

# A demographic prevalence of $\beta$ Thalassemia carrier and other hemoglobinopathies in adolescent of Tharu population

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## ABSTRACT

**Background and Aims:** Hemoglobinopathies and thalassemias are the commonest single gene disorders in India. In Terai region of India, Hemoglobinopathies and thalassemias are the most common in the Tharu community. Therefore, in this study, we aim to evaluate the Hb variant analysis of hemoglobinopathies and thalassemias in a Tharu population in Lakhimpur Kheri Districts of Uttar Pradesh, India. **Materials and Methods:** Total 493 individuals were recruited in this study. The demographic details and blood samples were collected from different location at Kheri district during mega health camp. Hb variant analysis was performed by high performance liquid chromatography (HPLC) system beta thalassemia short program in BIO-RAD VARIANT. **Results:** Out of 493, 108 (21.9%) individual suffers with abnormal haemoglobinopathies. In which  $\beta$ -thalassemia trait is the commonest haemoglobinopathy (12.98%), followed by HbE trait (7.50%), and compound heterozygous HbS/ $\beta$ -Thalassemia trait (1.42%) in overall population. The HbF was significantly greater in HbS heterozygous ( $1.45 \pm 1.41$ ), whereas mean HbA2 was significantly greater in  $\beta$ -Thalassemia trait ( $5.17 \pm 1.36$ ). **Conclusion:** The high incidence of hemoglobinopathies and thalassemias were observed in Tharu community in Lakhimpur Kheri districts of Uttar Pradesh, Indian.

**Keywords:** Hemoglobinopathies, high performance liquid chromatography, sickle cell anemia, thalassemia, Tharu community

## Introduction

Hemoglobinopathies and thalassemias are most common monogenic blood disorder.<sup>[1,2]</sup> Globally, approximately 5% people are thalassemias carriers.<sup>[1-3]</sup> These are commonly (37.1%)

seen in the Tharu community in Nepal.<sup>[4]</sup> The Tharu tribe is commonly found in Terai plains of India e.g., Lakhimpur Kheri Districts of Uttar Pradesh and Nepal border. The  $\beta$ -thalassemia major patients are blood transfusions dependent to sustain their life but it is very costly in developing countries.<sup>[5-8]</sup> The early diagnosis of these monogenic diseases helps to reduce the incidence of this disease. Therefore we aim to study HPLC based diagnosis of haemoglobinopathies and thalassemias in a Tharu population.

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## Methods

After obtaining institutional ethical committee approval (Ref. code: 86<sup>th</sup> ECM IIA/P22) 23/02/2018 the study was conducted in the Cytogenetics Lab, Department of Center for advance Research and Department of Pathology of King George's Medical University. This study was performed in association with Rashtriya Seva Sangh during 6-8 December, 2019, in a "Mega Health Camp". The proper health checkup was performed in various schools of the Lakhimpur Kheri district of Uttar Pradesh, India.

Out of the 600 individuals, 107 samples were hemolysed, and a total of 493 individuals were recruited in this study. The samples were collected from different school at Lakhimpur Kheri district. The samples were analyzed at the Department of Center for Advance Research (Cytogenetics Lab) and Pathology of King George's Medical University, Lucknow.

### Blood samples collection

In EDTA vials the 2 ml peripheral blood was collected after obtaining consent from the respective subjects.

### High performance liquid chromatography (HPLC) for haemoglobin

The HPLC tests were accomplished by using beta thalassemia short program BIO-RAD VARIANT, BIO-RAD laboratories, USA.<sup>[9]</sup> In every run in BIO-RAD HPLC, two levels of controls and HbF and HbA2 calibrator were analyzed. The various types of hemoglobin variants such as HbS was identified through retention time windows. These retention time windows are specified for hemoglobin variants.

The statistical package, SPSS (version 21) was used. The data are represented as number (%), mean  $\pm$  SD, median, minimum and maximum. Student "t" test and analysis of variance (ANOVA) were used to analyze overall difference in group means.

