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FULL PAPER

The efficacy of rbc indices as initial screening methods in detecting thalassemia and sickle cell anemia within the malayali tribes of the dharmapuri district, tamil nadu

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The study is done to find the effectiveness of preliminary tests on RBC indices in mass screening of thalassemia and sickle cell anemia. Cross-sectional design study was used with a structured questionnaire and laboratory evaluation to assess the effectiveness of using RBC indices as a preliminary test for the detection of hemoglobinopathies among Malayali Tribes in Dharmapuri District. RBC Parameters were immediately examined using a SYSMEX POCH-100 auto analyser, and samples were processed for HPLC Estimation the following day. The mean and standard deviation (SD) of quantitative data were used to describe the normally distributed data. Using the Chi-Square test, associations between demographic factors and hemoglobinopathies were examined. The T-test was used to compare the groups. Statistics were considered significant at $p < 0.05$. In MCH and MCV, there is found to be a significant difference between the normal and test with a p-value of 0.000. The RDW-CV mean values for β-thalassemia trait, Sickle cell trait, and normal are 17.792 ± 3.016, 15.833 ± 2.33, and 15.581 ± 2.807, respectively. HbA2 values above the mean score for Beta thalassemia are 4.514 ± 0.920 and for normal 2.601 ± 0.371. In the sickle cell trait case, the S. Window values for the mean are found to be 23.662 ± 0.867. There is minimal study about hemoglobinopathies in Tamil Nadu and with the huge population of tribes in Tamil Nadu. This study revealed the desired results on the effectiveness of preliminary tests. The people should be given proper genetic counselling on Thalassemia or sickle cell anemia for prevention.

KEYWORDS

β-Thalassemia; RBC Indices; sickle cell anemia; tribals.

Introduction

One of the common genetic disorders is Hemoglobinopathies worldwide. Pathogenic mutations in genes governing globin chain synthesis lead to hemoglobinopathies, such as sickle cell disease, by disrupting the production of functional hemoglobin molecules crucial for oxygen transport in red blood cells [1]. Sickle cell affects more than 44000 live births in India [2] and about 7500 to 12000 β-thalassemia major infants are born in India every year. The hemoglobinopathies are not just prevalent in tribal but also in Scheduled castes and other Hindu religions [3].

In India, the prevalence of β-thalassemia ranges from 5 to 15%. Thalassemia comes in three different forms: Heterozygous, intermediate, and homozygous. One normal and one mutated thalassemia globin chain is carried by the individual with thalassemia minor, also known as the thalassemia trait or carrier state. Any person could carry thalassemia minor without even being aware of it. A couple with one of the thalassemias has a 25% chance of having a homozygous thalassemic child [4].

Since hemoglobinopathies are considered a global burden and Tamil Nadu has a high prevalence rate of 4% it is also estimated that 45 million thalassemia carriers are there in the country. Indigenous communities are marginalized as a result of limited geographic accessibility, inadequate educational opportunities, and poor resource accessibility [5] are the reasons behind in selection of tribes in the Dharmapuri District.

High-performance liquid chromatography (HPLC) is the preferred method for controlling symptomatic hemoglobinopathies, but it is an expensive test for developing nations like India, so some kind of low-cost screening method is required. Previous research, primarily from northern India, has already discovered a strong correlation between hemoglobinopathies and red blood cell (RBC) indices [6].

Although the specific cut-off for hemoglobinopathies, most laboratories use low mean corpuscular volume (MCV), low mean corpuscular hemoglobin (MCH), or high microcytosis (MRC) as screening criteria. Numerous studies cite an MCH of 26 and an MCV of 78 [7].

Electrophoresis or HPLC analysis of hemoglobin for the diagnosis of β-thalassemia carriers is the gold standard (Hgb). To enable the selection of samples for additional HPLC analysis to confirm the diagnosis, a dependable and affordable method for mass screening of the population is required [8].

This study was carried out in Dharmapuri District of Tamil Nadu which is located in the southern parts of Tamil Nadu. Tamil Nadu had 1.1% of India's indigenous tribes as of the census conducted in 2011. The Malayali tribes residing in one of the hilly regions of Tamil Nadu's Eastern Ghats (mountains) are the subject of this study. Cross-cousin marriage, one of the risk factors for thalassemia, is one of the indigenous characteristics of this community [5] Among Malayali tribes in Dharmapuri district to understand the RBC Patterns and distribution of Hemoglobinopathies.

