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Coexistence of Haemoglobinopathies and Iron Deficiency in the Development of Anemias in the Tribal Population Eastern India

Sumana Chatterjee[1], Soujatya Dhar[1], Bani Sengupta[1], Sarthak Sengupta[2], Lakhsmi Mazumder [3] and Sila Chakarabarti[1]

[1]Thalassaemia Research Unit, [3] Department of Pathology, Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, Kolkata 700026, West Bengal, India
[2]Department of Anthropology, Dibrugarh University, Dibrugarh, Assam, India

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ABSTRACT Iron deficiency is the most prevalent nutritional deficiency and the most common cause of anemia worldwide. Several hemoglobin disorders have also been found to modify the morbidity and mortality of a population. It has been established that coexistence of á and â thalassaemia with abnormal HbS and E may modify the level of haemoglobin in blood. Tribal communities in India constitute the largest tribal population in the world. The present study is the first to report on the interaction of Haemoglobinopathies and Iron deficiency in the development anemias in the tribal population Eastern India. Methods: A total of 450 unrelated subjects of some tribal groups of two states of Eastern India were randomly selected for the study. Blood was collected from the subjects after obtaining consents. Prevalence of anemia was screened followed by tests for hemoglobin disorders and iron study of selected cases. Beta thalassaemia mutations were screened in selected cases. Age and sex matched control groups were chosen for the study. HbE was the most common haemoglobinopathy among the studied groups of the North-east. Prevalence of Beta thalassaemia (1.6%) is almost nil in the tribal groups. HbE in homozygous states, sometimes in heterozygous states induced anameia among the Mishings, Deoris and Ahoms, but not in Sonowals. Iron deficiency anemia was pronounced among the females of Mishings and Sonowals. Incidence of hemoglobin disorders is very low among the Santhal (1.12%) and this group was the victim of iron deficiency anemias.

INTRODUCTION

Anaemia is a major problem in India. A high incidence of anemia has been traced in the large multiethnic population of India (Sing and Toteja 2003). Micronutrient deficiency has been considered a prime factor for anaemia in India (Freedman et al. 2004). Iron deficiency anemia is characterized by a defect in hemoglobin synthesis, resulting in red blood cells that are abnormally small (microcytic) and contain a decreased amount of hemoglobin (hypochromic) (Provan 1999). Iron deficiency is the most common nutritional disorder in the world with a prevalence of 39.0% in non-industrialized countries among the under fives and 48.1% among the 5-14 years group (Oski 1993). Iron deficiency, defined by two or more abnormal measurements (serum ferritin, transferrin saturation and/or erythrocyte protoporphyrin), continues to be relatively prevalent in U.S. females, affecting 7.8 million adolescents and women of childbearing age (Looker et al. 1997). The thalassemias and the hemoglobinopathies are autosomal recessive conditions affecting the quantity and quality, respectively, of hemoglobin molecules within red blood cells. These disorders are found more commonly in certain ethnic groups, lending themselves to effective ethnicity-based population screening (Weatherall 1997). The clinical course of Eâ-thalassemia is punctuated by acute and chronic complications that may cause serious morbidity and mortality. The marked expansion of erythropoiesis is responsible for much of the pathology of the disease, including hepatosplenomegaly, extramedullary hematopoietic masses, growth retardation, delayed sexual maturation, and bone deformities (Fucharoen 2000). It has been established that coexistence of á and â thalassaemia with abnormal HbS and E may modify the level of haemoglobin in blood. In the seven North-Eastern states of India majority of the population comprise of tribes mostly of Tibeto-Mongoloid origin. In Assam, admixture with the surrounding Tibetian, Bengalee and Bangladeshi populations is evident from several studies (Chakraborty et al. 1996 ; Das et al.2000). Santal, the third larg-

Dr. Sila Chakrabarti
Scientist, Thalassaemia Research Unit,
Ramakrishna Mission Seva Pratishthan,
Vivekananda Institute of Medical Sciences,
99, Sarat Bose Road, Kolkata 700026,
West Bengal, India
Phone: 09831490395,
E-mail: Sheelachakrabarti@gmail.com

est tribe in India, lives in many states including Bihar, Jharkhand, Orissa, Tripura etc. In West Bengal, Santals represent 54.27% of total tribal population (Bagchi 1994).

Our earlier reports emphasized the prevalence of anameia in connection with hamoglobinopathies, different a and b thalassaemia mutations in several tribal and non-tribal population of Eastern India (Chakraborty et al. 1996 ; Das et al. 2000; Kukreti R et al. 2002; Sengupta Bani et al. 2002 ; Gajra et al. 2003a,b; Sen et al. 2005; De et al. 2006; Rudra 2008).

To the best of our knowledge this is the first report on the coexistence of haemoglobinopathies and iron deficiencies in the development of anemia in different ethnic groups of Eastern India.

METHODOLOGY

Selection of Cases: The study of genetic polymorphisms was performed from 2008-2009 amongst the tribal and non-tribal populations of West Bengal and Assam states of Eastern India. The studied unrelated tribal groups were Sonowal, Mishing, Deori and Santhals. In addition common Bengali and Ahom (a non-tribal community of Assam) and Assamese people from different parts of West Bengal and Assam respectively have also been included. Pregnant women and subjects with the history of any chronic disease were excluded from the study to rule out the effect of any chronic diseases on anaemia. All the participating subjects were given the questionnaires to have information regarding their diet, family history of chronic diseases and personal habits including tobacco and alcohol consumption. The ethnic origin of each subject was based on self-identification. Each case was interrogated about the family history, blood transfusion, presence of other chronic diseases etc. Written consents were collected from all the subjects. Free health check up, distribution of supplementary medicines and reports of Hemoglobin level and Hemoglobin electrophoresis were also given free to enable follow-up and counseling if necessary. In the present study, we have analyzed 876 subjects (450 tribals, 426 non-tribals) in total.

Sample: 5 milliliters of blood was collected by venipuncture.3ml of it was kept in an EDTA vacutainer and 2 ml of blood into plain vacutainer (without EDTA). Blood in plain container was allowed to clot and then spun to separate the serum. The serum was assayed.

