HEMATOLOGICAL PROFILES IN HEMOGLOBINOPATHY PATIENTS IN SOUTH WESTERN NEPAL

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ABSTRACT

INTRODUCTION

Homozygous inheritance of the hemoglobin results in sickle cell anemia (SCA), heterozygous inheritance results in sickle cell trait (SCT). Sickle cell anemia and β-thalasemia (β-TT) have been a major health threat for the Tharu living in the South -Western Terai of Nepal. This study is carried out to apply and optimise the phenotypic method and haematological profile to characterise the SCT, SCA and β-TT from suspected Tharu community dwellers.

MATERIAL AND METHODS

We enrolled 100 suspected cases of hemoglobinopathies of 12-14 years children and ≥15 years adults Tharu community dwellers from Dang, Kapilvastu, Nepalgunj, Rupandehi and Nawalparasi of South-Western Nepal from May 2018 to November 2018. Five mililitre of blood was collected in EDTA vial and transported to the laboratory maintaining cold chain. The hematological profile was recorded after investigations. The hemolysate from blood samples were subjected to phenotypic testing by adopting cellulose acetate electrophoresis at pH 8.6.

RESULTS

Our result showed the commonest hemoglobinopathy was SCT (38%) followed by β-TT (21%) and SCA (5%). Males were more affected with SCA (60%) while that of females were most affected with β-TT (57.1%). Only 44.7% females were affected with SCT while 42.9% males were affected with β-TT. The significant difference in mean was observed in Hb level (p=0.0001), RBC (p=0.004), MCHC (p=0.015) and RDW (p=0.028) whereas the non-significant difference in mean was observed for glucose 6 phosphate dehydrogenase (G6PD) level (p=0.063) in hemoglobinopathy patients.

CONCLUSION

Most cases develop severe type of anemia as shown by change in hematological parameters. This information could advocate for timely counselling before constellation of associated condition appeared in hemoglobinopathy patients.

KEYWORDS Cellulose acetate electrophoresis, Hematological parameters, Hemoglobinopathy, Sickle cell anemia

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DOI: http//doi.org/10.3126/jucms.v8i1.29813

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INTRODUCTION

Approximately 45% of the population, in some parts of Africa have sickle cell trait and 8% of blacks in United States carry sickle gene. It has been recognized that sickle cell trait has its highest in areas that are hyperendemic for malaria. It suggests that HbS afforded selective protection against lethal forms of malaria.[1] The new data has shown SCA and SCT frequency in Western part Nepal were 22% and 51.3% respectively in Tharu ethnicity.[2] The World Health Organisation (WHO) reports that the frequency of thalassemias and hemoglobinopathies carriers is 7% with nearly 226 million carriers worldwide.[3]

Hematological analyses are screening and readily available technique that provides a quick information about the different types of anemia and guides us towards the further diagnostic procedures limiting down the unnecessary expenses to the patients. Moreover, the hematological parameters give preliminary information about the patient's present hematological condition, so that we can go for further investigations accordingly to diagnose the actual cause of disease. The values discriminate towards different nutritional and genetic causes of anemia. The most successful and important application of the analyser principle is in the characterization of human blood cells.

Out of the many methods for separation of hemoglobin components, electrophoretic analysis remains the most effective and widely used technique. The method of cellulose acetate membrane electrophoresis introduced in recent years enabled confirmatory diagnosis of some of the abnormal haemoglobin like S and D.[4] Early detection and characterization of the hemoglobinopathies is essential so that appropriate counseling can be provided to couples and families who may be at risk of severe hematological consequences.[5]

This study is carried out to apply and optimise the phenotypic method to characterise the sickle cell trait, sickle cell anaemia and β-thalassemia for suspected sample that include Tharu community. Moreover, hematological profile was estimated in those hemoglobinopathy cases to observe the prevalence and vulnerability of condition associated with hemolysis.

MATERIAL AND METHODS

This community based cross-sectional study enrolled 100 suspected cases of hemoglobinopathies with age group 12-14 years children and adults ≥15 years Tharu community dweller from Dang, Kapilvastu, Nepalgunj, Rupandehi and Nawalparasi of South-Western Nepal from May 2018 to November 2018. The simple random sampling was employed to select sample from community. The ethical approval was obtained from Institutional Review Committee (IRC/73/18), UCMS-TH. The consent was obtained from children's guardians and community dweller adults.

