Original article

Haemoglobinopathies among the tribal and non-tribal antenatal mothers in a tertiary care hospital of rural West Bengal, India.

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Abstract:

Introduction: Anaemia in pregnancy is still a major concern in reducing maternal morbidity and mortality in India particularly in rural population. Haemoglobinopathies are important contributors to anaemia in pregnancy in rural India particularly in tribal population. Beta thalassemia is the commonest type of haemoglobinopathy all over the world. Thalassemia and other haemoglobinopathies are highly prevalent among the tribal communities in West Bengal. Bankura is one of the districts of West Bengal where more tribal population are present. So detection and prevention of thalassemias is one of the major public health problems in this part of the state of West Bengal.Study done by Manna et al[4] showed that about 10% of the population is carrier of haemoglobin disorder.This study was taken up to document the recent prevalence status of hemoglobinopathies particularly Thalassemias and coexistence of iron deficiency anaemias ***Objective:*** To find out prevalence of haemoglobinopathies and to compare the prevalence of different types of Thalassemias among the antenatal mothers. ***Materials and Methods:*** This study was carried out in Bankura Sammilani Medical College (BSMC), Bankura West Bengal among 3500 tribal and non-tribal antenatal mothers. Cation exchange-high performance liquid chromatography (CE-HPLC) is being used for investigation for hemoglobinopathies and thalassemias. Together with a complete blood count, the CE-HPLC is effective in categorizing hemoglobinopathies as traits, homozygous disorders and compound heterozygous disorders. ***Results:*** In our study 275 mothers had haemoglobinopathy. The commonest disorder we encountered was Beta Thalassemia trait (57.5%), followed by HbE carrier (36%), homozygous HbE disease (1%), HbS carrier (4%), HbE Beta Thalassemia (1.5%).

Keywords: hemoglobinopathies; Beta Thalassemia trait and major; antenatal mothers

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Introduction:

Anaemia is still a common disorder among pregnant mothers in our country particularly in rural setting and is a common cause of significant morbidity and mortality during ante-partum and post-partum period. In contrast to urban areas hemoglobinopathies of different types contributes significantly to anaemia in addition to iron deficiency disorder. In our country it is well known that Haemoglobinopathies particularly Thalassemias are more prevalent amongst tribal populations in different parts of the country. There is no study in this rural part of West Bengal State of India documenting the prevalence of Hemoglobinopathies and pattern of different types of Thalassemias. Detection of Beta Thalassemiatrait (BTT) is critical in preventing the birth of a child with thalassemia major and is of immense importance in preventing the propagation of the disease and will contribute to Thalassemia Control programme of Government of India.

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W.H.O estimate 4.5% of the world's population is carriers of different haemoglobinopathies. Thalassemias, particularly Beta variants are responsible for majority of them. Prevalence of BTT in West Bengal is about 3.5%. . Beta thalassemia is the commonest type of haemoglobinopathy in most the studies. Different types of deleterious mutations are restricted to some particular tribes. The high frequencies of these mutant alleles are maintained by the tribal populations probably due to consanguinity and endogenous mating for a long period of time. Ignorance, lack of awareness and conveyance, low income status, high cost of treatement and inadequate access of advanced diagnostic facilities make them more vulnerable. The co-existence of Beta and HbE gene makes the population of West Bengal vulnerable to get both Beta thalassemia and HbE Beta thalassemia. Therefore, Clinical identification of these carriers or trait is important particularly in antenatal mothers. Any difference in the prevalence of this hemoglobinopathies among tribal and non-tribal population in this backward districts will help us to give more attention to the vulnerable populations and in the long run to reduce maternal morbidity and mortality. Moreover,Iron deficiency anaemia frequently coexists with Beta Thalassemia trait. But this deficiency may be missed due to a reduction in value of HbA2. The present study was taken up to bringforth the prevalence of Hemoglobinopathies and co-existance of Iron deficiency anaemia with these disorders.

Objectives of this study:

i) To find out the prevalence of haemoglobinopathies among the tribal and non-tribal antenatal mothers attending Bankura Sammilani Medical College Hospital

ii) To compare the prevalence of Beta Thalassemia trait and other types of haemoglobinopathies among the tribal and non-tribal antenatal mothers attending Bankura Sammilani Medical College Hospital.