The research protocol was approved by the ethical and research advisory committee of the institution.

Tracing of β and E traits: Routine screening for thalassemia included detection of total blood counts and other indices (Sysmex K1000, Japan) from EDTA blood which was followed by hemoglobin electrophoresis at pH 8.6 (Chandra et al. 1987), fetal Hb estimation by alkali denaturation (Dacie 1994) and measurement of Hb A2 level by gel elution method. The standard diagnostic marker for b- thalassemia is elevation of the Hb A2 level >3.5 %.

Iron Study: Serum iron and total iron binding capacity were determined by colorimetric methods in full auto-analyser and serum ferritin was measured by standard Chemiluminescence methods for selected cases.

DNA Study: DNA was isolated from the peripheral leukocytes using standard salting out method (Miller et al. 1988).PCR-ARMS technique was used for identification of some common beta globin mutations (Newton et al. 1989).

Statistical Analysis: Results were analyzed with SPSS database (Version 14.0 for windows). Mean ± standard deviations were used to describe the continuous variables. Student's t test was used in the comparison of age and sex groups. There were age and sex dependent differences in the mean values of all parameters ($P < 0.01$). The statistically significant level was set at alpha 0.05 ($p \leq 0.05$).

RESULTS

A low serum ferritin (<15 µg/L), in addition to a low hemoglobin or hematocrit, confirms the diagnosis of iron deficiency anemia. The diagnostic range for total iron binding capacity was taken as above 400 ìg/dl, for serum iron concentration was less than 50 ìg/dl and for transferrin saturation was <16% .

All the demographic data for the studied population are shown in Table 1. In both states the tribal people were socio-economically weaker than the nontribal ones. The study shows that 39.92 % and 76.4 % of tribals are anemic (taking WHO standard for developing countries, Hb level<11gm/dl as standard) in Assam and West Bengal respectively and the prevalence is 1.92

and 2.26 times greater than the studied common people of the respective states. Table 2 represents the status of various haematological parameters with respect to sex, food-habit, coexistence of other diseases and haemoglobin abnormalities, which may modify the status of anaemia. The females were the main victim of anemia in all tribal groups, but intake of nonvegetarian diet did not improve the condition in all groups. It was confirmed from the lifestyle history of the study cases that, intake of fruits and fresh vegetables were significantly lower among the Santhals than other north-eastern tribals. Lower levels of Red Blood Cells (RBC), Packed Cell Volume (PCV), mean cell Volume MCV), Mean Cell Haemoglobin Concentration (MCHC) were found in the respective anemic cases.

Table 3 represents the results of iron study and the data were analysed with low Haemoglobin level, sex and co-existence of haemoglobin abnormalities. The study shows that the prevalence of anaemia was maximum (76.4%) in the tribal belt. The iron study confirmed that the Santhals are the major victims of iron deficiency anaemias, where prevalence of any Haemoglobin disorder (one HbS) is minimum (1.12%). In the north-east the Mishing and the Sonowal tribes were living in almost same location, but anaemia is more prevalent among the Mishing people (34.09%) that the Sonowals (30.0%). As expected, prevalence of HbE and EE disease was very high in Mishings, Sonowals, Deoris and Ahoms. But, high prevalence of HbE did not induce anaemia in these groups, the prevalence of anaemia was mainly associated with nutritional deficiencies (evident from food habits and iron data).Decrease in serum iron level is evident from the iron study among the people from both Sonowals and Mishings. From the life style history, greater incidence of anaemia among the Mishing people can be explained from the fact that they are economically weaker (Monthly income >Rs.3,000 in 20% people) than the Sonowals (Monthly income >Rs.3,000 in 45% people).

HbE was the most common haemoglobinopathy among the studied groups of the Northeast (Table 4). Prevalence of Beta thalassaemia trait was 5.84% and 15.07% in the two nontribal groups respectively, whereas its prevalence is minimum in the tribal groups. Presence of HbS was also very trace among the subjects. DNA analysis showed (Table 5) that IVS1-5(G'!C) is the most common mutation among the Beta thalassaemia traits followed by Frameshift 41-42 (-CTTT) and Frameshift 8-9 (+G) mutations. No mutations could be detected for two Beta traits. Codon26 (G→A) for HbE and Codon 6(G→A) mutation for HbS cases were also detected.

DISCUSSION

This is a well-established fact that socio-economic status and cultural norms has a direct impact on anemia (Devadas 1980; Kaur 1982). Few studies have reported the nutritional status of the tribal groups of India. One study (Rao et al. 2005) reported widespread undernutrition (60% underweight) among the preschool children of Gond tribe of Madhya Pradesh. In West Bengal, 54% of children (6-12 years of age) of Oraon tribe are suffering from severe malnutrition (Mittal 2006).Our study supports the data of Dutta Chowdhury et al. (2008). The results indicated that the prevalence of undernutrition was higher in Santhal girls than boys. Our study finds the Santhal group to be the victim of iron deficiency anaemia. Low serum ferritin and iron level and low transferring saturation were significantly associated with low Hb level in this study group. Hemoglobin disorders in no way modified their anemia status. Prevalence of low serum ferritin and iron among the females were found to be statistically significant.

No reliable report on iron deficiency anemia of the north-eastern tribal people is available. As the populations were chosen from the more or less same ethnical and nutritionally same background, the effects of all the related geographical, nutritional factors were thought to be same in all respects. Our study found no iron deficiency anemia among the Sonowals. But, among the Mishings serum iron level was significantly lower than the controls and the same was also lower among the males than females. Transferrin saturation was also low in this group. Surprisingly, its level was significantly lower among the non anemic people than the anemic people. Iron deficiency anaemia is most common cause of anaemia in India and this report appears to be contrary to the fact and difficult to explain. In India, the rural population are mostly subjected to low bio-availability of iron because of the cereal based diet and chronic blood loss from hookworm infestations which results in anemia