Five mililitre of blood was collected in EDTA vial and transported to the laboratory maintaining cold chain. The hematological profile was estimated in hematology analyzer, G6PD was estimated by G-6 Kit using spectrophotometric kinetics method by Human semi-automated analyzer. The plasma ferritin was estimated by competitive ELISA method.

Hematological parameters were obtained from five parts hematological cell counter by aspirating the blood sample of patients. While running electrophoresis, along with the patient sample, control lysate from healthy individual EDTA blood sample was also be prepared. These hemolysates were used to run electrophoresis of the sample in cellulose acetate membrane at pH 8.6 which was kept in a tris buffer for 10-15 min with repeated agitation in order to equilibrate the membrane with buffer before running of the sample.

The data were analysed by SPSS 22 IBM software. The categorical data were expressed in frequency (%) and were analysed by pearson's chi-square test. One way Analysis of Variance (ANOVA) was done to obtained p-value of quantitative data and p-value is set at <0.05 for statistical significance difference.

RESULTS

Table 1 shows the SCT was the commonest hemoglobinopathy (38%) followed by β-TT (21%) and SCA (5%). 36% of cases did not reveal any abnormality on detailed investigations and electrophoresis; hence labelled as normal cases. The mean±SD of G6PD of the population in Tharu community dwellers for Normal, SCT, SCA and β-TT was obtained to be statistically non-significant ($p=0.062$). However, significant difference was observed in Hb level ($p=0.0001$), RBC ($p=0.004$), MCHC ($p=0.015$) and RDW ($p=0.028$).

Table 1. Mean±SD of the hematological variables in normal and hemoglobinopathies cases

Variables	Hemoglobinopathies	N	Mean±SD	P value
Age (years)	Normal	36	37.53±17.99	
	SCT	38	33.75±18.52	
	SCA	5	18.80±5.45	0.053
	β-TT	21	27.38±15.99	
Hb (g/dl)	Normal	36	12.20 ±1.90	
	SCT	38	8.87± 2.91	
	SCA	5	8.28± 3.33	0.0001
	β-TT	21	7.56± 2.35	
RBC (10^6/µl)	Normal	36	4.58±0.57	
	SCT	38	3.91± 1.01	
	SCA	5	3.87± 0.94	0.004
	β-TT	21	3.94± 0.87	
Ferritin (ng/dl)	Normal	36	167.21±103.89	
	SCT	38	161.55± 108.80	
	SCA	5	120.30± 77.88	0.741
	β-TT	21	147.02± 91.79	
MCV (fl)	Normal	36	78.01±9.65	
	SCT	38	78.88± 10.47	
	SCA	5	80.24± 13.29	0.935
	β-TT	21	77.45± 12.68	
MCH (pg)	Normal	36	25.46±3.55	
	SCT	38	25.59± 3.83	
	SCA	5	23.42± 6.41	0.691
	β-TT	21	24.87± 5.19	
MCHC (g/dl)	Normal	36	32.62±1.74	
	SCT	38	31.85± 2.67	
	SCA	5	28.96± 4.27	0.015
	β-TT	21	31.81± 2.19	
RDW (%)	Normal	36	13.45±1.67	
	SCT	38	14.61± 2.23	
	SCA	5	15.42± 2.52	0.028
	β-TT	21	13.88±1.48	
G6PD (IU)/Hb	Normal	36	14.85±2.92	
	SCT	38	13.52±4.89	0.062
	SCA	5	9.67±8.39	
	β-TT	21	14.07±4.39	

In Table 2, out of the adult age group, maximum prevalent hemoglobinopathy was of SCT i.e. 47.1% cases in age group 45-60 years. But in the age >30 years, no one suffers from SCA. The maximum of 35% were affected with β-TT in age group 12-14 years.

Males were more affected with SCA (60%) while those of females were most affected with β-TT (57.1%). Out of five cases of SCA, 60% were males and 40% were females. Overall cases show male preponderance.

The most cases (26.3%) of hemoglobinopathies were prevalent for SCT in Nepalgunj followed by (23.7%) each in Dang and Rupandehi. Nawal Parasi and Kapilvastu were less affected i.e. 13.2% cases of SCT. 40% of SCA was observed from Dang and Nepalgunj region. 20% of SCA was obtained from Rupandehi. 38.1% of β-TT was prevalent in Rupandehi followed by 19% each in Dang and Nepalgunj with 14.3% in Nawal Parasi and 9.5% in Kapilvastu.