Experimental

Study design

It is a cross-sectional design study with a structured questionnaire and laboratory evaluation to assess the distribution of Hemoglobinopathies and the effectiveness of using RBC indices as a preliminary test for the detection of Hemoglobinopathies among Malayali Tribes in the Dharmapuri District. The study will identify the effectiveness of RBC indices in detecting thalassemia and sickle cell anemia at the field level in Tamil Nadu Tribal settlements.

Study settings

Dharmapuri's Harur and Poppyreddypalli were two of the randomly selected blocks. From those two blocks, 11 villages were randomly selected.

RBC Indices were used as a preliminary evaluation, and the RBC blood parameters were examined using the SYSMEX POCH 100I Instrument. High-performance liquid chromatography was used as a confirmatory test to identify hemoglobinopathies using a Bio-Rad Analyser.

This study was carried out as a part of the doctoral research of the first author, a public health specialist with an interest in public health genetics. The goal of the doctoral

research is to determine the prevalence of sickle cell anemia and thalassemia. In addition, the author applies the health belief model to people who have hemoglobinopathy. The author was involved in the ICMR project as a senior research fellow after receiving intensive training at the National Institute of Immunohematology in Mumbai on laboratory investigation of hemoglobinopathies. The study, entitled: "*Study on Hemoglobinopathies among Malayali Tribes in Dharmapuri District*", was approved by Institutional Ethics Committee. The respondents provided informed consent in their native language.

Sampling

Sample size was calculated based on the Probability Proportionate Sampling strategy, equal weightage was given for each village/Hamlet. The Cochran formula was utilized to determine a sample size of 475. This calculation is based on a certain 5% prevalence rate, a 95% confidence level, and a 2% margin of error. The formula used for calculating the sample size is n=p (1−p) z2e2n=e2p (1−p)z2, where n represents the sample size, p is the prevalence rate, z is the Z-score corresponding to the desired confidence level, and e is the margin of error. With these assumptions, 475 was determined to be the ideal sample size for the study.

The tribal people who were willing to participate in the study only were included and those people with a known history of chronic disorders and severe psychiatric illness were excluded from the study. The respondents from the age group above 3 years of age both Male and female were included in the study.

Sample collection

The respondents underwent a clean venipuncture, and 4 to 5 ml of venous blood was collected in EDTA Vacutainers using a 21-gauge needle under sterile conditions. For a week, these EDTA blood samples were kept at 2 to 8 C. RBC Parameters were examined immediately using a SYSMEX POCH-100 auto analyzer and samples were processed for HPLC estimation on a subsequent day. Microsoft Excel was used to enter each laboratory value.

Variables: All the basic demographic characteristics were included and the RBC Indices such as RCBs, Hb, PCV, MCH, MCV, MCH, and RDW were identified. Estimation of A2, HbF, S, and other values were determined using High-Performance liquid chromatography. Questions related to consanguinity and awareness about thalassemia and sickle cell anemia were studied.

Statistical analysis

The SPSS program version 16 was used for the study's statistical analysis. The mean and standard deviation (SD) of quantitative data were used to describe the normally distributed data. Using the Chi-Square test, associations between demographic factors and hemoglobinopathies were examined. A T-test was used to compare the groups. Statistics were considered significant at $p<0.05$.

Significance of the study

Since there have been very few studies on hemoglobinopathies in Tamil Nadu, particularly among the tribal population, this study will highlight the importance of preliminary tests on RBC indices in mass screening of thalassemia and sickle cell anemia.

Results

TABLE 1 Demographic characteristics included for testing a preliminary test

S. No.	Characteristics	Character	Frequency	Percentage
1	Gender	Female	263	54.3
		Male	221	45.7
		Total	484	100
2	Marital Status	Married	239	49.4
		Unmarried	245	50.6
		Total	484	100
3	Age Classification	0 to 11 Years	100	20.7
		12 to 23 Years	168	34.7
		24 to 35 Years	97	20
		36 to 47 Years	90	18.6
		48 to 59 Years	27	5.6
		Above 60Years	2	0.4
		Total	484	100
4	Education Qualification	Diploma/Higher Sec 11 to 12	9	1.9
		No Formal Education	119	24.6
		Primary (1to5th Std)	147	30.4
		Secondary(6th to 10th std)	151	31.2
		University	58	12
		Total	484	100
5	Occupation	Employed	72	14.9
		Farmer	22	4.5
		Others	114	23.6
		Peasant	64	13.2
		Student	212	43.8
		Total	484	100
6	Income	Below 5000	166	34.3
		5000 to 10000	82	16.9
		10000 to 20000	18	3.7
		Above 20000	6	1.2
		No Income	212	43.8
		Total	484	100