Table 1: Demography of the study population

Ethnicity, site	*Age (yrs) Mean ± SD*	*Gender (n,%)*		*Educational background (n,%)*		*Economic condition (n,%)*		*Presence of minor diseases n%*
		Male	*Female*	*literate*	*illiterate*	*Monthly income <Rs 5000*	*Monthly income >Rs 5000*	
Assam; Mishing, Ramailachan (N= 44)	32.84 ± 5.68	28(63.64)	16(36.36)	15(34.09)	29(65.91)	42(95.45)	2 (4.54)	6(13.64)
Sonowal, Chohorikota, (N= 100)	34.86 ± 12.06	67(67)	33(33)	74(74)	26(26)	88(88)	12(12)	10(10)
Deori, (N= 45)	32.02 ± 3.26	17(37.78)	28(62.23)	18(40)	27(60)	43(95.56)	3 (6.67)	1 (2.23)
Ahom, Kapoh (N= 54)	34.08 ± 11.09	18(33.34)	36(66.66)	26(48.15)	28(51.85)	47(87.04)	7(12.96)	4 (7.41)
Munda, Chyacha (N= 118)	31.99 ± 4.56	10(8.47)	108(91.53)	27(22.88)	91(77.12)	100(84.75)	8 (6.78)	2 (1.69)
Assamese; non-tribal, (N= 154)	33.09 ± 2.56	57(37.01)	97(62.99)	149(96.75)	5 (3.25)	131(85.06)	23(14.94)	6 (3.90)
West-Bengal; Santhals, Bolpur, Hooghly (N= 89)	34.34 ± 0.22	19(21.34)	70(78.65)	17(19.10)	72(80.89)	78(87.64)	11(12.35)	17(19.10)
Bengali non-tribals of Kolkata, Burdwan, Hooghly (N= 272)	32 ± 2.55	46(16.91)	226(83.09)	248 (91.18)	24 (8.82)	95(34.93)	177(65.07)	15 (5.51)

Table 2: Status of Hematological parameters w.r.t life style and haemoglobinopathies among tribals

Ethnicity	*Hb level (n,%)*			*RBC (x10⁶/ml)*		*PCV (%)*		*MCV(fl)*		*MCHC (gm/dl)*	
	<11gm/dl	*>11gm/dl*	*p*	*Mean ± S.D*	*p*	*Mean ± S.D*	*p*	*Mean ± S.D*	*p*	*Mean ± S.D*	*p*
1. Mishing (N=44)	15(34.09)	29(65.90)	<0.05	3.17 ± 1.1	<0.05	32.11 ± 0.33	<0.001	79.34 ± 10.1	NS	30.6 ± 4.5	<0.05
1a. Dietary Habits											
Vegetarian	4(9.09)	10(22.72)	NS	2.34 ± 2.34	<0.01	35.0 ± 2.66	NS	79.96 ± 9.87	NS	30.99 ± 5.78	<0.05
No vegetarian	11(25)	19(43.18)	-	3.54 ± 13.2	-	32.5 ± 1.11	-	79.06 ± 9.86	-	30.0 ± 8.72	-
1b. Sex											
Male	8(28.57)	20(71.42)	-	4.01 ± 0.04	-	35.0 ± 6.66	-	78.89 ± 8.56	-	30.94 ± 2.99	-
Female	7(43.75)	9(56.25)	<0.05	3.05 ± 1.4	<0.05	33.55 ± 4.89	<0.05	79.63 ± 1.22	NS	30.23 ± 9.86	<0.01
1c. Other Diseases											
Yes	2(4.54)	4(9.09)	NS	2.78 ± 7.08	<0.01	31.99 ± 2.45	NS	79.85 ± 6.69	NS	30.76 ± 8.76	<0.01
No	13(29.54)	25(56.81)	-	3.45 ± 1.23	-	36.98 ± 3.12	-	79.21 ± 1.43	-	30.11 ± 1.11	-
1d. Haemoglobinopathies											
Normal	4(9.09)	21(47.72)	-	3.54 ± 7.60	<0.05	37.90 ± 0.02	-	79.96 ± 0.03	-	30.87 ± 8.96	-
Heterozygous	9(20.45)	3(6.81)	<0.01	3.68 ± 0.67	<0.05	34.44 ± 1.43	NS	78.87 ± 9.5	<0.01	30.92 ± 9.21	<0.01
Homozygous	2(4.54)	5(11.36)	NS	3.05 ± 1.27	<0.05	33.75 ± 4.67	<0.05	78.67 ± 2.43	<0.01	30.0 ± 1.32	<0.01
2. Sonowal (N=100)	30(30)	70(70)	<0.01	4.17 ± 1.1	NS	34.4 ± 5.7	<0.01	80.24 ± 11.1	NS	30.1 ± 4.5	<0.05
2a. Dietary Habits											
Vegetarian	9(9)	16(16)	NS	4.08 ± 2.76	NS	29.87 ± 0.88	<0.001	79.85 ± 0.64	NS	30.87 ± 8.91	<0.01
No vegetarian	21(21)	54(54)	-	4.54 ± 1.11	NS	31.55 ± 0.55	-	80.59 ± 0.27	-	29.98 ± 9.74	-
2b. Sex											
Male	19(28.35)	48(71.64)	-	4.32 ± 4.12	NS	31.92 ± 0.22	-	80.0 ± 7.77	-	29.99 ± 9.99	-
Female	11(33.3)	22(66.6)	<0.05	4.05 ± 1.55	NS	29.96 ± 4.45	<0.001	79.68 ± 7.21	NS	30.04 ± 9.97	<0.01
2c. Other Diseases											
Yes	4(4)	6(6)	NS	3.78 ± 1.29	<0.05	29.99 ± 3.54	<0.05	80.74 ± 4.98	NS	30.98 ± 0.06	<0.001
No	26(57.78)	64(64)	-	4.19 ± 3.0	NS	31.44 ± 0.31	-	80.0 ± 0.99	-	30.0 ± 8.9	-
2d. Haemoglobinopathies											
Normal	8(8)	30(30)	-	4.56 ± 2.34	-	30.43 ± 4.56	-	79.55 ± 0.51	-	30.23 ± 3.33	-
Heterozygous	7(7)	31(31)	NS	4.4 ± 1.65	<0.05	32.5 ± 5.65	NS	78.02 ± 1.97	<0.01	31.66 ± 6.26	NS
Homozygous	6(6)	18(18)	NS	4.08 ± 2.48	<0.05	31.89 ± 1.19	NS	78.77 ± 2.85	<0.01	32.05 ± 1.17	NS
3. Deoris (N=45)	27(60)	18(40)	<0.001	3.01 ± 1.23	<0.05	30.4 ± 2.3	<0.001	78.81 ± 0.87	<0.01	31.21 ± 10.1	NS
3a. Dietary Habits											
Vegetarian	16(35.56)	9(20.0)	<0.001	2.99 ± 2.34	<0.01	33.77 ± 7.54	NS	78.90 ± 4.56	NS	31.0 ± 10.92	<0.05
No vegetarian	11(24.45)	9(20.0)	-	3.07 ± 1.01	<0.01	32.0 ± 11.11	-	78.54 ± 8.74	-	31.87 ± 9.8	-
3b. Sex											
Male	7(41.1)	10(58.82)	-	3.65 ± 2.56	<0.05	32.99 ± 9.99	-	77.98 ± 12.09	-	31.09 ± 6.65	-
Female	20(71.42)	8(28.57)	<0.001	3.00 ± 0.94	<0.01	31.74 ± 7.82	<0.001	78.03 ± 9.0	<0.01	31.03 ± 1.11	<0.05
3c. Other Diseases											
Yes	0(0)	1(2.23)	NS	2.99 ± 1.97	<0.01	31.89 ± 0.07	NS	77.96 ± 6.4	<0.01	31.44 ± 7.82	<0.05
No	27(60)	17(37.78)	-	3.09 ± 0.85	<0.05	30.99 ± 5.51	-	78.85 ± 9.8	-	31.10 ± 0.85	-
3d. Haemoglobinopathies											
Normal	4 (8.89)	6(13.34)	-	3.24 ± 3.00	<0.05	33.42 ± 2.25	-	78.62 ± 0.08	-	31.0 ± 9.92	-
Heterozygous	7(15.56)	11(24.45)	<0.01	3.11 ± 1.45	<0.05	31.25 ± 4.67	<0.001	78.32 ± 0.97	<0.01	31.76 ± 2.22	NS
Homozygous	16(35.56)	1(2.23)	<0.001	3.08 ± 2.48	<0.05	34.99 ± 0.89	NS	78.79 ± 0.91	<0.01	31.45 ± 3.97	NS

