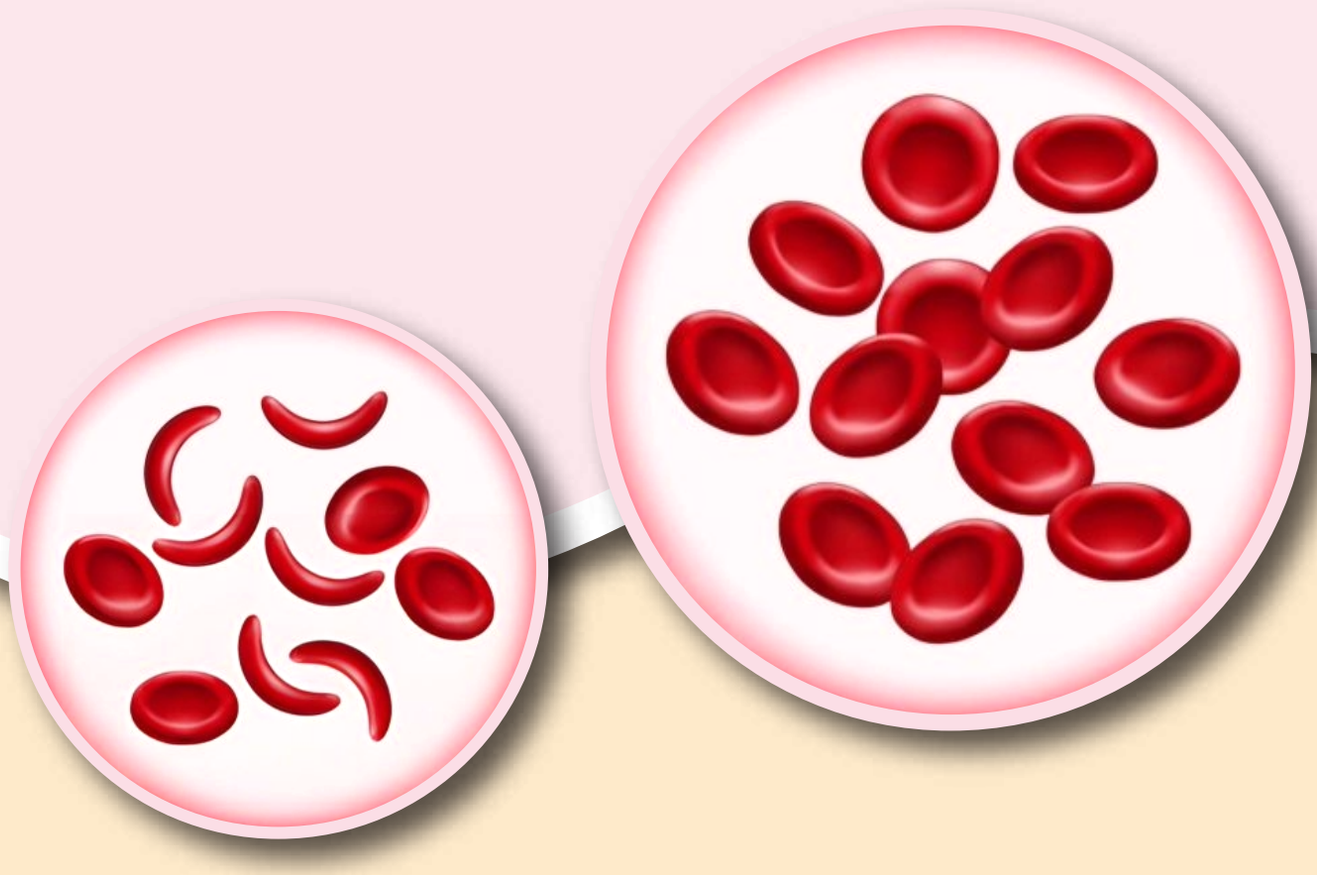
Study on Hemoglobinopathies and Glucose-6-Phosphate Dehydrogenase Deficiency in Terai Districts of Koshi, Bagmati and Gandaki Provinces in Nepal



Government of Nepal
Nepal Health Research Council (NHRC)
Ramshah Path, Kathmandu, Nepal

Study on Hemoglobinopathies and Glucose-6-Phosphate Dehydrogenase Deficiency in Terai Districts of Koshi, Bagmati and Gandaki Provinces in Nepal

2082 BS (2025 AD)

Government of Nepal

Nepal Health Research Council (NHRC)

Ramshah Path, Kathmandu, Nepal

Research report

Authors:

Pramod Joshi, Bishnu Prasad Marasini, Gunaraj Dhunganaa, Sujata Dahal, Suraj Chand, Rajina Shakya, Meghnath Dhimal

Suggested citation:

Joshi P, Marasini BP, Dhunganaa G, Dahal S, Chand S, Shakya R, and Dhimal M (2025) Study on Hemoglobinopathies and Glucose-6-Phosphate Dehydrogenase Deficiency in Terai Districts of Nepal: Nepal Health Research Council.

ISBN: 9789937185073

Preface

Health research stands as a cornerstone for informed policy and improved population health outcomes. The Nepal Health Research Council (NHRC), empowered by the NHRC Act, 2047 (1991), has been promoting health research throughout Nepal. NHRC always tends to ensure every province and community contributes to and benefits from advancing research capacity and addressing national priorities.

This report presents the results of a comprehensive study on hemoglobinopathies and glucose-6-phosphate dehydrogenase (G6PD) deficiency in Koshi, Bagmati, and Gandaki provinces. Because of the evolving burden of genetic disorders in the Terai regions of these provinces, this research sought to generate actionable evidence to inform local and federal health policy. The study involved collaborative fieldwork, meticulous laboratory analyses, and the dedication of a multidisciplinary team. The findings reveal a considerable prevalence of hemoglobin disorders, particularly among vulnerable Tharu subpopulations. It may depict a need for coordinated provincial interventions, screening, and genetic counseling. It is hoped that the evidence presented here will be instrumental for policymakers, health practitioners, and researchers—supporting both immediate action and long-term strategies to address hematologic diseases in Nepal.

Undertaking this research was both a challenge and an opportunity. The project benefited from the expertise and sustained support of NHRC staff, consultants, and field researchers, whose professionalism and commitment were indispensable. The NHRC remains steadfast in its objective to study pressing health issues, build research capacity, and provide knowledge that guides decision-making for better health across all provinces and for the Nation. May this publication inspire further research, dialogue, and innovation in Nepal's health sector.

Prof. Dr. Narayan Bikram Thapa

Chairman

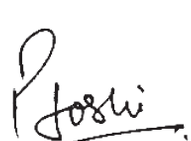
Nepal Health Research Council (NHRC)

Acknowledgments

It is our pleasure to publish this report of the study entitled "Study on Hemoglobinopathies and Glucose-6-Phosphate Dehydrogenase Deficiency in Terai Districts of Koshi, Bagmati and Gandaki Provinces in Nepal". On the successful completion of this study, I would like to express my heartfelt gratitude Dr. Meghnath Dhimal, Chief of Research Section, Nepal Health Research Council, for his technical input, constructive comments and coordination to make this study successful.

My sincere gratitude goes to Dr. Bishnu P. Marasini, Senior Research Associate, whose invaluable support in data collection, compilation, and analysis greatly contributed to the success of this report. His expertise and guidance throughout the research process were essential and deeply appreciated. Special thanks to Dr. Gunaraj Dhunganaa, Ms. Sujata Dahal, and Mr. Suraj Chand, Consultant, NHRC, Ms Rajina Shakya, Research Associate for their persistent support to report finalization. Without the field-based data collection, the study would not have been possible. I really owe all the field researchers who made this study successful even in the time of lockdown by gathering the scattered data all over seven provinces. So, my special thanks go to our field researchers. I am grateful towards our office staff Mr. Subodh Kumar Karn, Chief/Deputy Chief Account Controller; Mr. Saraswati Prasad Bhattarai, Store Officer for their support on financial aspects, administrative assistance and logistics related to the study, respectively.

I am also thankful to all the Female Community Health Volunteers (FCHVs), Nurses, Health Assistants (HAs), and other frontline health workers who played a crucial role in supporting field-level data collection and coordination efforts. Their dedication and cooperation were vital to the smooth execution of this study. I would like to extend my heartfelt thanks to all the members and representatives of the Provincial Public Health Laboratories of Koshi and Bagmati provinces for their continuous support, technical assistance, and collaboration throughout the project. Lastly, my heartfelt appreciation goes to all research participants, whose voluntary involvement and cooperation made this study possible. Their willingness to contribute their time and information is deeply valued.



Dr. Pramod Joshi
Member Secretary (Executive Chief), NHRC

Table of Content

List of Tables

List of Figures

List of Abbreviations

Hb	Hemoglobin
HbA	Hemoglobin A
HbA2	Hemoglobin A2
HbF	Hemoglobin F
HbE	Hemoglobin E
HbS	Hemoglobin S
SCD	Sickle Cell Disease
G6PD	Glucose-6-Phosphate Dehydrogenase
GSH	Glutathione (Reduced form)
HMP	Hexose Monophosphate (shunt)
WHO	World Health Organization
CBC	Complete Blood Count
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
RDW	Red Cell Distribution Width
RBC	Red Blood Cell (count)
WBC	White Blood Cell (count)
HCT	Hematocrit
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (Reduced form)
EDTA	Ethylenediaminetetraacetic Acid
PPS	Probability Proportional to Size
SPSS	Statistical Package for the Social Sciences
UL	Upper Limit
CBS	Central Bureau of Statistics
PPHL	Provincial Public Health Laboratory
UV	Ultraviolet (method)

Executive Summary

Hemoglobinopathies are a group of recessively inherited genetic disorders characterized by abnormalities in hemoglobin, the molecule in red blood cells the delivers oxygen throughout the body. So far, over 1,000 abnormalities genotypically and phenotypically such as thalassemia, sickle-cell disease (SCD), etc. has been found. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy worldwide, affecting an estimated 400 million people. Hemoglobinopathies and Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency is significant public health concerns, particularly in malaria-endemic regions. There is reported prevalence of these conditions among Nepalese population especially among Tharu residing in western and Far-Western regions. Despite a good number of researches performed in this area, the precise burden of these conditions in both Tharu and Non-Tharu populations remains uncertain. Thus, this study aims to estimate the prevalence of Hemoglobinopathies and G6PD deficiency and its associated clinical symptoms among 1-29 years population in the Terai districts of Nepal.

This nationwide study was planned to conduct in the Terai region of all provinces in Nepal. In this third phase of the study, a cross-sectional survey was carried out in selected districts of Koshi, Bagmati and Gandaki province. A total of 1,680 participants aged 1-29 years were enrolled in this study: 288 participants for Bagmati Province (Chitwan district) and 256 Gandaki Provicne (Nawalpur district) and 1,120 participants for Koshi Province (Jhapa, Morang and Sunsari districts). Data was collected through structured questionnaires and blood sample analysis, which included complete blood count, hemoglobin electrophoresis, and G6PD level assessment which were done in the months of Baisakh and Jestha, 2081. The blood samples were tested in Provincial Public health laboratories and Bir Hospital.

The study revealed a higher prevalence of Hemoglobinopathies among the Tharu population compared to the Non-Tharu population 16% in Chitwan and 25% in Koshi province. Thalassemia was the most common hemoglobinopathy identified, followed by Hemoglobin E (HbE) abnormality/trait. Few cases of Sickle cell trait (0.5%) were identified in Koshi Province while that was absent in Bagmati and Gandaki Province (Chitwan and Nawalpur). Anemia prevalence was significantly higher among Tharu participants (49.5-58.4%), especially younger age groups and females. A notable proportion of male participants (6.8%) in Chitwan exhibited G6PD deficiency. Most participants with Hemoglobinopathies or G6PD deficiency were asymptomatic. Among participants experiencing symptoms, headache and abdominal pain were the most commonly reported symptoms.

This study highlights the significant burden of Hemoglobinopathies especially thalassemia and HbE abnormality and G6PD deficiency in the Terai region of Nepal, particularly among the Tharu population. The findings underscore the need for targeted public health interventions, including awareness campaigns, genetic counseling, expanded screening programs, and improved healthcare infrastructure to address these conditions effectively.

CHAPTER I INTRODUCTION

1.1 Background

Hemoglobin (Hb) is an oxygen-binding protein within red blood cells that carries oxygen throughout the body (Pandya & Sharma, 2023). Its structure comprises an iron-containing heme molecule and globin chains, which are genetically determined. The main types of hemoglobin are Hemoglobin A (HbA), Hemoglobin A2 (HbA2), and Hemoglobin F (HbF), distinguished by different combinations of globin chains. HbA, the predominant adult hemoglobin, consists of two alpha (α) and two beta (β) chains and makes up 95-98% of adult hemoglobin. HbA2, composed of two α and two delta (δ) chains, represents 1-3% of adult hemoglobin, while HbF, with two α and two gamma (γ) chains, is dominant in fetal blood but accounts for 2-3% in adults. The specific proportions of these hemoglobin types vary with age, genetics, and health conditions (Harewood & Azevedo, 2025).

Hemoglobinopathies are recessively inherited genetic conditions that affect hemoglobin and are broadly classified into two categories: thalassemia syndromes and structural hemoglobin variants. Thalassemia, including α-thalassemia and β-thalassemia, is caused by mutations or deletions in the α- or β-globin genes, while structural variants like HbS, HbE, and HbC arise from specific gene mutations that alter the structure of hemoglobin (Kohne & Kleihauer, 2010; UK, 2018).

Thalassemia an inherited genetic disorder, is caused by reduced synthesis of alpha or beta globin chains in hemoglobin, leading to malformed red blood cells, inadequate oxygen transport, and lifelong anemia. Alpha thalassemia results from deletions in alpha-globin genes, with severity ranging from mild (one allele deletion) to lethal (four allele deletion, causing Hydrops fetalis). Beta thalassemia arises from mutations in the beta-globin gene and varies in severity: beta-thalassemia minor (mild, often asymptomatic), intermedia (moderate symptoms), and major (severe anemia, jaundice, growth delays, and dependency on lifelong transfusions). Symptoms in beta-thalassemia major typically manifest after six months of age as fetal hemoglobin transitions to adult hemoglobin (Bajwa & Basit, 2025) and causes transfusion-dependent anemia and complications from iron overload, leading to damage in vital organs such as the heart, liver, and endocrine glands (Angastiniotis et al., 2013).

Thalassemia is a globally prevalent genetic disorder, particularly common in Southeast Asia, with alpha thalassemia affecting individuals of African and Southeast Asian descent and beta thalassemia being most frequent among those of Mediterranean, African, and Southeast Asian ancestry (Colah et al., 2017). Approximately 5% of the global population carries a globin variant, but only 1.7% have an alpha or beta thalassemia trait, with an overall incidence of 4.4 per 10,000 live births (Herbert L. Muncie & Campbell, 2009).

Sickle hemoglobin (HbS) is an abnormal hemoglobin variant caused by a genetic mutation in the beta-globin gene, resulting in the substitution of valine for glutamic acid at the sixth position. This mutation leads to the formation of rigid, sickle-shaped red blood cells that block blood flow, causing pain, infections,

and complications such as stroke, acute chest syndrome, and organ damage (Kohne, 2011). Sickle cell disease (SCD), including variants like HbSC, HbSE, and HbS β-thalassemia, is a monogenic disorder with clinical heterogeneity, affecting approximately 70,000 to 100,000 Americans and causing high childhood mortality in low-income countries without treatment. Sickle cell trait, a milder condition with one defective gene, affects about 8% of African Americans and usually does not cause health problems, though extreme conditions can trigger complications (American society of Hematology, 2025; Makani et al., 2013).

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy affecting estimated 400 million people worldwide (Cappellini & Fiorelli, 2008; Richardson & O'Malley, 2025). It was discovered in 1956 after the development of hemolytic anemia following the administration of the anti-malarial primaquine. About 8% of the people who are exposed to malaria have an inherited disorder that impairs G6PD activity, leaving them at risk to develop different clinical consequences (hemolytic anemia) (Ghimire et al., 2017).

