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Pattern of hemoglobinopathies among Tharu population in and around Dhangadhi, Nepal

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Abstract

Hemoglobinopathies affect approximately 7% of the worldwide population and are a significant public health concern. This study, focusing on the Tharu community in Nepal, aims to assess the prevalence and patterns of hemoglobinopathies, utilizing High Performance Liquid Chromatography. This descriptive cross-sectional study conducted at Seti Zonal Hospital in Dhangadhi, Nepal, involved 369 individuals from the Tharu community. Blood samples were collected during a health camp in September 2019, with written consent obtained from participants. Sixty-six individuals were found to have hemoglobinopathies, with a prevalence of 17.9%. Sickle Cell Trait and Beta Thalassemia Trait were the most common variants, with 33 cases (8.9%) and 26 cases (7.0%), respectively. The study also revealed variations in hemoglobinopathy prevalence by gender, caste, and age range, with different chromatographic patterns observed for each hemoglobinopathy subtype. Findings of this study highlights the importance of significant prevalence of hemoglobinopathies, particularly Sickle Cell Trait and Beta Thalassemia Trait, within the Tharu community in and around the Dhangadhi, Nepal.

Keywords: Hemoglobinopathies, Sickle cell disorders, Thalassemia, Hemoglobin variants, Tharu communities, HPLC.

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INTRODUCTION

Hemoglobinopathies refer to a group of genetic disorders that affect the structure or production of hemoglobin in the blood. These disorders are predominantly caused by single amino acid substitutions in the hemoglobin protein, resulting in various clinical manifestations. These were originally characteristic of the tropics and subtropics but are now common worldwide due to the migration (1). Approximately 7% of people has found being carrier of hemoglobinopathies and is now one of the most common genetic diseases and major health problem in the world (2). Sickle cell disease (SCD) is the most common hemoglobinopathies followed by the thalassemia and other hemoglobin disorders like hemoglobin C (HbC), Hemoglobin D (HbD), persistence of fetal hemoglobin (HbF) and other rare ones (over 200 subtypes) (3). The HbS gene is predominantly found in regions where malaria falciparum has historically been endemic, including Saudi Arabia (up to 25% in the eastern sections), central India (20–30%), Equatorial Africa (10–30%), and the Mediterranean region (North Africa, Italy, Greece, Turkey) (4). Nepal is also a malaria endemic and HbS gene susceptible country where its western terai region is a high malarial zone (5,6). Hemoglobinopathies are also the major public health burdens in Nepal among which thalassemia and Sickle Cell Disease are the most predominant. The morbidity and mortality rate of these diseases is increasing day by day globally as well as in Nepal. Tharu communities in the western region of Nepal are living with different hemoglobinopathies has already been reported by WHO in 1990. Thousands of people in the Western Terai in Nepal are believed to have suffered from β- thalassemia which is more critical. The most prevalent inherited disorder in this group is Sickle cell disorder (SCD). One of the earliest ethnic groups still living in the terai is the Tharu people. Their socioeconomic status makes it extremely difficult to diagnose and treat this illness. (Gautam et al., 2019; Marchand et al., 2017). Moreover, we don't have well enough data and records about the status of hemoglobinopathies among this community, which seems to be increasing. So, this study was designed to find out the pattern of hemoglobinopathies among this population in and around Dhangadhi, Nepal, which is one of the major cities in western terai region of Nepal. A variety of hematological tests, including sickling tests, electrophoresis (alkaline and acid), isoelectric focusing (IEF), capillary electrophoresis, and DNA/protein analysis, can be employed to assess and diagnose hemoglobin abnormalities. However, definitive diagnosis often requires correlating these test results with the patient's peripheral blood smear findings. Many hemoglobin disorders exhibit characteristic abnormalities in blood smears, providing crucial diagnostic information. (7). In this study, High performance liquid chromatography (HPLC) technique was used to find out the pattern of hemoglobinopathies. Many structural hemoglobin disorders and rare hemoglobinopathies cannot be reliably identified or diagnosed using Hb electrophoresis. In these cases, HPLC is the preferred method due to its superior accuracy, sensitivity, and ability to detect a wider range of hemoglobin variants. It is considered the gold standard technique for such analyses (8). It has been proved that HPLC is very rapid, sensitive, specific, and reproducible replacing technique to conventional Hb electrophoresis (9).

