IJHOSCR

Original Article

International Journal of Hematology-Oncology and Stem Cell Research

Magnitude of Beta-Hemoglobinopathies through Biomarkers among the Selected Tribes of Dharmapuri, Tamil Nadu: A Community-Based Cross-Sectional Study

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Received: 19, Mar, 2024
Accepted: 27, Jul, 2024

ABSTRACT

Background: Hemoglobinopathies present a growing challenge to global healthcare resources. These disorders are monogenic: caused by a single gene, and they are inherited in an autosomal recessive manner from parents to offspring. Thalassemia and Sickle Cell Disease are the primary forms of Hemoglobinopathies. India has the highest prevalence of children affected by Thalassemia globally, with a population of 1-1.5 lakh children with Thalassemia, and every year, around 10,000-15,000 babies are born with this condition. This study attempted to estimate the disease burden of Beta**-**Hemoglobinopathies among the selected tribes residing in Dharmapuri, Tamil Nadu, India.

Materials and Methods: This cross-sectional study includes the data from 62 study participants belonging to the tribes residing in Sitteri hills and Balajangamanahalli – a village in the plains of Nallampalli, Dharmapuri. A semi–structured questionnaire was administered to collect socio–demographic details, and 5 ml of blood was collected for hematological tests: Complete Blood Count (CBC), Peripheral Smear, and High-Performance Liquid Chromatography (HPLC).

Results: Out of the 62 study participants, 43% (n=27) were anemic. Chi-square test of association revealed significant associations between Gender and Anemia, Mentzer's Index and Anemia, and Mentzer's Index and HbA2. The present study has reported the disease burden of Beta-Hemoglobinopathies to be 37.1%, in which beta-thalassemia trait/minor was 24.19%, sickle cell beta-thalassemia, beta-thalassemia intermedia, beta-thalassemia major/intermedia, and sickle cell disease were 3.23% each.

Conclusion: Family screening may be conducted to clarify the inheritance patterns of the disease, and genetic counseling should be offered to at-risk couples. To confirm the prevalence of hemoglobinopathies, genetic studies are required to confirm the type of mutations that cause Hemoglobinopathies.

Keywords: Hemoglobinopathies; Anemia; Tribes, Beta-Thalassemia; Sickle Cell Disease

INTRODUCTION

Hemoglobinopathies are hereditary conditions, usually monogenic and autosomal-recessive, that result in structural anomalies in hemoglobin, frequently due to mu tations. Thalassemia and Sickle Cell Anemia are the main group and Thalassemia is categorized into two: alpha and beta[1].

According to the "NHM Guidelines for Prevention and Control of Hemoglobinopathies in India 2016", the most prevalent genetic illnesses worldwide were hemoglobinopathies (that includes thalassemias and

sickle cell disease) and aberrant variant hemoglobins like Hemoglobin S, D, and E. They carried a significant load of illness. India has the largest number of children with Thalassemia in the world (1-1.5 lakh), 42 million carriers of beta thalassemia trait were identified and 10,000–15,000 babies with thalassemia were born every year[2].

Hemoglobinopathies, particularly beta-thalassemia, are significant health concerns in India, often overshadowed by other priorities such as protein energy malnutrition and communicable diseases[3].

Although the hemoglobinopathies occur at particularly high frequencies in the tropical regions like sub-Saharan Africa, Mediterranean regions, Middle East, South East Asia and the Indian subcontinent, they have been transported to most countries of the world by population migrations over many years[4].

Thalassemia

The production of one or more globin chains of hemoglobin tetramers is reduced or completely stopped in a class of congenital hemoglobin (Hb) disorders known as thalassemia. This leads to unchecked destruction of red blood cells (RBC) and severe anemia[5].

Alpha (α), Beta (β), and Delta Beta (δβ) are the primary forms of thalassemia that have clinical implications. HbS, HbE, HbC, and HbD are the most prevalent and clinically significant structural hemoglobin variants. One major factor in the global burden of disease is the expense towards patient care[6].

Sickle Cell Disease

Sickle cell disease (SCD) is among the most prevalent severe hereditary disorders worldwide. The condition, also known as sickle cell anemia, is characterized by serious pathophysiological effects because of the generation of HbS, which is mostly caused by genetic variation. The β globin gene has mutations (HBB; c.20A>T, p.Glu7Val), which forms sickle hemoglobin (HbS) in a tetramer with alpha globin chains, which form the primary source of sickle cell disease[7].

Hemoglobinopathies among the tribes in India

Tribal population of India constitutes approximately 8.5 per cent of the total population of India. With 36 different tribes residing within the state, Tamil Nadu has one of the most diverse tribal populations in the world[8].