Table 1 presents the demographic characteristics of selected 484 respondents for the study. Both genders were equally selected and about 50 percent of the respondents were married. About 75% of the respondents selected were in the age group below 35 years. It is noted that only 12% of the respondents completed university. The overall data obtained for testing preliminary is β-thalassemia and sickle cell trait is found to be 73(15.1%) and 13(2.7%) respectively which is listed in Table 2.

TABLE 2 MCV blood parameters comparison with Normal and Hemoglobinopathies

S. No.	Characteristics	Normal/Control	With Hemoglobinopathies/Test	Total
1	Below 65	71 59.2%	49 40.8%	120 100.0%
2	66 to 72	97 80.8%	23 19.2%	120 100.0%
3	73 to 79	117 90.7%	12 9.3%	129 100.0%
4	80 and above	113 98.3%	2 1.7%	115 100.0%
	Total	398 82.2%	86 17.8%	484 100.0%

Table 2 indicates that about 93.2% of respondents who had β-thalassemia trait were poorly informed or had never even heard of sickle cell anemia or thalassemia. About 53.3% of respondents believe in or act favourably toward early disease detection.

TABLE 3 MCH blood parameters compared with normal and hemoglobinopathies deficiency

S. No.	Characteristics	Normal/Control	With Hemoglobinopathies/Test	Total
1	Below 22	127	58	185
		68.6%	31.4%	100.0%
2	23 to 25	123	19	142
		86.6%	13.4%	100.0%
3	26 to 28	85	6	91
		93.4%	6.6%	100.0%
4	Above 29	63	3	66
		95.5%	4.5%	100.0%
Total		398	86	484
		82.2%	17.8%	100.0%

In Table 3, the mean MCV found in the cases is 65 fl with a minimum value of 22.1 fl and a maximum value of 81.7 fl. Among the controls the mean MCV was found to be 74 fl and the minimum and maximum values were 33.3 fl and 93.3 fl, respectively. There is a significant difference with a p-value of 0.0001.

TABLE 4 β-thalassemia, sickle cell anemia, and normal comparison with RBC indices

S. No.	Deficiency	Lab Parameters	Mean±SD	Minimum	Maximum
1	BTT(n=73)	RBC	5.329±0.7391	2.9	7
		MCV	65.596±6.645	52.9	81.7
		MCH	21.458±2.763	17	29.1
		RDW-CV	17.792±3.016	13.2	28.4
		HBA2	4.514±0.920	3.5	7.2
		HBF	0.936±0.309	0.8	2.2
2	HbAS(n=13)	RBC	5.082±0.708	4.4	7
		MCV	65.277±14.836	22.1	81.7
		MCH	24.054±4.561	13.9	31.1
		RDW-CV	15.833±2.333	13.2	20.3
		HBA2	3.585±0.877	2.2	4.9
		HBS	23.662±0.867	14.1	48.8
		HBF	1.392±1.386	0.8	5.4
3	Normal(n=398)	RBC	4.871±0.615	3.2	6.7
		MCV	74.781±9.575	33.3	104.7
		MCH	24.885±3.981	11.7	36.4
		RDW-CV	15.581±2.807	9.8	30
		HBA2	2.601±0.371	1.5	3.4
		HBF	0.833±0.354	0.8	6.7

Similarly, the mean for MCH is found to be 22 fl with the minimum and the maximum values of 13.9 fl and 31.1 fl, respectively. There is found to be a significant difference between the normal and the test with a p-value of 0.0001 summarized in Table 4.

The RDW-CV mean values for β-thalassemia trait, sickle cell trait, and normal are 17.792±3.016, 15.833±2.33, and 15.581±2.807, respectively. HbA2 values above the mean score for β-thalassemia are 4.514±0.920 and for normal 2.601±0.371. In

the sickle cell trait case, the S.Window values for the mean are found to be 23.662±0.867 (Table 5).

