NUTRITIONAL STATUS AND ASSOCIATED FACTORS IN TRANSFUSION-DEPENDENT BETA-THALASSEMIA MAJOR PEDIATRIC PATIENTS VISITING KANTI CHILDREN'S HOSPITAL

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Nutritional Status and Associated Factors in Transfusion-Dependent Beta-Thalassemia Major Pediatric Patients Visiting Kanti Children's Hospital

A dissertation submitted to the Department of Nutrition and Dietetics, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B.Sc. in Nutrition and Dietetics

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Approval letter

This *dissertation* entitled ***Nutritional Status and Associated Factors in Transfusion-Dependent Beta-Thalassemia Major Pediatric Patients Visiting Kanti Children's Hospital*** presented by **Arju Palikhey** has been accepted as the partial fulfillment of the requirement of the **B.Sc. degree** in **Nutrition and Dietetics**

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Arju Palikhey

Abstract

The objective of the study was to assess the nutritional status and associated factors among transfusion-dependent pediatric patients with β-thalassemia major (β-TM) visiting Kanti Children's Hospital in Nepal. A cross-sectional, hospital-based, descriptive study was conducted on 92 β-TM patients aged 1 to 14 years receiving regular blood transfusions. Data were collected using structured questionnaires, anthropometric assessments, biochemical evaluations, and dietary assessments. Anthropometric data were analyzed using WHO Anthro and WHO AnthroPlus software, dietary data using food composition tables and dietary intake references, and statistical analyses were performed to analyze the associated factors with the nutritional status.

Results showed that 27.2% of patients were stunted (moderate: 19.6%, severe: 7.6%) and 8.7% were underweight (moderate: 6.5%, severe: 2.2%), with stunting prevalence increasing significantly with age ($p<0.05$). Dietary analysis revealed inadequate caloric intake compared to age- and sex-specific requirements, suboptimal dietary diversity scores (mean: 4.230 ± 1.070), while protein intake exceeded estimated needs. Furthermore, body fat percentage showed significant sex-based differences and associations with age, chelation therapy ($p<0.001$), and total age of chelation ($p<0.05$). High serum ferritin levels (>2500 ng/ml) indicated a high prevalence of iron overload, persisting despite regular chelation therapy. Moreover, significant associations ($p<0.05$) were identified between nutritional status and various factors, including socioeconomic conditions, thalassemia-related aspects, and dietary practices. The study findings revealed compromised nutritional status in pediatric β-TM patients, influenced by disease-related factors, dietary inadequacies, and socioeconomic characteristics. The study underscores the need for comprehensive nutritional assessment and management as an integral part of β-TM care in this setting.

Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviations	Full form
ABCD	Anthropometric, biochemical, clinical, dietary
ANOVA	Analysis of variance
BIA	Bioelectrical impedance analysis
BMD	Bone mineral density
BMI	Body mass index
BMI/A	Body mass index-for-age
β-TM	Beta thalassemia major
DDS	Dietary diversity score
DEXA	Dual-energy x-ray absorptiometry
DFO	Deferoxamine
DFP	Deferiprone
DFTQC	Department of Food Technology and Quality Control
DFX	Deferasirox
DRI	Dietary reference intake
D2O	Deuterium oxide
EAR	Estimated average requirement
FANTA	Food and Nutrition Technical Assistance
FAO	Food and Agriculture Organization
FFQ	Food frequency questionnaire
FM	Fat mass
FFM	Fat free mass
GH	Growth hormone
GHD	Growth hormone deficiency
GoN	Government of Nepal
H/A	Height-for-age
Hb	Hemoglobin
ICMR	Indian Council of Medical Research
IGH-1	Insulin like growth hormone- 1
IOM	Institute of Medicine
IQR	Interquartile range