The tribal people of India are at high risk of developing blood-related genetic disorders. The prevalence of Sickle Cell Disease and Thalassemia among the various Indian tribes residing in different regions varied between 1 to 40%[9].

Considering the status in Tamil Nadu, the estimated prevalence of Sickle cell anemia varied between 1 to 31% and that of Thalassemia ranged from 1 to 3% and it was identified that these disorders were mostly prevalent among tribal population[2].

A significant emotional, psychological, and financial burden is placed on affected tribal members and their families by sickle cell disease (SCD) and thalassemia[10].

Tribal people are the ones who migrate to different places in search of jobs, better living conditions and many other necessities. Hence, the prevalence of Hemoglobinopathies tend to be higher among the tribal population when compared to the general population.

Aim

The main aim is to estimate the disease burden of Beta-Hemoglobinopathies among the selected tribes residing in Dharmapuri, Tamil Nadu, India, which includes the forms of Beta-Hemoglobinopathies, like: Trait, Major, Intermedia, Minor and Combined forms, if any.

MATERIALS AND METHODS

Study Design, Population and Setting

This is a cross-sectional study conducted in the Sitteri hills and Balajangamanahalli, a village in Nallampalli, a plain area from Dharmapuri district, where the subjects included 62 participants by adopting a simple random sampling technique. In the present study, 62 participants have been chosen to assess the feasibility of the larger, current, community-based cross-sectional study. As this study is time-bound and restricted by resources, the sample size is quite adequate for gathering preliminary

information, looking for any flaws that may occur in the ensuing study.

The target respondents of the survey were individuals between 18 and 60 years of age. This was done by first obtaining a written consent from every respondent with full consideration for his or her understanding and willingness to give blood for testing. Ages 18 to 60 were chosen because of appropriateness to the study's objectives: this age bracket will most likely express the clinical features of beta-hemoglobinopathies. Further, evaluation of the consequences of the condition and drawing inference that would be extrapolated to the working-age population of the chosen tribal communities would best fit in within this age group and would serve as an initiation for family screening and genetic counselling in the upcoming days. Once the proband/index cases were identified based on the hematological test results, family screening will be done, which will include all family members who are available at the time of screening, irrespective of their age.

Materials

Socio-demographic details were collected. Vital signs (Temperature, Pulse, Respiration and Blood Pressure) and Anthropometry (Height and Weight) were measured. 3 to 5 ml of blood was collected for performing Hematological Analysis like: Complete Blood Count (CBC), Peripheral Blood Smear and High Performance Liquid Chromatography (HPLC). Sterile precautions were followed while taking blood from the study participants. The blood was collected and stored in an Ethylene Diamine Tetra acetic Acid (EDTA) container and transported to the SRM Central Laboratory for carrying out the hematological tests.

Statistical analysis

Numbers and percentages were used to summarize the socio-demographic profile. The significant red blood cell indicators from Complete Blood Count, Vital signs, and Anthropometry were displayed as mean and standard deviation. Mentzer's Index was calculated to differentiate iron-deficiency anemia from beta-thalassemia. The HPLC results were computed as frequencies and percentages (for tests with categories) and as mean and standard deviation (for continuous values). Chi-square test of association was computed for the relevant categorical variables and comparisons were made between Mentzer's Index and the final diagnosis based on red blood cell parameters in concordance with the peripheral smear results. Hemoglobinopathies were counted and their disease burden were calculated as number and percentage.

Ethical considerations

The study was approved by SRM Medical College Hospital and Research Centre (Ethical Clearance Number: 8699/IEC/2023 dated 23.08.2023). Necessary permissions were sought from the Directorate of Tribal Welfare, Ezhilagam, Chennai (RC.No./TD/C2/7665/ 2023, Dated: 08.11.2023) and from the Village Heads of Sitteri and Nallampalli before carrying out the study.

RESULT

Socio-demographic characteristics

Out of the 62 study participants, the mean age was 37.68 (SD: 14.83) years. The average years of living in Sitteri and Nallampalli was 36.02 (SD: 14.58). The average family size was 3.76 (SD: 1.24).

Among the 62 study participants, the majority (n=35, 56%) were females and belonged to Malayali tribes from Sitteri hills (n=57, 92%). 90% of them (n=56) have not migrated from anywhere else, since they had been residing in their respective villages since their birth. Regarding their educational status, nearly one-third of the study participants were illiterates (n=17, 27%). One-third of the study participants were unemployed (n=20, 32%) and they had to depend on their family members for their livelihood and survival. 73% (n=45) of the study participants were either married/separated/divorced, out of which, 36% (n=16) had undergone consanguineous marriages, in which 56% (n=9) of marriages were among the first-degree relatives. 32% (n=20) of the study participants' parents had undergone consanguineous marriages (Table 1).