MATERIAL AND METHODS

This is a descriptive cross-sectional study was designed to perform in Tharu communities of western Nepal particularly in and around Dhangadhi. Approval to collect data was obtained with written consent from both the health workers and the medical officer at Seti Zonal Hospital in Dhangadhi, Nepal. Blood samples were collected from 369 individuals from the Tharu community during a free health camp held in Dhangadi, Nepal, in September 2018. Participants from the Tharu community residing in Dhangadhi and nearby rural municipalities of Kailali district attended the health camp. Written consent, either signed or through thumb

impression, was obtained from each participant, and questionnaires regarding relevant clinical symptoms were completed accordingly. Individuals with anemia, generalized weakness, fever, or splenomegaly, and those with suspected hemoglobinopathies based on clinical and family history, were included in the study. Individuals from non-Tharu populations and those with recent history of blood transfusion were excluded. A 3 ml EDTA-anticoagulated blood sample was collected from each participant and stored in a cool chain box at 4°C. Subsequently, the samples were transported to the Central Diagnostic Laboratory, a grade "A" reference laboratory located in Kathmandu, Nepal.

All collected samples were analyzed using a High-Performance Liquid Chromatography (HPLC) instrument manufactured by Bio-Rad Laboratories. Prior to each run, an HbA2/F calibrator and two levels of control were analyzed to ensure accuracy. Hemoglobin variants were diagnosed and their prevalence analyzed based on retention time and proportion.

RESULTS

This descriptive cross-sectional study was aims to assess the spectrum of hemoglobinopathies, as well as their distribution by age, gender, and caste. Hemoglobin variants of all 369 blood samples were analyzed by HPLC in Bio-Rsd-10(Manufactured by Bio-Rad, USA). Interpretation of all chromatograph was done by using the reference of manufacturer assigned chromatograph windows for Bio-Rad variant high-performance liquid chromatography system (Table 1).

Table 1 Manufacturer assigned windows for Bio- Rad variant high performance liquid chromatography system.

Analyte name	Retention time (min)	Band (min)	Window (min)
F	1.15	0.15	1.00- 1.30
P2	1.45	0.15	1.30- 1.60
P3	1.75	0.15	1.60- 1.90
A0	2.6	0.4	2.20- 3.30
A2	3.83	0.15	3.68- 3.98
D- window	4.05	0.07	3.98- 4.12
S- window	4.27	0.15	4.12- 4.42
C- window	5.03	0.15	4.88- 5.18
P2 and P3 are minor peaks associated with hemoglobin A.			

Among the 369 blood samples analyzed, 66 individuals were found to have hemoglobinopathies, resulting in a prevalence of 17.9%.

Table 2 Spectrum of Hemoglobinopathies.

Name	Number of cases	Percentage
Non hemoglobinopathies group	**303**	**82.1**
Hemoglobinopathies group	**66**	**17.9**
Sickle Cell Trait	33	8.9
Beta Thalassemia Trait	26	7.0
HPFH Trait	3	0.8
Hb E Heterozygous	2	0.6
Compound Heterozygous for HbS and Beta Thalassemia	1	0.3
Sickle Cell Homozygous	1	0.3
Total	**369**	**100.0**

The observed hemoglobinopathies included Sickle Cell Trait (33, 8.9%), Beta Thalassemia Trait (26, 7.0%), HPFH Trait (3, 0.8%), Hb E Heterozygous (2, 0.6%), Compound Heterozygous for HbS and Beta Thalassemia (1, 0.3%), and Sickle Cell Homozygous (1, 0.3%). (Table 2)

Among the all observed hemoglobinopathies; Sickle Cell Trait was found as the highest number of 33(8.9%). It was diagnosed on the basis of presence of HbS ranges between 20-40% (Figure 1). Beta thalassemia trait was diagnosed with the presence of high level of HbA2 ranges from 3-7 % (Figure 2) and was observed in the second highest number of 26(7.0%). Hereditary persistence of fetal hemoglobin (HPFH Trait) was present 3(0.8%), which was diagnosed on the basis of normal A2 values and high range of HbF (15-30%) (Figure 3). Two cases (0.6%) of Hb E Heterozygous was observed with the presence of about 30% of HbE, which was eluted from the high fraction (more than 9%) of HbA2 values (Figure 4). One case (0.3%) of Compound Heterozygous for HbS and Beta Thalassemia was found with elevated values of HbA2 and HbF along with presence of high level of HbS greater than 50% (figure 5). Similarly, one case (0.3%) of Sickle Cell Homozygous was also observed which was diagnosed with the presence of high HbF (more than 5%) and high