Table 2: Contd.....

Ethnicity	*Hb level (n,%)*			*RBC (x10⁶/ml)*		*PCV (%)*		*MCV(fl)*		*MCHC (gm/dl)*	
	<11gm/dl	*>11gm/dl*	*p*	*Mean ± S.D*	*p*	*Mean ± S.D*	*p*	*Mean ± S.D*	*p*	*Mean ± S.D*	*p*
4. Ahom (N=54)	29(53.7)	25(46.3)	<0.001	3.81 ± 3.23	<0.05	33.76 ± 4.56	<0.05	78.84 ± 8.77	<0.01	32.90 ± 9.82	NS
4a. Dietary Habits											
Vegetarian	6(11.12)	9(16.67)	<0.05	3.09 ± 1.04	<0.05	33.87 ± 8.82	NS	78.53 ± 1.22	<0.01	31.97 ± 0.99	<0.05
No vegetarian	23(42.59)	16(29.63)	-	3.97 ± 0.01	-	33.27 ± 7.54	-	78.95 ± 2.65	-	33.08 ± 0.07	-
4b. Sex											
Male	8(14.81)	12(22.23)	-	3.85 ± 0.56	<0.05	34.02 ± 2.35	-	78.29 ± 0.93	-	32.87 ± 6.68	-
Female	21(38.89)	13(24.07)	<0.001	3.80 ± 0.54	<0.05	33.70 ± 4.78	<0.01	78.91 ± 10.1	<0.01	31.08 ± 8.71	<0.05
4c. Other Diseases											
Yes	3 (5.56)	1 (1.85)	NS	3.99 ± 1.17	<0.05	33.86 ± 3.31	NS	79.02 ± 0.01	<0.05	32.08 ± 0.95	NS
No	26(48.15)	24(44.45)	-	3.79 ± 2.85	-	34.02 ± 3.41	-	78.61 ± 9.85	-	32.96 ± 10.94	-
4d. Haemoglobinopathies											
Normal	4 (7.41)	13(24.07)	-	3.14 ± 1.00	<0.05	34.0 ± 0.08	-	79.34 ± 9.62	-	32.85 ± 4.86	-
Heterozygous	17(31.48)	11(20.37)	<0.001	3.91 ± 7.45	NS	33.89 ± 2.34	<0.01	78.94 ± 4.78	<0.01	32.98 ± 9.87	NS
Homozygous	8(14.81)	1 (1.85)	<0.01	3.78 ± 4.48	<0.05	32.84 ± 4.87	<0.001	78.80 ± 0.05	<0.01	32.89 ± 0.05	NS
5. Munda (N=118)	64(54.24)	54(45.76)	<0.05	4.81 ± 0.23	<0.05	32.76 ± 4.56	<0.05	77.84 ± 8.17	<0.01	32.99 ± 1.82	NS
5a. Dietary Habits											
Vegetarian	5(4.24)	2(1.69)	<0.05	3.99 ± 5.0	<0.05	34.87 ± 0.82	NS	79.53 ± 0.02	<0.01	32.57 ± 0.79	<0.05
No vegetarian	59(50.0)	52(44.07)	-	3.90 ± 0.61	<0.05	33.97 ± 9.94	-	78.97 ± 8.95	-	33.8 ± 0.87	-
5b. Sex											
Male	8(80)	2(20)	-	3.95 ± 7.56	<0.05	34.22 ± 2.05	-	78.0 ± 0.83	-	33.07 ± 0.68	-
Female	56(51.85)	52(48.14)	<0.001	3.84 ± 0.04	<0.05	34.70 ± 3.78	<0.01	78.01 ± 0.1	<0.01	32.88 ± 0.72	<0.05
5c. Other Diseases											
Yes	2 (1.69)	0(0)	NS	3.55 ± 0.17	<0.05	34.76 ± 2.01	NS	78.92 ± 3.01	<0.05	32.88 ± 0.65	NS
No	62(52.54)	54(45.76)	-	3.79 ± 2.85	-	34.02 ± 3.41	-	77.91 ± 8.75	-	32.86 ± 0.94	-
5d. Haemoglobinopathies											
Normal	62(52.54)	50(42.37)	-	3.74 ± 1.60	<0.05	34.08 ± 0.08	-	79.44 ± 0.62	-	32.85 ± 4.86	-
Heterozygous	2 (1.69)	4 (3.39)	<0.001	3.91 ± 7.45	NS	33.89 ± 2.34	<0.01	78.94 ± 4.78	<0.01	32.98 ± 9.87	NS
Homozygous	-	-	-	-	-	-					
6. Assam Control (N=154)	32(20.78)	122(79.22)	-	4.91 ± 2.33	-	35.4 ± 7.3	-	80.91 ± 2.87	-	32.23 ± 9.1	-
6a. Dietary Habits											
Vegetarian	16(10.39)	19(12.34)	-	3.99 ± 2.04	-	35.77 ± 0.54	-	80.97 ± 2.76	-	32.0 ± 0.92	-
No vegetarian	16(10.39)	103(66.88)		5.07 ± 1.91		35.02 ± 1.18		81.14 ± 0.64		32.87± 0.8	
6b. Sex											
Male	7(12.28)	50(87.71)	-	4.65 ± 3.56	-	35.99 ± 0.99	-	79.98 ± 1.09	-	32.09 ± 0.65	-
Female	25(25.77)	72(74.22)		5.00 ± 1.94		35.04 ± 1.82		80.99 ± 6.0		32.53 ± 4.11	
6c. Other Diseases											
Yes	0(0)	6(3.90)	-	2.99 ± 1.97	-	31.89 ± 0.07	-	80.16 ± 0.4	-	32.44 ± 5.82	-
No	32(20.78)	116(75.32)		3.09 ± 0.85		30.99 ± 5.51		80.85 ± 9.8		32.10 ± 9.55	
6d. Haemoglobinopathies											
Normal	9(5.84)	81(52.60)	-	4.24 ± 7.00	-	35.0 ± 6.25	-	80.62 ± 3.08	-	33.0 ± 0.72	-
Heterozygous	17(11.04)	31(20.93)		5.11 ± 8.95		35.25 ± 4.67		81.32 ± 8.97		31.76 ± 8.22	
Homozygous	6(3.90)	10(6.49)		4.08 ± 1.48		34.99 ± 0.69		80.79 ± 2.91		32.45 ± 2.97	