IRC	Institutional review committee
IYCF	Infant and young child feeding
IYCF-MDD	Infant and young child feeding minimum dietary diversity
KCH	Kanti Children's Hospital
LIC	Liver iron concentration
MAM	Moderate acute malnutrition
MDD-W	Minimum dietary diversity for women
MoH	Ministry of Health
MUAC	Mid-upper arm circumference
NHANES	National health and nutrition examination survey
NIN	National Institute of Nutrition
NPR	Nepalese rupee
NTBI	Non-transferrin bound iron
NTDT	Non-transfusion dependent thalassemia
NTS	Nepal thalassemia society
PIS	Patient information sheet
RBC	Red blood cells
RDA	Recommended dietary allowances
SD	Standard deviation
SF	Serum ferritin
SFT	Skin fold thickness
SPSS	Statistical package for social sciences
SSFT	Subscapular skin fold thickness
TEE	Total energy expenditure
TEF	Thermic effect of food
TDT	Transfusion dependent thalassemia
TIF	Thalassemia international federation
TSFT	Triceps skin fold thickness
UNICEF	United Nations International Children's Emergency Fund
USD	United States Dollar
W/A	Weight-for-age
WHO	World Health Organization
25-OHD	25-hydroxyvitamin D

Part I

Introduction

1.1 General introduction

Thalassemias are inherited heterogeneous group of hemoglobin disorders in which the production of normal hemoglobin is partly or completely suppressed due to defective synthesis of one or more of the normal globin chains, resulting in chronic anemia (MoH, 2017; MoHP, 2019; Origa, 2021). Clinically, thalassemias are primarily classified into two types: α-thalassemia and β-thalassemia. Among these, β-thalassemia major (β-TM), also known as transfusion-dependent β-thalassemia (TDT), represents the most severe form. Patients with β-TM require regular blood transfusions for survival due to their inability to produce sufficient normal hemoglobin (Galanello and Origa, 2010; WHO, 2021).

In the under-resourced healthcare setting of Nepal, β-TM poses a significant challenge, including both medical and nutritional complexities. Growth failure, a common issue in β-TM patients, stems from chronic anemia, chelation toxicity, and iron-induced endocrinopathies (Fung, 2010, 2016; Arab-Zozani *et al.*, 2021; Soliman *et al.*, 2023). Furthermore, the nutritional management of β-TM is complicated by socio-cultural and economic factors, inadequate nutrient intake, stress, poor appetite, and medication-induced gastrointestinal issues (Fung *et al.*, 2012; Goldberg *et al.*, 2018). These factors collectively impact the overall health and well-being of β-TM patients (Porter, 2021b).

Despite these evident challenges, the lack of comprehensive understanding and effective management of nutritional challenges of β-TM in Nepal contributes to suboptimal health outcomes, impacting growth and quality of life. The current standard of care involves regular blood transfusions and iron chelation therapy, but nutritional management often receives insufficient attention (MoH, 2017). To address this critical gap, this study aims to evaluate the nutritional status and related health impacts in pediatric patients with transfusion-dependent β-TM.

1.2 Statement of the problem

Thalassemia, particularly β-thalassemia, is one of the most common hemoglobinopathies in Nepal, affecting all ethnic groups nationwide. Despite its widespread occurrence, β-thalassemia shows significant regional disparities, with Province 3 recording the highest

proportion (86.3%) (Shrestha *et al.*, 2020). This condition is also particularly prevalent in the Terai region, notably among the Tharu ethnic group (Jha and Jha, 2014).

Growth failure, a common and serious complication in β-TM patients, arises from multifaceted factors, including ineffective erythropoiesis, chronic hypoxia, and iron overload (Fung, 2016). These factors contribute to the development of endocrinopathies, such as hypogonadism, hypothyroidism, and growth hormone deficiency, as well as low bone mass, further exacerbating growth impairment (Elalfy *et al.*, 2020; Soliman *et al.*, 2023). Among these factors, iron overload emerges as a critical challenge in managing β-TM (Fung, 2010; Arab-Zozani *et al.*, 2021).

In Nepal's under-resourced healthcare landscape, β-TM poses a significant clinical challenge. This is exemplified by the findings of Sharma Poudyal *et al.* (2023), which show that 93% of patients with β-TM registered at the Nepal Thalassemia Society (NTS) have stunted growth.