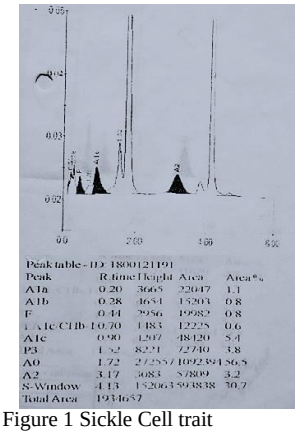

Figure 1 Sickle Cell trait

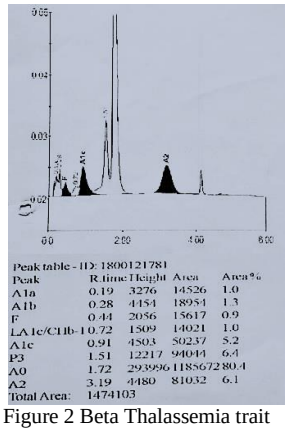

Figure 2 Beta Thalassemia trait

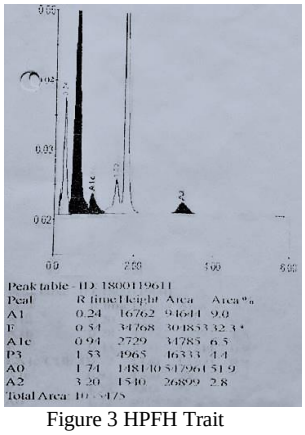

Figure 3 HPFH Trait

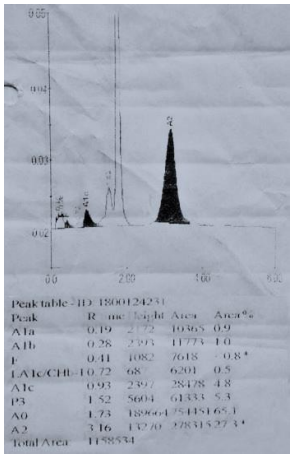

Figure 4 HbE heterozygous

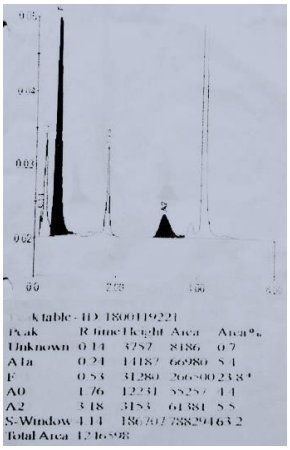

Figure 5 Compound Heterozygous for HbS and Beta Thalassemia

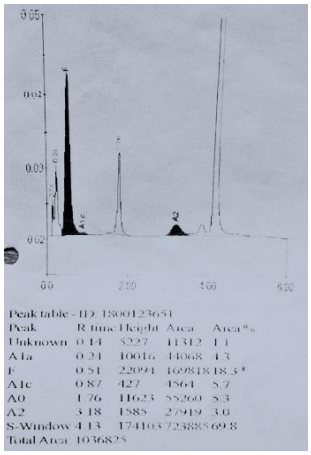

Figure 6 Sickle cell Homozygous

HbS level (69.8%) (Figure 6) Among the hemoglobinopathies group, there were 39 females and 27 males. The numbers of females and males with Sickle Cell Trait, Beta Thalassemia Trait, HPFH Trait, Hb E

Heterozygous, Compound Heterozygous for HbS and Beta Thalassemia, and Sickle Cell Homozygous were 21, 14, 0, 2, 2, 0 and 12, 12, 1, 0, 1, 1 respectively (Figure 7).