Table 2: Contd.....

Ethnicity	*Hb level (n,%)*			*RBC (x10⁶/ml)*		*PCV (%)*		*MCV(fl)*		*MCHC (gm/dl)*	
	<11gm/dl	*>11gm/dl*	*p*	*Mean ± S.D*	*p*	*Mean ± S.D*	*p*	*Mean ± S.D*	*p*	*Mean ± S.D*	*p*
7. Santhal (N=89)	68(76.4)	21(23.6)	<0.001	2.60 ± 0.45	<0.001	29.88 ± 8.01	<0.001	77.03 ± 3.66	<0.001	29.97 ± 4.67	<0.001
7a. Dietary Habits											
Vegetarian	14(15.73)	1(1.12)	<0.01	2.53 ± 0.56	<0.001	27.27 ± 7.22	<0.001	77.99 ± 5.47	<0.001	29.87 ± 7.78	<0.001
No vegetarian	54(60.67)	20(22.47)	-	2.64 ± 2.4	-	29.95 ± 8.21	-	76.89 ± 0.95	-	28.85 ± 8.88	-
7b. Sex											
Male	2(10.52)	17(89.47)	-	2.76 ± 0.99	-	29.97 ± 11.43	-	77.46 ± 6.96	-	29.66 ± 1.28	-
Female	66(94.28)	4(5.71)	<0.001	2.41 ± 1.56	<0.001	29.62 ± 0.94	<0.001	76.99 ± 0.87	<0.001	28.05 ± 9.01	<0.001
7c. Other Diseases											
Yes	2 (2.25)	1 (1.12)	NS	2.58 ± 2.03	NS	29.87 ± 0.85	NS	77.65 ± 1.01	NS	28.96 ± 5.55	NS
No	66(74.16)	20(22.47)	-	2.67 ± 1.40	-	29.81 ± 1.55	-	77.0 ± 0.01	-	29.99 ± 6.1	-
7d. Haemoglobinopathies											
Normal	46(51.69)	21(23.6)	-	2.17 ± 1.1	-	32.4 ± 2.3	-	78.34 ±12.1	-	30.1 ± 4.5	-
Heterozygous	1(1.12)	-	NS	2.67 ± 0.62	NS	33.2 ± 1.23	NS	80.11 ± 0.12	NS	29.7 ± 2.3	NS
Homozygous	-	-	-	-	-	-	-	-	-	-	-
8. West Bengal Control (N=272)	92(33.82)	180(66.18)	-	4.96 ± 8.33	-	36.4 ± 8.9	-	81.41 ± 3.17	-	32.73 ± 7.1	-
8a. Dietary Habits											
Vegetarian	26(9.56)	69(25.37)	-	4.99 ± 1.04	-	35.47 ± 0.44	-	80.97 ± 2.76	-	32.0 ± 0.92	-
No vegetarian	66(24.26)	111(40.81)		5.04 ± 3.31		36.07 ± 0.68		81.14 ± 0.64		32.87 ± 0.8	
8b. Sex											
Male	17(36.95)	29(63.04)	-	4.65 ± 3.56	-	35.99 ± 1.49	-	79.98 ± 1.09	-	32.09 ± 0.65	-
Female	75(33.18)	151(66.81)		5.00 ± 1.94		36.04 ± 1.02		80.99 ± 6.0		32.53 ± 4.11	
8c. Other Diseases											
Yes	0(0)	15(5.51)	-	2.99 ± 1.97	-	35.89 ± 0.87	-	80.16 ± 0.4	-	32.44 ± 5.82	-
No	92(33.82)	64(23.53)		3.09 ± 0.85		36.09 ± 6.61		80.85 ± 9.8		32.10 ± 9.55	
8d. Haemoglobinopathies											
Normal	73(26.84)	138(50.74)		4.24 ± 7.00		36.1 ± 7.23		82.62 ± 3.18		33.9 ± 6.32	
Heterozygous	17 (6.25)	41(15.07)		5.11 ± 8.95		35.65 ± 0.87		80.02 ± 5.37		33.76 ± 8.02	
Homozygous	2 (0.74)	1 (0.37)		4.08 ± 1.48		36.99 ± 0.79		80.79 ± 0.91		32.05 ± 0.57	