Glucose is the main source of energy for the red cell, which is metabolized by two major routes; the glycolytic pathway and the hexose monophosphate (HMP) shunt. Glucose-6-phosphate-dehydrogenase (G6PD) is an X-linked enzyme that catalysis the first step in the HMP pathway of glucose metabolism and it produces NADPH, which is required for the maintenance of reduced glutathione (GSH). GSH is essential for protecting red cells from oxidative damage. The major clinical manifestations of this disorder are hemolytic anemia and/or neonatal jaundice and a small proportion of G6PD deficient individuals have chronic non-spherocytic hemolytic anemia (Class I G6PD deficiency) (Mukherjee et al., 2015). Hemolysis can be induced by infection, hyperglycemia, certain foods, and certain medications (Bubp et al., 2015).

G6PD deficiency is an X-linked genetic disorder with 187 known allelic mutations (Minucci et al., 2012). The large majority of affected individuals are males as G6PD deficiency is linked to the X-chromosome although there are affected females where the prevalence is high. As the G6PD deficiency is transmitted X-chromosomally, the deficiency can be detected reliably in homozygous women and homozygous males with a number of tests. More than 80 % of all patients suffering from sickle cell anemia live in Central Africa, but it is also prevalent in the Arabian Peninsula, in the Middle East, the Indian subcontinent and the eastern part of the Mediterranean (Theodorsson et al., 2007).

Hemoglobinopathies including beta-thalassemia and sickle cell anemia, are among the most significant inherited hemoglobin disorders due to their high prevalence, morbidity, and mortality, with approximately 7% of the global population being carriers, 10-20% in Central Asia, and 5-40% in Southeast Asia (Kohne, 2011; Minucci et al., 2012). According to the World Health Organization (WHO), approximately 5% of the global adult populations are carriers for a hemoglobin disorder, including 2.9% for thalassemia and 2.1% for sickle cell disease (SCD) (Memish et al., 2011). Each year, over 300,000 babies are born with severe forms of Hemoglobinopathies, with 80% of these births occurring in low- and middle-income countries (Weatherall, 2011). About 3.5% of the deaths in children below 5 years are due to hemoglobin disorders (Modell & Darlison, 2008). Of the children born with major Hemoglobinopathies annually, 70% are affected by SCD, while 30% have thalassemia.(Aguilar Martinez et al., 2014) Hemoglobinopathies are most prevalent in tropical regions, although population migration has facilitated their spread to countries worldwide (Aguilar Martinez et al., 2014; Kohne & Kleihauer, 2010).

The HbS gene, associated with sickle cell disorders, shows its highest prevalence in the Middle East, Mediterranean regions, Southeast Asia, and sub-Saharan Africa, with Nigeria having particularly high rates. (Makani et al., 2013). It has been observed that sickle cell trait offers selective protection against lethal forms of malaria, contributing to its high prevalence in malaria-endemic regions (Allison, 1954; Hebbel, 2003). Similarly, glucose-6-phosphate dehydrogenase (G6PD) deficiency is most common in sub-Saharan Africa, where its prevalence averages 7.5%, followed by the Middle East (6.0%) and Asia (4.7%) (Nkhoma et al., 2009). The global population affected by G6PD deficiency in malaria-endemic

regions includes an estimated 220 million males and 133 million females (Howes et al., 2012, 2013). These data emphasize the global burden of Hemoglobinopathies and G6PD deficiency, particularly in regions with a high prevalence of malaria, highlighting the importance of early detection and targeted interventions to mitigate their impact.

It is assumed that the global spread of these inheritable diseases might be due to international migration occurring in different locations of the world (Kohne & Kleihauer, 2010). One of the reasons for increasing number of hemoglobinopathy related diseases is high number of consanguineous marriages in some under-resource countries and poor public health measures to control this issue. Nepal being located in the south-eastern part of Asia is suspected to have higher number of hemoglobinopathies cases which is also supported by some of the previous studies in different districts of Nepal (Bhusal et al., 2022).

Hemoglobinopathies and related genetic disorders are significant public health concerns in Nepal, particularly in specific regions and ethnic groups. A retrospective study conducted in five sites across Nepal identified sickle cell disease as the most predominant hemoglobinopathy, affecting the western provinces and predominantly the Tharu community (Shrestha et al., 2020), while thalassemia was prevalent throughout the country and across all ethnic groups (Roma et al., 2023; Shrestha et al., 2020). a hospital based study in western Nepal found prevalence of sickle cell disease to be 14.7% which is higher than previous studies (Pandey & Shrestha, 2022). Supporting this finding, another study in western Nepal revealed that 97.7% of patients with sickle cell disease were from the Bardiya district and the Tharu ethnic group, with joint pain being the most common symptom (Pande et al., 2019). Although most studies focus on the Terai region, a study conducted in Pokhara found that 7% of participants were suspected to have beta-thalassemia traits based on Hemoglobin A2 (HbA2) levels. Additionally, some participants were identified as heterozygous for Hemoglobin E (Bastola et al., 2017).

Glucose-6-phosphate dehydrogenase (G6PD) deficiency, another significant genetic condition, has also been studied extensively. National-level data indicate a prevalence of 3.5%, with higher rates among males (4.1%) than females (2.1%). The prevalence was notably high among Janajati groups (6.2%), particularly in the Mahato (17.6%), Chaudhary (7.7%), and Tharu (7.5%) communities, but was lower among Brahman and Chhetri populations (1.3%) (Marasini et al., 2020). Similarly, a study conducted in an eastern Terai district reported a 7.2% prevalence of G6PD deficiency, with higher proportions among the Rajbanshi and Santhal communities (Lamichhane et al., 2017).

Despite a good number of researches performed in Terai region, the exact number of Tharu with Hemoglobinopathies and G6PD is yet to be determined thus making it a matter of utmost importance for the management of this disorder. Similarly; besides the Tharu population, studies have shown that Hemoglobinopathies and G6PD are also prevalent among other ethnic groups residing in the Terai region. It is known that Terai people share common socio- economic conditions as well as belonging to malaria endemic areas. Hence, it is of utmost importance to determine the exact prevalence of hemoglobin disorder and G6PD among the population of Terai district of Nepal. Thus, this study aims to estimate prevalence of Hemoglobinopathies (Thalassemia and Sickle Cell disease) and G6PD and its associated clinical symptoms among 1-29 years of population residing in Terai district of Nepal.

1.2 Rationale

Hemoglobinopathies, among the most common monogenic diseases, pose a significant global health challenge. Studies indicate that sickle cell disease affects diverse ethnic and tribal populations globally (Mohanty et al., 2022). These genetic disorders, originally concentrated in the Mediterranean, Asia, and Africa, have now spread worldwide, largely due to international migration and high rates of consanguineous marriages (Aguilar Martinez et al., 2014; Kohne & Kleihauer, 2010). Factors such as high rates of consanguineous marriages in some regions and have contributed to their increasing prevalence (Bhusal et al., 2022).

According to the Central Bureau of Statistics (CBS, 2011), the Terai region of Nepal is home to approximately 13.31 million people. Several hospital-based and community-based studies have reported the prevalence of Hemoglobinopathies and G6PD deficiency among the Nepalese population, particularly among the Tharu community residing in the western and far western regions of the country. Sickle cell disease is the most common Hemoglobinopathy, predominantly affecting the Tharu community in western Nepal, while thalassemia has been reported across all ethnic groups (Pande et al., 2019; Shrestha et al., 2020) Similarly, G6PD deficiency has been reported at a national prevalence of 3.5%, with higher rates among Janajati groups, including the Tharu, Mahato, and Chaudhary communities (Lamichhane et al., 2017; Marasini et al., 2020). However, due to cultural practices such as consanguineous marriage, the increasing trend of inter-caste marriages, and inadequate public health measures, the prevalence of Hemoglobinopathies is reportedly rising among other ethnic groups (Bhusal et al., 2022). While prior research has shed light on these conditions in the Terai region, the exact prevalence among the Tharu and other ethnic groups remains unclear. Considering the shared socioeconomic conditions and the malaria-endemic status of the Terai region, this study aims to estimate the prevalence of Hemoglobinopathies (thalassemia and sickle cell disease) and G6PD deficiency, along with their associated clinical symptoms, among the population aged 1–29 years in the Terai districts of Nepal.

1.3 Objectives

1.3.1 General Objective:

- To determine the prevalence of Hemoglobinopathies and G6PD among the population of Terai district of Nepal.

1.3.2 Specific Objective:

1. To determine the prevalence of Hemoglobinopathies and G6PD in 1-29 years' age group among the population of Terai district of Nepal.
2. To compare the Hemoglobinopathies and G6PD among Tharu and other Non-Tharu population.
3. To determine the relation of socio demographic factors to Hemoglobinopathies.
4. To determine the association of clinical symptoms to Hemoglobinopathies.

1.4 Variables

1.4.1 Independent variable: Socio-demographic Variable, History of Medical Illness.

1.4.2 Dependent Variables: The primary dependent variable in this study is the presence of anemia, determined based on hemoglobin (Hb) concentration levels according to established clinical thresholds. Additional dependent variables include the presence or absence of G6PD (Glucose-6-Phosphate Dehydrogenase) deficiency, as well as the measured levels of various hemoglobin variants and subtypes, specifically:

HbA (Hemoglobin A) – the most common adult hemoglobin,

HbA2 (Hemoglobin A2) – a minor adult hemoglobin component, useful in the diagnosis of beta-thalassemia,

HbE (Hemoglobin E) – a structural hemoglobin variant common in Southeast Asia,

HbF (Fetal Hemoglobin) – normally present in infants and certain hemoglobinopathies,

HbS (Hemoglobin S) – associated with sickle cell disease,

HbH (Hemoglobin H) – found in some alpha-thalassemia syndromes, and

Quantitative G6PD enzyme level – used to assess the severity of G6PD deficiency.

(HbA, HbA2, HbE, HbF, HbS, HbH and G6PD level).

CHAPTER II METHODOLOGY

2.1 Research Design

A cross-sectional, quantitative study design was conducted for this study. This study determined the prevalence of the hemoglobin disorder among the Tharu and Non-Tharu population in the Terai districts of Nepal.

2.2 Study Site

This multi-year study aims to include samples from all Terai districts of Nepal to provide a comprehensive understanding of Hemoglobinopathies and G6PD deficiency prevalence. The Terai is a lowland region in southern Nepal, extending from the Indian border to the easternmost parts of the country. It is characterized by a tropical savanna climate with dry winters, hot summers, and annual rainfall ranging from 1,600–1,800 mm in the west to 2,500–3,000 mm in the east. The mean annual temperature ranges between 20–28 °C, creating conditions conducive to the persistence of malaria, which is a risk factor for Hemoglobinopathies (Terai, 2024).

The Tharu community, the largest ethnic group in the Terai region, is known to have a higher risk of Hemoglobinopathies due to genetic predisposition and environmental factors. Previous evidence highlights the association between the region's geographical structure, endemic malaria, and the prevalence of these conditions.

This study is being conducted in multiple phases. The first phase included all districts of Madhesh Province, while the second phase focused on districts from Sudurpashchim Province. The current third phase targets Terai districts in Koshi, Bagmati, and Gandaki Provinces. To ensure robust and comparative analysis, the study enrolled participants from both Tharu and non-Tharu populations, allowing for a broader understanding of the condition's prevalence and its variation across ethnic groups. The inclusion of diverse districts and communities enhances the study's generalizability and provides valuable insights for targeted public health interventions.

2.3 Study population, Sampling Technique and Sample size

2.3.1 Study Population

The study population included participants of both sexes of Tharu and Non-Tharu population, aged 1–29 years, who have been residing at the study site for six months.

2.3.2 Sampling Technique

The study used probability sampling to select participants. Wards, as defined in the 2011 census, served as primary sampling units. Seventeen wards were selected from 190 wards in Chitwan and Nawalpur districts (Bagmati and Gandaki provinces), and 35 wards from 414 wards in Jhapa, Morang, and Sunsari districts (Koshi province) using Probability proportional to Size (PPS) sampling, with Tharu and non-Tharu households as measures of size. From each ward, 32–34 households were selected and only one eligible participant from each household was enrolled in the study.

2.3.2 Sample Size

The sample size was calculated using the WHO STEPS sample size calculator for reliable national-level estimates, with a 95% confidence level, 5% margin of error, and a 37% prevalence of Hemoglobinopathies in Nepal, resulting in 358. Adjustments for non-response (20%) and a design effect of 2 increased it to 900. To analyze data by sex and two age groups (0–15, 15–30), the sample size was quadrupled to 3600. An equal number of Non-Tharu participants were included as a comparison group so the final sample size was 7200.

In the third phase of the study, the calculated sample sizes were 288 participants for Bagmati Province (Chitwan district) and 256 Gandaki Provicne (Nawalpur district) and 1,120 participants for Koshi Province (Jhapa, Morang and Sunsari districts). To accommodate potential data loss due to incomplete responses or inadequate blood samples, slightly more participants were enrolled during data collection. As a result, data were collected from 288 participants in Bagmati Province, 258 in Gandaki Province and 1,148 participants in Koshi Province.

Following quality checks, including the completeness of participant information and adequacy of blood sample volume for laboratory testing, a total of 546 samples from Bagmati Province and 1,136 samples from Koshi Province were deemed valid and included in the final analysis.

2.4 Criteria for Selection

2.4.1 Inclusion Criteria

- Both men and women aged 1–29 years who had been living at their place of residence for at least six months and who are willing to participate in the study

2.4.2 Exclusion Criteria

- Aged less than 1 year and aged more than 29 years
- Too frail to participate in the study.
- History of blood transfusion in the past three months
- Pregnant women

2.5 Ethical Consideration

Ethical approval was obtained from the Ethical Review Board of the Nepal Health Research Council (Ref. no. 191-2024). Participants were informed about the study procedure, confidentiality, and their right to refuse or withdraw without penalty. Written assent/consent was obtained, ensuring ethical standards were maintained.

2.6 Data Collection Tools

A structured questionnaire was used to collect detailed information on participants' socio-demographic characteristics, including age, sex, ethnicity, and marital status, as well as their family history of blood disorders, medical history of diseases, and any history of blood transfusion. Multiple tools were utilized for data collection in this study. Socio-demographic and clinical history data were obtained through face-to-face interviews using a structured questionnaire. For laboratory assessments, a Complete Blood Count (CBC) test was conducted to measure various hematological parameters such as hemoglobin (Hb), hematocrit (HCT), red blood cell count (RBC), white blood cell count (WBC), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW), using a fully automated hematology analyzer. The Glucose-6-Phosphate Dehydrogenase (G6PD) activity level, expressed in units per gram of hemoglobin (U/g Hb), was measured using the Randox Monza Analyzer. Additionally, hemoglobin electrophoresis was performed to detect various hemoglobinopathies, including Hemoglobin A1 (HbA1), Hemoglobin A2 (HbA2), Hemoglobin E (HbE), Hemoglobin F (HbF), and Hemoglobin S (HbS), through capillary electrophoresis using the SEBIA Minicap Flex Piercing system. The tools used for the data collection are given in table 1.

Table 1: Instruments used in study and data collection

Study Components	Measurements	Study Instruments
Interview for background information and history of diseases	Socio-demographic information (age, sex, ethnicity, marital status), Clinical symptoms	Structured questionnaire
CBC Test	CBC parameters: Hb, HCT, RBC, WBC, Platelet count, MCV, MCH, MCHC, RDW	Fully automated Hematology Analyzer
G6PD Test	G6PD activity/gm/Hb	Randox Monza Analyzer for G6PD
Hb electrophoresis	Hemoglobinopathies (HbA1, HbA2, HbE, HbF, HbS)	Capillary Electrophoresis through SEBIA Minicap Flex piercing

2.7 Validity and Reliability of Tool

A structured questionnaire was developed with the input of experts, and modifications were made based on feedback obtained during the second phase of the study to ensure content validity.

To minimize primary errors in data entry, blood samples were labeled using specific participant code numbers. Enumerators were trained and recruited based on their experience with blood collection, ensuring procedural reliability. They were provided with detailed guidelines for blood drawing and instructed to adhere strictly to these guidelines throughout the study.

