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Research Article

Hematological and Biochemical Alterations in School-Aged Children with Sickling Disorders Indicate an Elevated Risk of Liver and Cardiovascular Diseases

Roshan Pandit[1,2], Harish Chandra Upreti[1,3], Rekha Manandhar Shrestha[1], Uday Kumar Yadav[1,4], Rameshwar Das[1,4] and Binod Kumar Yadav[2,5]*

[1]National Public Health Laboratory, Department of Health Services, Ministry of Health and Population, Kathmandu, Nepal

[2]Madhesh Institute of Health Sciences, Janakpurdham, Madhesh Province, Nepal

[3]Devdaha Medical College and Research Institute Pvt. Ltd., Kathmandu University, Nepal

[4]Province Public Health Laboratory, Janakpurdham, Madhesh Province, Nepal

[5]Department of Biochemistry, Maharajgunj Medical Campus, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal

***Corresponding Author:** Binod Kumar Yadav, Department of Biochemistry, Maharajgunj Medical Campus, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal.

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Abstract

Introduction: Sickling disorder is an umbrella term comprising all pathologies that arise due to the presence of sickle hemoglobin (HbS). HbS has been reported to cause several changes to the red blood cells leading to the development of different clinical syndrome. This study aimed to assess the associated risks in school-going children with sickling disorder in a steady state.

Methods: A cross-sectional study was carried out at National Public Health Laboratory (NPHL) for six months (March – July 2019) and samples were collected from patients visiting NPHL for hemoglobinopathies diagnosis. A total of sixty case subjects and fifty, age and sex matched control subjects were included. Following laboratory investigation, the data was analyzed using a statistical package for social sciences (SPSS) software. ANOVA and Student's t-test were used to compare between case and control subjects, and a p-value less than 0.05 at 95% confidence interval was considered statistically significant.

Results: School-aged sickling disorder patients showed a significant difference in the level of hemoglobin, PCV, MCV, MCHC, RDW-CV, and platelets. The level of triglyceride (p-value 0.000) was significantly higher while the level of HDL cholesterol (p-value 0.012) was significantly lower in sickling disorder patients suggesting an increased risk for cardiovascular disease. Likewise, the level of total bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) were also significantly increased in sickling disorder patients indicating an increased risk for liver disease.

Conclusions: This study suggested an increased risk for liver and cardiovascular disease in school-aged children with sickling disorder in steady state.

Keywords: Sickle Cell; Hemoglobinopathies; Cardiovascular Risk; Liver Dysfunction; Nepal

Introduction

Sickling disorder is an umbrella term comprising all pathologies that arise due to the presence of sickle hemoglobin (HbS) in at least one beta chain of hemoglobin molecule [1]. It includes all asymptomatic sickle cell trait and sickle cell anemia cases [1]. It is estimated that approximately 5% of the world's population carries a gene for sickle cell disease or thalassemia, and 300000 children are born with hemoglobin disorder every year globally [2,3]. Previous study from Nepal has also suggested sickling disorder as the most common form of a hemoglobinopathies especially in the western part among the Tharu population having an incidence rate of 17.4% [4].

Sickling disorder-related complications may begin at an early age when the concentration of HbS predominates over HbF. Therefore, early diagnosis and management of such cases seems important.

This study aimed to assess the associated risks of sickling disorder in school-going children.

Materials and Methods

A cross-sectional study was conducted at the National Public Health Laboratory (NPHL) over six months (March–July 2019). Samples were collected from patients below 18 years visiting NPHL with their parents for hemoglobinopathy diagnosis. Informed consent was taken from the guardian of each patient enrolled in the study. Ethical approval was granted by Nepal Health Research Council (Ref. No. 2761) before carrying out this study.

Patients who tested positive for sickling disorders during screening were invited to provide a fresh blood sample for this study. Prior to blood collection, a questionnaire was filled that consists of detail information of patient's health status and symptoms related to sickle cell disorders. The study included only patients with sickle cell disorders in a steady state and under 18 years of age. A total of 60 case subjects (patients with sickling disorders) and 50 age- and sex-matched control subjects were enrolled in this study. Control subjects were selected from apparently healthy volunteers aged 10–20 years with no personal or family history of hemoglobin-related disorders.

