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Research Article

Biochemistry for Better Diagnosis and Therapy

Status of Sickle Cell Disease Among Tharu Population In Banke District of Nepal.

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Abstract: Sickle cell disease (SCD) is prevalent in malaria-endemic areas because the gene for sickle cell provides its carrier with resistance against malaria. In Nepal, malaria is prevalent in Terai, hence the susceptibility of SCD is high in this region. Being indigenous to the Terai, thousands of people in the Tharu communities of the Banke districting Nepal are believed to have suffered from sickle cell disease. The objective of this study was to find out the status of sickle cell disease among the Tharu population of Banke district, Nepal. A cross-sectional, experimental study was performed among systematically randomly selected 275 samples from 3 Village Development Committee (VDCs). All the samples were first screened for the presence of sickle hemoglobin using the sickle solubility test method in Bheri Zonal Hospital. Then all sickle solubility positive samples were further processed for alkaline hemoglobin electrophoresis by using Interlab GenioS electrophoresis instrument. Out of a total 275 samples, 33 (12.0%) samples were confirmed as sickle solubility test positive. Among which, sickle cell trait was the most common disorder found grossing to 81.8%, followed by homozygous sickle cell disease; (15.2 %). One case (3.0%) of compound heterozygous sickle beta-thalassemia was also found.The Males were found to be more affected than females with ratio of 1.4:1.1. The highest frequency of SCD was found to be in 11-20 age groups comprising about 36.4%. Dangaura Tharu (51.5%) was the most common ethnic group with this disorder. The findings of this study indicate SCD is prevalent among the Tharu population in Banke district of Province-5, Nepal.

Keywords: Sickle Cell Disease, Sickle Cell Trait, Sickle Solubility Test, Hemoglobin Electrophoresis, Tharu Communities, SCD Prevalence.

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1. INTRODUCTION

Sickle cell disease (SCD) is a group of autosomal inherited structural disorders of hemoglobin caused by a point mutation in the beta-globin chain. A glutamic acid to valine substitution at the 6th amino acid of the β-globin chain of human adult hemoglobin (Hb A) results in the formation of sickle hemoglobin.[1] Sickle cell disease results from homozygosity for this mutation, in which the production of HbS leads to pathophysiological consequences, which is also referred as 'SCD SS' or as 'sickle cell anemia (SCA). The heterozygosity of this mutation is known as sickle cell trait (SCT). SCT has been viewed as a benign condition, a non-disease status. People with SCT may not experience any painful episodes which are the characteristic of homozygous SCD. Population with SCT has no visible impact on life expectancy.[2] Vaso-occlusive events and chronic hemolytic anemia are the major symptoms of Sickle cell disease (SCD). There are more than 300 million carriers of sickle cell trait worldwide. Moreover, compound heterozygosity for sickle hemoglobin and β-thalassemia or another β-globin variant are also common in different ethnic group.[3,4] Sickle - shaped RBC can be observed in SCD, because of the polymerization of HBS in deoxygenated conditions. The primary pathophysiology of SCD is based on the hemolysis and vaso-occlusion of sickle-shaped RBC. However, the clinical presentation of SCD patients can show anemia and different organ injury. Vaso-occlusive events result in tissue ischemia leading to acute and chronic pain as well as organ damage that can affect any organ system, including the bones, spleen, liver, brain, lungs, kidneys, and joints. Dactylitis (pain and/or swelling of the hands or feet) is often the earliest manifestation of SCD.[5] Sickle cell disorders were originally characteristics of the tropics and subtropics, but are now common worldwide due to the migration of people from affected areas. The highest prevalence of HbS is seen in blacks from tropical Africa who participated in the slave trade. HbS is also seen in the Mediterranean basin, Saudi Arabia, and parts of India.[6]In India, 1-20 % of sickle cell trait has been estimated in different tribal groups. The highest number of sickle cell traits has been found in Madhya Pradesh, Assam, Gujarat, Maharastra, Tamilnadu, and Kerala.[1] It has been found that HbAS can also provide protection against malaria caused by *P.falciparum*. On the other hand, SCD patients are more susceptible to the lethal effects of malaria.[7]In Nepal, malaria is mostly prevalent in the western Terai region, the southern foothills of the Himalayas. Hence the susceptibility of SCD is high in this region. In the context of Nepal, Sickle cell anemia is most common among the indigenous Tharu population of the western Terai region. In 1990, it was reported by WHO that most of the Tharu communities in the western region of Nepal are living with different hemoglobinopathies, SCD being the commonest inherited disorder in this population. The Tharu people are one of the oldest ethnic groups indigenous to the terai. Their socio-economic background is so poor for the diagnosis and treatment of this disease.[8] The estimated SCD is found to be 30,000 and most of Tharu's have sickle cell throughout the country with heavy prevalence in mid and far western regions of Nepal.[9] So far, the spectrum of hemoglobinopathies among this community is still not well documented and seems to be increasing. So, this study was designed to find out the status of sickle cell disease among the Tharu population of Banke district of Nepal. Study data of this area and results of this study will be helpful to the national health management system.