To maintain instrument reliability, blood testing equipment was calibrated at specific intervals. Quality control measures for Complete Blood Count (CBC) and hemoglobin electrophoresis were implemented daily before running samples. Cross-validation of the samples was conducted with internal laboratories to ensure the accuracy of results. Additionally, the instruments used for testing were regularly checked for consistency and adequacy, ensuring reliable and valid data collection and analysis.

2.8 Data Collection Technique

Firstly, the enumerators were trained to collect structured questionnaires on socio-demographic information and family history of the participants. This was followed by phlebotomy of 3 mL of blood in an EDTA vial. The participants were given code no. based on the district name, ward no. and serial no. for their unique identification. All the collected samples were maintained at 4-8°C in cold chain box. Complete blood count (CBC) and G6PD test were done at PPHL, Biratnagar and Chitwan. Complete blood cell count (CBC) was analyzed by using fully automated hematology analyzer which relies on two principles: electrical resistance or impedance and optical analysis. Similarly, G6PD test was analyzed using Randox Monza analyzer which is based on UV method. This method is used to determine enzyme activity by measuring the rate of absorbance change at 340 nm due to the reduction of NADP+.

Capillary electrophoresis for detection of Hemoglobinopathies was done at the Genetics Lab, Bir Hospital, Kathmandu. The samples were analyzed using SEBIA Minicap Flex piercing that uses the principle of capillary electrophoresis to separate different hemoglobin variants. With this technique, charge molecules are separated by their electrophoretic mobility in an alkaline buffer with a specific PH. Separation also occurs according to electrolyte pH and electro-osmotic flow. All the samples were processed within a week from the date of collection following instructions manual of SEBIA Minicap Flex piercing and the diagnosis of cases were made based on electro-phoretogram generated by the instrument and complete blood count parameter.

Table 2: Reference Range of Complete Blood Count (Barbara J. Bain et al., 2017)

Parameters	Reference	Unit
Red Blood Count (RBC)	Male: 4.7-6.1 $x10^{12}$ Female: 4.2-5.4$x10^{12}$	Per litre (/l)
Hematocrit (HCT)	Male: 40-54 Female: 36-46	%
White Blood Count (WBC)	4-11$x10^{9}$	Per litre(/l)
Platelets	150-400 $x10^{9}$	Per litre(/l)
Mean Corpuscular Volume (MCV)	80-94	fL
Mean Corpuscular Hemoglobin (MCH)	28-33	pg
Mean Corpuscular Hemoglobin Concentration (MCHC)	31-38	%

Table 3: Parameters and Reference Range of Hemoglobin. (Aggarwal et al., 2020)

Age group (years)	Grading of anemia based on Hb level (WHO)			
	Normal	Mild	Moderate	Severe
Under 5 years	≥11 g/dl	10-10.9 g/dl	7-7.9 g/dl	<7 g/dl
5-11 years	≥11.5 g/dl	11-11.4 g/dl	8-10.9 g/dl	<8 g/dl
12-14 years	≥12 g/dl	11-11.9 g/dl	8-10.9 g/dl	<8 g/dl

Age group (years)	Grading of anemia based on Hb level (WHO)			
	Normal	Mild	Moderate	Severe
Adult Male (15 years and above)	≥13 g/dl	11-12.9g/dl	8-10.9 g/dl	<8 g/dl
Adult Female (15 years and above)	≥12 g/dl	11-11.9 g/dl	8-10.9 g/dl	<8 g/dl

Table 4: Reference values for Capillary electrophoresis (Kim et al., 2011)

Hb Types	Normal Reference Range
HbA1	96.8-97.8%
HbA2	2.2-3.2%
HbE	<1%(absent)
HbS	<1%(absent)
HbF	<0.2-1%
G6PD-UL*	Men 7.0 to 16.0 U/g of hemoglobin* Women 5.0 to 12.0 U/g of hemoglobin* Men 4 U/g of hemoglobin**

**Based on protocol of manufacturer* (Gandaki and Bagmati Province)

***Based on protocol of manufacturer* (Koshi Province)

2.9 Data Management and Analysis

Socio-demographic information and laboratory data were entered in Microsoft Excel and checked for completeness and outliers. Incomplete or inconsistent data were removed. The cleaned datasets were compiled and imported into SPSS version 25 for analysis. Descriptive statistics, including frequencies, percentages, and means, were calculated and presented in tables and graphs.

Table 5: Diagnostic Criteria for Hemoglobinopathies (Kohne, 2011)including Germany, due to immigration.\n\nMethod\nSelective review of the literature with consideration of national guidelines.\n\nResults\nThe hemoglobinopathies encompass all genetic diseases of hemoglobin. They fall into two main groups: thalassemia syndromes and structural hemoglobin variants (abnormal hemoglobins

Conditions	Criteria
Hemoglobinopathies	Those meeting criteria for Thalassemia trait, HbE abnormalities/disease/ trait, Sickle cell trait/disease and having low HbA
Thalassemia trait	HbA2 >3.5% MCV <80fL MCH <27pg
HbE abnormality	Presence of HbE

Conditions	Criteria
HbE trait	Presence of HbE Hb: normal to low HbA2 >3.5 % MCV <80fL MCH <27pg
Sickle cell trait	HbA (60–80%) HbS (20–40%). No or very low HbF
Sickle cell disease	HbS (80–100%) and no HbA. May have elevated HbF and HbA2.
Low HbA	<95%

2.10 Limitations

This study relied on CBC and Hb electrophoresis tests, but the absence of additional assessments, such as iron and vitamin B12 levels, may have limited the accurate determination of Hemoglobinopathies. Additionally, low Hemoglobin A levels were considered indicative of Hemoglobinopathies, though this may not always be the case. Further confirmatory tests are necessary for a more precise diagnosis.

Ideally, all samples should have been tested in a single laboratory under uniform conditions. However, due to distance and challenges in sample transportation from Primary Sampling Units (PSUs), testing was conducted in two provincial laboratories, which may have introduced variability. Blood sample collection in infants was constrained by the quantity available (less than 3 ml), which may have affected the completeness of testing in this age group. Timely conduction of laboratory testing was challenging, potentially affecting the accuracy and reliability of some results.

CHAPTER III RESULTS (FINDINGS)

3.1 Findings from Bagmati and Gandaki Province

A total of 549 participants were enrolled in the study, and blood samples were collected and tested from individuals in Bagmati and Gandaki provinces. However, three participants were excluded from the analysis due to incomplete data and insufficient blood sample volume for testing. As a result, data from 546 participants were included in the final analysis.

3.1.1 Geographical Distribution of Participants

Out of 546 participants, 53% (288) participants were enrolled from Chitwan and remaining (258) from Nawalpur district. In Nawalpur participants were enrolled from eight different wards of Madhyabindu Municipality, Kawasoti Municipality and Gaidakot Municipality. In Chitwan, participants were enrolled from five municipalities namely Bharatpur Sub-Metropolitan City, Ichhyakamana, Kalikamuni, Khairahani and Ratnanagar.

Table 6: Distribution of participants based on local levels **N =546**

SN.	District	Municipality	Number of wards	Frequency (n)	Percentage (%)
1	Chitwan	Bharatpur	4	128	23.5
2		Ratnanagar	1	31	5.7
3		Ichhyakamana	1	32	5.9
4		Kalikamuni	1	32	5.9
5		Khairahani	2	65	11.9
6	Nawalpur	Madhyabindu	4	129	23.6
7		Kawasoti	2	65	11.9
8		Gaidakot	2	64	11.7

3.1.2 Socio-Demographic Characteristics of Participants

The table presents the socio-demographic characteristics of the participants where majority of participants (51.6%) were females. Around 30% participants belong to age group 10 -14 years with mean age of 13.6 ± 6.7years. More than half (55.5%) participants have primary level education while only 1.5% reported

having no formal education. Majority (77.9%) of participants aged 15 years and above were unmarried and only 20.7% were married. About 44% participants were Tharu and among those majority belonged to Kathoriya Tharu (66.3%) followed by Nawalpuria tharu (13.6%).

Table 7: Socio-demographic characteristics of participants **N=546**

Variables	Frequency	Percentage
Gender		
Male	264	48.4
Female	282	51.6
Age group		
1-4 years	26	4.8
5-9 years	140	25.6
10-14 years	163	29.9
15-19 years	104	19.0
20-24 years	64	11.7
25-29 years	49	9.0
Mean [SD]		13.6 [6.7]
Education level		
No education	8	1.5
Primary	303	55.5
Secondary	133	24.4
Higher Secondary	72	13.2
Bachelor and above	30	5.5
Marital Status (15 years and above) N= 217		
Married	45	20.7
Refused to answer	3	1.4
Unmarried	169	77.9
Ethnicity		
Non-Tharu	305	55.9
Tharu	241	44.1

Variables	Frequency	Percentage
Sub Group of Tharu		
Kathoriya tharu	160	66.3
Nawalpuriya tharu	33	13.6
Rajput tharu	30	12.8
Aarkutwa or chitwania tharu	5	2.1
Other *	13	5.3

**Others: Jimdar, Kochila, Kumar, Lalpuria, Rana tharu, Paschuhan tharu*

The figure 1 displays the age distribution of participants among Tharu and Non-Tharu groups. Majority of under five children (61.5%) belonged to Non-Tharu community followed by participants aged 25-29 years (51%). However, most of the participants in age group 5-9 years, 10-14 years, 15-19 years and 20-24 years belonged to Tharu community.

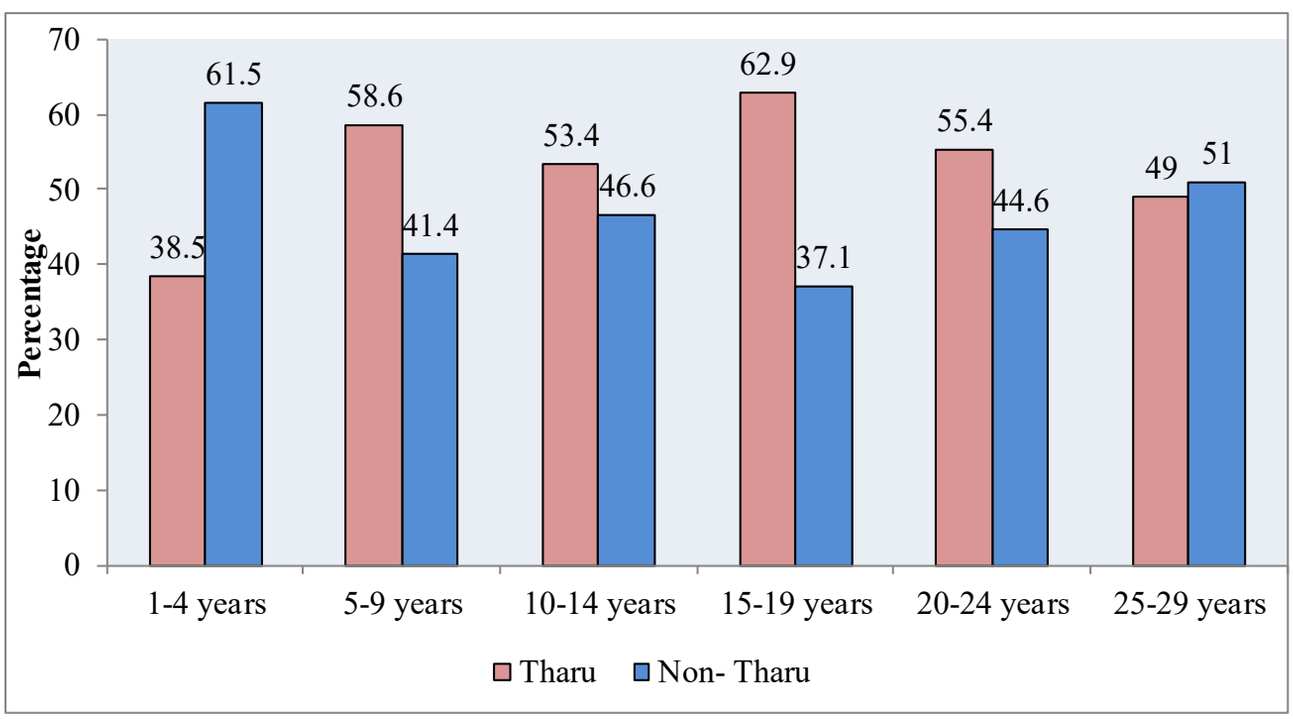


Figure 1: Distribution of participants based on age and ethnicity

3.1.3 History of Hemoglobinopathies

The table represents the clinical and family history of Hemoglobinopathies among participants. A small proportion (3.8%) of respondents had been screened or diagnosed for a hemoglobin disorder, with anemia or iron deficiency anemia being the most common condition (37.9%), followed by low hemoglobin or vitamin B12 deficiency (31.0%), blood infections (17.2%), and other conditions such as cancer, dengue, jaundice, and menorrhagia (13.8%). Only 1.1% of respondents reported having ever received a blood transfusion, with reasons including iron deficiency anemia (33.3%), pneumonia (16.7%), pregnancy (16.7%), blood loss during menstruation (16.7%), and unspecified causes (16.7%). Additionally, 4.0% of participants reported having family members with a hemoglobin disorder, most commonly their mother (50.0%), followed by siblings (22.7%), grandmothers (9.1%), aunties (9.1%),

and others such as wives, mothers-in-law, or uncles (13.6%). Most of these family members (86.4%) were alive, with reported disorders including anemia or iron deficiency anemia (31.8%), blood deficiency (22.7%), sickle cell anemia (9.1%), aplastic anemia (4.8%), and other unspecified or pregnancy-related conditions (9.0%).

Table 8: Clinical and family history of Hemoglobinopathies of participants **N = 546**

Variables	n (%)
Respondents screened/diagnosed for hemoglobin disorder (SCD and Thalassemia)	21 (3.8)
If yes, what (N=21)	
Anemia/Iron deficiency Anemia	11 (37.9)
Low Hemoglobin/Vitamin B12 deficiency	9 (31.0)
Blood infection	5 (17.2)
Other (Cancer, Dengue, Jaundice, Menorrhagia)	4 (13.8)
Respondents ever received blood transfusion	6 (1.1)
Reason for blood transfusion (N= 6)	
Iron deficiency anemia	2 (33.3)
Pneumonia	1 (16.7)
Pregnancy	1 (16.7)
Blood loss during period	1 (16.7)
Not mentioned	1 (16.7)
Family members with presence of hemoglobin disorder	22 (4.0)
If yes, relation (N=22)	
Mother	11 (50.0)
Siblings	5 (22.7)
Grand mother	2 (9.1)
Aunty	2 (9.1)
other (Wife, mother-in-law, uncle)	3 (13.6)
Are they alive? (N =22)	
Yes	19 (86.4)
Type of blood disorder among family member (N=22)	
Anemia/Iron deficiency anemia	7 (31.8)
Blood deficiency	5 (22.7)
Sickle cell anemia	2 (9.1)
Aplastic anemia	1 (4.8)
Other (Pregnancy, don't know)	6 (9)

3.1.4 History of Clinical Symptoms

Headache was the most frequently reported symptom (11.4%), followed by abdominal pain (6.6%) and vision problems (4.6%). Less frequently reported symptoms included chest pain and joint pain (4% each), extreme tiredness (3.3%), difficulty in breathing (2.9%), and pale discoloration of the palm or skin (1.3%). Rare symptoms included frequent infections and trouble with physical activities (0.5% each).

Table 9 : Clinical Symptoms **N=546**

Clinical symptoms	Frequency	Percentage
Headache	62	11.4
Abdomen pain	36	6.6
Joint pain	22	4.0
Chest pain	22	4.0
History of loss of consciousness or seizure	20	3.7
Extreme tiredness	18	3.3
Difficulty in breathing	16	2.9
Yellowish discoloration of eye	13	2.4
Pain in back of trunk	9	1.6
Pale discoloration of palm or skin	7	1.3
Painful swollen tips of fingers and toes	4	0.7
Others *	8	1.4

***** *Frequent infection, Trouble with physical activities, Vision problems*

3.1.5 Hematological Parameters of Participants

The table presents the hematological parameters of participants, detailing the mean, standard deviation, and their classification as low, normal, or high based on reference values. The mean white blood cell (WBC) count was 7829.2 [3496.2], with most participants (91.9%) having normal levels. Neutrophils had a mean of 52.2 [10.0], with 85.3% having normal range, while lymphocytes had a mean of 41.1 [9.5], and majority (51.1%) have high levels of lymphocytes.