The nutritional management of β-TM in Nepal is further complicated by socio-cultural factors such as traditional eating habits, economic limitations, and healthcare factors, significantly influencing food intake and nutritional status. Compounding these challenges is the issue of low nutrient intake, arising from psychosocial and physiological factors like stress, poor appetite, and medication-induced gastrointestinal disturbances (Fung *et al.*, 2012; Goldberg *et al.*, 2018).

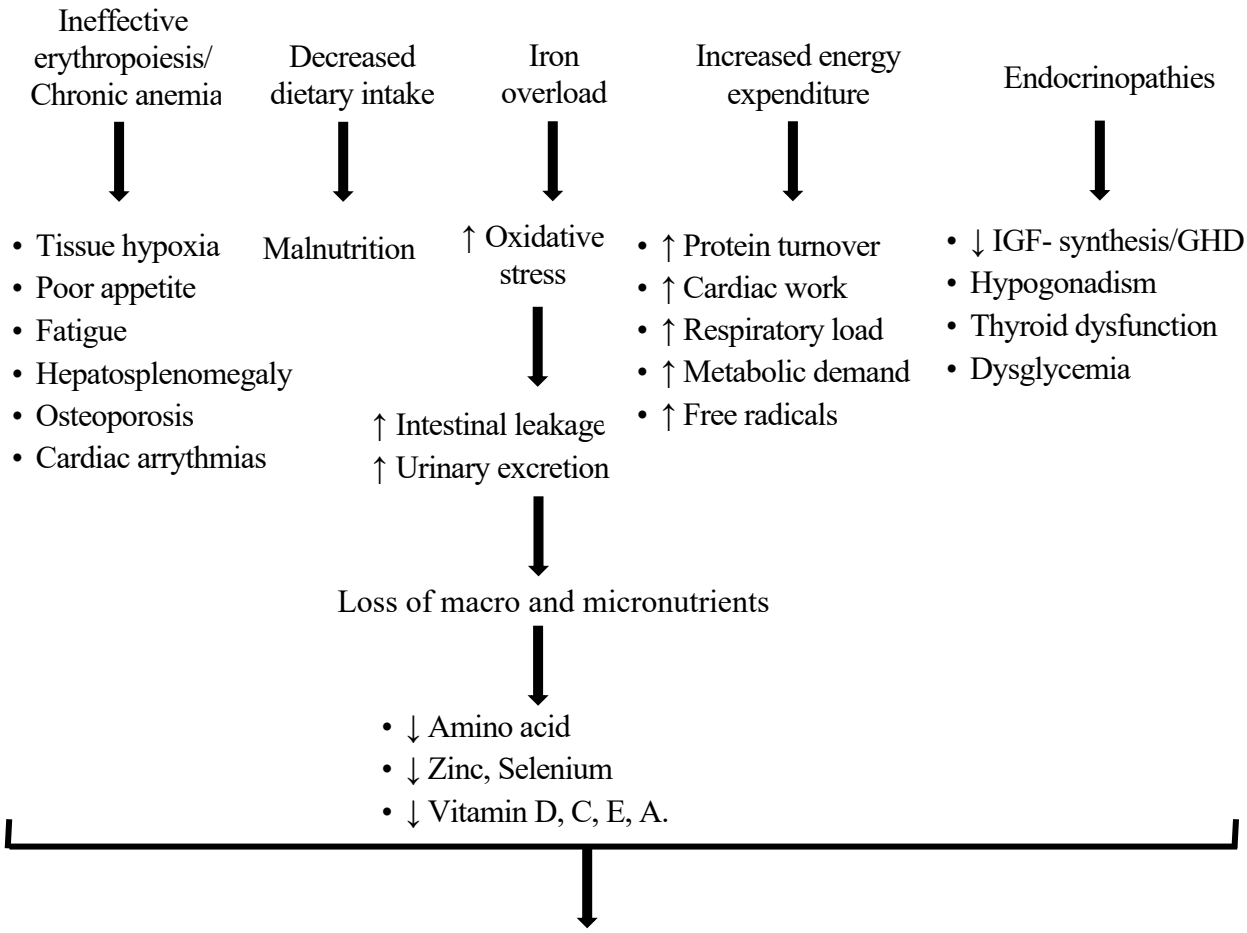
Moreover, current nutritional guidelines in Nepal recommend a low-iron diet, stemming from outdated perceptions of the disease and its management (MoH, 2017). This approach may be less relevant for transfusion-dependent β-TM patients, as dietary iron contributes minimally to their total iron burden (Fung, 2010; Fung *et al.*, 2023; Fung and Yardumian, 2024). This discrepancy between guidelines and current best practices highlights the need for updated, evidence-based nutritional recommendations.