Blood samples were collected in a K3EDTA vial and a clot activator gel tube. Serum was separated from clot activator gel tube by centrifuging at 1500 rcf for 5 minutes and used for biochemical analysis while K3EDTA blood was used for hematological investigation. Screening for sickling disorder was carried out with the help of capillary electrophoresis (Sebia Minicap Flex-Piercing) by interpreting electrophoretogram in conjunction with peripheral blood smear and complete blood count findings. Figure 1 An ancillary sickling test was also performed using sodium metabisulphite for the confirmation of sickling disorder cases.

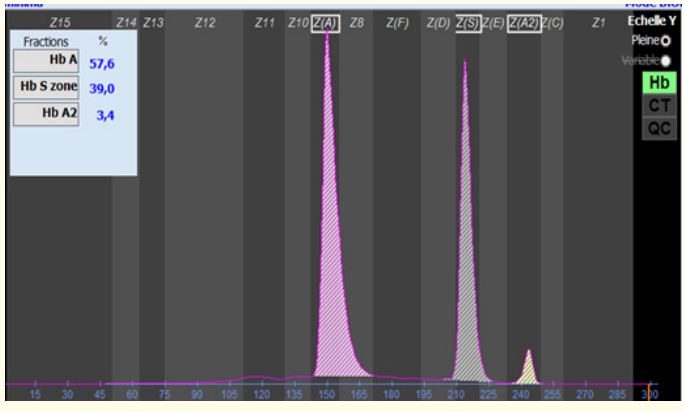

Figure 1: Electrophoretogram generated during screening for sickling disorder.Doppler imaging in the fundus of a patient with TA.

Hematological tests were performed using ABX Pentra XL80 Hematology Analyzer (Horiba Medical Ltd. Kyoto, Japan). K3EDTA blood sample was employed for testing complete blood count (CBC) parameters including Hemoglobin (Hb), Red Blood Cell count (RBC), Hematocrit (Hct), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), Red Cell Distribution Width-coefficient of variation (RDW-CV), White Blood Cell count (WBC), Granulocytes percentage (Gran. %) and Platelet count (Plt). The analyzer uses the principle of impedance to calculate blood count and, spectrophotometry method to calculate hemoglobin level. Blood indices (MCV, MCH and MCHC) were computed from the RBC histogram and hematocrit by numeric integration of pulses by the instrument.

Biochemical investigation was performed using AU480 Chemistry Analyzer (Beckman Coulter, Inc., USA) that uses the principle of spectrophotometry and potentiometry for several biochemical assay. The analyzer was validated using standard quality control (QC) reference material and commercial kits before performing biochemical tests. The samples were prepared and tested strictly adhering to the manufacturer's guidelines. The tests performed included lipid profile parameters; triglycerides (TG), total cholesterol (T. Cho.), high-density lipoproteins (HDL) and low-density lipoproteins (LDL) and, liver function test parameters; total bilirubin (T. Bil), direct bilirubin (D. Bil), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP).

The obtained data were entered and analyzed using statistical package for social sciences (SPSS) software version 20.0 (IBM Armonk, NY, USA). Mean and standard deviation was calculated for each parameter separately for case and control subjects. ANOVA and Student's t-test were used to understand the difference between two groups. A p-value of less than 0.05 was considered statistically significant at 95% confidence interval.

Results

A total of sixty sickling disorder patients of age 10 – 20 years were enrolled in the study. The median age of patients was 13 years. The total number of control subjects was fifty, with a median age of 18 years. There were 32 males and 28 females among case subjects, and 22 males and 28 females among control subjects. Among the total 60 sickling disorder cases, the highest number had heterozygous sickle cell (83.3%) followed by compound heterozygous sickle cell/β-thalassemia (15.0%) and homozygous sickle cell (1.7%) (Table 1).

Table 1: Types of sickling disorder among case subjects.