The mean value of RBC was 4.8 [0.6] with 32.6% male participants and 20.6% female participants having RBC lower than normal range. Nearly half (48.9%) male participants and 30% female participants had low Hematocrit levels. The mean corpuscular volume (MCV) was 81.2 [9.4] and four out of ten participants had low MCV levels. The Mean corpuscular hemoglobin (MCH) was lower than normal range 25.5[5.0] with seven out of ten participants having lower MCH values. About 40% participants had mean corpuscular hemoglobin concentration (MCHC) less than normal range.

Table 10: Clinical parameters of participants **N= 546**

Parameters	Mean ±SD	Low n (%)	Normal n (%)	High n (%)	Reference value
WBC	7829.2± 349.6	4 (0.7)	502 (91.9)	40 (7.4)	4000-11000 cells/µL
Neutrophils	52.2±10.0	66 (12.1)	466 (85.3)	14 (2.6)	40-70%
Lymphocytes	41.1±9.5	9 (1.6)	258 (47.3)	279 (51.1)	20-40%
Eosinophil	2.9±1.6	4 (0.7)	524 (96.0)	18 (3.3)	1- 6%
Monocyte	3.6±2.9	76 (13.9)	442 (81.0)	28 (5.1)	2-8%
Basophil	-	-	546 (100)	-	<1%
RBC	4.8±0.6				
Male		86 (32.6)	165 (62.5)	13(4.9)	4.7 -6.1 million/mm3
Female		58 (20.6)	198 (70.2)	26 (9.2)	4.2-5.2 million/mm3
HCT	39.1±4.5				
Male		129(48.9)	134 (50.8)	1 (0.4)	40-54 %
Female		82 (29.1)	197 (69.9)	3 (1.1)	36-46%
MCV	81.2±9.4	214 (39.2)	296 (54.2)	36 (6.6)	80-94 fL
MCH	25.5±5.0	397 (72.7)	142 (26.0)	7 (1.3)	28- 33 pg
MCHC	31.1±1.5	224 (41.0)	322 (59.0)	-	31-38%
RDWCV	13.4±2.1	62 (11.3)	368 (67.5)	116 (21.2)	11.5 -14.5%
Platelets	226080 ± 99226	108 (19.7)	408 (74.8)	30 (5.5)	150000-400000/mcL

3.1.6 Hemoglobin Level of Participants

The majority of non-Tharu participants (68.2%) had normal hemoglobin levels, whereas nearly half of the Tharu participants were found to be anemic. Among the Tharu participants, 25% had mild anemia, 23% had moderate anemia, and 0.8% had severe anemia. Notably, females above 15 years of age exhibited the highest prevalence of anemia, with 31% classified as moderately anemic, 30% as mildly anemic, and 1% as severely anemic. Similarly, a comparatively higher proportion of children aged 12–14 years experienced mild (19.8%) and moderate anemia (22.9%). In contrast, the majority of males above 15 years of age (74.8%) and children aged 6–11 years (67.6%) had normal hemoglobin levels.

Table 11: Distribution of participants based on hemoglobin grading **N= 546**

Variables	Hemoglobin Grading				Total
	Severe n (%)	Moderate n (%)	Mild n (%)	Normal n (%)	
Ethnic Group					
Non- Tharu	0 (0.0)	47 (15.4)	50 (16.4)	208 (68.2)	305
Tharu	2 (0.8)	56 (23.2)	59 (24.5)	124 (51.5)	241
Age Group					
Under 5 children	-	4 (15.4)	5 (19.2)	17 (65.4)	26

Variables	Hemoglobin Grading				Total
	Severe n (%)	Moderate n (%)	Mild n (%)	Normal n (%)	
6-11 years children	-	39 (18.8)	28 (13.5)	140 (67.6)	207
12-14 years children	-	19 (19.8)	22 (22.9)	55 (57.3)	96
>15 years women	1(0.9)	36 (31.6)	34(29.8)	43(37.7)	114
>15 years men	1 (1.0)	5 (4.9)	20 (19.4)	77 (74.8)	105

Figure 2 illustrates the proportion of low hemoglobin anemia among Non-Tharu and Tharu ethnic groups across different age and gender categories. Among children under five years of age, anemia was more prevalent in Non-Tharu participants (50.0%) compared to Tharu participants (25.1%). However, for all other age groups, the prevalence of anemia was higher in the Tharu community. Specifically, 44.6% of Tharu children aged 6–11 years were anemic compared to 24.2% of Non-Tharu children in the same age group. Notably, among adult females above 15 years, 74.5% of Tharu participants were anemic, which is higher than the 52.4% observed in Non-Tharu females. In adult males above 15 years, anemia was also more prevalent in the Tharu group (31.0%) compared to Non-Tharu males (20.6%).

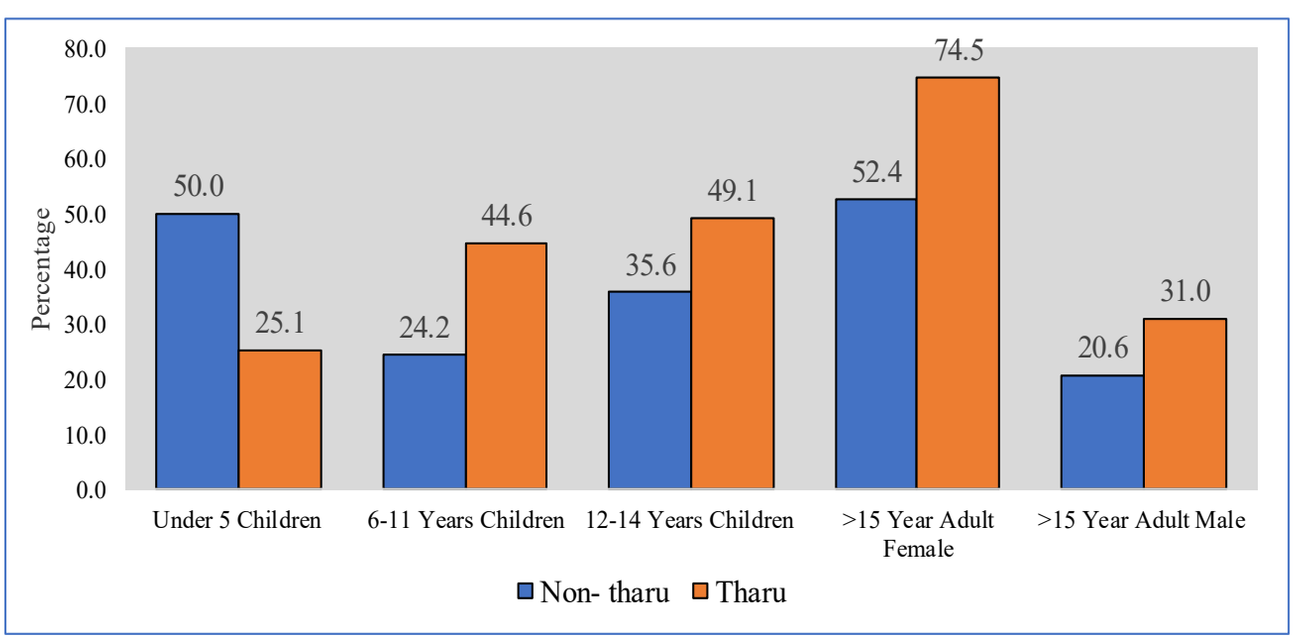


Figure 2: Proportion of low hemoglobin anemia among participants with low hemoglobin

3.1.7 Red Blood Cell (RBC) Level

Table 10: presents the distribution of RBC levels among participants based on age, gender, and ethnicity. Among Non-Tharu participants, 45.6% of males had low RBC levels compared to 28.5% of females. In contrast, only 16.2% of males and 10.5% of females in the Tharu community had low RBC levels. Overall, a higher proportion of males (32%) had low RBC levels compared to females (20.5%). When analyzed by age groups, the highest proportion of males with low RBC levels (33.8%) was observed in the 10–14 years age group. Among females, the majority (36.1%) with low RBC levels were in the 25–29 years age group. Conversely, 80% of males aged 15–19 years and 79.1% of females aged 10–14 years had normal RBC levels.

Table 10: Distribution of participants based on Age, Gender, Ethnicity and RBC level **N= 546**

Variables	RBC Level							
	Male				Female			
	Low n (%)	Normal n (%)	High n (%)	N	Low n (%)	Normal n (%)	High n (%)	N
Ethnicity								
Non-Tharu	67(45.6)	79(53.7)	1(.7)	147	45(28.5)	108(68.4)	5(3.2)	158
Tharu	19(16.2)	86(73.5)	12(10.3)	117	13(10.5)	90(72.6)	21(16.9)	124
Age group								
1-4 years	2 (18.2)	8(72.7)	1(9.1)	11	1(6.7)	10(66.7)	4(26.7)	15
5-9 years	36(14.3)	35(47.9)	2(2.7)	73	10(14.9)	51(76.1)	6(9.0)	67
10-14 years	26(33.8)	48(62.3)	3(3.9)	77	12(14.0)	68(79.1)	6(7.0)	86
15-19 years	8(13.3)	47(80.0)	4(6.7)	59	12(26.7)	30(66.7)	3(6.7)	45
20-24 years	10(32.3)	18(58.1)	3(9.7)	31	10(30.3)	21(63.6)	2(6.1)	33
25-29 years	4(30.8)	9(69.2)	0(0.0)	13	13 (36.1)	18 (50.0)	5 (13.9)	36

3.1.8 Hematocrit (HCT) Level

In terms of hematocrit levels, the majority of male participants from both the Tharu (52.1%) and Non-Tharu (46.3%) groups had low hematocrit levels compared to their female counterparts. Age-wise analysis revealed that a significant proportion (89%) of male children aged 5–9 years had low hematocrit levels. Similarly, the majority (77.9%) of females aged 15–19 years also exhibited low hematocrit levels.

Table 11: Distribution of participants based on Age, Ethnicity and HCT level **N= 546**

Variables	HCT Level							
	Male				Female			
	Low n (%)	Normal n (%)	High n (%)	N	Low n (%)	Normal n (%)	High n (%)	N
Ethnicity								
Non-Tharu	68(46.3)	78(53.1)	1(0.7)	147	44(27.8)	111(70.3)	3(1.9)	158
Tharu	61(52.1)	56(47.9)	-	117	38(30.6)	86(69.4)	-	124
Age group								
1-4 years	9(81.8)	2(18.2)	-	11	5(33.3)	10(66.7)	-	15
5-9 years	65(89.0)	8(11.0)	-	73	21(31.3)	45(67.2)	1(1.5)	67
10-14 years	37(48.1)	40(51.9)	-	77	21(24.4)	65(75.6)	-	86
15-19 years	6(10.0)	53(90.0)	-	59	9(20.0)	35(77.8)	1(2.2)	45
20-24 years	8(25.0)	22(71.9)	1(3.1)	31	12(36.4)	21(63.6)	-	33
25-29 years	4(30.8)	9(69.2)	-	13	14(38.9)	21(58.3)	1(2.8)	36

3.1.9 Mean Corpuscles Volume (MCV) Level

The Non-Tharu group demonstrated a higher proportion of participants with normal MCV levels, with 70.7% of males and 70.3% of females falling within the normal range. In contrast, the Tharu group had a significantly higher percentage of participants with low MCV levels, particularly among males (59.8%) and females (70.2%). Overall, a greater proportion of females in both ethnic groups exhibited low MCV levels compared to male counterparts. Age-wise analysis revealed that males in the 1–4 years' age group had the highest prevalence of low MCV levels (72.7%), followed by those in the 5–6 years' age group (42.5%). Similarly, among females, more than half (53%) of children aged 1–5 years had MCV levels below the normal range.

Table 12: Distribution of participants based on Age, Ethnicity and MCV level **N= 546**

Variables	MCV level							
	Male				Female			
	Low n (%)	Normal n (%)	High n (%)	N	Low n (%)	Normal n (%)	High n (%)	N
Ethnicity								
Non-Tharu	24(16.3)	104(70.7)	19(12.9)	147	33(20.9)	111(70.3)	14(8.9)	158
Tharu	70(59.8)	45(38.5)	2(1.7)	117	87(70.2)	36(29.0)	1(0.8)	124
Age Group								
1-4 years	8(72.7)	3(27.3)	-	11	8(53.3)	7(46.7)	-	15
5-9 years	31(42.5)	41(56.2)	1(1.4)	73	25(37.3)	41(61.2)	1(1.5)	67
10-14 years	29(37.7)	48(62.3)	-	77	45(52.3)	38(44.2)	3(3.5)	86
15-19 years	13(22.0)	36(61.0)	10(16.9)	59	16(35.6)	28(62.2)	1(2.2)	45
20-24 years	10(32.3)	13(41.9)	8(25.8)	31	12(36.4)	15(45.5)	6(18.2)	33
25-29 years	3(23.1)	8(61.5)	2(15.4)	13	14(38.9)	18(50.0)	4(11.1)	36

3.1.10 Mean Corpuscular Hemoglobin (MCH) Level

The majority of males (58.5%) and females (61.4%) in the Non-Tharu community were found to have low MCH levels. In comparison, an even higher proportion of Tharu males (85.5%) and females (91.9%) exhibited low MCH levels. Only 12.8% of Tharu males and 7.3% of Tharu females had normal MCH levels. All children under the age of five were reported to have low MCH levels. A trend of decreasing MCH levels was observed with younger age groups. Among both males and females, most children aged 5–9 years and 10–14 years had low MCH levels. However, 53.8% of males aged 25–29 years had normal MCH levels, whereas only 27.8% of females in the same age group exhibited normal MCH values.

Table 13: Distribution of participants based on Age, Ethnicity and MCH level **N= 546**

Variables	MCH level							
	Male				Female			
	Low n (%)	Normal n (%)	High n (%)	N	Low n (%)	Normal n (%)	High n (%)	N
Ethnicity								
Non-Tharu	86(58.5)	58(39.5)	3(2.0)	147	97(61.4)	60(38.0)	1(.6)	158
Tharu	100(85.5)	15(12.8)	2(1.7)	117	114(91.9)	9(7.3)	1(.8)	124
Age group								
1-4 years	11(100.0)	-	-	11	15(100.0)	-	-	15
5-9 years	62(84.9)	9(12.3)	2(2.7)	73	56(83.6)	11(16.4)	-	67
10-14 years	60(77.9)	17(22.1)	-	77	65(75.6)	21(24.4)	-	86
15-19 years	27(45.8)	31(52.5)	1(1.7)	59	28(62.2)	16(35.6)	1(2.2)	45
20-24 years	21(67.7)	9(29.0)	1(3.2)	31	21(63.6)	11(33.3)	1(3.0)	33
25-29 years	5(38.5)	7(53.8)	1(7.7)	13	26(72.2)	10(27.8)	-	36

3.1.11 Mean Corpuscular Hemoglobin Concentration (MCHC) Level

The Tharu group exhibited a significantly higher proportion (58.2%) of participants with low MCH levels. Among females, a larger percentage (47.5%) had low MCHC levels compared to males. Age-wise analysis revealed that more than half (53.8%) of participants aged 1–4 years had low MCHC levels, followed by those in the 20–24 years' age group. In contrast, approximately 65% of participants aged 15–19 years had normal MCHC levels.