## Results

A total of 493 individuals were enrolled in this study. A total of 96 (19.47%) individuals are male and 297 (80.53%) individuals are female [Table 1]. The HPLC data are shown in Table 1. The values are expressed as mean, median,  $\pm$ SD, minimum and maximum. Range of HbF, HbA2, HbA0 and S windows ( $n = 44$ )

were 0.00 to 11.00, 0.70 to 7.80, 25.70 to 94.20 and 19.60 to 36.90, respectively. The mean  $\pm$  SD value of HbF, HbA2, HbA0 and S windows ( $n = 44$ ) were  $0.69 \pm 0.83$ ,  $3.19 \pm 1.01$ ,  $84.24 \pm 8.10$  and  $27.12 \pm 3.74$ , respectively.

Table 2 and Figure 1 show the wide spectrum of various types of haemoglobinopathies of Tharu population by HPLC. In this study, out of 493, 108 (21.9%) individual suffers with abnormal haemoglobinopathies, in which  $\beta$ -thalassemia trait is most common type of haemoglobinopathy (12.98%), HbE trait (7.50%) and compound heterozygous HbS and  $\beta$ -Thalassemia trait (1.42%) respectively in overall population. However, this study was conducted in Lakhimpur Kheri District, Uttar Pradesh, India, and the data represent an overall picture of hemoglobinopathies and thalassemia in Tharu tribes of India.

Table 3 shows the distribution of HPLC spectrum and Types of hemoglobinopathies and thalassemia in male and female gender. The HPLC spectrum such as HbF, HbA2, HbA0 and HbS were statistically similar in male and female. Furthermore, the distributions of hemoglobinopathies and thalassemia were not depend on types of gender. The  $\beta$ -Thalassemia trait are more common in male whereas HbS heterozygous in female.

HbF was significantly greater in HbS heterozygous ( $1.45 \pm 1.41$ ), after that compound heterozygous HbS/ $\beta$ -Thalassemia trait ( $1.34 \pm 0.68$ ), followed by  $\beta$ -Thalassemia trait ( $1.04 \pm 1.45$ ) as shows in Table 4. Whereas mean HbA2 was significantly greater in  $\beta$ -Thalassemia trait ( $5.17 \pm 1.36$ ), after that compound heterozygous HbS/ $\beta$ -Thalassemia trait ( $3.91 \pm 0.53$ ), followed by HbS heterozygous ( $3.56 \pm 0.33$ ). Moreover, the mean HbA0 was significantly lower in HbS heterozygous ( $62.41 \pm 5.08$ ), after that compound heterozygous HbS/ $\beta$ -Thalassemia trait ( $63.90 \pm 2.47$ ), followed by  $\beta$ -Thalassemia trait ( $84.78 \pm 3.55$ ) as shows in Table 4. The S window was significantly higher in HbS heterozygous ( $27.84 \pm 3.49$ ) as compared to compound heterozygous HbS/ $\beta$ -Thalassemia trait ( $23.31 \pm 2.66$ ) as shows in Table 4.

## Discussion

In developing countries, health is one of the prime concerns of the human community. In India, mostly tribal community has various types of disorders and illnesses.<sup>[10]</sup> The evaluated predominance of  $\beta$ -thalassemia minor (carriers) in India is 3-8%,<sup>[11]</sup> which transforms to 35-45 million carriers in

Table 1: Demographic and HPLC finding in participants

Parameter	Mean	Median	$\pm$ SD	Minimum	Maximum
Gender					
Male	96 (19.47%)				
Female	397 (80.53%)	-	-	-	-
HbF	0.69	0.50	0.83	0.00	11.00
HbA2	3.19	2.90	1.01	0.70	7.80
HbA0	84.24	86.60	8.10	25.70	94.20
S windows ( $n=44$ )	27.12	26.20	3.74	19.60	36.90

