh and Orissa); Western (Maharashtra and Gujrat) and Northern Zone (Rajasthan, Uttar Pradesh, Bihar and Bengal) in a decreasing orders.

The annual report of ICMR (1995-96) revealed 14.53% sickle cell haemoglobin among children and adolescent from two tea gardens of Upper Assam of which 12.79% were with sickle cell trait (SA) and 1.74% with sickle cell disease (SS).

A study carried out in ICMR (1989-1990 Annual Report) revealed a high prevalence of sickle cell haemoglobin (13.1%) in tea garden population of Dibrugarh District of Assam.

The present study was undertaken to find out the frequency of distribution of abnormal haemoglobin in the tea garden community around Dibrugarh.

In the present study 250 healthy, asymptomatic individuals belonging to tea garden community near Dibrugarh Town were examined. For the detection of haemoglobinopathies electrophoresis was done.

Frequency Distribution

In the present study, total 155 male individuals and 95 female individuals were studied; 30 cases of HbS trait (12%), 5 cases of HbS disease (2%) and 4 cases of HbE trait (1.6%) were detected.

In this study not a single case of β -thalassemia trait or any other abnormal haemoglobin such as HbD, HbC, etc. were detected.

Age Distribution

In this study, maximum numbers of cases of haemoglobinopathies were found in the age group 10-19 years. In this group 16 cases (18.82%) were found to be HbS trait, 2 cases (2.35%) were HbS disease and only 1 case (1.18%) was HbE trait. Similar age wise study was also observed by Batabyal and Wilson, 1958, Bora (1978), Talukdar (1984).

Sex Distribution

The present study shows slightly higher frequency of haemoglobinopathic disorder in males 25 (16.13%) than

females 14 (14.74%). The result of this study is comparable with the reports cited by Dutta A. 1974.²

Haematological Parameters

Significant differences of different haematological parameters have been observed between adult haemoglobin type and different haemoglobinopathic disorders.

Peripheral blood smear showed mild anisocytosis, mild poikilocytosis in both HbE trait and HbS trait, whereas moderate and variable in case of HbS disease. Few target cells were seen in case of HbE trait and HbS trait, but it was plenty in HbS disease. Few normoblasts were seen in HbSS, but seen occasionally in HbSA. Few sickle cells were seen in HbSS. But no sickle cells were seen in HbSA.

Another feature was basophilic stippling, which were prominent in HbS disease and occasional in HbS trait.

Sickling test was positive in both Sickle cell trait and Sickle cell anaemia.

Reduction in haemoglobin percentage was more pronounced in HbSS with mean value of 5.53 ± 0.86 than HbSA and HbEA, where mean values were 9.74 ± 1.89 and 10.62 ± 2.14 respectively.

The levels of Hb%, MCV, MCH and MCHC in cases with HbE trait, HbS trait and HbS disease were similar to the reports done by several authors (Wasi P et al 1981.³; Chernoff 1956.⁴; Chatterjee 1965.⁵; Wintrobe's Clinical Haematology, 11th Edn: 2004.⁶; De Gruchy's Clinical Haematology in Medical Practice, 5th Edn:2005.⁷).

CONCLUSION

From the present study, it has been observed that haemoglobinopathic disorder is prevalent in all age group of individuals among the Tea Garden Community around Dibrugarh Town and among the haemoglobinopathic disorder high frequency of HbS disorders has been observed.

The present study was carried out with a limited numbers of individuals in a short span of time. So, it may not reflect the exact scenario in most of the observations. Elaborate study covering a wider population and topographical distribution over a much longer period is suggested to focus the actual prevalence of these common and important problems in this ethnic group.

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