Table 14: Distribution of participants based on Age, Ethnicity and MCHC level **N= 546**

Variables	MCHC level		Total
	Low n (%)	Normal n (%)	
Age group			
1-4 years	14 (53.8)	12 (46.2)	26
5-9 years	51 (36.4)	89 (63.6)	140
10-14 years	70 (42.9)	93 (57.1)	163
15-19 years	37 (35.6)	67 (64.4)	104
20-24 years	30 (47.7)	34 (52.3)	64
25-29 years	22 (44.9)	27 (55.1)	49

Variables	MCHC level		Total
	Low n (%)	Normal n (%)	
Gender			
Male	90 (34.1)	174 (65.9)	264
Female	134 (47.5)	148 (52.5)	282
Ethnicity			
Non-Tharu	84 (27.5)	221 (72.5)	305
Tharu	140 (58.4)	101 (41.6)	241

3.1.12 Platelets Level

The proportion of male participants with low platelet levels was 19.2%, while 20.2% of female participants had low platelet levels. More than 70% of both male and female participants had normal platelet levels. Additionally, 30% of Tharu participants and 11.5% of non-Tharu participants exhibited platelet values lower than normal. Regarding age, the highest proportion of low platelet levels was observed in the 20-24 years' age group (31.3%), followed by the 25-29 years' age group (28.6%).

Table 15: Distribution of participants based on Age, Ethnicity and platelets level **N= 546**

Variables	Platelets level			Total
	Low n (%)	Normal n (%)	High n (%)	
Gender				
Male	51 (19.2)	201 (76.3)	12 (4.5)	264
Female	57 (20.2)	207 (73.4)	18 (6.4)	282
Ethnicity				
Non-Tharu	35 (11.5)	247 (81.0)	23 (7.5)	305
Tharu	73 (30.0)	161 (67.1)	7 (2.9)	241
Age Group				
1-4 years	2 (7.7)	20 (76.9)	4 (15.4)	26
5-9 years	20 (14.3)	108 (77.1)	12 (8.6)	140
10-14 years	29 (17.8)	125 (76.7)	9 (5.5)	163
15-19 years	23 (22.1)	81 (77.9)	0 (0.0)	104
20-24 years	20 (31.3)	40 (62.5)	4 (6.3)	64
25-29 years	14 (28.6)	34 (69.4)	1 (2.0)	49

3.1.13 G6PD Level and Clinical Symptoms among G6PD Deficient Participants

A total of 250 male participants underwent the G6PD test to assess for G6PD deficiency, with 17 participants (6.8%) identified as having the condition. Among the Tharu participants, 8.4% were found to have G6PD deficiency, while 5.6% of non-Tharu participants exhibited the condition. No cases of G6PD deficiency were observed in children under five years of age or in participants from the 25-29 years' age group. The highest proportion of G6PD deficiency was recorded in the 20-24 years' age group (10.3%), followed by the 15-19 years' age group (8.9%).

Table 16: Distribution of participants based on Age, Ethnicity and G6PD level **N= 250**

Variables	G6PD level		Total
	Low (%)	Normal/ High (%)	
Ethnicity			
Non-Tharu	8 (5.6%)	135 (94.4%)	143
Tharu	9 (8.4%)	98 (91.6%)	107
Total	17 (6.8%)	233 (93.2%)	250
Age group			
1–4 years	0 (0.0%)	11 (100.0%)	11
5–9 years	5 (7.2%)	64 (92.8%)	69
10–14 years	4 (5.5%)	69 (94.5%)	73
15–19 years	5 (8.9%)	51 (91.1%)	56
20–24 years	3 (10.3%)	26 (89.7%)	29
25–29 years	0 (0.0%)	12 (100.0%)	12
Total	**17 (6.8%)**	**233 (93.2%)**	**250**

Among 17 participants with G6PD deficiency, 15 participants had no any symptoms, while one participant had yellowish discoloration of skin and one participant have headache and abdominal pain.

Table 17 : Clinical Symptoms among G6PD deficiency participants **N=17**

Symptoms	Frequency	Percentage
No any symptoms	15	88.2
One (Yellowish discoloration of skin)	1	5.9
Two (Headache and abdominal pain)	1	5.9

3.1.14 Hemoglobinopathies Analysis Using Capillary Electrophoresis

Capillary electrophoresis was conducted to identify Hemoglobinopathies among 546 samples. The analysis detected the presence of hemoglobin variants, including HbA, HbA2, HbE, HbF, and HbS. Any abnormalities in these hemoglobin types were classified as Hemoglobinopathies. Hemoglobin abnormalities were identified in 17.4% (95 samples) of the total, with thalassemia trait being the

most common disorder. Additionally, 0.2% (1 sample) exhibited the HbE trait. No cases of HbS trait were found. A significant proportion (14.6%) showed low HbA levels, classified as undetermined Hemoglobinopathies.

Table 18 : Distribution of Hemoglobinopathies among participants **N=546**

Variables	Frequency	% among total Population	% among Hemoglobinopathies
Presence of Hemoglobinopathies	95	17.4	
Categories of Hemoglobinopathies (N=95)			
1. Thalassemia trait	14	2.6	14.7
1. HbE trait	1	0.2	1.1
2. Sickle cell	-	-	-
3. Low HbA (<95%)	80	14.6	84.2

The prevalence of Hemoglobinopathies was higher among females (20.2%) compared to males; however, this association was not statistically significant. Age-wise distribution indicated that participants aged 25–29 years exhibited the highest prevalence (24.5%), followed by those aged 5–9 years (20%). Ethnicity-wise analysis revealed that 22% of Tharu participants had Hemoglobinopathies, and this association was statistically significant ($p < 0.05$). Nonetheless, a notable proportion (14%) of non-Tharu participants also exhibited some forms of Hemoglobinopathies.

Table 19: Association of Hemoglobinopathies with socio-demographic variables **N=546**

Variables	Presence of Hemoglobinopathies		Chi-square	p-value
	Yes	No		
Gender			3.212	0.073
Male	38 (14.4)	226 (85.6)		
Female	57 (20.2)	225 (79.8)		
Age group			0.064	0.801
1-4 years	5 (19.2)	21 (80.8)		
5-29 years	90 (17.3)	430 (82.7)		
Ethnicity			6.331	0.012*
Non-Tharu	42 (13.8)	263 (86.2)		
Tharu	53 (22.0)	188 (78.0)		

Table 20 presents the distribution of participants diagnosed with various Hemoglobinopathies. A higher proportion of females (3.6%) were found to have thalassemia trait/disease compared to males. Age-wise distribution showed that 2.5% participants aged 5–29 years had thalassemia trait/disease. Ethnically, 4.6% of Tharu participants were identified with thalassemia trait/disease compared to Non-Tharu participants. Additionally, HbE abnormality was observed among Tharu females aged 5–29 years, while Low HbA was more among Tharu, females and 5-29 years of age.

Table 20: Association of various types of Hemoglobinopathies and socio-demographic variables

Variables	Thalassemia trait/disease		HbE abnormality		Low HbA	
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
Gender						
Male	4 (1.5)	260 (98.5)	-	264(100.0)	38 (14.4)	226(85.6)
Female	10 (3.6)	271(96.4)	2 (0.7)	280(99.3)	57(20.2)	225(79.8)
Age group						
1-4 years	1(3.8)	25 (96.2)	-	26 (100.0)	5 (19.2)	21 (80.8
5-29 years	13(2.5)	507 (97.5)	2 (0.4)	518(99.6)	90(17.3)	430 (82.7)
Ethnicity						
Non tharu	3(1.0)	302 (99.0)	-	305(100.0)	42(13.8)	263(86.2)
Tharu	11(4.6)	1(0.4)	2(0.8)	239 (99.2)	53 (22.0)	188(78.0)

Among 95 participants with Hemoglobinopathies, 83 participants do not have experienced any symptoms and 5 of them have experienced any one symptom related to Hemoglobinopathies. Among the symptoms headache was most commonly experienced symptoms by the participants followed by chest pain.

Table 21: Clinical symptoms among participants with Hemoglobinopathies **N= 95**

Variables	Frequency	Percentage
Number of symptoms		
No any symptoms	83	87.4
Any one of the symptoms	5	5.3
Any two of the symptoms	4	4.2
Any three of the symptoms	1	1.1
Any four of the symptoms	2	2.1
Most commonly experienced Symptoms		
Headache	8	8.4
Chest pain	4	4.2
Abdomen pain	2	2.1
Joint pain	2	2.1
Unconsciousness/seizure	2	2.1
Vision problems	1	2.1
Extreme tiredness	1	2.1
Back pain	1	2.1
Painful swelling of fingers/toes	1	2.1
Difficulty breathing	1	2.1
Pallor skin	1	2.1

3.2 Findings of Koshi Province

A total of 1140 participants were enrolled in the study, and blood samples were collected and tested from individuals in Koshi province. However, six participants were excluded from the analysis due to incomplete data and insufficient blood sample volume for testing. As a result, data from 1,134 participants were included in the final analysis.

Analysis of total 1,134 participants from three districts of Koshi province namely Sunsari (34.6%), Morang (34.1%), and Jhapa (31.3%) was done.

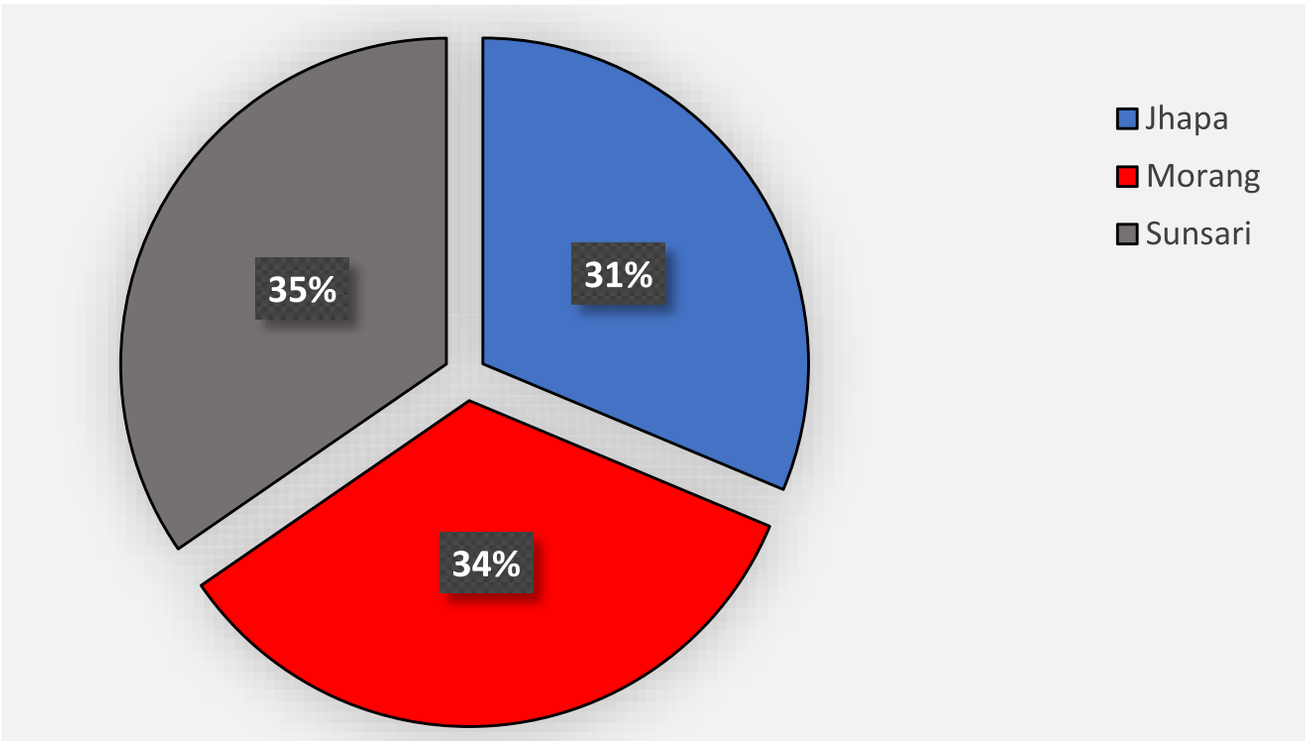


Figure 3: Research participant and geographical distribution of Koshi province

The study included a total of 29 municipalities from three districts in Koshi Province namely Jhapa, Sunsari and Morang. Specifically, three wards were selected from Belbari, and two wards each were selected from Biratnagar, Rangeli, Mechinagar, and Duhabi. Additionally, one ward from each of the remaining municipalities was included in the study. About 30-35 participants from each ward were enrolled in the study (Table 22).

3.2.1 Socio-Demographic Information of Participants

Among the total participants, females constituted a higher proportion (54.5%) compared to males (45.5%). The majority of participants belonged to the 10–14 years age group (25.7%), with a mean age of 14.9 ± 7.2 years. Among participants aged above 15 years, 72.2% were unmarried. Regarding educational status, 4.6% of participants were illiterate, while the majority (37.9%) had primary-level education, followed closely by secondary-level education (36.7%). Ethnic composition showed that a significant portion of participants (72.2%) were from Non-Tharu ethnic groups, while Tharu individuals comprised 27.8%. Within the Tharu subgroup, Kochila Tharu represented the largest group (39.9%), followed by Morangya Tharu (18.7%) (Table 23).

Table 22 : Distribution of participants based on local level **N =1134**

S.N	District	Municipality	Number of Wards	Frequency	Percentage %
1	Morang	Belbari	3	97	8.5
2		Biratnagar	2	66	5.8
3		Rangeli	2	66	5.8
4		Dhampal	1	32	2.8
5		Gramthan	1	32	2.8
6		Katahari	1	32	2.8
7		Sundarharaicha	1	31	2.9
8		Sunwarshi	1	31	2.7
9	Jhapa	Mechinagar	2	65	5.7
10		Birtamode	1	34	3
11		Kanchana Kawal	1	34	3
12		Jahada	1	33	2.9
13		Arjundhara	1	32	2.8
14		Bahradashi	1	32	2.8
15		Bhadrapur	1	32	2.8
16		Damak	1	32	2.8
17		Shiva Shatakshi	1	31	2.7
18		Gauradaha	1	30	2.6
19	Sunsari	Harinagara	1	33	2.9
20		Paanwari	1	34	3
21		Dakha	1	32	2.8
22		Barahakshetra	1	34	3
23		Dewangung	1	32	2.8
24		Gadhi, Aaurabani	1	34	3
25		Duhabi	2	64	5.6
26		Inaruwa	1	32	2.8
27		Ramdhuni	1	32	2.8
28		Dharan	1	31	2.7
29		Itahari	1	35	3.1

Table 23: Socio-demographic information of participants **N=1134**

Variables	Frequency (n)	Percentage (%)
Gender		
Female	618	54.5
Male	516	45.5
Age		
1-4 years	62	5.5
5-9 years	227	20.0
10-14 years	292	25.7
15-19 years	250	22.0
20-24 years	154	13.6
25-29 years	149	13.1
Mean [SD]	14.9 ± 7.2	
Marital status (15 years above, N=553)		
Married	154	27.8
Unmarried	399	72.2
Education status		
Illiterate	52	4.6
Primary Level	432	37.9
Secondary Level	416	36.7
Higher Secondary Level	164	14.4
Bachelor level and above	7	6.6
Ethnicity		
Non-Tharu	819	72.2
Tharu	315	27.8
Sub group of tharu (N=316)		
Kochila Tharu	126	39.9
Morangya Tharu	58	18.7
Saptariya Tharu	35	11.1
Kharel Tharu	31	9.8
Purbahatharu	26	8.2
Danuwar	25	7.9
Other*	14	4.4

**Majhi tharu, Bhagat khawas, Chaudhary morangia*,

3.2.2 Socio-Demographic Information of Participants

The table 24 highlights key findings related to hemoglobin disorder screening, blood transfusions, and family history among respondents. Among the total participants, 2.6% were screened or diagnosed with a hemoglobin disorder. Anemia or iron deficiency emerged as the most common condition (37.9%), followed by low hemoglobin levels or vitamin B-12 deficiency (31%). Regarding blood transfusions, approximately 2% of participants reported recent transfusions, the majorities (62.5%) of which were attributed to conditions other than Hemoglobinopathies. Family history analysis revealed that 1.8% (21 participants) reported a history of hemoglobin disorders among family members. Among these cases, mothers constituted the majority (57%). Anemia was the most frequently reported hemoglobin disorder (38.1%) among family members, followed by blood deficiencies (28%).