NS= Not significant

Table 3: Association of iron deficiency with different parameters in connection with anaemia for selected cases

Ethnicity	*Serum ferritin level*			*Serum iron level*			*Total iron binding capacity*			*Transferrin saturation*		
	<10 µg/lt	*p*	*>10 µg/lt*	*<50 µg/dl*	*p*	*>50 µg/dl*	*<250µg/dl*	*p*	*>250µg/dl*	*<16%*	*p*	*>16%*
1. Mishing (N=35)	0(0)	NS	35(100)	10(28.57)	<0.05	25(71.43)	0(0)	NS	35(100)	21(60)	<0.01	14(40)
1a. Presence of Hb Abnormalities												
Normal trait	0(0)	NS	18(51.43)	4(11.43)	NS	12(34.29)	0(0)	NS	18(51.43)	14(40)	NS	4(11.43)
Heterozygous/ homozygous thalassaemia	0(0)	NS	17(48.57)	6(17.14)	<0.05	13(37.47)	0(0)	NS	17(48.57)	7(20)	NS	10(28.57)
1b. Hb Level												
<11gm/dl	0(0)	NS	26(74.29)	2(5.71)	NS	7(20)	0(0)	NS	9(25.71)	7(20)	NS	6(17.14)
>11gm/dl	0(0)	NS	9(25.71)	8(22.86)	NS	18(51.43)	0(0)	NS	9(25.71)	14(40)	<0.05	8(22.86)
1c. Sex												
Male	0(0)	NS	22(62.86)	10(28.57)	NS	12(34.29)	0(0)	NS	22(62.86)	13(37.14)	<0.05	7(20)
Female	0(0)	NS	13(37.14)	0(0)	<0.01	13(37.47)	0(0)	NS	13(37.14)	8(22.86)	NS	7(20)
2. Sonowal (N=52)	0(0)	NS	52(100)	19(28.57)	<0.05	33(71.43)	0(0)	NS	52(100)	2(3.85)	NS	50(96.15)
2a. Presence of Hb Abnormalities												
Normal trait	0(0)	NS	1(40.38)	5(9.62)	NS	15(28.85)	0(0)	NS	21(40.38)	2(3.85)	NS	14(26.92)
Heterozygous/ homozygous thalassaemia	0(0)	NS	31(59.62)	14(26.92)	<0.05	18(34.62)	0(0)	NS	31(59.62)	0(0)	NS	36(69.23)
2b. Hb Level												
<11gm/dl	0(0)	NS	17(13.46)	8(15.38)	<0.05	12(23.08)	0(0)	NS	17(13.46)	1(1.92)	NS	12(23.08)
>11gm/dl	0(0)	-	35(67.31)	11(21.15)	-	21(40.38)	0(0)	-	35(67.31)	1(1.92)	-	38(73.08)
2c. Sex												
Male	0(0)	-	39(75)	19(28.57)	-	20(38.46)	0(0)	-	39(75)	2(3.85)	-	17(32.69)
Female	0(0)	NS	13(25)	10(19.23)	<0.05	13(25)	0(0)	NS	13(25)	0(0)	-	33(63.46)
3. Nontribal Assam (N=72)	0(0)	-	72(100)	21(29.17)	-	51(70.84)	0(0)	-	72(100)	32(76.19)	-	40(55.56)
3a. Presence of Hb Abnormalities												
Normal trait	0(0)	-	58(80.56)	16(22.23)	-	42(58.34)	0(0)	-	58(80.56)	26(36.12)	-	32(44.45)
Heterozygous/ homozygous thalassaemia	0(0)		14(19.45)	5(6.95)		9(12.5)	0(0)		14(19.45)	6(8.34)		8(11.12)
3b. Hb Level												
<11gm/dl	0(0)	-	12(16.67)	7(9.72)	-	5(6.95)	0(0)	-	12(16.67)	9(12.5)	-	3(4.17)
>11gm/dl	0(0)		60(83.34)	14(19.45)		46(63.89)	0(0)		60(83.34)	23(31.95)		37(51.39)
3c. Sex												
Male	0(0)	-	1(1.39)	0(0)	-	1(1.39)	0(0)	-	1(1.39)	0(0)	-	1(1.39)
Female	0(0)		71(98.62)	21(29.17)		50(69.45)	0(0)		71(98.62)	32(44.45)		39(54.17)
4. Santhal (N=50)	31(62)	<0.05	19(38)	7(14)	NS	43(96)	0(0)	NS	50(100)	31(62)	<0.01	19(38)
4a. Presence of Hb Abnormalities												
Normal trait	31(62)	NS	18(36)	6(12)	<0.01	43(96)	0(0)	NS	49(98)	29(58)	<0.05	20(40)
Heterozygous/ homozygous thalassaemia	0(0)	NS	1(2)	1(2)	NS	0(0)	0(0)	NS	1(2)	0(0)	NS	1(2)
4b. Hb Level												
<11gm/dl	22(44)	<0.01	9(18)	7(14)	<0.05	4(8)	0(0)	NS	21(42)	27(54)	<0.01	15(30)
>11gm/dl	9(18)	NS	10(20)	0(0)	NS	39(78)	0(0)	NS	29(58)	4(8)	NS	4(8)
4c. Sex												
Male	1(2)	NS	1(2)	0(0)	NS	2(4)	0(0)	NS	2(4)	0(0)	NS	2(4)
Female	30(60)	<0.01	18(36)	7(14)	<0.01	41(82)	0(0)	NS	48(96)	31(62)	NS	17(34)

Table 3: Contd.....