The lack of comprehensive understanding and effective management of nutritional challenges in pediatric patients with β-TM in Nepal contributes to suboptimal health outcomes, impacting not just growth but also overall development and quality of life. Inadequate nutritional management can result in long-term consequences, such as delayed puberty, impaired bone health, and reduced quality of life (Porter, 2021b; Fung *et al.*, 2023).

To the limits of our knowledge, no other study has specifically addressed the nutritional status and dietary intake of β-TM patients in Nepal, making this research at the forefront of this important healthcare challenge.

1.3 Conceptual framework

Fig. 1.1 shows the potential mechanisms that contribute to malnutrition and abnormal body composition in β-TM.



IGF-1: Insulin-like growth factor 1
GHD: Growth hormone deficiency
BMI: Body mass index
BMD: Bone mineral density

Redrawn from: (Soliman *et al.*, 2023; El-Beshlawy *et al.*, 2024)

Fig. 1.1 Potential mechanisms that contribute to malnutrition and abnormal body composition in β- thalassemia major

1.4 Objectives of the study

1.4.1 General objective

i. To assess the nutritional status and identify factors influencing it among transfusion-dependent pediatric patients with β-TM visiting Kanti Children's Hospital.

1.4.2 Specific objectives

i. To assess the anthropometric measurements, viz., height, weight, mid-upper arm circumference (MUAC), and skin fold thickness.
ii. To assess the biochemical parameters, viz., pre-transfusion hemoglobin, serum ferritin, serum vitamin D, and serum calcium level.
iii. To assess the dietary intake, viz., 24-hour dietary recall, food frequency questionnaire (FFQ), and dietary diversity score (DDS).
iv. To assess the prevalence of malnutrition (undernutrition, stunting, and wasting).
v. To assess the knowledge and awareness of patients' families regarding the nutritional needs and dietary management of β-TM.

1.5 Research questions

i. What is the nutritional status of transfusion-dependent pediatric patients with β-TM aged 1 to 14 years visiting Kanti Children's Hospital?
ii. What are the associated factors that influence the nutritional status of these patients?

1.6 Significance of the study

The findings of the study will be helpful to:

i. Identify gaps in the nutritional management of thalassemic patients and assess the influence of socio-cultural practices, economic limitations, and disease severity on the dietary habits and nutritional status of β-TM patients in Nepal.
ii. Highlight the need for revising current dietary guidelines for β-TM patients in Nepal, in light of recent international recommendations.
iii. Enhance the understanding of healthcare providers and caretakers about the nutritional needs of β-TM patients, potentially leading to improved dietary practices and better health outcomes.
iv. Serve as a foundational study for future research, particularly focusing on patients with transfusion-dependent β-TM.

1.7 Limitations of the study

i. Mid-Upper Arm Circumference (MUAC) and Skinfold Thickness (SFT) could not be measured on the same side or dominant hand for all participants, potentially affecting consistency in the data collection process.
ii. Energy obtained from breast milk could not be calculated for breastfeeding participants, possibly leading to an underestimation of their total calorie intake.
iii. Due to the relatively rare nature of β-TM, the sample size may be limited, potentially affecting statistical power.