Table 1: Frequency and Percentage distribution of Socio-demographic characteristics (n=62)

Gender	**Frequency**	**Percent**
Female	35	56
Male	27	44
Total	62	100
Migrated	Frequency	Percent
No	56	90
Yes	6	10
Total	62	100
Tribe	Frequency	Percent
Kurumba	5	8
Malayali	57	92
Total	62	100
Education	Frequency	Percent
High School (9-10)	11	18
Higher Secondary (11-12)	3	5
Illiterate	17	27
Intermediate/Diploma	6	10
Middle School (6-8)	8	13
Primary School (1-5)	9	15
Under Graduate/Post Graduate	8	13
Total	62	100
Occupation	Frequency	Percent
Clerk	4	7
Craft and related trade workers	14	23
Elementary occupation	3	5
Plant and machine operators and assemblers	2	3
Professionals	1	2
Skilled agricultural and fishery workers	4	7
Student	9	15
Technicians/associate professionals	5	8
Unemployed	20	32
Total	62	100
Marital Status	Frequency	Percent
Married	39	63
Separated/Widowed	6	10
Single	17	27
Total	62	100
Consanguineous Marriages (n = 45)	Frequency	Percent
No	29	64
Yes	16	36
Total	45	100
Degree of Consanguinity	Frequency	Percent
1st degree relative	9	56
2nd degree relative	3	19
3rd degree relative	4	25
Total	16	100
Parents: consanguinity	Frequency	Percent
No	42	68
Yes	20	32
Total	62	100

Previous History of Anemia, Jaundice and Blood Transfusion

Based on the self-reporting of the study participants, it was found that none of them reported they had a previous history of anemia. Only 8% of the 62 study participants (n=5) reported that they had a prior history of Jaundice, in which, two of them (3.2%) had swelling of face and extremities at the time of diagnosis. Upon observation and by scleral examination, 25.8% (n=16) looked pale, weak and tired. While enquiring about their history of blood transfusion, nearly 10% (n=6) of them said that they had undergone blood transfusion.

Clinical Parameters (Vital Signs)

The vital signs: temperature (degree Fahrenheit), pulse (beats per minute), respiration (breaths per minute) and blood pressure (systolic and diastolic: millimeter of Mercury) were measured for all the study participants. The mean and standard deviation of these clinical parameters were 97.33±0.68 degree Fahrenheit, 77.74±5.50 beats per minute, 21.16±1.90 breaths per minute, 128.47±14.66 mm of Hg and 81.84±10.09 mm of Hg, respectively.

Anthropometric Measurements

The anthropometric measurements: Height (in centimeter) and Weight (in Kilograms) were measured for all the study participants and the body mass index (BMI) was calculated by dividing weight (in kilograms) by Height (in meter square). The mean and standard deviation of these anthropometries: Height, Weight and BMI were 158.29±10.93 cm, 54.13±11.23 kg and 21.49±3.33 kg/m^2, respectively.

Laboratory Parameters

Table - II comprises of four parameters of the Complete Blood Count (CBC) test: Total RBC Count, Hemoglobin levels, Mean Corpuscular Volume and Red Cell Distribution Width.

Table 2: Descriptive Statistics of Red Blood Cell Indices (n=62)

Parameter	Normal Range (Clinical)	Mean±SD
Total RBC Count (10^6/uL)	4.5-5.5	5.06±0.84
Hb (HGB: g/dl)	Male: 13-17 Female: 12-14	12.30±2.79
MCV (fL)	83-101	78.67±12.97
RDW-CV (%)	11 to 15%	16.17±5.66
HbA (%)	96.8-97.8	79.55±14.13
HPLC: HbA2 (%)	</=5 months: 0.1 to 2.7; 6 months to 1 year: 1.3 to 3.1; >1 year: 2.0 to 3.3	3.51±1.38

Distribution of Anemia based on Gender

According to the World Health Organization (WHO), anemia is defined as hemoglobin (Hb) levels <12.0 g/dL in women and <13.0 g/dL in men[11]. As per this criterion, 43% (n=27) were anemic, in which, 21 (78%) were females and the rest (n=6, 22%) were males. Chi-square test of association revealed that there is a highly significant association of gender with anemia ($p<0.01$), with a Chi-square value of 7.38 with a p-value of 0.007 (Table 3).