Table 2. Distribution of normal cases and spectrum of hemoglobinopathy into demographic variables

Demographic Variables		Normal Cases (n=36)	SCT (n=38)	SCA (n=5)	β-TT (n=21)	Total	p-value
Age groups (yrs)	12-14	2 (10%)	13(45%)	2 (10%)	7 (35%)	20	0.120
	15-29	13 (43.3%)	9(30%)	3 (10%)	5 (16.7%)	30	
	30-44	6 (30%)	8 (40%)	0 (0%)	6 (30%)	20	
	45-60	7 (41.2%)	8 (47.1%)	0 (0%)	2 (11.8%)	17	
	≥60	8 (61.5%)	4 (30.8%)	0 (0%)	1 (7.7%)	13	
Gender	Male	16 (44.4%)	1 (55.26%)	3 (60%)	9 (42.9%)	49	0.148
	Female	20 (55.6%)	17 (44.7%)	2 (40%)	12 (57.1%)	51	
Regions	Dang	6 (16.7%)	9(23.7%)	2(40%)	4 (19%)	21	0.003
	Kapilvastu	13 (36.1%)	5 (13.2%)	0 (0%)	2 (9.5%)	20	
	Nepalgunj	5(13.9%)	10 (26.3%)	2 (40%)	4 (19%)	21	
	Nawal Parasi	12(33.3%)	5 (13.2%)	0 (0%)	3 (14.3%)	20	
	Rupandehi	0(0%)	9 (23.7%)	1(20%)	8 (13.8%)	18	

Table 3 shows that maximum cases of hemoglobinopathies were in the RBC groups 3.5-4.5 lakhs followed by 4.5-5.5 lakhs, 2.5-3.5 lakhs and 1.5-2.5 lakhs in which frequency observed were 52%, 31%, 11% and 6% respectively. β-TT, SCA and SCT were predominant in 66.7%, 40% and 47.4% in RBC group 3.5-4.5 lakhs.

Table 3. Distribution of normal cases and spectrum of hemoglobinopathy in different hematological profiles

Types	RBC Category				p-value
	1.5-2.5	**2.5-3.5**	**3.5-4.5**	**4.5-5.5**	
Normal	0 (0%)	1 (2.8%)	18 (50%)	17 (47.2%)	
SCT	4 (10.5%)	6 (15.8%)	18 (47.4%)	10 (26.3%)	0.043
SCA	0 (0%)	2 (40%)	2 (40%)	1 (20%)	
β-TT	2 (9.5%)	2 (9.5%)	14 (66.7%)	3 (14.3%)	
Total	**6**	**11**	**52**	**31**	
	Anemia				
	Normal	**Mild**	**Moderate**	**Severe**	**p-value**
Normal	15 (41.6%)	10 (27.8%)	0 (0%)	11 (30.6%)	
SCT	5 (13.2 %)	20 (52.6%)	10 (26.3%)	3 (7.9%)	
SCA	0 (0%)	1 (20%)	3(60%)	1(20%)	0.0001
β-TT	0(0%)	13(61.9%)	5(23.8%)	3(14.3%)	
Total	**20**	**44**	**18**	**18**	
	MCV Category				
	<78	**78-100**	**>100**		**p-value**
Normal	20 (55.6%)	16 (44.4%)	0 (0%)	--	
SCT	20 (52.6%)	17 (44.7%)	1 (2.6%)		
SCA	3 (60%)	1 (20%)	1 (20%)		
β-TT	13 (61.9%)	7 (33.3%)	1 (4.8%)	--	0.922
Total	**56**	**41**	**3**		
	MCH Category				
	<26	**26-32**	**>32**		**p-value**
Normal	22 (61.1%)	12 (33.3%)	2 (5.6%)	--	
SCT	23 (60.5%)	11 (28.9%	4 (10.5%)		
SCA	3 (60%)	1 (20%)	1 (20%)	--	0.30
β-TT	13 (61.9%)	5 (23.8%)	3 (14.3%)		
Total	**61**	**29**	**10**		
	RDW Group				**p-value**
	Normal	**Increase**	--	--	
Normal	31 (86.1%)	5 (13.9%)			
SCT	27 (71.1%)	11 (28.9%)		--	0.246
SCA	3 (60%)	2 (40%)			
β-TT	18 (85.7%)	3 (14.3%)			
Total	**79**	**21**			

The most of the patients with hemoglobinopathy had mild anemia 44% in which 61.9% had β-TT, 52.6% had SCT and 20% had SCA. This was followed by moderate and severe anemia (18%) in which 23.8% and 14.4% patients were of β-TT, 60% and 29% were SCA and only 26.3 % and 7.3% were of SCT respectively.