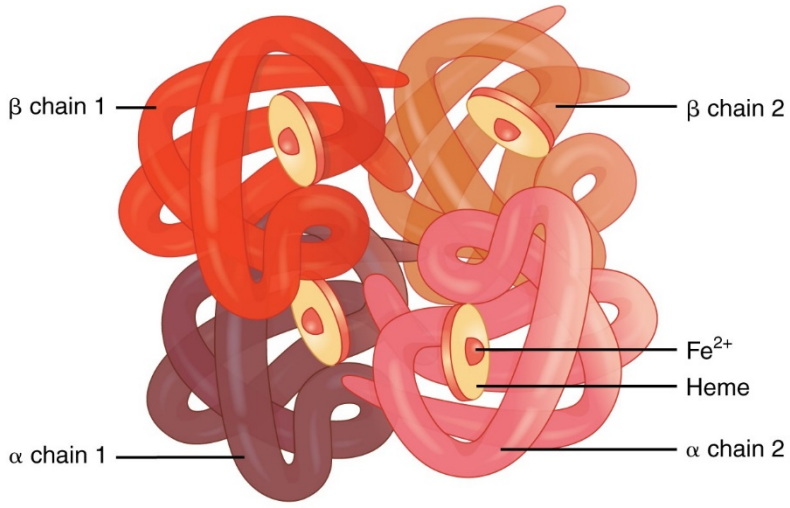
Part II

Literature review

2.1 Hemoglobinopathies

Hemoglobinopathies (Hemoglobin disorders) are hereditary disorders affecting the structure, function, or production of hemoglobin (Hb) (Fig. 2.1) and are among the most common clinically significant monogenic disorders (Williams and Weatherall, 2012). Hemoglobin disorders can be broadly classified into two general categories (Forget and Bunn, 2013), i.e.,

i. Those in which there is a quantitative defect in the production of one of the globin subunits, either total absence or marked reduction. These are called the thalassemia syndromes.
ii. Those in which there is a structural defect in one of the globin subunits.



Source: Betts *et al.* (2013)

Fig. 2.1 Structure of a hemoglobin molecule

2.2 Thalassemia

The term 'thalassemia' originates from the Greek words 'Thalassa' (sea) and 'haima' (blood) (Galanello and Origa, 2010). Thalassemias are recessively inherited heterogeneous group of hemoglobin disorders in which the production of normal hemoglobin is partly or completely suppressed due to defective synthesis of one or more of the normal globin chains, resulting in chronic anemia (MoHP, 2019; Origa, 2021; Taher *et al.*, 2021).

2.2.1 Types of thalassemia

There are many different types of thalassemias, but there are two major classifications from a clinical point of view (Williams and Marks, 1994; Origa, 2021):

i. the α-thalassemias, in which there is a defect in the synthesis of the α-chain with consequent excess of β-chains, and
ii. the β-thalassemias, in which there is a deficit in β-chain synthesis and hence an excess of α-chains.