Table 3: Association of Gender with Anemia (n=62)

Gender	Anemic		Total	Chi-square (Continuity Correction), df	p
	No	Yes		7.378, 1	0.007**
Female	14	21	35		
Male	21	6	27		
Total	35	27	62		

**Chi-square is significant at 1% level of significance df – degrees of freedom

The Mentzer Index, invented in 1973, is a commonly used tool to differentiate between iron deficiency anemia (IDA) and beta thalassemia trait (BTT/βTT) due to the inability to differentiate them based on blood pictures and the affordability of tests like Hb electrophoresis and iron studies. Mentzer Index <13 points to diagnosis of βTT and >13 indicates IDA[12]. Mentzer's Index is calculated by dividing the Mean Corpuscular Volume (MCV) with the total Red Blood Cell (RBC) count. The average values of Mentzer's Index were 16.45 with a standard deviation of 7.32.

Based on the cut-off values, this study has 29% (n=18) of the study participants fall into the category of Beta-thalassemia trait. High Performance Liquid Chromatography (HPLC) is considered to be the gold standard test for ruling out abnormal hemoglobin patterns. Quantitative HbA2 determination is the most valuable test for beta-thalassemia carrier detection[13]. HbA2 levels above 4% are diagnostic for beta thalassemia trait with HbF levels that may or may not be slightly elevated. Some cases of beta thalassemia trait may, however, be present with HbA2 levels lower than 4%[14]. Table 4 depicts the findings of HPLC.

Table 4: Findings from High Performance Liquid Chromatography

Fetal Hemoglobin (HbF)	**Frequency**	**Percent**
No	18	29
Yes	44	71
Total	62	100
S Window	**Frequency**	**Percent**
No	58	93
Yes	4	7
Total	62	100
HbA2	**Frequency**	**Percent**
Susceptive Beta-thalassemia carriers	19	31
Normal/Trait	43	69
Total	62	100

Table 5 revealed significant associations of Mentzer's Index with Anemic condition as well as Hemoglobin A2 (HbA2) levels at 1% level of significance.

Table 5: Association of Mentzer's Index with Anemia and HbA2

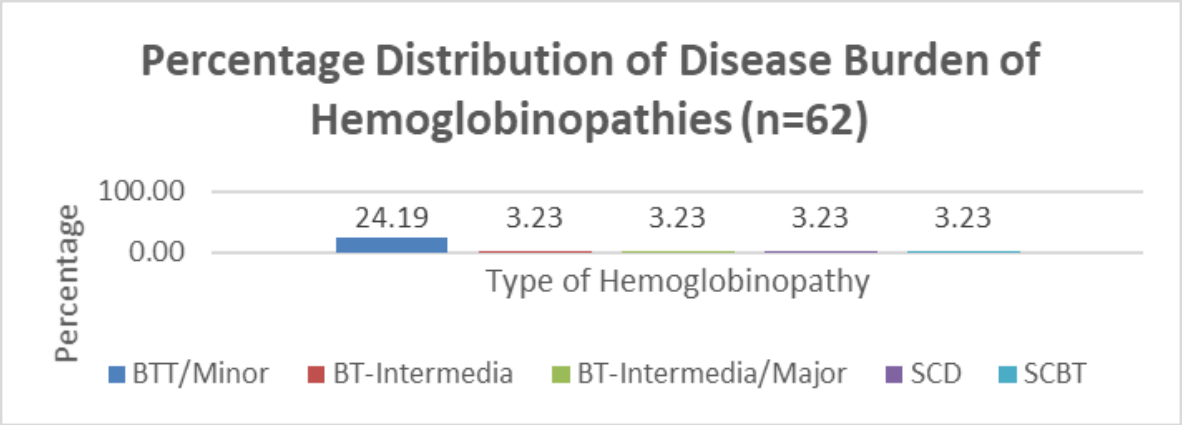
Mentzer's Index	**Anemic**		**Total**	**Chi-square: Continuity Correction (df, p)**	**HbA2**		**Total**	**Chi-square: Continuity Correction (df, p)**
	No	**Yes**			**Susceptive Beta thalassemia carriers**	**Normal/Trait**		
BTT	3	15	18	14.130 (1, 0.000**)	11	7	18	7.693 (1, 0.006**)
IDA	32	12	44		8	36	44	
Total	35	27	62		19	43	62	

**Chi-square is significant at 1% level of significance; HbA2 – Hemoglobin A2; BTT – Beta-Thalassemia Trait; IDA – Iron Deficiency Anemia

Peripheral smear was carried out by using Methyl Blue stain and through the smears, the presence of target cells, tear drop cells and early sickling formation were identified. As per the results of smear in concordance with red blood cell indices, 31% (n=23) were found to have Beta-Hemoglobinopathies.