S.N.	Diagnosis	Number	Percentage (%)
1.	Homozygous sickle cell	1	1.7
2.	Heterozygous sickle cell	50	83.3
3.	Sickle cell/β-thalassemia	9	15.0
	Total	60	100

Hematological finding shows significant difference in hemoglobin, PCV, MCV, MCHC, RDW-CV and platelets among case and control subjects. Sickling disorder patients had significantly lower values for hemoglobin (10.96 ± 1.96), PCV (33.48 ± 6.04), MCV (68.41 ± 9.49), MCHC (32.76 ± 1.65) and platelets (137.33 ± 73.35) compared to the control subjects, hemoglobin (13.71 ± 2.17), PCV (40.32 ± 6.35), MCV (83.14 ± 10.40), MCHC (34.06 ± 1.42) and platelets (253.04 ± 90.07) (p-value 0.000). However, RDW-CV for case subjects was significantly higher (16.61 ± 3.11) than the control subjects (13.87 ± 1.99) (p-value 0.000) (Table 2).

Table 2: Hematological findings in case and control subjects.

S.N.	Parameters	Case (mean ± s.d)	Control (mean ± s.d)	p-value
	RBC (millions/**µl**)	4.90 ± 0.65	4.86 ± 0.59	0.720
	Hemoglobin (gm/dl)	10.96 ± 1.96	13.71 ± 2.17	0.000
	PCV (%)	33.48 ± 6.04	40.32 ± 6.35	0.000
	MCV (fl)	68.41 ± 9.49	83.14 ± 10.40	0.000
	MCH (pg)	25.46 ± 12.01	28.30 ± 3.59	0.410
	MCHC (%)	32.76 ± 1.65	34.06 ± 1.42	0.000
	RDWcv	16.61 ± 3.11	13.87 ± 1.99	0.000
	Platelets (*10^3cells/**µl**)	137.33 ± 73.35	253.04 ± 90.07	0.000
	WBC (*10^3 cells/**µl**)	7.05 ± 1.85	7.10 ± 2.01	0.902

Some lipid profile test parameters showed significant differences among case and control subjects. Triglyceride level was significantly higher in the case subjects (95.70 ± 38.15) as compared to that of control groups (67.30 ± 29.47) (p-value 0.000). On the contrary, HDL cholesterol level was significantly lower in case subjects (37.93 ± 9.35) than that of control subjects (42.64 ± 10.01) (p-value 0.012) (Table 3).

Most of the liver function test parameters were relatively elevated in sickling disorder cases compared to healthy controls. Total bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) were significantly increased in case subjects. The mean value for total bilirubin, ALT, AST, and ALP of case subjects was (1.01 ± 1.01), (28.33 ± 14.83), (34.53 ± 11.60) and (320.96 ± 158.76), respectively while that for control subjects was (0.52 ± 0.33), (11.72 ± 7.12), (17.26 ± 12.39) and (201.00 ± 82.15), respectively. The p-value for total bilirubin was 0.002, and that for ALT, AST and ALP were 0.000 (Table 3).

Table 3: Comparisons of lipid profile and liver function test parameters

	Parameters	Case (mean ± s.d)(N = 60)	Control (mean ± s.d)(N = 50)	p-value
Lipid profile	Triglycerides (mg/dl)	95.70 ± 38.15	67.30 ± 29.47	0.000
	Total Cholesterol (mg/dl)	119.45 ± 25.50	128.50 ± 25.91	0.069
	HDL-cholesterol (mg/dl)	37.93 ± 9.35	42.64 ± 10.01	0.012
	LDL-cholesterol (mg/dl)	62.06 ± 20.37	69.48 ± 23.03	0.076
Liver function test	T. Bilirubin (mg/dl)	1.01 ± 1.01	0.52 ± 0.33	0.002
	D. Bilirubin (mg/dl)	0.18 ± 0.15	0.16 ± 0.14	0.533
	ALT (U/L)	28.33 ± 14.83	11.72 ± 7.12	0.000
	AST (U/L)	34.53 ± 11.60	17.26 ± 12.39	0.000
	ALP (IU/L)	320.96 ± 158.76	201.00 ± 82.15	0.000

Discussion

Sickling disorder has been shown to alters several biological phenomena inside our body characterized by vaso-occlusive crisis, hemolytic anemia and organ damage [5]. Due to the genetic cause of the disease, its effect begins at an early age and continues throughout life. Sickling disorder cases are distributed heterogeneously worldwide, with a significant number found in sub-Saharan Africa, parts of the Mediterranean, the Middle East, and India [3,6]. In Nepal, a higher prevalence is observed in the western region [4]. In this study we assessed the risks associated with sickling disorder in Nepal through hematological and biochemical analysis and, found that these population faces an increased risk for liver and cardiovascular diseases.