2. MATERIALS AND METHODS

A cross-sectional, experimental study was performed among systematic randomly selected 275 individuals of Tharu populations from Phattepur, Baijapur and Binauna Village Development Committee (VDCs) of Banke district, Nepal. The selection of these VDCs was based on the majority of the Tharu population's resides. Systematic random sampling was done on the basis of the total number of household and average family members. The sampling process was done after taking in confidence the health workers and the Medical Officer at the Primary Health Center of each VDC. Villagers were first educated in groups, with the help of booklets and posters. A Performa and a consent form and questionnaire of related clinical symptoms were duly filled, which was signed (or thumb impression) by the individual. Non- Tharu population and people with less than 3 years age group wasexcluded in this study. 3 ml EDTA anti-coagulated blood was collected from each sampled individual. Samples were stored in a cool chain box maintained at 4°C and were transported to the base laboratory at Bheri Zonal Hospital, Nepalgunj, Banke, Nepal.[10] All collected samples were first processed for sickle solubility test by using Sicklevue solubility test kit (Tulip diagnostic Pvt. Ltd, India). Hemoglobin lysate was prepared by using 2 ml of solubility test reagent and 100 µl of the whole blood sample. Tubes with sample and reagent mixture were vortexed for 10-15 seconds and were allowed to stand for 10 minutes in a standing position. Reaction tubes were then centrifuged at 5000 rpm for 5 minutes in a laboratory centrifuge and were then carefully removed without disturbing the contents. Tubes were again centrifuged for another 5 minutes if the lower layer is not clear. After the centrifugation process, a precipitation pattern in the reaction tube was observed. If the lower layer is clear and dark red with grey precipitate in the upper layer was considered normal. Reaction tube showing clear and light red to pink to colorless lower layer with red precipitate upper layer was considered as sickle solubility positive. All sickle solubility positive samples were further processed for alkaline hemoglobin electrophoresis by using Interlab GenioS electrophoresis apparatus and commercially available Interlab Master Kit at pH 8.6. This automated system incorporates both hemoglobin electrophoresis and densitometry quantification.

3. RESULTS

3.1. Analysis of samples

Out of a total of 275 samples; 33 (12.0%) were found to have sickle solubility test positive and the remaining 242 samples (88%) were found negative. All sickle solubility test positive samples were further analyzed by a fully automated alkaline hemoglobin electrophoresis test. Among all 33 samples, 27 samples were confirmed as sickle cell trait, which is 88 % of all sickle solubility positive samples. Out of 33 positive samples, 5 samples were confirmed as homozygous sickle cell disease, which is 15.2 % of all positive samples. One sample, which contributes 3 % of total positive cases, was also confirmed as compound heterozygous of sickle cell beta-thalassemia (Fig.1,2,3).

