A demographic prevalence of β Thalassemia carrier and other hemoglobinopathies in adolescent of Tharu population

Nitu Nigam1, Rashmi Kushwaha2, Geeta Yadav2, Prithvi K. Singh1, Nitin Gupta1, Bhupendra Singh3, Monica Agrawal4, Pooran Chand5, Shailedra K. Saxena6, Madan Lal Brahma Bhatt7

1Department of Center for Advance Research (Cytogenetics Lab), 2Pathology, 3Clinical Hematology, 4Obstetrics and Gynecology, 5Prosthodontics 6Center for Advance Research and 7Vice Chancellor, King George’s Medical University, Lucknow, Uttar Pradesh, India

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 β-thalassemia trait is the commonest haemoglobinopathy (12.98%), followed by HbE trait (7.50%), and compound heterozygous HbS/β-Thalassemia trait (1.42%) in overall population. The Hbf was significantly greater in HbS heterozygous (1.45 ± 1.41), whereas mean HbA2 was significantly greater in β-Thalassemia trait (5.17 ± 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.[1,2] Globally, approximately 5% people are thalassemias carriers.[3] These are commonly (37.1%) seen in the Tharu community in Nepal.[4] The Tharu tribe is commonly found in Terai plains of India e.g., Lakhimpur Kheri Districts of Uttar Pradesh and Nepal border. The β-thalassemia major patients are blood transfusions dependent to sustain their life but it is very costly in developing countries.[5-8] The early diagnosis of these monogenic diseases helps to reduce the incidence of this disease. Therefore we aim to study HLPC based diagnosis of haemoglobinopathies and thalassemias in a Tharu population.

Address for correspondence: Dr. Nitu Nigam, Cytogenetics Lab, Department of Center for Advance Research, King George’s Medical University, Lucknow - 226 003, Uttar Pradesh, India. E-mail: nigamnitu@gmail.com

Received: 16-05-2020 Revised: 14-06-2020 Accepted: 02-07-2020 Published: 25-08-2020

How to cite this article: Nigam N, Kushwaha R, Yadav G, Singh PK, Gupta N, Singh B, et al. A demographic prevalence of β Thalassemia carrier and other hemoglobinopathies in adolescent of Tharu population. J Family Med Prim Care 2020;9:4305-10.
Methods

After obtaining institutional ethical committee approval (Ref. code: 86th 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.[9] 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 ± 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, ±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 ± SD value of Hbf, HbA2, HbA0 and S windows (n = 44) were 0.69 ± 0.83, 3.19 ± 1.01, 84.24 ± 8.10 and 27.12 ± 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 β-thalassemia trait is most common type of haemoglobinopathy (12.98%), HbE trait (7.50%) and compound heterozygous HbS and β-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 β-Thalassemia trait are more common in male whereas HbS heterozygous in female.

Hbf was significantly greater in Hbs heterozygous (1.45 ± 1.41), after that compound heterozygous Hbs/B−Thalassemia trait (1.34 ± 0.68), followed by B−Thalassemia trait (1.04 ± 1.45) as shows in Table 4. Whereas mean HbA2 was significantly greater in B−Thalassemia trait (5.17 ± 1.36), after that compound heterozygous Hbs/B−Thalassemia trait (3.91 ± 0.53), followed by Hbs heterozygous (3.56 ± 0.33). Moreover, the mean HbA0 was significantly lower in Hbs heterozygous (62.41 ± 5.08), after that compound heterozygous Hbs/B−Thalassemia trait (63.90 ± 2.47), followed by B−Thalassemia trait (84.78 ± 3.55) as shows in Table 4. The S window was significantly higher in Hbs heterozygous (27.84 ± 3.49) as compared to compound heterozygous Hbs/B−Thalassemia trait (23.31 ± 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.[10] The evaluated predominance of B−thaalassemia minor (carriers) in India is 3.8%,[11] which transforms to 35-45 million carriers in

| Table 1: Demographic and HPLC finding in participants |
|-----------------------------------------------------|
| Parameter   | Mean | Median | ±SD   | Minimum | Maximum |
| Gender      |      |        |       |         |         |
| Male        | 96 (19.47%) |        |       |         |         |
| Female      | 307 (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   |
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. It was documented that approximately 7,500-12,000 β-thalassemia major kids are born in India each year which encompasses 10% of the World total number. 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 Uttrakhand, 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.