This study found significant differences in most hematological parameters between sickling disorder patients and control subjects. Hemoglobin, PCV, MCV, MCHC, and platelet levels were notably lower in the case group compared to the control group. A study carried out in India by Nagose., *et al*. also showed decreased value for hematological parameters among sickle cell anemia subjects in accordance to our study [7]. Similarly, a survey by Akinbami., *et al*. observed decreased levels of Hemoglobin, PCV and MCV in homozygous sickle cell disease patients, aligning with our results [8]. However, the same survey reported increased levels of MCHC and platelets in sickle cell disease patients, which contrasts with our findings [8]. Additionally, a survey by Antwi-Boasiako., *et al*. in Ghana noted decreased Hemoglobin and PCV levels but increased MCV, MCHC, and Platelets levels in sickle cell disease patients compared to controls [9]. These differences may stem from variations in the selection criteria for case subjects between our study and that of Akinbami., *et al*. [8]. Our finding is also comparable to the result of Clarence., *et al*. that reported decreased value of PCV in patients with sickle cell anemia [10]. Overall, hematological values appear to differ significantly between individuals with sickling disorders and healthy controls. These variations may be attributed to factors such as the sickling phenomenon, the presence of other hemoglobin variants, hypoxia, dehydration, and acidosis in affected patients.

This study also noted significant changes in liver function test parameters among case and control subjects. Total bilirubin, ALT, AST, and ALP were significantly increased in sickling disorder patients compared to those of controls. Increased value for total bilirubin was also reported among sickle cell disease children by a study carried out in India by Biswal., *et al*. similar to our study [11]. The increased value for total bilirubin, ALT, and AST in our study is further supported by Clarence., *et al*. who found similar results in sickle cell anemia patients [10]. Likewise, a similar finding was also observed in a study carried out in Nigeria by Yahaya I. among sickle cell anemia patients [12]. Additionally, a study by Kotila., *et al*. also suggests an increased value of ALT, AST, and ALP in some portions of the study population [13]. The evidence gained from our study population indicates that sickling disorder patients have significantly elevated levels of liver function test parameters. Abnormally elevated levels of such parameters might be the indication for liver dysfunction that puts sickling disorder patients at an elevated risk for the development of liver disease.

Sickling disorder patients in this study were also found to have significantly different levels of lipid profile parameters. The study noted a considerably lower value for HDL cholesterol but a substantially higher value for triglyceride in sickling disorder patients compared to control subjects. They also had a decreased levels of total cholesterol and LDL cholesterol compared to control groups. These findings are comparable to the study of Yalcinkaya., *et al*. and Hazmi., *et al*. who also demonstrated lower values for total, HDL and LDL cholesterol in sickle cell disease patients [14,15]. A case-control study conducted in the Tema metropolitan similarly reported significantly lower HDL cholesterol levels, consistent with our findings. Other findings from that study are also comparable to ours [16]. Minor variations in certain parameters may be attributed to differences in the age groups of study participants or the selection of case subjects based on disease severity. Overall, this study accounted for significantly increased triglyceride and decreased HDL cholesterol value, which is a significant predictor of cardiovascular disease. This suggests that sickling disorder patients aged 10 – 20 years in steady-state are at higher risk for cardiovascular disease.

Conclusion

This study concludes that patients with sickling disorders exhibit significantly altered hematological, lipid profile, and liver function test parameters putting the patients at a higher risk of developing liver and cardiovascular complications. Therefore, it is recommended that patients with sickling disorders undergo regular monitoring, with a focus on preventive measures to address liver and cardiovascular complications.

Ethical Approval and Consent to Participate

The study was approved by Nepal Health Research Council (Ref. No.: 2761). Informed consent was obtained from parents of each patient enrolled in the study.

Data Availability

The datasets obtained in this study can be made available from the corresponding author on reasonable request.

Consent for Publication

All the authors read the manuscript and agreed for publication.

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Authors' Contribution

RP reviewed the literature, performed laboratory tests, analyzed the data and wrote the manuscript. BKY designed the study and supervised the work. RMS and HCU supervised during drafting of manuscript. UKY and RD helped in data collection and analysis. All authors read and approved the final version of manuscript.

Conflict of Interest

None.

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