3.2.3 History of Clinical Symptoms

Among the total participants, 30.4% reported having experienced symptoms associated with Hemoglobinopathies. Among those who reported symptoms, headaches were the most commonly experienced (40%), followed by abdominal pain (30.4%) and vision problems (18.3%). Additionally, among 320 male participants aged above 15 years, only 2 individuals reported experiencing involuntary penile erection. [Table 25]

Table 24: Clinical and Family History Hemoglobinopathies of Respondents **N=1,134**

Variables	**n (%)**
Respondents screened for hemoglobin disorder (SCD and Thalassemia)	29 (2.6)
Type of hemoglobin disorder (N=29)	
Anemia/Iron Deficiency	11 (37.9)
Low HB/Vit B12 Deficiency	9 (31.0)
Blood Infection	5 (17.2)
Other (Cancer, Dengue, Jaundice, Menorrhagia)	4 (13.8)
Respondents ever received blood transfusion	24 (2.1)
Reason for blood transfusion (N= 24)	
Conditions other than Hemoglobinopathies (Hospitalization, Blood Loss, pregnancy, surgery)	15 (62.5)
Iron Deficiency Anemia	5 (20.8)
Sickle Cell Anemia	4 (16.7)
Family members with presence of hemoglobin disorder	21 (1.8)
If yes, relation (N=21)	
Mother	12 (57.1)
Father	1 (4.8)
Grand Mother	2 (9.5)

Variables	n (%)
Siblings	2 (9.5)
Other (Cousin, Daughter, Uncle)	4 (19.0)
Type of Hemoglobin disorder among family member (N=21)	
Anemia	8 (38.1)
Blood Deficiency	6 (28.6)
Sickle Cell Anemia	4 (19.0)
Aplastic Anemia	1 (4.8)
Other (Cancer, Surgery)	2 (9.5)

Table 25: Clinical symptoms experienced by participants

Variables	Frequency	Percentage
Ever experienced symptoms of Hemoglobinopathies	345	30.4
Commonly experienced symptoms (N = 345)		
Headache	138	40.0
Abdomen pain	105	30.4
Vision problems	63	18.3
Joint pain	54	15.7
Extreme tiredness	45	13.0
Chest pain	38	11.0
Frequent infection	35	10.1
Difficulty in breathing	29	8.4
Pain in back of trunk	29	8.4
Pale discoloration of palm or skin	28	8.1
Yellowish discoloration of eye	21	6.1
Trouble with physical activities	15	4.3
History of loss of consciousness or seizure	14	4.1
Painful swollen tips of fingers and toes	9	2.6
Leg ulcer	7	2.0
Delayed growth or puberty	6	1.7
Involuntary penile erection (N=320) only male above 13 years)	2	0.2

The table 26 presents clinical parameters for 1134 respondents, categorized as low, normal, or high. Most parameters, including WBC (87.9%), neutrophils (84.2%), and monocytes (64.4%), were within the normal range. High eosinophil levels were observed in 31.3%, while high RDWCV was noted in 45.8%. For RBC, normal levels were prevalent in both males (77.9%) and females (76.7%), with low HCT levels more common in males (40.1%) compared to females (18.1%). Low MCV (44.6%) and MCH (84.4%) were frequently observed, while MCHC was predominantly within the normal range (65.8%). Normal platelet counts were recorded in 80.4% of respondents with mean value of 262111.0 ± 97004.6.

Table 26: Hematological Parameters of participants **N= 1,134**

Parameters	Mean ± SD	Low n (%)	Normal n (%)	High n (%)	Reference value
WBC	8848.7 ± 7132.7	15 (1.3)	997 (87.9)	122 (10.8)	4000-11000 cells/µL
Neutrophils	56.5 ± 10.6	79 (7.0)	955 (84.2)	100 (8.8)	40-70%
Lymphocytes	30.2 ± 9.2	142 (12.5)	849 (74.9)	143 (12.6)	20-40%
Eosinophil	5.4 ± 4.6	45 (4.0)	737 (64.7)	355 (31.3)	1- 6%
Monocyte	7.4 ± 2.8	2 (0.2)	734 (64.4)	404 (35.4)	2-8%
Basophil	-	-	1134 (100)	-	<1%
RBC	5.01 ± 0.6				
Male		82 (15.9)	402 (77.9)	32 (6.2)	4.7 -6.1 million/ mm3
Female		66 (10.7)	474 (76.7)	78 (12.6)	4.2-5.2 million/ mm3
HCT	39.95 ± 4.39				
Male		207 (40.1)	306 (59.3)	3 (0.6)	40-54 %
Female		112 (18.1)	502 (81.2)	4 (0.6)	36-46%
MCV	80.28 ± 9.52	505 (44.6)	596 (52.6)	32 (2.8)	80-94 fL
MCH	24.55 ± 3.34	957 (84.4)	177 (15.6)	-	28- 33 pg
MCHC	30.46± 1.24	746 (65.8)	388 (34.2)	-	31-38%
RDWCV	15.30 ± 3.04	-	615 (54.2)	519 (45.8)	11.5 -14.5%
Platelets	262111.0 ± 97004.6	133 (11.4)	912 (80.4)	89 (7.8)	150000-400000/mcL

3.2.4 Hemoglobin Level

The hemoglobin grading analysis revealed disparities in anemia prevalence across ethnic and age groups. Among the Non-Tharu population, the majority (64.3%) had normal hemoglobin levels, while the Tharu group showed a higher prevalence of anemia, with only 41.6% having normal levels and 24.4% and 33.3% exhibiting moderate and mild anemia, respectively. Age-wise, anemia was more prevalent among women over 15 years (53.1%) and children under 5 years (47.9%), with lower rates among men over 15 years, where 81.5% had normal hemoglobin. Severe anemia was rare but slightly higher in Tharu individuals and specific subgroups like women and children.

Table 27: Distribution of participants based on Age, Ethnicity and Hemoglobin Level **N=1,134**

Variables	Hemoglobin Grading				Total
	Severe n (%)	Moderate n (%)	Mild n (%)	Normal n (%)	
Ethnic Group					
Non- Tharu	-	110 (13.3)	184 (22.3)	527 (64.3)	819
Tharu	2 (0.6)	77 (24.4)	105 (33.3)	131 (41.6)	315
Age Group					
Under 5 children	-	7 (11.3)	23 (36.6)	32 (51.8)	62
6-11 years children	1(0.3)	81 (24.0)	66 (19.6)	190 (56.4)	338
12-14 years children	-	30 (16.5)	56 (30.8)	96 (52.7)	183
>15 years women	1(0.3)	63 (19.7	106 (33.1)	150 (46.9)	320
>15 years men	1 (0.4)	6 (2.6)	36 (15.5)	190 (81.5)	233

Figure 4: illustrates the proportion of anemia (low hemoglobin) among Non-Tharu and Tharu ethnic groups across different age and gender categories. Overall, Tharu participants had a higher prevalence of anemia compared to Non-Tharu participants across all age groups. The highest proportion of anemia among Tharu participants was observed in children aged 12–14 years (72.9%), while the lowest proportion was seen in males aged 15 years and above (27.6%). Similarly, among females aged 15 years and above, approximately 64% of Tharu and around 50% of Non-Tharu participants were found to be anemic. These findings indicate a higher burden of anemia among the Tharu population, particularly among younger age groups and females.

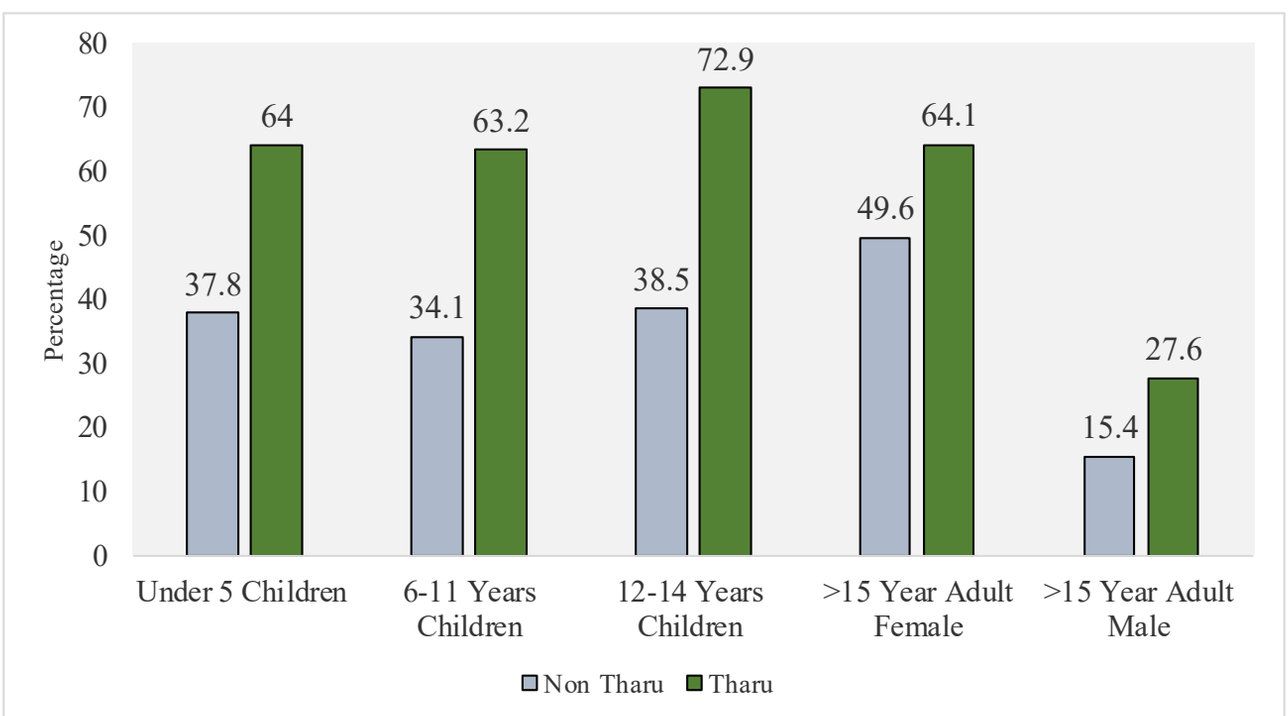


Figure 4: Age-wise Proportion of Tharu and Non-Tharu Individuals among Confirmed Anemia (Low Hemoglobin) Cases

The table presents RBC levels among 1134 participants based on age, gender, and ethnicity. Among males, low RBC levels were more common in Non-Tharu (20.4%) compared to Tharu (4.7%), while high RBC levels were more frequent in Tharu (16.1%). Among females, low RBC levels were also higher in Non-Tharu (14.2%) than in Tharu (1.2%), but high RBC levels were significantly higher in Tharu females (35.5%). Age-wise, low RBC levels were most frequent in males aged 5-9 years (24.3%) and females aged 15-19 years (16.7%), while high RBC levels were highest in males aged 20-24 years (15.6%) and females aged 1-4 years (23.7%). Normal RBC levels dominated across all groups.

Table 28: Distribution of RBC level among participants based demographic variables

Variables	RBC Level							
	Male				Female			
	Low n (%)	Normal n (%)	High n (%)	N	Low n (%)	Normal n (%)	High n (%)	N
Ethnicity								
Non-Tharu	75(20.4)	284(77.4)	8(2.2)	367	64(14.2)	369(81.6)	19(4.2)	452
Tharu	7(4.7)	118(79.2)	24(16.1)	149	2(1.2)	105(63.3)	59(35.5)	166
Age group								
1-4 years	2(8.0)	22(88.0)	1(4.0)	25	2(7.8)	26(68.5)	9(23.7)	37
5-9 years	26(24.3)	79(73.8)	2(1.9)	107	7(5.8)	95(79.2)	18(15)	120
10-14 years	31(20.5)	117(77.5)	3(2.0)	151	9(6.4)	117(83.0)	15(10.6)	141
15-19 years	10(8.1)	105(84.7)	9(7.3)	124	21(16.7)	89(70.6)	16(12.7)	126
20-24 years	5(7.8)	49(76.6)	10(15.6)	64	13(14.4)	70(77.8)	7(7.8)	90
25-29 years	8(17.8)	30(66.7)	7(15.6)	45	14(13.5)	77(74.0)	13(12.5)	104

The HCT (hematocrit) level analysis reveals significant variations across ethnicity, gender, and age groups. Among males, low HCT levels were higher in Tharu (51.0%) compared to Non-Tharu (35.7%), while normal levels were more prevalent in Non-Tharu (63.8%). Among females, low HCT levels were also higher in Tharu (22.9%) than Non-Tharu (16.4%), with normal levels dominating in both groups. Age-wise, low HCT levels were most frequent in males aged 1-4 years (88.0%) and females in the same group (35.1%), while normal HCT levels were highest in males aged 15-19 years (93.5%) and females aged 10-14 years (85.8%). High HCT levels were rare across all groups.

Table 29: Distribution HCT level among participants based on demographic variables

Variables	HCT Level							
	Male				Female			
	Low n (%)	Normal n (%)	High n (%)	N	Low n (%)	Normal n (%)	High n (%)	N
Ethnicity								
Non Tharu	131(35.7)	233(63.8)	3(0.8)	367	74(16.4)	377(83.4)	1(.2)	452
Tharu	76(51.0)	73(49.0)	-	149	38(22.9)	125(75.3)	3(1.8)	166

Variables	HCT Level							
	Male				Female			
	Low n (%)	Normal n (%)	High n (%)	N	Low n (%)	Normal n (%)	High n (%)	N
Age group								
1-4 years	22 (88.0)	3 (12.0)	-	25	13(35.1)	24(64.9)	-	37
5-9 years	82 (76.6)	25 (22.4)	-	107	25 (20.8)	95(79.4)	-	120
10-14 years	88 (58.3)	63(41.7)	-	151	20 (14.2)	121(85.8)	-	141
15-19 years	8(6.5)	116(93.5)	-	124	23 (18.3)	101(80.2)	2(1.6)	126
20-24 years	4(6.3)	60(93.8)	1(3.1)	64	12(13.3)	77(85.6)	1(1.1)	90
25-29 years	3(6.7)	39 (86.7)	3(6.7)	45	19(18.3)	84(80.8)	1(1.0)	104

The table 30 outlines MCV (mean corpuscular volume) levels among participants based on age, gender, and ethnicity. Low MCV levels were significantly higher in Tharu males (83.2%) and females (85.0%) compared to Non-Tharu males (32.7%) and females (26.8%). Normal MCV levels were predominant in Non-Tharu males (62.4%) and females (69.7%), while high MCV levels were rare across all groups, with the highest observed in males aged 15-19 years (5.6%). Age-wise, low MCV levels were most frequent in males aged 1-4 years (96.0%) and females in the same group (73.0%), while normal MCV levels were highest in males aged 15-19 years (65.3%) and females aged 10-14 years (56.0%).

Table 30: Distribution of MCV level among participants based on demographic variables

Variables	MCV level							
	Male				Female			
	Low n (%)	Normal n (%)	High n (%)	N	Low n (%)	Normal n (%)	High n (%)	N
Ethnicity								
Non-Tharu	120(32.7)	231(62.4)	16(4.4)	367	121(26.8)	315(69.7)	16 (3.5)	452
Tharu	124(83.2)	25(16.8)	-	149	141(85.0)	25(15.0)	-	166
Age Group								
1-4 years	24 (96.0)	1 (4.0)	-	25	27(73.0)	10(27.0)	-	37
5-9 years	72 (67.3)	35 (32.7)	-	107	68 (56.7)	51(42.5)	1(0.8)	120
10-14 years	82 (53.9)	68 (45.4)	1 (0.7)	151	60 (42.6)	79 (56.0)	2(1.4)	141
15-19 years	36(29.0)	81(65.3)	7 (5.6)	124	45(35.7)	75(59.5)	6(4.8)	126
20-24 years	21 (32.8)	41(64.1)	2(3.1)	64	29(32.2)	60(66.7)	1(1.1)	90
25-29 years	9(20.0)	30 (66.7)	6(13.3)	45	33 (31.7)	65 (62.5)	6 (5.8)	104

The table 31 highlights the distribution of MCH (mean corpuscular hemoglobin) levels among participants by age, gender, and ethnicity. Low MCH levels were highly prevalent across all groups, particularly in Tharu (98.4%) compared to Non-Tharu (79.0%). Age-wise, low MCH levels were most common in children aged 1-4 years (98.4%) and least common in individuals aged 25-29 years (64.4%). Normal MCH levels were highest in the 25-29 age groups (35.6%). By gender, low MCH levels were similar in males (84.9%) and females (84.0%), with slightly higher normal levels in females (16.1%).