Materials and methods:

This Cross-Sectional study was conducted at the Department of Pathology, BSMC after receiving approval from the Ethical Committee of BSMC, Bankura, West Bengal. The present study includes 3500 voluntary participants for the screening of haemoglobinopathies from January 2011 to June 2012.All pregnant women both tribal and non-tribal attending the outpatient department of thalassemia unit and antenatal clinic of BSMC during the period of January 2011 to June 2012 were enrolled by Census method(requires no sampling technique) for this study. Data Collection was done with filling up of Bio-data, detailed history and physical examination of the patients in a recognized format as per international standard. Tools and Techniques: Tools used are Haemoglobin estimation, Complete Hemogram,__Peripheral Blood Smear (PBS), Hemoglobin Electrophoresis,Serum Ferritin. Patients suffering from anaemia at defined level are screened by HPLC. The following techniques were used for different tools. Haemoglobin estimation was done by cyanmethaemoglobin method as it is the best and standard method. In this method Test was done by using Drabkin's reagent and Colorimetric determination of absorption peak of Cynamethemoglobin at 530-550 nm.Total count of RBC ,Total count of WBC ,Platelet count ,PCV,MCV,MCH, MCHC, RDw was done by KX21 Hematology Analyzer made by SYSMEX Corporation JAPAN CARF COMPANY LTD.

Peripheral blood smears examination-by using Leishman's stain.

Reticulocytes count by supravital staining (with patients with MCV less than 80 fl).

HPLC by BIORAD technique using catalog No 788-0000,flow rate 0.001-10 ml/min/0.01-10ml/ min. Study of haemoglobin type was determined by BIORAD variant haemoglobin testing system-which works on cation exchange high performance liquid chromatography (CE-HPLC) method. Hemoglobin separates into major and minor hemoglobins when subjected to CE-HPLC. The order of elution of the various components are HbA1a, HbA1b, HbF, LA1c/CHb-1, LA1c/CHb-2, HbA1c, P3 (Hbd component), HbA0, and HbA2. The minor hemoglobins A1a, A1b, A1C, F1, and the P3 component are posttranslational modifications of the globin chains. HbA2, minor hemoglobin, however, is composed of two alpha and two delta chains. HbA0 and HbF are the major hemoglobins in a normal hemolysate.An elevated HbA2 with an average value of about 5%, along with microcytic hypochromic indices, is characteristic of Beta Thalassemiatrait[1]. HbE trait is diagnosed by the presence of a high HbA2 (E+A2), approximately 30%[2]. Homozygous HbE patients have approximately 90% HbE+A2 with minor elevation of HbF[2]. HbE+A2 levels of 40–60% with marked elevation of HbF are seen in HbE-Beta -thalassemias [2]. HbS is around 40% in sickle cell trait, 90–95% in sickle cell anemias (which varies inversely with HbF proportion), and less than 50%

Table 1. Distribution of different type of haemoglobinopathies among the tribal and non tribal mothers (n =275) (Total nontribal mothers 2625 Total tribal mothers 875)

Type of haemoglobinopathies	Non tribal (%) (n=193)	Tribal (%) (n=82)	Total (%) (n=275)
BTT*	107 (55.44)	51 (63.42)	158 (57.50)
HbE carrier**	72 (37.30)	27 (32.53)	99 (36)
HbS carrier	07 (3.63)	04 (4.05)	11 (04)
HbE Beta thal	04 (2.07)	00	04 (1.50)
HbE homozygous	03 (1.56)	00	03 (01)
Total	193 (70.18)	82 (29.82)	275 (100)

Total nontribal mothers 2625: Total Tribal mothers 875

*χ^2 = 4.675, df=1, p= 0.031

**χ^2 = 0.281, df=1, p= 0.596

Table 2. HPLC parameter of antenatal mothers with Beta thalassaemia trait (BTT) and BTT with increased HbF

Presumptive HPLC diagnosis	B thalassaemia trait (%)	B thalassaemia traitWith ↑ed HbF (%)
HbA2(%)	4.95	81.40
HbF(%)	1.03	5.10
HbA(%)	84.44	5.30

It is seen that HbA2 is 4.95% and HbF value is 1.03% in BTT carrier and these patients not symptomatic. In BTT with increased HbF, HbA is very low only 5.30%.