Ethnicity	*Serum Ferritin level*			*Serum Iron level*			*Total Iron Binding Capacity*			*Transferrin saturation*		
	<10 µg/lt	*p*	*>10 µg/lt*	*<50 µg/dl*	*p*	*>50 µg/dl*	*<250µg/dl*	*p*	*>250µg/dl*	*<16%*	*p*	*>16%*
5. *Nontribal West Bengal (N= 48)*	0(0)	-	48(100)	10(20.84)	-	38(79.17)	0(0)	-	48(100)	21(43.75)	-	27(56.25)
5a. Presence of Hb Abnormalities												
Normal trait	0(0)	-	40(83.34)	9(18.75)	-	32(66.67)	0(0)	-	40(83.34)	20(41.67)	-	25(52.08)
Heterozygous/ homozygous thalassaemia	0(0)		8(16.67)	1(2.08)		6(12.5)	0(0)		8(16.67)	1(2.08)		2(4.17)
5b. Hb Level												
<11gm/dl	0(0)	-	14(29.17)	3(6.25)	-	13(27.08)	0(0)	-	15(31.25)	9(18.75)	-	5(10.42)
>11gm/dl	0(0)		34(70.84)	7(15.58)		25(52.08)	0(0)		33(68.75)	12(25)		22(45.83)
5c. Sex												
Male	0(0)	-	2(4.17)	0(0)	-	1(2.08)	0(0)	-	2(4.17)	1(2.08)	-	5(10.42)
Female	0(0)		46(95.84)	10(20.84)		37(77.08)	0(0)		46(95.84)	20(41.67)		22(45.83)

(Malville 1991). Still our results indicate the significant statistical difference in the prevalence of anemia among both the tribal and nontribal rural people.

Several reports have identified some tribal groups to be at high-risk for thalassaemias and other haemoglobinopathies, causing high incidence of mortality among them (Das et al. 2000; Sengupta et al. 2002; Balgir 2003). We did not have much data for homozygous conditions of haemoglobinopathies except the HbE diseases. One report on Ahoms indicated the frequency of HbE to be 1.55 (Flatz et al. 1972). In the Northeast homozygous HbE induced anaemia in Deoris and Ahoms, not in Sonowals. HbE trait induced anemia in Mishings, Deoris and Ahoms. But, this significant association did not increase the morbidity and mortality rate among the study groups. The prevalence rate of HbE among the Northeastern tribes does not exactly support some previous reports, however all report high indicence of HbE among them (Chakraborty et al. 1996 ; Das et al. 2000; Krishnamurti 2000). Such localization of the same hemoglobin variant in almost same geographic location in various isolated groups may be explained from the fact that migration from higher risk zone and non-random mating pattern for a long time, particular mutations are restricted to some specific groups (Varawalla et al. 1991). No statistical analysis was possible for the beta and HbS traits due its low availability in studies tribal groups. It was found that hemoglobin disorders did not alter the hematological parameters significantly in the heterozygous state. Though an earliest report of Choudhury et al. observed the indicence of HbE among the Bengali Santhals, we found no case with HbE (Choudhary et al. 1967).

Maternal malnutrition resulting in iron deficiency anaemia leads to miscarriages, premature birth of child and even risk of hypertension, diabetes and obesity later in life. Anaemia among the males also imparts a socio-economic burden of the country. The co-existence of micronutrient deficiency and inherited anaemias makes diagnosis delayed and management difficult. The screening camps arranged for the study have increased awareness of inherited anaemias popularizing premarital and prenatal screening. The study traced significant prevalence of iron-deficiency anemia and hemoglobinopathies in some tribal groups of Eastern India.

Table 4: Incidence of abnormal haemoglobin variants among the studied subjects

Results of Hb electrophoresis	*Studied groups*							
	Mishing (N=44) (n%)	*Sonowal (N=100) (n%)*	*Deori (N=45) (n%)*	*Ahom (N=54) (n%)*	*Munda (N=118) (n%)*	*Santhal (N=89) (n%)*	*Assamese (Non-tribal) (N=154) (n%)*	*Bengali (Non-tribal) (N=272) (n%)*
Normal	21(47.72)	38(38)	10(22.23)	17(31.48)	112(94.92)	88(98.87)	90(58.44)	211(77.57)
Beta Carrier	-	-	-	-	5 (4.24)	-	9 (5.84)	41(15.07)
Hb E	16(36.36)	36(36)	18(40)	28(51.85)	1 (0.85)	-	38(24.68)	17 (6.25)
Homozygous E	6(13.63)	24(24)	17(37.78)	9(16.67)	-	-	16(10.39)	3 (1.10)
E-Beta thalassemia	-	2 (2)	-	-	-	-	-	-
HbS	-	-	-	-	-	1 (1.12)	1 (0.65)	-
ES (Compound homozygous)	1 (2.27)	-	-	-	-	-	-	-

Table 5: Prevalence of beta-globin gene mutations among the haemoglobinopathy cases

Results of ARMS-PCR	*Tribal group*	*Non-tribal group*
IVS1-5(G'!C)/Normal	1	3
Frameshift 41-42 (-CTTT)/Normal	-	-
Frameshift 8-9 (+G)/ Normal	-	-
Codon26 (G→A)	30 (Homo-zygous) 20 (Homo-zygous)	56 (Hetero-zygous) 9 (Hetero-zygous)
Codon6 (G→A) /Normal	1	6
Codon 15 (G→A) /Normal	-	-
Codon30 (G→C) /Normal	-	-
Unknown	1	1

Extensive family screening for hemoglobin disorders and mass screening for presence of anaemia should be the strategy for the management of anaemia among the isolated tribal groups. We believe that our study provides enough information in tracing the causes of anaemia in some tribal groups of Eastern India which may help in the management of this severe health care problem for vulnerable population groups.

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REFERENCES

Bagchi T 1994. *Profile of Some Indian Tribes*. 1st Edition. Calcutta: Punthi Pustak.

Balgir RS, Mishra RK, Murmu B 2003. Clinical and hematological profile of hemoglobinopathies in two tribal communities of Sundargarh district in Orissa, India. *International Journal of Human Genetics*, 3(4): 209-216.