Table 31: Distribution of MCH level among Participants Based on Demographic Variables

Variables	MCH level		Total
	Low n (%)	Normal n (%)	N= (1134)
Age group			
1-4 years	61 (98.4)	1 (1.6)	62
5-9 years	221 (97.4)	6 (2.6)	227
10-14 years	269 (92.1)	23 (7.9)	292
15-19 years	191 (76.4)	59 (23.6)	250
20-24 years	119 (77.3)	35 (22.7)	154
25-29 years	96 (64.4)	53 (35.6)	149
Gender			
Male	438 (84.9)	78 (15.1)	516
Female	519 (84.0)	99 (16.1)	618
Ethnicity			
Non Tharu	647 (79.0)	172 (21.0)	819
Tharu	310 (98.4)	5 (1.6)	315

The table 32 presents the distribution of MCHC (mean corpuscular hemoglobin concentration) levels among 1134 participants by age, gender, and ethnicity. Low MCHC levels were more prevalent in Tharu participants (81.3%) compared to Non-Tharu (59.8%). Age-wise, low MCHC levels were highest in children aged 1-4 years (83.9%) and lowest in individuals aged 25-29 years (57.7%). Normal MCHC levels increased with age, peaking in the 25-29 age group (42.3%). By gender, low MCHC levels were higher in females (68.9%) compared to males (62.0%), with males showing higher normal levels (38.0%) than females (31.1%).

Table 32: Distribution of MCHC level among participants based on demographic variables

Variables	MCHC level		Total (N= 1,134)
	Low n (%)	Normal n (%)	
Gender			
Male	320 (62.0)	196 (38.0)	516
Female	426 (68.9)	192 (31.1)	616
Age group			
1-4 years	52 (83.9)	10 (16.1)	62
5-9 years	158 (69.6)	69 (30.4)	227
10-14 years	189 (64.7)	103 (35.3)	292
15-19 years	163 (65.2)	87 (34.8)	250
20-24 years	98 (63.6)	56 (36.4)	154
25-29 years	86 (57.7)	63 (42.3)	149

Variables	MCHC level		Total (N= 1,134)
	Low n (%)	Normal n (%)	
Ethnicity			
Non Tharu	490 (59.8)	329 (40.2)	819
Tharu	257 (81.3)	59 (18.7)	316

The table 33 displays platelet levels among 1134 participants, categorized by age, gender, and ethnicity. Low platelet levels were more common in females (12.4%) than in males (11.0%). Ethnically, low platelet levels were higher in Tharu (14.0%) compared to Non-Tharu (10.9%). Age-wise, low platelet levels were most prevalent in individuals aged 15-19 years (18.8%) and least common in 1-4 years (0.0%). Normal platelet levels were highest in children aged 10-14 years (86.0%) and lowest in those aged 1-4 years (64.5%). High platelet levels were more frequent in males (6.4%) and Non-Tharu (7.4%).

Table 33: Distribution of Platelets level among participants based on demographic variables

Variables	Platelets level			Total (N =1,134)
	Low n (%)	Normal n (%)	High n (%)	
Gender				
Male	57 (11.0)	426 (82.6)	33 (6.4)	516
Female	76 (12.4)	486 (12.4)	56 (9.3)	618
Age Group				
1-4 years	-	40 (64.5)	22 (35.5)	62
5-9 years	12 (5.5)	181 (79.7)	34 (15.0)	227
10-14 years	23 (7.9)	251 (86.0)	18 (6.1)	292
15-19 years	47 (18.8)	200 (80.0)	3 (1.2)	250
20-24 years	25 (16.2)	124 (80.5)	5 (3.2)	154
25-29 years	26 (17.4)	116 (77.9)	7 (4.7)	149
Ethnicity				
Non-Tharu	89 (10.9)	669 (81.7)	61 (7.4)	819
Tharu	44 (14.0)	243 (77.1)	28 (8.9)	315

3.2.5 Status of Glucose-6-Phosphate Dehydrogenase (G6PD)

Glucose-6-Phosphate Dehydrogenase (G6PD) is a crucial enzyme that plays a protective role in red blood cells, helping them manage oxidative stress. A deficiency in G6PD can predispose individuals to hemolytic anemia, particularly when exposed to certain foods, infections, or medications. Out of the 516 male participants, G6PD status was recorded for 487 individuals among them, 346 were non-Tharu and 141 were Tharu. Of these, 105 were found to have low G6PD activity, indicating a deficiency, while 382 had normal levels. There were 29 cases with missing data. This indicates that approximately 21.6% of males with recorded data (105 out of 487) were G6PD deficient, a relatively high prevalence that warrants attention in clinical and public health settings.

Previous studies in South Asia have shown that certain populations, including indigenous groups like the Tharu, may have different prevalence rates for G6PD deficiency due to genetic and evolutionary factors, such as protection against malaria.

In conclusion, this dataset highlights a notable burden of G6PD deficiency among males, which could have implications for screening policies, especially in areas where oxidative stress-inducing drugs (like certain antimalarials) are commonly used. Further analysis breaking down G6PD status by ethnicity would provide a more complete picture and guide targeted interventions.

Table 34: Distribution of participants based on Age, Ethnicity and G6PD level **N= 487**

Variables	G6PD level		Total
	Low	normal	
Ethnicity			
Non-Tharu	48 (13.9%)	298 (86.1%)	346
Tharu	19 (13.5%)	122 (86.5%)	141
Total	67 (13.8%)	420 (86.2%)	487
Age Group			
1-4 years	2 (8.3%)	22 (91.7%)	24
5-9 years	9 (8.8%)	93 (91.2%)	102
10-14 years	18 (12.5%)	126 (87.5%)	144
15-19 years	19 (16.2%)	98 (83.8%)	117
20-24 years	9 (15.0%)	51 (85.0%)	60
25-29 years	10 (25.0%)	30 (75.0%)	40
Total	67 (13.8%)	420 (86.2%)	487

3.2.6 Hemoglobinopathies Analysis Using Capillary Electrophoresis

Capillary electrophoresis was conducted to identify Hemoglobinopathies among 1134 samples. The analysis detected the presence of hemoglobin variants, including HbA, HbA2, HbE, HbF, and HbS. Any abnormalities in these hemoglobin types were classified as Hemoglobinopathies. Hemoglobin abnormalities were identified in 25% (283 samples) of the total, with thalassemia trait being the most common disorder. Specifically, 6.5% (74 samples) had thalassemia trait. About 5% (58 sample) had presence of HbE, however only 35 (3.1%) met the criteria for HbE trait. Six (0.5%) cases of HbS trait were found. A significant proportion (11.7%) showed low HbA levels. [Table 35]

Table 35: Distribution of Hemoglobinopathies among Participants

Variables	Frequency	% among total Population	% among Hemoglobinopathies
Presence of Hemoglobinopathies	283	25.0	
Types of Hemoglobinopathies			
1. Thalassemia trait	74	6.5	26.1
2. HbE abnormalities/disease	58	5.1	20.5
a. HbE trait	35	3.1	12.4
3. Sickle cell	6	0.5	2.1
4. Low HbA	133	11.7	47.0

Table 36: illustrates the association between Hemoglobinopathies and various socio-demographic variables. Although a higher proportion of males (27.3%) were identified with Hemoglobinopathies, the association was not statistically significant. In terms of age distribution, Hemoglobinopathies were most prevalent among children aged 1–4 years (37.1%) compared to older age groups, and this association was statistically significant (P-value < 0.05). Similarly, ethnicity-wise analysis showed that a higher proportion of Tharu participants (31.1%) had Hemoglobinopathies, with the association being statistically significant (P-value < 0.05).

Table 36: Association of Hemoglobinopathies with socio-demographic variables

Variables	Presence of Hemoglobinopathies		Chi- square	P-value
	Yes	No		
Gender			2.839	0.092
Male	141 (27.3)	375 (72.7)		
Female	142 (23.0)	476 (77.0)		
Age group			5.162	0.023*
1-4 years	23 (37.1)	39 (62.9)		
5-29 years	260 (24.3)	812(75.7)		
Ethnicity			8.823	0.003*
Non-Tharu	185 (22.6)	634 (77.4)		
Tharu	98 (31.1)	217(8.9)		

The table 37 presents the distribution of Hemoglobinopathies—thalassemia trait, HbE abnormatlity, and HbS trait—by gender, age, and ethnicity. Thalassemia trait was more prevalent among males (7.9%) than females (5.3%) and was most common in the 10–14 years' age group (8.6%). HbE abnormality was most prevalent in the 10–14 years' age group (6.8%). HbS trait exhibited a very low prevalence, with similar rates in males (0.6%) and females (0.5%). It was most commonly observed in the 5–9 years (1.3%) and 15–19 years (1.2%) age groups. Overall, Hemoglobinopathies were more prevalent among Tharu participants compared to Non-Tharu participants across all types of disorders.

Table 37 : Association of various types of Hemoglobinopathies and socio-demographic variables

Variables	Thalessemia trait		HbE abnormality		HbS trait/disease	
	Yes	No	Yes	No	Yes	No
Gender						
Male	41 (7.9)	475 (92.1)	28 (5.4)	488(94.6)	3 (0.6)	513 (99.4)
Female	33 (5.3)	585 (94.7)	30 (4.9)	588 (95.1)	3 (0.5)	615 (99.5)
Age group						
1-4 years	3 (4.8)	59 (95.2)	4 (6.5)	58 (93.5)	-	62 (100.0)
5-9 years	18 (7.9)	209(92.1)	13(5.7)	214 (94.3)	3(1.3)	224 (98.7)

Variables	Thalessemia trait		HbE abnormality		HbS trait/disease	
	Yes	No	Yes	No	Yes	No
10-14 years	25 (8.6)	267 (91.4)	20 (6.8)	272 (93.2)	-	292(100.0)
15-19 years	12 (4.8)	238 (95.2)	11 (4.4)	239 (95.6)	3 (1.2)	247 (98.8)
20-24 years	7 (4.5)	147 (95.5)	7 (4.5)	147 (95.5)	-	154 (100.0)
25-29 years	9 (6.0)	140 (94.0)	3 (2.0)	146 (98.0)	-	149 (100.0)
Ethnicity						
Non-Tharu	39 (4.8)	780 (95.2)	36(4.4)	783 (95.6)	2(0.2)	817 (99.8)
Tharu	40 (12.7)	275 (87.3)	22 (7.0)	293 (93.0)	4 (1.3)	311 (98.7)

Among participants diagnosed with Hemoglobinopathies, 71.7% reported no symptoms, while approximately 16% experienced at least one symptom. A small proportion (1.8%) reported experiencing five or more symptoms associated with Hemoglobinopathies. The most commonly reported symptom was headache (9.9%), followed by abdominal pain (6.7%) and chest pain (5.3%). [Table 38]

Table 38: Clinical symptoms among participants with Hemoglobinopathies **N=283**

Variables	Frequency	Percentage
Number of symptoms experienced		
No any symptoms	203	71.7
Any one of the symptoms	45	15.9
Any two of the symptoms	16	5.7
Any three of the symptoms	9	3.2
Any four of the symptoms	5	1.8
Five or more Symptoms	5	1.8
Most commonly experienced symptoms		
Headache	28	9.9
Abdomen pain	19	6.7
Chest pain	15	5.3
Vision problems	14	4.9
Frequent infection	13	4.6
Extreme tiredness	13	4.6
Joint pain	11	3.9
Pallor skin	9	3.2
Back pain	8	2.8
Yellowish discoloration of eye	7	2.5
Difficult breathing	7	2.5
Trouble with physical activities	4	1.4
Others (unconscious/seizure, pain/swelling of finger/toes, Delayed growth/puberty, leg ulcer)	7	2.5

CHAPTER IV DISCUSSION

This study found prevalence of Hemogloninpathies 17.4% in central Terai districts (Bagmati and Gandaki province) and 25% in Koshi province. This study included cases meeting the criteria for Hemoglobinopathies, such as thalassemia traits, HbE abnormalities (disease or trait), sickle cell traits/ disease, and those with low HbA levels. This prevalence is lower than a lab based retrospective study conducted in Terai regions of Nepal (Shrestha et al., 2020). This difference may be due the fact mostly sick people go to lab and the current study was community based. Recognizing that prior studies have established a higher prevalence of Hemoglobinopathies in the Terai region and among specific ethnic groups, particularly the Tharu community (Ghimire et al., 2017; Mohanty et al., 2022; Pande et al., 2019) such as primaquine. G6PD deficiency (G6PDd. This research focused on populations from the Terai, with a special emphasis on the Tharu ethnic group. To allow for comparative analysis, non-Tharu participants residing in the same region were also enrolled. This approach enabled the study to identify and compare the prevalence of Hemoglobinopathies among non-Tharu populations in the Terai. Studies have found that prevalence of Hemoglobinopathies is increasing in Terai due to high number of consanguineous marriages and inter-caste marriages (Bhusal et al., 2022).

This study identified thalassemia as the most common type of Hemoglobinopathy, with a prevalence of 2.6% in Bagmati and Gandaki Provinces and 6.5% in Koshi Province. These findings are consistent with previous study conducted in the southwestern region of Nepal that reported thalassemia as the predominant Hemoglobinopathy) (Roma et al., 2023). In contrast, a retrospective study found that sickle cell disease was the most common Hemoglobinopathy, followed by thalassemia (Shrestha et al., 2020).

In this study, only 0.5% of participants in Koshi Province were found to have sickle cell trait, while no cases were identified in the central Terai districts. This prevalence is notably lower than findings from previous studies conducted in the western Terai districts of Nepal (Pande et al., 2019). Similarly, another study conducted in Kanchanpur and Dang reported sickle cell prevalence ranging from 8.3% to 9.3% (Ghaju et al., 2022; Marchand et al., 2017)which is an indigenous and minority group mostly residing in the Terai region of Nepal. They are also considered as the most vulnerable group for inheriting Sickle cell anemia.Methods: Purposive sampling, which included 130 Tharu individuals of Kanchanpur district of Nepal, was considered for the study. The survey was conducted using a descriptive questionnaire that contained relevant information including the family history of Sickle cell anemia. This was followed by the analysis of blood samples to determine the prevalence of Sickle cell anemia and Sickle cell traits. Primer-mediated enzymatic amplification of target sequences in genomic DNA followed by restriction endonuclease assay with an enzyme DdeI was carried out for the confirmation.Results: Among 130 individuals, only 55.4% had basic knowledge about Sickle cell anemia. After screening for sickle cell anemia from 60 participants, 27 (45%. Given that these studies were conducted in western Nepal, the

lower prevalence observed in the present study may be attributed to regional differences, as the current study was conducted in the eastern part of the country.

Although sickle cell disease has been predominantly reported among the Tribal/Tharu community (Mohanty et al., 2022; Pande et al., 2019), this study also identified a few cases of sickle cell trait among non-Tharu participants. This suggests the possibility of a wider distribution of the condition beyond traditionally affected ethnic groups, highlighting the need for further investigation into genetic and demographic variations in Hemoglobinopathies across Nepal.