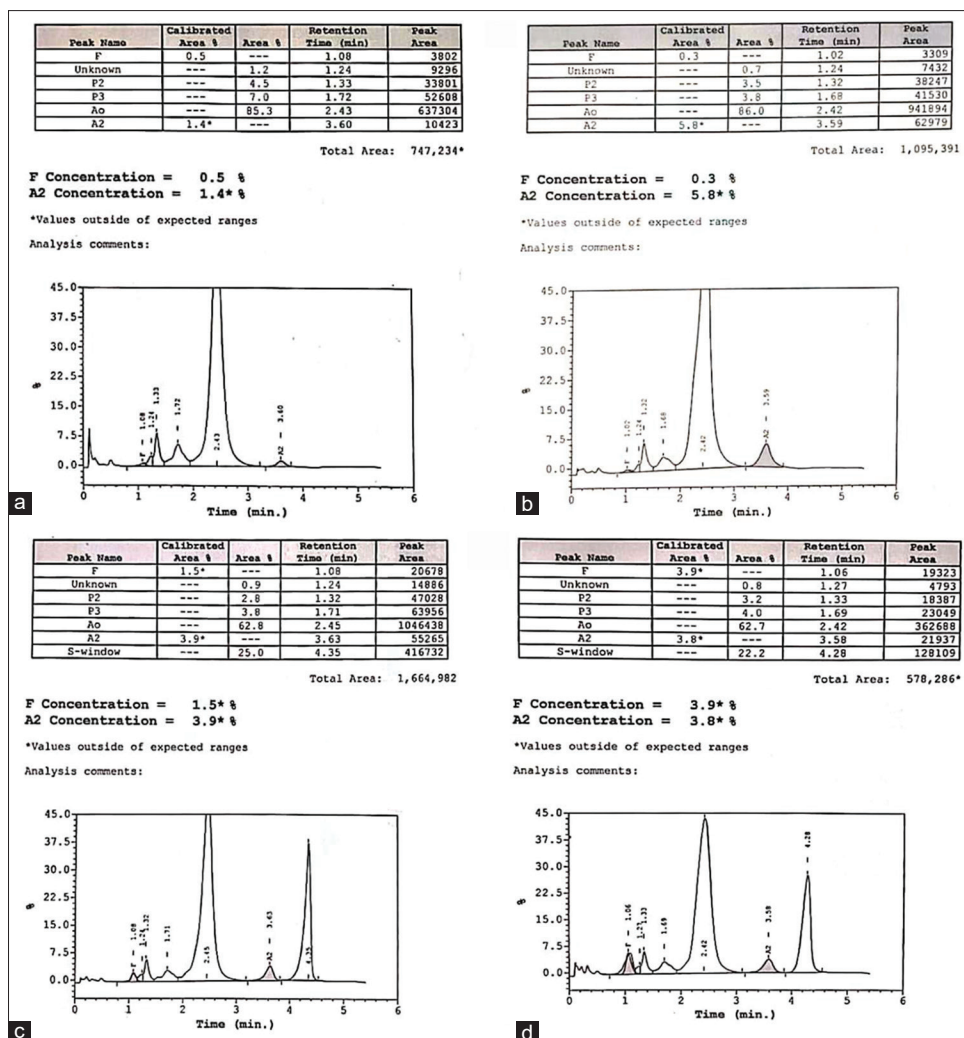


Figure 1: HPLC of various types of haemoglobinopathies and thalassemia; (a) Normal hemoglobin (Hb); (b)  $\beta$ -thalassemia trait with A2 fraction on 5.8%; (c) and (d) Compound heterozygous HbS and  $\beta$ -Thalassemia Trait

Table 2: Distribution of normal and abnormal Hemoglobinopathies and thalassemia

	n	Percentage
<b>Hemoglobinopathies</b>		
Normal	385	78.1
Abnormal	108	21.9
<b>Types of abnormal hemoglobinopathies</b>		
$\beta$ -Thalassemia Trait	64	59.26
HbS Heterozygous	37	34.26
Compound heterozygous HbS and $\beta$ -Thalassemia trait	7	6.48

1.3 billion diverse population with culturally and linguistically and multi-ethnic (about 8% of tribal groups). Various Tribal groups have a greatly higher 4 to 17% prevalence.<sup>[12,13]</sup> It was documented that approximately 7,500-12,000  $\beta$ -thalassemia major kids are born in India each year which encompasses 10% of the World total number.<sup>[12,14]</sup> The different tribe communities are live, one of them is Tharu. This is a most common tribe living in the Terai plains of India and Nepal border. A few

numbers of Tharus live in India, frequently in Lakhimpur Kheri, Pilibhit, Balrampur, Gonda, Bahirayach, Gorakhpur districts of Uttar Pradesh, Udham Singh Nagar districts of Uttarakhand, and Champaran districts of Bihar. In India, the total Tharu tribe population is 169,209, of which an approximate 83,544 Tharus live in Uttar Pradesh.<sup>[15]</sup>