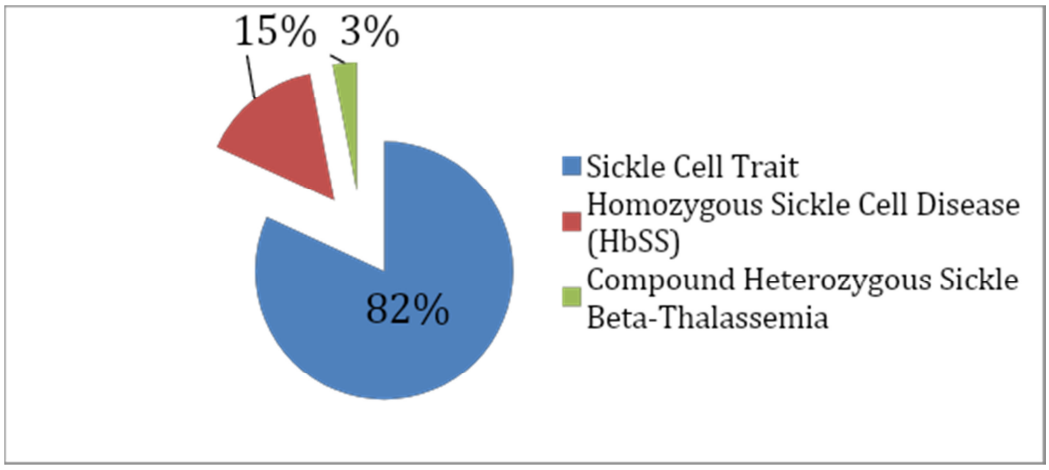

Fig 1. Sickle cell traits and SCDbased on the spectrum of hemoglobin electrophoresis.

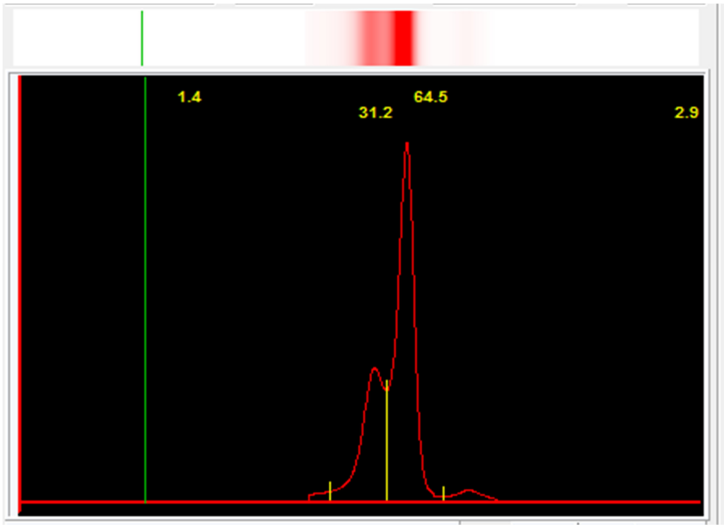

Fig 2. Densitometer graph of a sample showing HbA-1.4%, HbF-31.2%, HbS-64.5% and HbA2-2.9%. Suggestive of homozygous sickle cell disease.