Chakraborty G, De M , Das SK, Das N, Bhattacharya DK, Talukder G 1996. Screening for haemoglobin variants by molecular study in tribal population of Tripura. *Nucleus*, 39: 148-150.

Chandra S, Rao A, Dutta A, Bhattacharya DK 1987. A simple and inexpensive method of mass screening for abnormal Haemoglobins. *Current Science*, 56**:** 411- 412.

Choudhary S, Ghosh H, Mukherjee BN, Roychoudhury AK 1967. Study of blood groups and haemoglobin variants among the Santal Tribe in Midnapore District of West Bengal. *American Journal of Physical Anthropology*, 26(3): 307-312.

Dacie JV, Lewis SM 1994. *Practical Haematology*. 8th Edition, London: Churchill Livingstone.

Das SK, De M, Bhattacharya DK, Talukder G 2000. Interaction of different haemoglobinopathies in Eastern India with a view to establish genotype-phenotype correlation. *American Journal of Human Biology*, 12: 454-459.

De M, Halder A, Podder S, Sen R, Chakrabarty S, Sengupta B, Chakraborty T, Das U, Talukder G 2006. Anemia and hemoglobinoptahies in tribal population of eastern and north-eastern India. *Hemoglobinopathy*, 11(5/6): 371-373.

Devadas RP, Vijayalakshmi P, Chandy A 1980. Nutritional status of the expectant mothers and their offspring. *Indian Journal of Nutritional Diet*, 17: 275-280.

Dutta Chowdhury Sutanu, Chakraborty Tarun, Ghosh Tusharkanti 2008. Prevalence of undernutrition in Santal children of Puruliya district, West Bengal. *Indian Pediatrics*, 43: 45.

Flatz G, Chakravarthy MR, Das BM, Delbrück H 1972. Genetic survey in the Population of Assam. ABO blood groups, G6PD and Haemoglobin types. *Human Heredity*, 22: 323-330.

Freedman LP, Waldman R, Wirth M, Rosenfield A, Chowdhury M. 2004. *Millenium Project Task Force 4: Child Health Interim Report.* NY: Millenium Project.

Fucharoen S, Winichagoon P 2000. Clinical and hematologic aspects of hemoglobin E beta-thalassemia. *Current Opine of Hematology,* 7: 106-112.

Gajra B, Chakrabarty S, Sengupta B, Bose S, Dasgupta I, De M, Talukder G. 2003. Molecular heterogenity of alpha thalassaemia and its relationship with other haemogloginopathies in a tribal population from Sian village, Birbhum, West Bengal. *Nucleus,* 46(1and 2): 29-33.

Gajra B, Chakrabarti S, Sengupta B, De M, Mukherjee S, Talukder G 2003. Prevention of beta thalassaemia major and E beta thalasseamia by prenatal diagnosis in Eastern India. *International Journal of Human Genetics*, 3(4): 225-235.

Kaur YP, Sagar V, Khirwar SS 1982. Nutritional ecology and food consumption pattern of expectant mothers in selected villages and suburbs of Hissar. *Indian Journal of Nutritional Diet*, 19: 50-58.

Krishnamurti L 2000. Few reports of hemoglobin E/ Beta thalassaemia in Northeast India: underdiagnosis of complete exclusion of beta thalassaemia by hemoglobin E. *Journal of Paediatric Hematology and Oncology*, 22: 558-563.

Kukreti R, Rao CB, Das SK, De M, Talukder G, Vaz F, Verma I C, Brahmachari S K. 2002. Spectrum of b thalassaemia mutations and their association with allelic sequence polymorphisms at the b-Globin gene cluster in an Eastern Indian population. *American Journal of Hematology*, 70: 269-277.

Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL 1997. Prevalence of iron deficiency in the United States. *JAMA,* 277(12): 973-976.

Malville NJ 1991. Haemoglobin concentration and working capacity of non-pregnant rural women of the Katmandu valley of Nepal. *American Journal of Human Biology,* 3: 337-387.

Miller S.A, Dykes D.D, Polesky H.F 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Research*, 18(3): 1215.

Mittal PC, Srivasatava S 2006. Diet, nutritional status and food related traditions of Oraon tribes of New Mal (West Bengal), India. *Rural Remote Health,* 6: 385.

Newton CR et al 1989. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Research*, 17**:** 2503-2516.

Oski F 1993. Iron deficiency in infancy and childhood. *New England Journal of Medicine,* 329: 190-193.

Provan D 1999. Mechanisms and management of iron deficiency anaemia. *British Journal of Haematology,* 105: 19-26.

Rao VG, Yadav R, Dolla CK, Kumar S, Bhondeley MK, Ukey M. 2005. Undernutrition and childhood morbidities among tribal preschool children. *Indian Journal of Medical Research*, 122: 43-47.

Rudra T, Chakrabarti S, Sengupta B 2008. Alpha Thalassaemia: Experience of Referral cases in Kolkata, India. *International Journal of Human Genetics*, 8(3): 295-299.

Sen R, Chakrabarti S, Sengupta B, De M, Haldar A, Poddar S, Gajra B, Talukder G, Sengupta S. 2005. Alpha thalassemia among tribal population of Eastern India. *Hemoglobin*, 29(4): 270-280.

Sengupta B, De M, Dasgupta I, Poddar S, Chakrabarty S, Talukder G, Kukreti R, Brahmachari S K. 2002. Camparative study of Haemoglobinopathies in Tribal populations of Arunachal Pradesh and Tripura (North-East India). *International Journal of Human Genetics*, 2(3):169-172.

Sing P, Toteja GS 2003. Micronutrient profile of Indian children and women: Summary of available data for iron and vitamin A. *Indian Pediatrics*, 40:477-479.

Varawalla NY, Old JM, Venkateshanz SR, Weatherall DJ 1991. The spectrum of beta thallassaemia mutations in Indian subcontinent on the basis if prenatal diagnosis. *British Journal of Hematology,* 78: 242-247.

Weatherall DJ 1997. The thalassaemias. *BMJ,* 314: 1675–8.