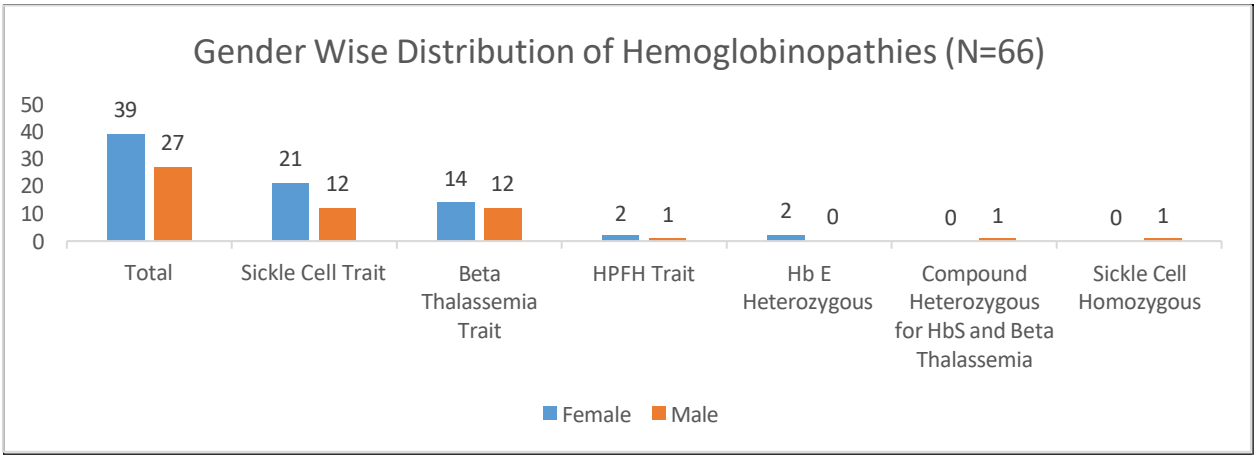

Figure 7. Gender-wise distribution of Hemoglobinopathies

Different Tharu casts (Chaudhary, Dangaura, Katharia,Rana and Tharu) were found Among the hemoglobinopathies group, there were 51(77%) Chaudhary, 1(1.5%) Dangaura, 3(4.54%) Katharia, 11(16.66%) Rana, and 0 cases from Tharu sub cast, within the Tharu community (Figure 8).

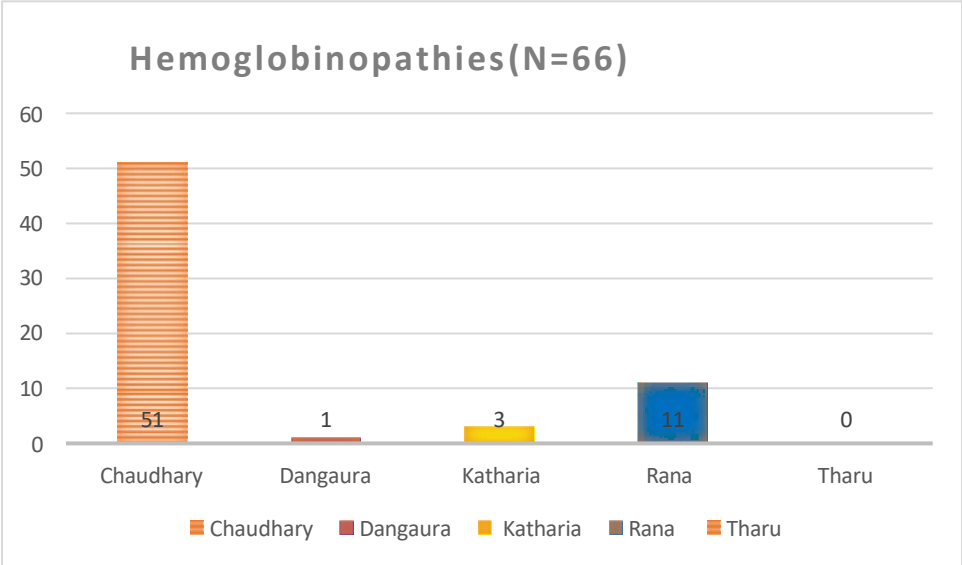

Figure 8. Cast-wise distribution of Hemoglobinopathies

The age wise distribution of hemoglobinopathies shows the high in the age ranges from ≤10, 10- 20, 20-30, 30-40, 40-50, and ≥50. Highest frequency of 21(31.84%) hemoglobinopathies was observed in ≤10 age group, followed by 13 (19.69%), 18 (27.27%), 11 (16.66%), 2 (3.03%), and 1 (1.51%), respectively (Table 3).