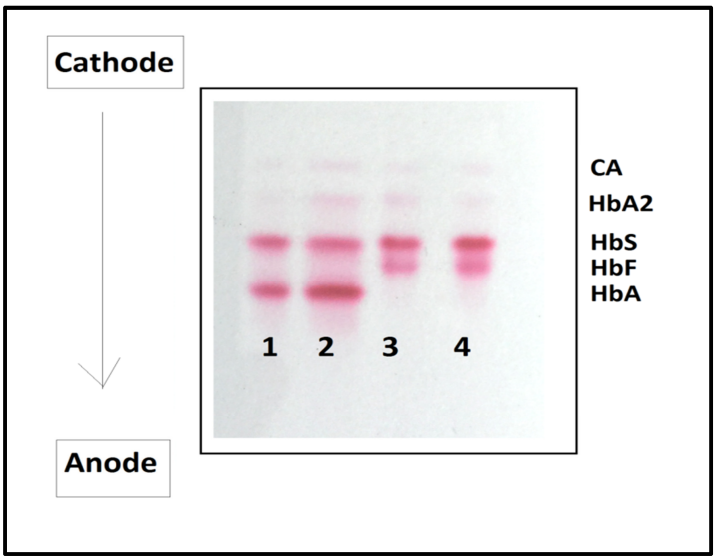

Fig 3. Hemoglobin electrophoresis strip. Sample numbers 1 and 2 show bands in A and S position, which is suggestive of the Sickle cell trait. Sample numbers 3 and 4 show bands in S and F position, which is suggestive of homozygous sickle cell disease.

3.2. Analysis of samples on the basis of gender

Among all 275 samples, 87 (31.6 %) were males and 188 (68.4%) were females. Out of 87 males, 12 samples were found positive and out of 188 females, 21 cases were found positive. It showed that the ratio of affected males and females was 1.4:1.1 with the male being more affected than females. Out of a total of 27 sickle cell trait cases, 11 (40.7%) were male and 16 (59.3%) were female. The incidence of homozygous sickle cell disease (HbSS) was found more in females (4 out of 5 cases) than males (1 out of 5 cases). The only case of compound heterozygous sickle cell beta-thalassemia (HbS/β -Thal) was of a female (Fig. 4).

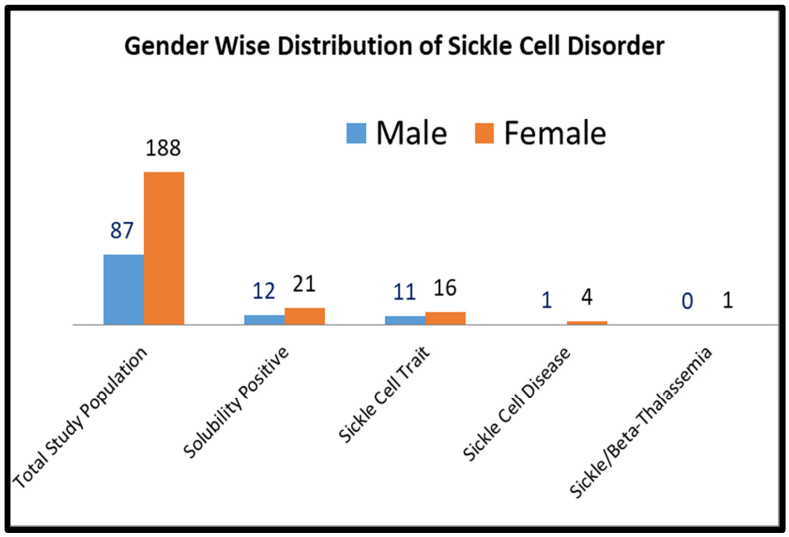

Fig 4. Gender-wise distribution of sickle cell disease.

3.3. *Analysis of samples on the basis of different casts among Tharu communities*

Different Tharu castes were included and classified as Dangaura, Desauria, and others. Out of 33 positive cases, 17 (51.5%) were from Dangaura, 3 (9.1%) were from Desauri and the remaining 13 (39.4 %) positive cases were from other Tharu ethnicities. Out of 27 sickle cell trait cases, a maximum frequency of 48.2 % (13 cases) was seen among Dangaura Tharu (Fig. 5).