The health status is very poor among tribal population, which is dependent upon socio-cultural, socio-economic, and ecological factors.<sup>[16]</sup> The Tharu population is the oldest and leading endogenous group, living in dense forest of Himalayan village. Malnutrition is more common in tribe communities. Anemia is a major health concern in the developing country affecting maximum people.<sup>[17]</sup> In India the haemoglobinopathies mainly  $\beta$ -thalassemia, HbS and HbE are significant provocation for tribal community.<sup>[18]</sup>

The prevalence of thalassemias and hemoglobinopathies differs with geographic region. The frequency of Hb disorder encountered in India comprise  $\beta$ -thalassemia, sickle cell anemia,

HbE/ $\beta$ -thalassemia, HbE and HbD. HbE is generally limited in Odisha, West Bengal, North-East and Andaman and Nicobar islands whereas HbS has widespread and predominant in North-East region.<sup>[18-20]</sup>

A previous study has reported that the commonest hemoglobinopathies such as Hb E trait, homozygous E, beta-thalassemia trait, E- $\beta$ -thalassemia sickle cell- $\beta$ -thalassemia and  $\beta$ -thalassemia major have 34.4%, 25.3%, 17.8%, 15.1%, 3.4% and 1.5%, respectively.<sup>[21]</sup> Another study reported that the  $\beta$ -thalassemia trait was commonest hemoglobinopathies, followed by  $\beta$ -thalassemia major and HbS in Gujarat state of India.<sup>[22]</sup>

The Tharu tribe generally lives in the Terai plains of India such as Lakhimpur Kheri, Pilibhit, Balrampur, Gonda, Bahirayach, Gorakhpur districts of Uttar Pradesh, Udham Singh Nagar district of Utrakhhand and Champaran district of Bihar. In the tharu population the hemoglobinopathies and thalassemias were commonly found.<sup>[22]</sup> In India, hemoglobinopathies were generally found in other castes also. Previously a study reported that the HbS was commonly found in the Tharu population.<sup>[23]</sup> Therefore in this survey, we are trying to evaluate the incidence rate of HbS, HbE and thalassemias in Tharu population in Lakhimpur Kheri districts of Uttar Pradesh, India.

In this study, total 493 individuals were enrolled in which 19.47% male and 80.53% female. Total 108 (21.9%) individual suffers with abnormal haemoglobinopathies. Therefore, the prevalence of abnormal haemoglobinopathies was 21.9% in Tharu community

in Lakhimpur Kheri district of Uttar Pradesh, India. Out of 108 (21.9%) individual with abnormal haemoglobinopathies,  $\beta$ -thalassemia trait is the most common (59.26%). The other hemoglobinopathies such as HbS trait and compound heterozygous thalassemia (HbS- $\beta$ ) have 34.26% and 6.48% respectively in Tharu community in this HPLC based study.

In our study we also found that the  $\beta$ -Thalassemia Trait are more common in male whereas HbS heterozygous in female but not statistically different.