2.2.2 Prevalence

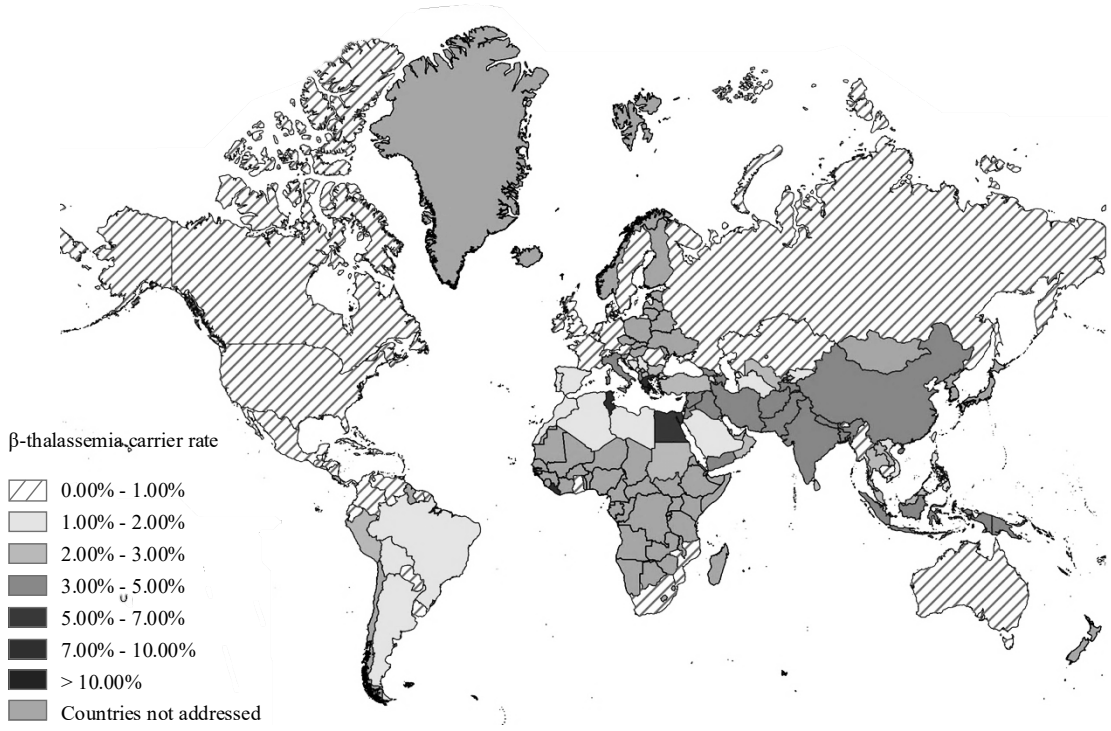
Globally, between 5-7% of the population is estimated to carry gene mutations that impair hemoglobin synthesis or function, leading to the birth of about 330,000 infants with hemoglobinopathies each year, with thalassemia syndromes accounting for 17% of these cases (Modell and Darlison, 2008; Eleftheriou and Angastiniotis, 2022).

The highest prevalence of β-thalassemia is observed within the "thalassemia belt," which extends from Africa to Southern Europe and the Middle East, toward Southeast Asia. This geographical distribution is primarily attributed to the evolutionary advantage of resistance to *Plasmodium falciparum* malaria, historically prevalent in these regions (Taher *et al.*, 2018; El-Beshlawy *et al.*, 2024).

A significant proportion of the global thalassemia burden is concentrated in the Indian Subcontinent, encompassing India, Pakistan, Nepal, Bangladesh, Sri Lanka, and the Maldives. This region alone is estimated to account for 35-40% of all global cases of thalassemia (Eleftheriou and Angastiniotis, 2022) (Fig. 2.2)

Each year, over 40,000 new cases of severe thalassemia, including β-thalassemia major (β-TM), are diagnosed. Remarkably, nearly 80% of these cases are reported in developing countries, underscoring the disproportionate burden these nations face (Modell and Darlison, 2008).

Thalassemia has been recognized by the World Health Organization (WHO) as a major global health problem, with its prevalence intensified by factors such as population migration and intermarriage among diverse ethnic groups (WHO, 2021).



Source: Angastiniotis *et al.* (2023)

Fig. 2.2 β-thalassemia carrier rates across the World, based on data gathered by Thalassemia International Federation

2.3 β-thalassemia and its types

β-thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent beta globin chain synthesis of hemoglobin, the iron-containing protein in red blood cells (RBCs) that carries oxygen to cells in the body (Galanello and Origa, 2010). The major forms of β-thalassemia, classified based on their clinical severity and treatment requirements, are (Galanello and Origa, 2010; MoHP, 2019; WHO, 2021):

i. β-thalassemia major (Cooley's Anemia or transfusion-dependent β-thalassemia (TDT)): This is the most severe form of β-thalassemia leading to a heavy reliance on regular blood transfusions for management.
ii. β-thalassemia intermedia (Non-transfusion-dependent β-thalassemia (NDT)): It exhibits milder clinical findings. Patients may require occasional blood transfusion and may become transfusion-dependent later in life.
iii. β-thalassemia minor (β-thalassemia carrier/trait): It is asymptomatic and has mild or absent anemia.

2.4 Pathophysiology and clinical presentation of β-thalassemia

In β-thalassemia, the accumulation of excess α chains forms insoluble complexes in erythroid precursors, causing oxidative damage to cell membranes and apoptosis, a phenomenon known as ineffective erythropoiesis. Although hemolysis also occurs, it is less prominent compared to ineffective erythropoiesis. The combined effect results in the immediate manifestation of chronic anemia, leading to symptoms such as fatigue and leg ulcers, as well as long-term detrimental effects on growth and developmental delays in children, and an increased risk of organ failure in adolescents and young adults.