Table 3. HPLC parameter of antenatal mothers with HbE thalassaemia syndrome:

Presumptive HPLC diagnosis	HbE thalassaemia trait	HbE Beta thal-assaemia	HbE homozy-gous
HbA2 (%)	26.13	48.17	76.32
HbF (%)	0.79	21.50	3.38
HbA (%)its	63.65	22.28	5.68

Table-3 shows that in HbE-beta thalassaemia HbA2 and HbF both are raised (48.17%, 21.50% respectively). In HbE homozygous HbA2 is very high (76.32%).

in sickle Beta -thalassemias [3]. Approximately less than 50% of abnormal hemoglobin is seen in HbD traits[3].The present study highlights the detection of the hemoglobinopathies and thalassemias by CE-HPL Difficult cases were further analysed by agarose gel electrophoresis at appropriate pH. Agarose gel electrophoresis was done according to the manufactures guidelines. Accu-Bind ELISA Microwells were used for measurements of serum ferritin level in BTT patients.

Master Chart was prepared collecting datas from all the patients in our study putting each data in a separate column. Then the datas were analyzed. Data processing was done by Tally, Tabulation and drawing of Pictograms.

Statistical analysis was done by using the software SPSS-17 Version. Correlation, association were assessed by applying suitable statistical method like p Value, chi square test and percentage (%).

Observations:

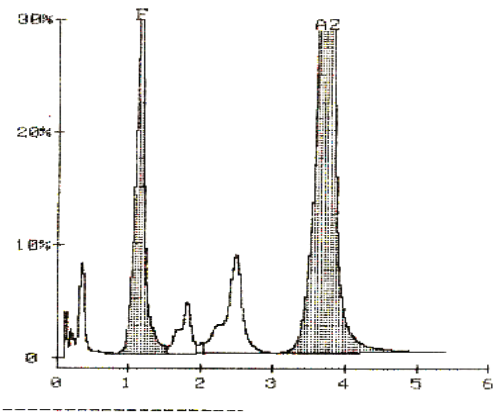
Out of 3500 antenatal mothers included in this study 875 were tribal mothers and 2625 were nontribal mothers. Of these 275 (7.86%) mothers had different types of haemoglobinopathies and 3225 (92.14%) mothers were normal. In our study it was found that number of mothers with BTT was 158 (57.50%). Of these no of nontribal mothers were 107 (55.44%) and tribal mothers were 51 (63.42%). Number of mothers with HbE trait was 99 (36%) of which nontribal mothers were 72 (37.30%) and tribal mothers were 27 (32.53%), Number of mothers with HbE homozygous were 03 (1%), and all were nontribal mothers-03 (1.56%). Numbers of mothers with HbE beta thalassaemia were 04 (1.50%) and all were nontribal mothers 04(2.07%).Number of mother with HbS traits

were 11(4%) of which nontribal mothers were 07(3.63%) and tribal mothers were 04(4.05%). Therefore, among total haemoglobinopathies (n = 275) BTT is more prevalent in tribal mothers (n =82) than nontribal mothers (n =193) and the difference is statistically significant(α= 0.05, p= 0.031). HbE carrier is more prevalent in nontribal mother(n= 193) than tribal mother(n = 82) and the difference is statistically insignificant(α= 0.05, p =0.596).

This study shows that in cases of BTT mild anaemia(Hb-10.98gm%) with decreased MCV(67.16fl) and MCH(20.50pg) is found and RDW(cv) shows mild elevation (normal range <14.5) and there is more anaemia in BTT with increased HbF. Patients with HbE-beta thalassaemia have very low level haemoglobin (6.25gm%) and high RDW (cv) 30.31. Patients with HbE homozygous have mild anaemia (Hb-9.96 gm%) and have low MCV(59.22fl).Patient with sickle cell trait suffer from mild anaemia (Hb 11.83gm%) and have normal RDW value . In HbS trait HbA is very high(53.92%) and HbS is mildly elevated(35.41%) and HbA2 is within normal value (3.31%).