Table 3 Age-wise distribution of Hemoglobinopathies.		
Age Range	**Hemoglobinopathies** (N=66)	
	Number of cases	**Percentages**
≤10	21	31.84
10-20	13	19.69
20-30	18	27.27
30-40	11	16.66
40-50	2	03.03
≥50	1	01.51

Clinical symptoms in all positive cases were observed (figure 9). Fatigue was observed in 32 cases (48.48 %), shortness of breath was found in 24 cases (36.36%), Jaundice and joint pain were found in 12 cases (18.18%), fever was observed in 10 cases (15.15%). Pallor was also observed in 8(12.12 %) and Splenomegaly was found in 2 cases (3%).

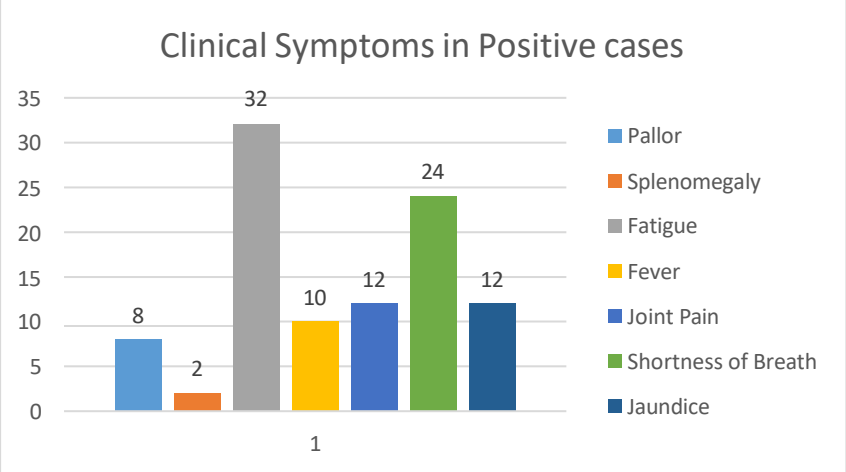

Figure 9. Clinical symptoms observed in all positive cases of hemoglobinopathies.

DISCUSSION

Hemoglobinopathies encompass inherited disorders primarily affecting hemoglobin of red blood cells. These conditions are characterized by abnormal hemoglobin (Hb) variants resulting from mutations in globin genes and reduced production of specific hemoglobin chains. These mutations, which can manifest as point mutations or deletions, disrupt normal hemoglobin synthesis, leading to a range of clinical manifestations (10). Hemoglobinopathies contribute to disability and reduced quality of life for affected individuals. Hemoglobinopathies are recognized as important contributors to the global burden of disease, with varying prevalence, impact, and trends across different populations and over time (11). Indeed, the

geographic location of Nepal in South Asia contributes to a notable prevalence of hemoglobinopathies within its population. Hemoglobinopathies are a common occurrence in Nepal, particularly among the Tharu population. However, the majority of those affected are still undiagnosed because there aren't any specialized laboratory testing available in that area. This is supported by multiple studies that have documented the occurrence of hemoglobinopathies (12,13).

The prevalence of hemoglobinopathies within the studied population, which was found to be 17.9% signifies burden of these inherited disorders among the individuals whose blood samples were analyzed. Sickle Cell Trait and Beta Thalassemia Trait was found to be higher compared to HPFH Trait, Hb E Heterozygous, Compound Heterozygous for HbS and Beta Thalassemia, and Sickle Cell Homozygous. Each of these hemoglobinopathies has unique genetic and clinical characteristics, requiring specific management strategies and genetic counseling for affected individuals and their families. Sickle Cell Trait is an autosomal recessive blood disorder characterized by individuals being heterozygous carriers for hemoglobin S (HbAS) and typically remaining asymptomatic (12, 14). Findings of this study reveals the prevalence of Sickle Cell Trait was the most frequent hemoglobinopathy. The thalassemia comprise a group of inherited hematologic disorders resulting from defects in the synthesis of one or more hemoglobin chains. Alpha thalassemia arises from reduced or absent synthesis of alpha globin chains, while beta thalassemia stems from reduced or absent synthesis of beta globin chains. Imbalances in globin chains lead to hemolysis and hinder erythropoiesis. Asymptomatic silent carriers of alpha thalassemia and individuals with alpha or beta thalassemia trait typically require no treatment (15). Hereditary Persistence of Fetal Hemoglobin (HPFH) is a benign condition characterized by the continued production of significant levels of fetal hemoglobin into adulthood, despite the usual cessation of fetal hemoglobin synthesis after a certain developmental stage (16).