The 56% of cases of normal and hemoglobinopathy have MCV value less than 78 fl. The rest of the cases i.e. 41% show MCV value in between 78-100 whereas there are 3% cases with MCV value > 100 fl. Although MCV is severely low (78 fl) in hemoglobinopathy but the greatest frequency is seen in β-TT which is 61.9%. MCH is severely low (<26 pg) in 61% cases of hemoglobinopathies. 29 % cases show MCH range in between 26-32 pg. the lowest frequency i.e. 14.3 % cases are of MCH value >32. Majority of cases of β-TT i.e. 61.9% have lowest MCH value (< 26 pg). RDW is increase in only 21% cases of hemoglobinopathies. 79 % cases show RDW in normal range. Majority of cases of SCT and SCA i.e. 28.9% and 40% have increase RDW value.

Table 4 and Figure 1 shows the different patterns of hemoglobin bands (by cellulose acetate electrophoresis at alkaline pH) observed in different hemoglobinopathies. HbA1 band is normal in case of β-TT and it is decreased in Sickle cell disease (both homozygous and heterozygous). Similarly HbA2 band is normal in Sickle cell disease and is increased in case of thalassemia. Fetal hemoglobin (HbF) band is normal in 53.96% cases of sickle cell trait and increased in rest of the cases of sickle cell disorder and thalassemia trait. HbS band is only present in sickle cell disorders and is absent in thalassemia disorder.

Table 4. Distribution according to different hemoglobin bands observed from cellulose acetate electrophoresis

Bands	Normal Cases (n=36)	SCT (n=38)	SCA (n=5)	β-TT (n=21)	Total	P value
HbA1						
Normal	36 (67.9%)	1 (1.9%)	0 (0%)	16 (30.2%)	53	
Increased	0	0	0	5 (100%)	5	**0.0001**
Decreased	0	37 (88.1%)	5 (11.9%)	0 (0%)	42	
HbA2						
Normal	36 (45.6%)	37 (46.8%)	5 (6.3%)	1 (1.3%)	79	
Increased	0	0	0	20 (100%)	20	**0.0001**
Decreased	0	1 (100%)	0	0	1	
HbF						
Normal /ab	36 (57.14%)	34 (53.96%)	3 (4.76%)	0	63	
Increased	0	4 (14.8%)	2 (7.4%)	21 (77.8%)	27	**0.0001**
HbS						
Absent	36 (63.2%)	0	0	21 (36.8%)	57	
Present	0	38 (88.4%)	5 (11.6%)	0	43	**0.0001**

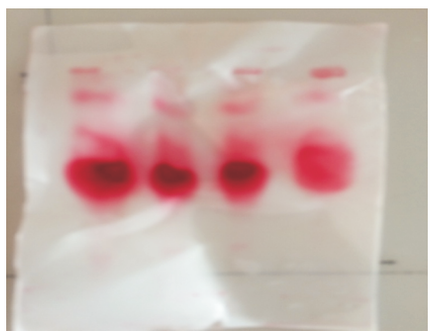

Figure 1. Electropherogram in cellulose acetate mem brane pH 8.6 showing various band positions (HbA2 minor, HbA1 major and HbS)

DISCUSSION

Haemoglobinopathies are monogenic disorders of erythrocyte formation that has a widespread prevalence extending from Mediterranean zone, Middle East, Indian subcontinent, and parts of South-East Asia.[6]

In our study, hemoglobinopathy was found to be SCT 38%, β-TT 21 % followed by SCA 5%. The SCT and β-TT are very common in Western Nepal in Tharu community and similar result was obtained in the study by Balgiret et al[7], sickle cell trait was noted to be common as the study included tribes from Orissa where this gene is prevalent. The Study done in Nepal on sickle cells anemia by Shrestha A and Karki S also showed that sickle cell disorder was most common in the Tharu community.[8]

Our study revealed that, 49% were males and 51% were females showing male and female distribution to be comparable. This is similar to the study conducted by Jha et al[9], RS Balgir[7] and Brig GS Chopra et al.[10]

Our study revealed that majority of the patients with hemoglobinopathies (80%) was aged above 15 years i.e. adult age group followed by (20%) children age group 12-14 years. This is compared with the study conducted by Balgir RS[11] which shows that that a majority of the cases of haemoglobinopathy belongs to the reproductive age group, i.e. 15 to 29 years, followed by children (12-14 years), and only a few cases of old age (≥ 60 years). It may be because of the most of the cases of haemoglobinopathy, in general, are detected accidentally when the couple is advised by the physician to go for laboratory investigations to find the cause of anemia.