SAMPLE CHROMATOGRAMS (HPLC)

ANALYTE ID	%	TIME	AREA
F	20.4	1.14	618229
P3	3.6	1.80	103525
Unknown 1	0.4	2.01	10895
Ao	10.1	2.48	292526
A2	66.4	3.71	2051073
	TOTAL AREA		3076248
F	20.4%	A2	66.4%



HPLC: HbE-Beta thalassaemia

Discussion:

Comparison with other studies outside West Bengal in percentage (%)

	Present study	Sachdev et al. [9]	Rao et al. [10]	Balgir [6]
Beta thal trait	57.5	8.9	18.1	18.2
HbE trait	36.0	0.19	1.1	0.9
HbS trait	4.0	—	1.4	29.8
E Beta -thalassemia	1.5	0.23	1.3	0.7
S Beta -thalassemia	-	0.07	0.8	1.7
HbE homo	1.0	-	-	-
Hb SS	-	0.03	0.5	7.5

In the present study out of 3500 antenatal mothers screened for hemoglobinopathies only 275 mothers has hemoglobinopathies.Among antenatal mothers with hemoglobinopathies, prevalence of BTT 57.50%, HbE trait 36%, Sickle cell trait 4%, HbE homozygous is 1% , HbE-Beta thalassaemia (1.50%). **Study of Dr A K Manna et al[4]** in an urban population **around Kolkata** showed prevalence of Beta thalassaemia major 30%, E-Beta thalassaemia 5.3%, BTT 10.73%, HbE traits0.35% other small proportion of cases were sickle cell and HbD diseases. **In Apollo Hospitals Chennai Chandrasekhar et al[5]** studied 543 abnormal chromatogram patterns in **Southern parts of India**. In their study they found Beta Thalassemiatrait (37.9%), followed by HbE trait (23.2%), homozygous HbE disease (18.9%), HbS trait (5.3%), HbE Beta Thalassemia(4.6%), HbS Beta Thalassemia(2.5%), Beta Thalassemiamajor (2.3%), HbH (1.6%), homozygous HbS (1.4%), HbD trait (0.7%). The average value of HbA2 in Beta Thalassemiaminor was 5.4%. Among the HbE disorders the HbA2 + HbE was 30.1% in the heterozygous state, 90.8% in the homozygous state and 54.8% in HbE Beta -thalassemia. In the sickle cell disorders, HbS varied from 30.9% in the trait to 79.9% in the homozygous state to 65.6% in HbS Beta -thalassemia. In the **study by Balgir RS[6] from Orissa**, sickle cell trait was noted to be common as the study included tribes from Orissa where this gene is prevalent. Study by S.S Ambekar et al[7] showed BTT 7%, Thal major 5.9%,Sickle cell disease2.3%,HbE disease0.6%,HbD disease0.2% **A study was done by B M Das et al[8] from Assam** in 2 groups of tribal communities in Assam, India. Among 80 Khasi tribal group (Bhoy subgroup showed 41% HbE heterozygous), and in 82 Ahom Tribal group 58% of HbE carriers were found.

We are presenting a comparison between our study and other three studies

I.F. Estevao et al[11] in a study from Brasil showed no significant difference in serum ferritin and transferrin saturation levels in Beta ^{0} and Beta $^{+}$ thalassaemia patient.

Our study shows body iron status of BTT antenatal mothers as determined by serum ferritin assay were also within normal range.

Therefore from our study it is evident that prevalence of different types of Hemoglobinopathies are higher in this part of rural west Bengal compared to southern part as the tribal population is more in this part of West Bengal. Studies outside India are very much limited. The prevalence and pattern is also higher compared to other studies in available literatures till date.

Conclusion:

Beta thalassaemia traits comprises 57.50% and HbE traits comprises 36% whereas HbS carrier is 4% followed by HbE Beta thal is 1.50% and HbE homozygous (01%) amongst patients of hemoglobinopathies in ante-natal mothers . Beta thalassaemia traits are higher in tribal (63.42%) than nontribal mothers (55.44%). No α thalassaemia was detected. HPLC is the gold standard for the diagnosis of haemoglobinopathy. Serum ferritin assay reflects the body iron status and it remains within normal range in case of Beta thalassaemia traits. The frequency of haemoglobinopathy and thalassaemia comparatively high in Bankura district of West Bengal. Genetic counseling is essential for certain communities that show higher frequency of haemoglobin disorders. "thalassaemia control is an urgent need and we dream of a thalassaemia free west bengal".

Limitations of the study: As it is a Cross-Sectional study more no of patients from different health facilities both Govt and Non-Govt Institutions would have been more informative. Follow up of the patients could not be done because of socio-economic reasons. More importantly, Genetic Studies are important to compare the mutation pattern of patients with other studies. Genetic studies could not be done in our study because of financial reasons and non-availability of resources.

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