In this study, we also found that the HbF level was significantly higher in HbS heterozygous followed by in compound heterozygous HbS/ $\beta$ -Thalassemia trait, followed by  $\beta$ -Thalassemia Trait. Whereas mean HbA2 was significantly greater in  $\beta$ -Thalassemia Trait followed by in compound heterozygous HbS/ $\beta$ -Thalassemia trait, then HbS heterozygous. Moreover, the mean HbA0 was significantly lower in HbS heterozygous, after that compound heterozygous HbS/ $\beta$ -Thalassemia trait, followed by  $\beta$ -Thalassemia Trait. The increased level of HbF may help to ameliorate the severity of illness.<sup>[24]</sup> A recent study reported that the level of HbF was highest in  $\beta$ -Thalassemia major and compound heterozygous for HbE/ $\beta$ -thalassemia.<sup>[4]</sup> He also reported that the increased level of HbF was found in sickle cell anemia. Whereas Shrikhande et al. (2007) demonstrated that that the average HbF was found in sickle cell disease.<sup>[25]</sup> The increased level HbA2 is associated with  $\beta$ -thalassemia trait.<sup>[26-30]</sup> The high HbA2 (E + A2) is used for the diagnosis of HbE trait.<sup>[28,31]</sup> In sickle cell trait, approximately 40% HbS, 90-95% in HbS (which differs inversely proportion to HbF), and <50% in sickle  $\beta$ -thalassemias.<sup>[32]</sup> In our study the mean level of HbA2 was  $5.17 \pm 1.36$  in  $\beta$ -thalassemia traits. Similarly various studies reported that HbA2 was 5% in  $\beta$ -thalassemia traits.<sup>[26,27,29]</sup> The present study similar to previous study, they reported that the HbF level was slightly high in  $\beta$ -thalassemia trait patients and was around 1.04%.<sup>[26,27]</sup>

This study provides better knowledge about hemoglobinopathies in Tharu population. As we know that the HbA2 is a gold standard analysis for the thalassemia diagnosis. In this study we try to find out the incidences of hemoglobinopathies/thalassemia in Tharu population. Our data will be helpful for clinicians to make an early diagnosis of thalassemia minor (carrier) to provide primary care to Tharu population. Further the clinician may correlate the level of HbA2 with the thalassemia. Therefore this information is helpful for the early diagnosis of thalassemia trait and provides the genetic counselling to affected families of Tharu population.

**Table 3: Distribution of HPLC finding and normal and abnormal hemoglobinopathies and thalassemia in male and female**

	Male (n=96)	Female (n=397)	P
HbF	0.64±0.70	0.70±0.85	0.523
HbA2	3.29±1.16	3.17±0.97	0.303
HbA0	84.18±8.77	84.25±7.94	0.936
S Window	24.70±1.59	27.58±3.86	0.158
Hemoglobinopathies			
No	76 (79.2%)	309 (77.8%)	0.884
Yes	20 (20.8%)	88 (22.2%)	
Types of hemoglobinopathies			
$\beta$ -Thalassemia Trait	13 (65%)	51 (57.95%)	0.915
HbS Heterozygous	4 (20%)	33 (37.5%)	0.270
Compound heterozygous HbS and $\beta$ -Thalassemia trait	3 (15%)	4 (4.55%)	0.300

**Table 4: Distribution of HPLC spectrum in hemoglobinopathies**

	Normal (n=385)	$\beta$ -Thalassemia Trait (n=64)	HbS Heterozygous (n=37)	Compound heterozygous HbS and $\beta$ -Thalassemia trait (n=7)	P
HbF	0.55±0.49	1.04±1.45	1.45±1.41	1.34±0.68	<0.001*
HbA2	2.82±0.40	5.17±1.36	3.56±0.33	3.91±0.53	<0.001*
HbA0	86.61±4.50	84.78±3.55	62.41±5.08	63.90±2.47	<0.001*
S Window	-	-	27.84±3.49	23.31±2.66	0.002*

\*= Significant

The HbA2 analysis is necessary for the early diagnosis of thalassemia minor (carrier) in Tharu population, which may reduce or eradicate the burden of thalassemia from this society.

## Conclusion

We have observed that the Tharu community in Lakhimpur Kheri districts of Uttar Pradesh, India is a rich reservoir of thalassemias and hemoglobinopathies. Approximately 21.9% population of Tharu community was showed the hemoglobinopathies and thalassemias. In which  $\beta$ -thalassemia carrier is the commonest hemoglobinopathies (59.26%) followed by HbS (34.26%). Moreover, the compound heterozygous HbS/ $\beta$ -Thalassemia trait was found 6.48% in Tharu community. Furthermore, the larger sample size with multicentric studies is required to get the actual prevalence of the hemoglobinopathies and thalassemias carrier in Tharu population.

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## Conflicts of interest

There are no conflicts of interest.

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