Since G6PD deficiency is an X-linked genetic disorder and more commonly manifests symptoms in males, this study focused exclusively on analyzing blood samples from male participants to assess G6PD enzyme activity. This approach is consistent with existing literature indicating that males are more likely to exhibit symptoms of G6PD deficiency (Ghimire et al., 2017)such as primaquine. G6PD deficiency (G6PDd. The findings of this study indicate that the prevalence of G6PD deficiency among male participants in the central Terai districts (Gandaki and Bagmati Provinces) was lower than previously reported in studies conducted in other Terai districts of Nepal (Ghimire et al., 2017; Lamichhane et al., 2017 (Ghimire et al., 2017; Lamichhane et al., 2017)such as primaquine. G6PD deficiency (G6PDd. In contrast, the national-level prevalence of G6PD deficiency among males is reported to be much lower than the findings of the current study. However, when compared at the district level, the prevalence observed in this study is nearly similar to previous district-specific reports (Marasini et al., 2020).

The study also sought to assess symptoms among individuals who tested positive for Hemoglobinopathies. However, it was observed that most symptoms reported by participants, such as fatigue and general weakness, were nonspecific and were also described by individuals without Hemoglobinopathies. Only a small proportion of participants with positive Hemoglobinopathy results reported experiencing symptoms directly attributable to their condition. This highlights the challenge of relying solely on symptomatic presentations for identifying Hemoglobinopathies and underscores the importance of laboratory-based diagnostic approaches.

Overall, the findings reinforce the need for targeted screening programs in regions with a high prevalence of Hemoglobinopathies and G6PD deficiency, particularly among at-risk populations such as the Tharu community, while also emphasizing the role of genetic and laboratory testing in identifying and managing these conditions effectively.

CHAPTER V CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study, conducted in the Terai districts of Gandaki, Bagmati, and Koshi Provinces in Nepal, highlighted a significant prevalence of Hemoglobinopathies, with thalassemia trait being the most common. HbE abnormality/trait was observed in a substantial proportion of participants, particularly in Koshi Province, while sickle cell trait/disease was rare, with no cases detected in Chitwan and only a small number identified in Koshi Province. The prevalence of Hemoglobinopathies was notably higher among the Tharu ethnic group compared to the Non-Tharu population. Additionally, G6PD deficiency was identified in a significant proportion of male participants, particularly in Chitwan, underscoring the need for targeted screening in malaria-endemic areas. While the condition was more prevalent among Tharu participants, a considerable number of Non-Tharu participants were also affected, with the highest prevalence observed among those aged 15–24 years. Most individuals with Hemoglobinopathies or G6PD deficiency remained asymptomatic, though headache and abdominal pain were the most commonly reported symptoms among symptomatic participants.

Anemia was found to be highly prevalent, especially among Tharu participants and women aged 15 years and above. In Chitwan, nearly half of the Tharu participants were anemic, while in Koshi Province, the prevalence rose to six out of ten Tharu participants. These findings emphasize the need for targeted screening and intervention programs to address Hemoglobinopathies, G6PD deficiency, and anemia, particularly among vulnerable populations such as the Tharu community and individuals in malaria-endemic regions. Strengthened public health efforts, including early detection and management strategies, are essential to mitigating the long-term health impacts of these conditions.

5.2 Recommendation:

- There is need to establish specific guidelines for premarital, post-marital and antenatal counseling among Tharu community/ Terai region for early identification and management of risk population. Making laboratory facilities available for genetic testing in all districts of Nepal is essential for timely and accurate diagnosis of Hemoglobinopathies and G6PD deficiency which can reduce complications.
- Expanding this research to all provinces will help to get a complete picture of the prevalence and distribution of Hemoglobinopathies and G6PD deficiency throughout the country study showed that diverse ethnic groups also have prevalence of Hemoglobinopathies.
- Awareness campaigns to educating the public about Hemoglobinopathies and G6PD deficiency, their symptoms, and prevention strategies.
- Expanding screening programs for Hemoglobinopathies and G6PD deficiency, particularly in malaria-endemic regions is essential for early identification of risk population

References

Aggarwal, A., Aggarwal, A., Goyal, S., & Aggarwal, S. (2020). Iron-deficiency anemia among adolescents: A global public health concern. *International Journal of Advanced Community Medicine*, *3*(2), 35–40. https://doi.org/10.33545/comed.2020.v3.i2a.148

Aguilar Martinez, P., Angastiniotis, M., Eleftheriou, A., Gulbis, B., Mañú Pereira, M. D. M., Petrova-Benedict, R., & Corrons, J.-L. V. (2014). Haemoglobinopathies in Europe: Health & migration policy perspectives. *Orphanet Journal of Rare Diseases*, *9*, 97. https://doi.org/10.1186/1750-1172-9-97

Allison, A. C. (1954). Protection Afforded by Sickle-cell Trait Against Subtertian Malarial Infection. *British Medical Journal*, *1*(4857), 290–294.

American society of Hematology. (2025). *Sickle Cell Disease*. https://www.hematology.org/education/patients/anemia/sickle-cell-disease

Angastiniotis, M., Eleftheriou, A., Galanello, R., Harteveld, C. L., Petrou, M., Traeger-Synodinos, J., Giordano, P., Jauniaux, E., Modell, B., & Serour, G. (2013). *Prevention of Thalassaemias and Other Haemoglobin Disorders: Volume 1: Principles* (J. Old, Ed.; 2nd ed.). Thalassaemia International Federation. http://www.ncbi.nlm.nih.gov/books/NBK190481/

Bajwa, H., & Basit, H. (2025). Thalassemia. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK545151/

Barbara J. Bain, Ilmeda Bates, & Mike A. Laffan. (2017). *Dacie and Lewis Practical Haematology* (12th edition). Elsevier.

Bastola, G., Acharya, R., Dhakal, N., & Gupta, U. P. (2017). Study of Thalassemia and Haemoglobinopathies in Pokhara, Nepal. *JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH*. https://doi.org/10.7860/JCDR/2017/29845.10916

Bhusal, A., Bhandari, S., & Sah, R. P. (2022). Can HPLC be used as an ideal methodology instead of Hb Electrophoresis for the diagnosis of hemoglobinopathies in a routine clinical laboratory of under-resource country like Nepal? Is the change necessary? *Annals of Medicine and Surgery*, *82*, 104367. https://doi.org/10.1016/j.amsu.2022.104367

Bubp, J., Jen, M., & Matuszewski, K. (2015). Caring for Glucose-6-Phosphate Dehydrogenase (G6P-D)-Deficient Patients: Implications for Pharmacy. *P & T: A Peer-Reviewed Journal for Formulary Management*, *40*(9), 572–574.

Cappellini, M. D., & Fiorelli, G. (2008). Glucose-6-phosphate dehydrogenase deficiency. *The Lancet*, *371*(9606), 64–74. https://doi.org/10.1016/S0140-6736(08)60073-2

Colah, R., Italia, K., & Gorakshakar, A. (2017). Burden of thalassemia in India: The road map for control. *Pediatric Hematology Oncology Journal*, *2*(4), 79–84. https://doi.org/10.1016/j.phoj.2017.10.002

Ghaju, S., Khadayat, K., Shrestha, R., Ghaju, S., Manandhar, T., & Marasini, B. P. (2022). Knowledge and DdeI Based Confirmation of Sickle Cell Anemia Among the Tharu Community. *Journal of Nepal Health Research Council*, *20*(02), 454–459. https://doi.org/10.33314/jnhrc.v20i02.4158

Ghimire, P., Singh, N., Ortega, L., Rijal, K. R., Adhikari, B., Thakur, G. D., & Marasini, B. (2017). Glucose-6-phosphate dehydrogenase deficiency in people living in malaria endemic districts of Nepal. *Malaria Journal*, *16*(1), 214. https://doi.org/10.1186/s12936-017-1864-2

Harewood, J., & Azevedo, A. M. (2025). Alpha Thalassemia. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK441826/

Hebbel, R. P. (2003). Sickle hemoglobin instability: A mechanism for malarial protection. *Redox Report: Communications in Free Radical Research*, *8*(5), 238–240. https://doi.org/10.1179/135100003225002826

Herbert L. Muncie, J., & Campbell, J. S. (2009). Alpha and Beta Thalassemia. *American Family Physician*, *80*(4), 339–344.

Howes, R. E., Battle, K. E., Satyagraha, A. W., Baird, J. K., & Hay, S. I. (2013). G6PD deficiency: Global distribution, genetic variants and primaquine therapy. *Advances in Parasitology*, *81*, 133–201. https://doi.org/10.1016/B978-0-12-407826-0.00004-7

Howes, R. E., Piel, F. B., Patil, A. P., Nyangiri, O. A., Gething, P. W., Dewi, M., Hogg, M. M., Battle, K. E., Padilla, C. D., Baird, J. K., & Hay, S. I. (2012). G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: A geostatistical model-based map. *PLoS Medicine*, *9*(11), e1001339. https://doi.org/10.1371/journal.pmed.1001339

Kim, J.-E., Kim, B.-R., Woo, K.-S., Kim, J.-M., Park, J.-I., & Han, J.-Y. (2011). Comparison of Capillary Electrophoresis with Cellulose Acetate Electrophoresis for the Screening of Hemoglobinopathies. *The Korean Journal of Laboratory Medicine*, *31*(4), 238–243. https://doi.org/10.3343/kjlm.2011.31.4.238

Kohne, E. (2011). Hemoglobinopathies. *Deutsches Ärzteblatt International*, *108*(31–32), 532–540. https://doi.org/10.3238/arztebl.2011.0532

Kohne, E., & Kleihauer, E. (2010). Hemoglobinopathies: A longitudinal study over four decades. *Deutsches Arzteblatt International*, *107*(5), 65–71. https://doi.org/10.3238/arztebl.2010.0065

Lamichhane, N., Adhikari, N., Shrestha, U. T., Rijal, K. R., Banjara, M. R., & Ghimire, P. (2017). Population Based Survey of Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency among People Living in Terai Districts of Nepal. *Tribhuvan University Journal of Microbiology*, *4*, 73–78. https://doi.org/10.3126/tujm.v4i0.21680

Makani, J., Ofori-Acquah, S. F., Nnodu, O., Wonkam, A., & Ohene-Frempong, K. (2013). Sickle Cell Disease: New Opportunities and Challenges in Africa. *The Scientific World Journal*, *2013*(1), 193252. https://doi.org/10.1155/2013/193252

Marasini, B., Lal, B. K., Thapa, S., Awasthi, K. R., Bajracharya, B., Khanal, P., Neupane, S., Jha, S. N., Acharya, S., Iama, S., Koirala, M., Koirala, D., Bhandari, S., Mahato, R. K., Chaudhary, A., Ghimire, P., Magar, R. G., Bhattarai, R. K., Gornsawun, G., … Acharya, B. P. (2020). G6PD deficiency in malaria endemic areas of Nepal. *Malaria Journal*, *19*(1), 287. https://doi.org/10.1186/s12936-020-03359-6

Marchand, M., Gill, C., Malhotra, A. K., Bell, C., Busto, E., McKeown, M. D., Cherukupalli, A., Yeo, J., Arnold, B., & Kapoor, V. (2017). The Assessment and Sustainable Management of Sickle Cell Disease in the Indigenous Tharu Population of Nepal. *Hemoglobin*, *41*(4–6), 278–282. https://doi.org/10.1080/03630269.2017.1414058

Memish, Z. A., Owaidah, T. M., & Saeedi, M. Y. (2011). Marked regional variations in the prevalence of sickle cell disease and β-thalassemia in Saudi Arabia: Findings from the premarital screening and genetic counseling program. *Journal of Epidemiology and Global Health*, *1*(1), 61–68. https://doi.org/10.1016/j.jegh.2011.06.002

Minucci, A., Moradkhani, K., Hwang, M. J., Zuppi, C., Giardina, B., & Capoluongo, E. (2012). Glucose-6-phosphate dehydrogenase (G6PD) mutations database: Review of the "old" and update of the new mutations. *Blood Cells, Molecules & Diseases*, *48*(3), 154–165. https://doi.org/10.1016/j.bcmd.2012.01.001

Modell, B., & Darlison, M. (2008). Global epidemiology of haemoglobin disorders and derived service indicators. *Bulletin of the World Health Organization*, *86*(6), 480–487. https://doi.org/10.2471/BLT.06.036673

Mohanty, S. S., Parihar, S., Huda, R. K., Toteja, G. S., & Sharma, A. K. (2022). Prevalence of sickle cell anemia, β-thalassemia and glucose-6-phosphate dehydrogenase deficiency among the tribal population residing in the Aravali hills of Sirohi region of Rajasthan state. *Clinical Epidemiology and Global Health*, *13*, 100916. https://doi.org/10.1016/j.cegh.2021.100916

Mukherjee, M. B., Colah, R. B., Martin, S., & Ghosh, K. (2015). Glucose-6-phosphate dehydrogenase (G6PD) deficiency among tribal populations of India—Country scenario. *The Indian Journal of Medical Research*, *141*(5), 516–520. https://doi.org/10.4103/0971-5916.159499

Nkhoma, E. T., Poole, C., Vannappagari, V., Hall, S. A., & Beutler, E. (2009). The global prevalence of glucose-6-phosphate dehydrogenase deficiency: A systematic review and meta-analysis. *Blood Cells, Molecules & Diseases*, *42*(3), 267–278. https://doi.org/10.1016/j.bcmd.2008.12.005

Pande, R., Ghimire, P. G., Chand, P. B., & Gupta, S. (2019). Sickle cell disease in Western Nepal. *Nepal Journal of Medical Sciences*, *4*(1), Article 1. https://doi.org/10.3126/njms.v4i1.24121

Pandey, S., & Shrestha, N. (2022). Sickle Cell Anaemia among Tharu Population Visiting the Outpatient Department of General Medicine in a Secondary Care Centre: A Descriptive Cross-sectional Study. *JNMA; Journal of the Nepal Medical Association*, *60*(253), 774–776. https://doi.org/10.31729/jnma.7651

Pandya, N. K., & Sharma, S. (2023). Capnography and Pulse Oximetry. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK539754/

Richardson, S. R., & O'Malley, G. F. (2025). Glucose-6-Phosphate Dehydrogenase Deficiency. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK470315/

Roma, K., Pande, R., & Shrestha, D. L. (2023). Genotype-Phenotype Profile of Beta-thalassemia. *Journal of Nepal Health Research Council*, *20*(4), 834–837. https://doi.org/10.33314/jnhrc.v20i4.3719

Shrestha, R. M., Pandit, R., Yadav, U. K., Das, R., Yadav, B. K., & Upreti, H. C. (2020). Distribution of Hemoglobinopathy in Nepalese Population. *Journal of Nepal Health Research Council*, *18*(1), 52–58. https://doi.org/10.33314/jnhrc.v18i1.2303

Terai. (2024). In *Wikipedia*. https://en.wikipedia.org/w/index.php?title=Terai&oldid=1247146269

Theodorsson, E., Birgens, H., & Hagve, T. A. (2007). Haemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency in a Scandinavian perspective. *Scandinavian Journal of Clinical and Laboratory Investigation*, *67*(1), 3–10. https://doi.org/10.1080/00365510601046359

UK, G. (2018). *Understanding haemoglobinopathies. In Handbook for sickle cell and thalassaemia screening.* GOV.UK. https://www.gov.uk/government/publications/handbook-for-sickle-cell-and-thalassaemia-screening/understanding-haemoglobinopathies

Weatherall, D. (2011). The inherited disorders of haemoglobin: An increasingly neglected global health burden. *The Indian Journal of Medical Research*, *134*(4), 493–497.

Annex

Study Team

Dr. Pramod Joshi, Executive Chief, NHRC

Dr. Meghnath Dhimal, Chief/Senior Research Officer, NHRC

Dr. Bishnu P. Marasini, Senior Research Associate, NHRC

Dr. Gunaraj Dhunganaa, Consultant, NHRC

Ms. Sujata Dahal, Consultant, NHRC

Mr. Suraj Chand, Consultant, NHRC

Report Prepared by

Dr. Meghnath Dhimal, Chief/Senior Research Officer, NHRC

Dr. Bishnu P. Marasini, Senior Research Associate, NHRC

Ms. Rajina Shakya, Research Associate, NHRC

Nepal Health Research Council (NHRC)
P.O. Box: 7626, Ramshah Path, Kathmandu, Nepal
Tel. : +977-1-5354220, 5328460
E-mail : nhrc@nhrc.gov.np
Website: www.nhrc.gov.np

9789937185073