Out of the 23 study participants who were found to have Beta-Hemoglobinopathies, beta-thalassemia trait (minor) was more prevalent (n=15, 65%).

Out of the 62 study participants, the disease burden of Beta-Hemoglobinopathies was found to be 37.1% and its various forms were given in Figure 1.



*BTT – Beta Thalassemia Trait; BT – Beta Thalassemia; SCD – Sickle Cell Disease; SCBT – Sickle Cell Beta Thalassemia

Figure 1: Disease Burden of Beta-Hemoglobinopathies (n=62)

DISCUSSION

In the present study, anemia was reported to be 43%, which might be a confounding factor for Hemoglobinopathies. The study has also reported the disease burden of Beta-Hemoglobinopathies to be 37.1% in which beta-thalassemia trait/minor was 24.19%, sickle cell beta-thalassemia, sickle cell disease, beta-thalassemia intermedia and beta-thalassemia major/intermedia were 3.23% each. In a recent ICMR study by Balasubramaniam Ganesh and team in 2023, the prevalence of the α-thalassemia trait was found among 0.68%, the β-thalassemia trait was 13.99% and the borderline was 2.03% among the Malayali tribes of Jawadhu hills, Thiruvannamalai[10]. A study carried out by Kavitha and team to analyze the incidence of Hemoglobinopathies in the South Indian population revealed an incidence of beta-thalassemia trait to be 69.13%[15]. Many such studies were carried out in North India. In a study by Mohanty et al., that was carried out in Sirohi district of Rajasthan in 2022, the prevalence of sickle cell anemia (SCA) was 8.51% and it was found to be higher in Garasia tribes and the prevalence of β-thalassemia was recorded as 7.25%, which was higher in the Bhil community[16]. Another study carried out by Mohanty et al., among the tribal students of southern Rajasthan to determine the prevalence of sickle cell disorder reported its prevalence to be 5.8%[17]. A similar study was carried out by Sujata Dixit and team among the tribes in Odisha which reported the prevalence of sickle cell heterozygotes (AS) to be 3.4%, sickle cell homozygous (SS) to be 0.1%, β-thalassaemia heterozygotes to be 0.3%, HbS/β-thalassaemia compound heterozygote to be 0.07% and HbS-α-thalassaemia to be 2.1%[18]. Chourasia et al., in 2020 carried out a similar kind of study among the tribes of Madhya Pradesh – the findings revealing high prevalence (76.7%) of anemia, the prevalence of sickle cell trait and sickle cell disease varied from 10.7 to 15.6% and 0.4 to 0.8%, respectively, β-Thalassemia (β-thal) trait was only 1.4% of the screened population and α (alpha) Gene deletions were observed in 84.7% individuals[19]. Yet another study carried out among the young tribes of West Bengal revealed 9.09% to be the carriers of beta-globin gene mutations. specifically, beta trait is 5.3%, sickle or HbS trait is 2.35% and HbE trait is 1.4%[20]. Hemoglobinopathy is thought to be more common in the tribes due to high consanguinity[20]. Further research is needed in Tamil Nadu to determine the extent of the issue.

CONCLUSION

This study reported the burden of Beta-Hemoglobinopathies to be 37.1% in which beta-thalassemia trait/minor was the highest, i.e., 24.19%. Creating awareness of Hemoglobinopathies, its risk factors and preventive measures should be undertaken on a large scale with the help of tribal leaders by collaborating with Governmental and Non-Governmental organizations. Family screening on a large-scale might help in identifying couples at-risk at an early stage itself.

WAY FORWARD

This is an ongoing research for which a preliminary study was carried out to understand the disease burden of Beta-Hemoglobinopathies among the tribes residing in Dharmapuri district. Inclusion of large samples and molecular studies of families may be further performed to identify the defective genes, the DNA sequences and the type of mutation involved in these hematological disorders. Genetic counseling could be given for people at risk to reduce the burden of Hemoglobinopathies in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest

SOURCE OF FUNDING

None

ACKNOWLEDGEMENT

The author extends her sincere gratitude to the local authorities of the villages for providing the necessary permissions and support, the study participants for their kind cooperation and her fellow scholars: Ms. Indra S for helping in sample collection and Mr. Saravanan C for giving his valuable inputs in drafting the manuscript. She is thankful to her research supervisor, the pathologist and the lab technician of SRM Medical College Hospital and Research Centre, who helped her in carrying out the laboratory tests, interpreting the results and shaping this manuscript. She also extends her heartfelt gratitude to her family, friends and well-wishers.

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