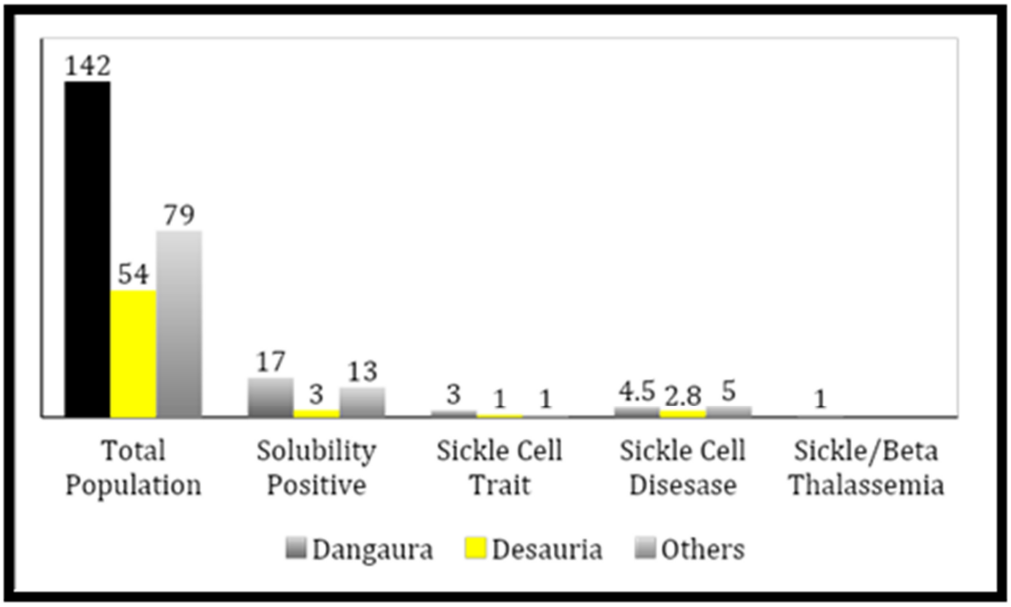

Fig 6. Cast wise distribution of sickle cell disease.

3.4. *Age Wise Analysis Of Samples*

The age-wise distribution showed the highest frequency of SCD 36.4% (12 out of 33) was found in 11-20 age groups and all of them are sickle cell traits. One case of HbSS andHbS/β-Thal was found in the 2-10 years of age group. One HbSS was also found in the 41-50 years of age group (Table1).

Table 1 Age wise distribution of sickle cell disease among all positive samples.

Age Range	Total	Solubility Positive	Electrophoresis Results		
			Trait	HbSS	HbS/β-Thal
2-10 years	37 (13.5%)	6 (18.2%)	5 (18.5%)	1 (20%)	1 (100%)
11-20 years	57 (20.7%)	12 (36.4%)	11 (40.8%)	0	0
21-30 years	66 (24%)	6 (18.2 %)	3 (11.1 %)	3 (60%)	0
31-40 years	61 (22.2%)	5 (15.1%)	5 (18.5%)	0	0
41-50 years	48 (17.4%)	4 (12.1%)	3 (11.1%)	1 (20%)	0
>50 years	6 (2.2%)	0	0	0	0

The samples were randomly selected from three different VDCs namely; Phattepur, Binauna, and Baijapur of Banke district on the basis of the average household and family members. 109 out of 275 people were included from Phattepur VDC, followed by Baijapur (97 out of 275) and the remaining 69 were from Binauna VDC (Table 2). This showed that most of the positive cases were contributed from Phattepur VDC followed by Binauna and Baijapur.

Table 2 VDC-wise distribution of sickle cell disease.

VDC	Total Cases	Solubility Positive Cases	Electrophoresis Results		
			Trait	HbSS	HbS/β-Thal
Phattepur	109 (39.6%)	16 (48.5%)	13 (48.2%)	2 (40%)	1 (100%)
Binauna	69 (25.1%)	8 (24.2%)	6 (22.2%)	2 (40%)	0
Baijapur	97 (35.3%)	9 (27.3%)	8 (29.6%)	1 (20%)	0

Note: Phattepur VDC has the highest sickle cell disease, patients.

Patterns of clinical symptoms in SCD positive cases were analyzed from questionnaire form duly filled during blood collection time. Main complaints included recurrent fatigue (45.4 %), shortness of breath (36.4%), Eye problem (21.2%), and fever (21.2%) joint and musculoskeletal pain (18.2%). Pallor was also observed in 9.1 % of cases.