The health status is very poor among tribal population, which is dependent upon socio-cultural, socio-economic, and ecological factors. The Tharu population is the oldest and leading endogenetic 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. The prevalence of thalassemias and hemoglobinopathies differs with geographic region. The frequency of Hb disorder encountered in India comprise β-thalassemia, sickle cell anemia,
HbE/β-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.[18-20]

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

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 Uttarkhand and Champaran district of Bihar. In the tharu population the hemoglobinopathies and thalassemias were commonly found.[23] In India, hemoglobinopathies were generally found in other castes also. Previously a study reported that the HbS was commonly found in the Tharu population.[23] Therefore in this study, 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 this study, we also found that the β-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 compound heterozygous HbS/β-Thalassemia trait, followed by β-Thalassemia Trait. Whereas mean HbA2 was significantly greater in β-Thalassemia Trait followed by in compound heterozygous HbS/β-Thalassemia trait, then HbS heterozygous. Moreover, the mean HbA0 was significantly lower in HbS heterozygous, after that compound heterozygous HbS/β-Thalassemia trait, followed by β-Thalassemia Trait. The increased level of HbF may help to ameliorate the severity of illness.[24] A recent study reported that the level of HbF was highest in β-Thalassemia major and compound heterozygous for HbE/β-thalassemia.[4] 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.[25] The increased level HbA2 is associated with β-thalassemia trait.[26-28] The high HbA2 (E + A2) is used for the diagnosis of HbE trait.[29,30] In sickle cell trait, approximately 40% HbS, 90-95% in HbS (which differs inversely proportion to HbF), and <50% in sickle β-thalassemias.[23] In our study the mean level of HbA2 was 5.17 ± 1.36 in β-thalassemia traits. Similarly various studies reported that HbA2 was 5% in β-thalassemia traits.[29,27] The present study similar to previous study, they reported that the HbF level was slightly high in β-thalassemia trait patients and was around 1.04%,[26,27]

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 cancelling to affected families of Tharu population.

| Types of hemoglobinopathies | 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    | 0.956 |
| S Window                   | 24.70±1.59 | 27.58±3.86    | 0.158 |

| Types of hemoglobinopathies |  |
|----------------------------|---|
| β-Thalassemia Trait        | 13 (65%)  51 (57.95%)  0.915 |
| HbS Heterozygous           | 4 (20%)  33 (37.5%)  0.270 |
| Compound heterozygous      | 3 (15%)  4 (4.55%)  0.300 |

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

| Types of hemoglobinopathies | Normal (n=385) | β-Thalassemia Trait (n=64) | HbS Heterozygous (n=37) | Compound heterozygous HbS and β-Thalassemia trait (n=7) | P value |
|----------------------------|---------------|---------------------------|-------------------------|--------------------------------------------------------|---------|
| 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 β-thalassemia carrier is the commonest hemoglobinopathies (59.26%) followed by HbS (34.26%). Moreover, the compound heterozygous HbS/β-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.