Discussion

This study aimed to investigate the distribution of hemoglobinopathies among one of the primitive tribes in the Dharmapuri District of Tamil Nadu. Data related to the prevalence of hemoglobinopathies in this region were scarce. A study in Tamil Nadu revealed a significant burden of thalassemia with a prevalence of 4% [4]. However, this study found an even higher estimation of thalassemia at 15.1% and sickle cell trait at 2.7% among Malayali tribes. Malayali tribes are a cultural group classified as a Scheduled Tribe [9] by the Indian government, characterized by economic backwardness and low literacy rates [10]. The high prevalence of thalassemia and sickle cell anemia among these tribal peoples could have adverse effects on their health and social conditions. Preventing the birth of affected children is considered the best way for India to manage hemoglobinopathies and β-thalassemias [11].

The birth of a thalassemic child, especially among vulnerable groups, causes families significant stress due to the need for frequent hospital visits, costly treatment, and invasive procedures. Only 10-15% of all thalassemic infants born each year receive optimal care, which includes frequent blood transfusions and chelation therapy. Only a fortunate few can afford bone marrow transplants, the only curative treatment currently available in India [12].

A study conducted in Indonesia found that the most effective first-round mass screening technique for carriers of thalassemia in a small facility area is MCV 80 fl and MCH 27 pg [13]. In this study, the mean MCV found for all respondents was 65 fl, with approximately 40% and 19.2% of thalassemia trait values falling below 65 fl and 72 fl, respectively. Only 1.7% of respondents with thalassemia carriers had MCV values above 80 fl. About 31.4% and 13.4% of respondents with thalassemia carriers had MCH values below 22 and 25, respectively. Only 4.5% of respondents fell out of the MCH range above 27 fl. According to the literature, the results are aligned with the prescribed cut-off values of MCV 80 fl and MCH 27 fl.

According to census data from 2011, Tamil Nadu has a sizable tribal population of 794,697 people from 36 different tribes [14]. High-performance Liquid Chromatography (HPLC) is considered one of the costliest tests for the detection of thalassemia and sickle cell anemia compared to CBC [15]. Therefore, it is not feasible to conduct HPLC for the entire tribal population. Instead, basic and effective preliminary tests for the detection of thalassemia are necessary. RBC indices such as MCV and MCH, according to our study, proved effective in shortlisting respondents for confirmatory HPLC tests.

Individuals with β-thalassemia carriers will not exhibit any symptoms and may not be aware that they have the disease. The most prevalent hemoglobinopathies are caused by the β-thalassemia trait [16]. This study identified 73 cases of β-thalassemia trait and 13 sickle cell trait individuals. These identified individuals carry out their normal activities and live life normally.

The high prevalence of sickle cell trait and β-thalassemia among Malayali tribes in the Dharmapuri district calls for immediate intervention and preventive measures to lessen the burden of hemoglobinopathies. The study's findings highlight the critical importance of targeted screening programs and awareness campaigns, especially among vulnerable populations.

Remarkably, as high as 93.2% of the participants in this study who exhibited β-thalassemia trait knew very little or nothing about sickle cell anemia or thalassemia. This ignorance may affect family planning

decisions by causing missed opportunities for genetic counselling and delayed diagnosis. Community education and raising awareness about the inherited nature of hemoglobinopathies should be the focus of efforts, particularly given the cultural customs of cross-cousin marriage of the Malayali tribes [17].

Preliminary screening for hemoglobinopathies can be completed with the use of easily accessible and reasonably priced RBC indices, like MCV and MCH. This strategy is especially helpful in environments with limited resources, where it might not be possible to perform costly tests like HPLC on the whole population. The observed lower mean values of MCV and MCH in the β-thalassemia trait group are consistent with previous research, suggesting these indices as potential markers of thalassemia carriers. These parameters are useful for preliminary screening due to their simplicity and dependability, enabling medical professionals to rank cases for additional examination.

Conclusion

The high prevalence of sickle cell trait and β-thalassemia among the Malayali tribes in Dharmapuri district calls for immediate intervention and preventive measures to alleviate the burden of hemoglobinopathies. Targeted screening programs and awareness campaigns, especially focusing on vulnerable populations, are imperative. The community's lack of knowledge about hemoglobinopathies suggests a pressing need for education and genetic counselling initiatives. In addition, utilizing accessible and reasonably priced RBC indices for preliminary screening can be a valuable strategy in resource-limited settings, facilitating the identification of individuals for further evaluation and intervention. Collaboration among healthcare professionals, policymakers, and community stakeholders is essential to address the challenges posed by hemoglobinopathies effectively.

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Authors' Contributions

All Authors contributed in the study.

Conflict of Interest

The authors declare no conflict of interest.

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