Hemoglobin E (Hb E) is prevalent in many Asian populations, particularly in Southeast Asia. As a β-hemoglobin variant, it is produced at a slightly reduced rate, leading to a mild form of β thalassemia. This condition can manifest as mild anemia due to decreased production of normal hemoglobin. In the heterozygous state for Hb E, minimal morphological abnormalities of red blood cells are typically observed, and red cell indices remain within the normal range (17). The compound heterozygous condition of Hb S and Beta results from the combination of the sickle cell mutation and a beta thalassemia mutation. This condition was first described in 1944 and has since been recognized for its variable clinical manifestations and genetic complexity (18). Sickle Cell Homozygous otherwise referred to as sickle cell anemia is the most predominant form of Sickle Cell Disease (19).

The analysis of hemoglobinopathies revealed a gender disparity, with 39 females and 27 males identified. This observation prompts further exploration into potential factors influencing prevalence and presentation across sexes. These findings indicate the importance of considering gender-specific differences in the

prevalence and manifestation of hemoglobinopathies (20,21).

Among castes of Tharu Community, Chaudhary was found to have hemoglobinopathies followed by Rana, Katheria and Dangoura in our study. These screening data can also be utilized to determine whether certain caste groups have a higher prevalence rate, because they may reflect patterns of intermarriage within the same community. This screening may help in identifying high risk individuals so can serve as a model for community-based surveillance for hemoglobinopathies and may lay the foundation for education, counseling and management (22). Among the age group, the highest number of cases were observed in age group of ≤10 in our study followed by age group of 20-30, 10-20, 30-40, 40-50 and ≥50. Hemoglobinopathies are diagnosed across a wide age range, with significant proportions of both pediatric and adult patients being observed before the age of 30 years (23).

In a comprehensive analysis of hemoglobin variants among Asian Indians using high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE). The prevalence of different hemoglobinopathies, particularly in a diverse population holds epidemiological significance. Among the findings, Hb S trait emerged as the most frequent mutation. The detection of various hemoglobin variants, including compound heterozygous cases, underscores the complexity of these disorders (24). HPLC is a crucial tool for accurately diagnosing hemoglobinopathies and thalassemias. The advantages of HPLC are need of small sample quantities, cost-effectiveness, short turnaround time, and accurate categorization of hemoglobin fractions whereas disadvantage is associated with variant hemoglobin within the same retention windows. Utilizing the BIO RAD D10 hemoglobin analyzer found that sickle cell syndromes, including double heterozygous states, accounted for the majority of cases (56.13%). Specific hemoglobin variants, such as HbSS, HbS/β0-thal, HbS/β+-thal, and β-thal trait, were identified (25). Analytical methods for human hemoglobin analysis started from Alkaline cellulose acetate electrophoresis to modern high-performance liquid chromatography (HPLC) techniques. Electrophoretic methods have been widely used; they have limitations in resolving certain hemoglobin variants. Isoelectric focusing is labor-intensive and lacks accurate quantitation. HPLC methods offers both screening and confirmation of hemoglobinopathies with high sensitivity and specificity (26).

CONCLUSION

This study provides a comprehensive assessment of hemoglobinopathies, revealing a prevalence of 17.9% among the Tharu Community in Nepal, with Sickle Cell Trait and Beta Thalassemia Trait being the most common variants. Analysis by gender, caste, and age range signifies the distribution patterns within the community, while chromatographic patterns obtained from blood sample analysis with HPLC aid in the accurate diagnosis of various hemoglobinopathies. These findings suggest the importance of targeted screening and diagnostic approaches for effective management and intervention strategies.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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