**Acknowledgement**

This study was conducted with general support by Rashtriya Swayamsevak Sangh, Eklavya Model Awasiya Vidyalaya, Rajkiya Ashram Padaksh Balika Inter College and Pratap Narayan Shishu Sadan Vidyalaya, Chandan Chowki Kheri Lakhempur, Uttar Pradesh.and Point-of-care testing (POCT) for technical and material support.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Weatherall DJ. Hemoglobinopathies worldwide: Present and future. Curr Mol Med 2008;8:592-9.
2. Iolascon A, De Franceschi L, Muckenthaler M, Taher A, Rees D, de Montalembert M, et al. EHA research roadmap on hemoglobinopathies and thalassemia: An update. Hemasphere 2019;3:e208.
3. Hossain MS, Raheem E, Sultana TA, Nahar N, Islam S, et al. Thalassemias in South Asia: clinical lessons learnt from Bangladesh. Orphanet J Rare Dis 2017;12:93.
4. Jha R. Distribution of hemoglobinopathies in patients presenting for electrophoresis and comparison of result with High performance liquid chromatography. J Pathol Nepal 2015;5:850-8.
5. Succar J, Musallam KM, Taher AT. Thalassemia and venous thromboembolism. Mediterr J Hematol Infect Dis 2011;3:e2011025.
6. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis 2010;5:11.
7. Bouva MJ, Mohrmann K, Brinkman HB, Kemper-Proper EA, Elvers B, Loeber JG, et al. Implementing neonatal screening for haemoglobinopathies in the Netherlands. J Med Screen 2010;17:58-65.
8. Sharma SK, Choudhary D, Gupta N, Dhamija M, Khandelwal V, Kharya G, et al. Cost of hematopoietic stem cell transplantation in India. Mediterr J Hematol Infect Dis 2014;6:e2014046.
9. Bravo-Urquiuola M, Arends A, Montilla S, Velasquez D, Garcia G, Alvarez M, et al. Advantages in the use of high performance chromatography technique for screening hemoglobinopathies in Venezuela. Invest Clin 2004;45:309-15.
10. Rajpoot A, Kumar VP, Sharma J. Current health status of Uttarakhand, Tharu TRIBE on the basis of blood clinical parameters: A bio-cultural perspective. Int Clin Pathol J 2016;3:219-3.
11. Verma IC, Choudhry VP, Jain PK. Prevention of thalassemia: A necessity in India. Indian J Pediatr 1992;59:649-54.
12. Madan N, Sharma S, Sood SK, Colah R, Bhatia LH. Frequency of β-thalassemia trait and other hemoglobinopathies in northern and western India. Indian J Hum Genet 2010;16:16-25.
13. Colah KI, Gorakshakar A. Burden of thalassemia in India: The road map for control. Pediatr Hematol Oncol J 2018;2;79-84.
14. Varawalla NY, Old JM, Sarkar R, Venkatesan R, Weatherall DJ. The spectrum of beta thalassaemia mutations on the Indian subcontinent: The basis for prenatal diagnosis. Br J Haematol 1991;78:242-7.
15. Verma SC. The eco-friendly Tharu tribe: A study in socio-cultural dynamics. J Asia Pac Stud 2010;1:177-87.
16. Balgir RS. Health care strategies, genetic load, and prevention of hemoglobinopathies in tribal communities in India. South Asian Anthropologist 2004;4:189-98.
17. Singh IP, Bhasin MK. Anthropometry. Delhi, India: Kamla-Raj Enterprises; 1989.
18. Ghosh K, Colah RB, Mukherjee MB. Haemoglobinopathies in tribal populations of India. Indian J Med Res 2015;141:505-8.
19. De M, Halder A, Podder S, Sen R, Chakrabarty S, Sengupta B, et al. Anemia and hemoglobinopathies in tribal population of Eastern and North eastern India. Hematology. 2006;11:371-3.
20. Bhatia HM, Rao VR, editors. Genetic Atlas of the Indian Tribes. Mumbai: Institute of Immunohaematology, Indian Council of Medical Research; 1986.
21. Goswami BK, Pramanik R, Chakrabarty S, Pal PP, Banerjee S, Bandyopadhyay A. Spectrum of hemoglobin variants in the population of northern region of West Bengal: An ethnogenetic proposition. J Family Med Prim Care 2014;3:219-23.
22. Mohanty D, Colah RB, Gorakshakar AC, Patel RZ, Master DC, Mahanta J, et al. Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: A multicentre study. J Community Genet 2013;4:33-42.
23. Shrestha A, Karki S. Analysis of sickle hemoglobin. J Pathol Nepal 2013;3:437-40.
24. Paunipagar PV, Vaidya SM, Singh CM. Changing pattern of Hb electrophoresis and HbA2 levels in β thalassemia major. Indian J Prev Soc Med 2010;4:148-51.
25. Shrikhande AV, Dani AA, Tijare JR, Agrawal AK. Hematological profile of sickle cell disease in central India. Indian J Hematol Blood Transfus 2007;23:92-8.
26. Borgen-Pignatti C, Galanello R. Thalassemias and related disorders. Quantitative disorders of hemoglobin synthesis. In: Greer JP, Foerster J, Lukens JN, Rodgers GM, Parakkavas F, Glader B, editors. Wintrobe's Clinical Hematology. 11th ed.
Philadelphia, Pa, USA: Lippincott Williams and Wilkins; 2004. p. 1319-65.

27. Chandrashekar V, Soni M. Hemoglobin disorders in South India. ISRN Hematol 2011;2011:748939.

28. Pornprasert S, Thichak S, Kongthai K, Wangchauy C. Comparison of HbA2, E, F and red cell parameters in homozygous HbE with and without α0-thalassemia trait. Lab Med 2018;49:118-22.

29. Needs T, Gonzalez-Mosquera LF, Lynch DT. Beta Thalassemia. [Updated 2020 May 23]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020

30. Aslan D. “Silent” β-thalassemia mutation (promoter nt-101 C>T) with increased hemoglobin A2. Turk J Pediatr 2016;58:305-308.

31. Vichinsky E. Hemoglobin e syndromes. Hematology Am Soc Hematol Educ Program 2007;79-83.

32. Bain BJ. Sickle Cell Haemoglobin and Its Interactions with Other Variant Haemoglobins and with Thalassaemias, in Haemoglobinopathy Diagnosis. 2nd ed. Oxford, UK: Blackwell; 2006. p. 139-89.