4. DISCUSSION

Sickle cell disease is major hemoglobinopathies invariably occurring in Nepal, especially among Tharu ethnicity, but most of the affected people are still remain undiagnosed due to lack of specific laboratory tests availability in that region.[8] Despite the fact, no significant governmental or non-governmental efforts are done to find out the actual status of SCD and hence there is no single authentic data regarding the prevalence of SCD among Tharu communities in Nepal. This cross-sectional study is the first time carried out in this region especially in Banke district confined to a small geographical area. Children below 3 years of age were excluded in this study. Since a large amount of HbF is present at that age group, which may interfere with the test result analysis and may lead to false-negative test results.[11]Moreover, a higher number of female samples were included than males with M: F ratio of 1:2.2. This is because the majority of the population belongs to low-income families and the male population was out of the village for employment during the period of study. A simple and rapid sickle solubility method was used for the mass screening of SCD.[12,13]Results showed males are more prevalent than females (1.4:1.1). It is very similar to the result find out by Shrestha *et al*, 2013.[6]Hemoglobin electrophoresis technique was used to confirm and differentiate sickle cell disease and trait along with other hemoglobinopathies.[14]SCD is found predominant amongst certain high-risk communities belonging to schedule caste, schedule tribe and other backward classes in India.[10] Similarly majority of Tharu communities in the western region of Nepal belong to the deprived group. They are the oldest ethnic group indigenous to this malaria-endemic region and are more susceptible to SCD. Results showed the most affected age group of this community lies between 11-20 years and most of them are sickle cell traits. This finding correlates with the result reported by Shrestha *et al*, 2013.[6] It was also observed that most the sickle cell traits were asymptomatic, but few of them presented with some clinical manifestations, such as joint pain, eye problem, fatigue, and shortness of breath. Similar results were observed in the study done at Southern Libia.[15] Finding of this study showed that screening by the solubility test with the confirmation of genotype by alkali hemoglobin electrophoresis is a feasible method for mass screening of population with limited resources. This has been proven in a similar study done in state Chhattisgarh of India.[16] This cross-sectional study is the first time carried out in this region especially in the Banke district of Nepal. Ethical clearance (No: 171/075/76) for this study was taken from Pokhara University Research Center. In low-resource countries like Nepal, population screening can identify individuals with SCD and can lead them to comprehensive care clinics to receive the appropriate medicine and follow-up. This Study suggests population-wide screening must be targeted to specific ethnic groups and populations in which prevalence is found. This will help to raise awareness levels about screening, health implications, treatment, management, and pre-marital counseling. Moreover, Sickle cell disease (SCD) is an important, hidden cause of childhood mortality worldwide.[17] SCD birth prevalence study is also important in high prevalent Tharu communities of Nepal. WHO recommends that countries, where the SCD birth prevalence exceeds 0.5 per 1000 births, should develop separate SCD programs

5. CONCLUSIONS

Tharu people in Banke district of Nepal are living with a predominantly high risk of SCD. 12% prevalence of SCD has been observed in this community and most of them are sickle cell traits without clinical systems. Young age groups of

individuals, between the age group of 11-20 years are more prevalent with sickle cell traits. Among different castes in Tharu community, Dangaura are more affected with SCD. Moreover, males were found more prevalent than females. Government level community-based mass screening and sickle cell card distribution is required to reduce the mortality rate of SCD patients.

6. AUTHOR CONTRIBUTION STATEMENT

Mr. Umesh Prasad Gupta has conceptualized and given inputs in sample analysis, gathered the data, and manuscript designing. Dr. Kapilesh Jadhav and Dr. Rajendra Kumar BC have analyzed these data and necessary inputs were given towards the designing of the manuscript. Mr. Amrit Bhandari, Mr. Dhruba Giri, Ms. Sushmita Adhikari, Ms.Sangita Paudel, and Ms. Aarati Pokhrel have contributed to the sample analysis and gathered the data. All authors discussed the methodology and results and contributed to the final manuscript. All the authors read and approved the final version of the manuscript.

7. ACKNOWLEDGMENTS

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8. CONFLICTS OF INTEREST

Conflictof interest declared none

9. REFERENCES

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