Our study showed significantly decreased level of MCV (< 78 fl) and MCH (< 26 pg) in majority of cases of β-TT, SCT & SCA. 61.9% β-TT, 61.1% SCT and 60% SCA patients have lowest level of MCV and MCH. Majority of the patients (38%) of SCA have normal RBC counts whereas only 21% of patients of β-TT have normal RBC counts and rest have low RBC counts. Similar results were also found in the study of Jha R et al.[9] Their study shows that MCV and MCH were low in thalassemia while RBC count was normal in sickle cell anemia (mean 3.4 million/cumm), it was elevated in case of β-TT (mean 5.2 million/cu mm). The red cell count was increased in cases of thalassemias while it was not much affected in sickle cell disorders. The indices were lower in sickle cell and thalassemia trait.[12] In another study, Mehadi et al also concluded that moderate degree of microcytosis (MCV ≤ 78 fl) and hypochromia (MCH ≤ 27pg) was a feature of β-thalassemia trait and homozygous α-thalassemias.[13] RDW is increase in only 21% cases of hemoglobinopathies. 79 % cases show RDW in normal range. Majority of cases of SCT and SCA i.e. 28.9% and 40% have increase RDW value.

Vehapoglu A et al compared different mathematical indices and found that MCV and RBC counts and their related indices (Mentzer index and Ehsani index), have good discrimination ability in diagnosing β-TT.[14] In Mentezer index, if the quotient of the mean corpuscular volume (MCV fl) divided by the red blood cell count (RBC, in millions per microlitre) is less than 13, thalassemia is said to be more likely.

If the result is greater than 13, then iron-deficiency anemia is said to be more likely. In a lot of cases, the index may fall in between 11 and 13, such cases a peripheral blood smear and iron or ferritin studies would help to differentiate iron deficiency from thalassemia.[15] The most cases (26.3%) of hemoglobinopathies were prevalent for SCT in Nepalgunj followed by (23.7%) each in Dang and Rupandehi. Nawalparasi and Kapilvastu were less affected i.e. 13.2% cases of SCT. 40% of SCA was observed from Dang and Nepalgunj region. 20% of SCA was obtained from Rupandehi. 38.1% of β-TT was prevalent in Rupandehi followed by 19% each in Dang and Nepalgunj with 14.3% in Nawalparasi and 9.5% in Kapilvastu. So it is clear that Tharu communities residing in those regions of Nepal are mostly vulnerable groups of having sickle cell disorder and β-TT. The study done by Pande R has shown the prevalence of sickle cell disorders and carrier to be 51.3 % in Western part of Nepal.[2]

In the present study, cellulose acetate electrophoresis at alkaline pH was done to view the thickness of hemoglobin bands, from which, we can see the various abnormal bands of hemoglobin variants of patient sample as compared with the normal bands of control sample. We found that the HbA1 band was normal in case of β-TT and it was decreased in sickle cell disease (both homozygous and heterozygous).Similarly HbA2 band is normal in sickle cell diseases and is increased in case of thalassemia. Fetal hemoglobin (HbF) band was normal in 50% cases of sickle cell trait and increased in rest of the cases of sickle cell disorder and thalassemia trait. Increase in HbF keeps HbS more soluble in the deoxygenated state, and the illness is thus less severe.[16]

The abnormalities in the hematological profiles enable the rapid counselling, awareness about the consequences of consanguineous marriages, early diagnosis in Tharu community before it becomes cumbersome to control.

CONCLUSION

Most cases of suspected hemoglobinopathy develop severe type of anemia as shown by change in hematological parameters. This information could advocate for timely counseling before constellation of associated condition appeared in hemoglobinopathy patients.

ACKNOWLEDGEMENTS

Our sincere acknowledgement goes to Prof. Dr. Anand Kumar, Principal and Finance Department, UCMS, Bhairahawa for faculty project grant as well as to all patients from different regions and finally to those who have directly or indirectly involved in this study for its successful accomplishment.

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