In response to the anemia and ineffective erythropoiesis, erythropoietin production increases, stimulating red blood cell production and causing marrow expansion. This expansion can result in craniofacial deformities, bone thinning, fractures, pain, altered immune function, and splenomegaly (Taher *et al.*, 2018; Origa, 2021).

Increased erythropoiesis and apoptosis elevate the demand for folate, often leading to folic acid deficiency. The breakdown of red cells releases free hemoglobin and heme into the circulation, which can exceed detoxification capacities, causing inflammation and potential vascular dysfunction (Vinchi *et al.*, 2021).

The clinical presentation of β-TM typically occurs between 6 and 24 months of age and is characterized by severe microcytic, hypochromic anemia, and hepatosplenomegaly. Affected infants become pale, have poor appetite, grow slowly, and experience failure to thrive, feeding difficulties, irritability, recurrent fevers, and abdominal enlargement due to spleen and liver enlargement. Adolescents with the most severe form of the disease may also experience delayed puberty (Origa, 2021).

Early diagnosis is essential for the effective management of β-TM. Timely diagnosis helps in the identification and prevention of complications, thereby minimizing unnecessary healthcare expenditures (TIF, 2021).

2.5 Management of transfusion-dependent β-thalassemia major

The Thalassemia International Federation (TIF) has published guidelines for the clinical management of transfusions. The current TIF (2021) management of β-TM includes regular transfusion programs and chelation therapy to control the effects of iron overload.

2.4.1 Blood transfusion

The goals of blood transfusion therapy in children with transfusion-dependent β-TM are to correct anemia, suppress ineffective erythropoiesis, and prevent splenomegaly and skeletal abnormalities (Trompeter, 2021). The TIF (2021) recommends lifelong regular blood transfusions administered every two to four weeks to maintain pre-transfusion hemoglobin levels at 95–105 g/l. This regimen aims to minimize transfusional iron overload while promoting normal growth and well-being.

In Kanti Children's Hospital (KCH), the same protocol is followed, with a transfusion volume of 15 ml per kg of body weight per session (Thakali, 2024).

2.4.2 Iron chelation

Iron overload is the most relevant clinical concern in TDT, resulting from the cumulation of iron due to regular red blood cell transfusions. While transfusions are important for managing anemia in β-TM patients, they inevitably lead to iron overload because the human body lacks a mechanism to excrete excess iron. Each unit of red blood cells contains approximately 200 to 250 mg of iron, leading to a daily accumulation of about 0.3 to 0.6 mg/kg in various organs, while the body can only excrete about 1 mg of iron per day (Mishra and Tiwari, 2013; Arab-Zozani *et al.*, 2021).

Despite improved life expectancy due to hyper-transfusion protocols, iron overload remains an unavoidable complication. This condition is so common that it is often referred to as the "second disease", leading to significant morbidity and mortality if untreated (Fink, 1964; Cohen, 1987).

2.4.2.1 Iron chelation therapy

The primary strategy for managing iron overload in β-TM involves chelation therapy. This therapeutic approach aims to maintain safe body iron levels by balancing iron intake from blood transfusions with iron excretion through chelation. The rate of iron excretion via chelation must exceed the rate of iron accumulation from transfusions. Therapy should be initiated before toxic levels of iron accumulate, as tissue damage from iron deposition is often irreversible (Porter, 2021a).

Chelation therapy involves administering iron chelating agents to bind and excrete excess iron, thereby preventing or reducing iron accumulation and related organ damage. Effective

chelation decreases the incidence of new endocrine disorders, while poor chelation increases the risk of growth and pubertal disorders, as well as glucose homeostasis issues affecting body composition (Soliman *et al.*, 2023).

A significant challenge in chelation therapy is balancing the removal of excess iron while maintaining essential physiological iron levels, requiring careful dose adjustment. Ensuring adherence to treatment regimens is also critical, as interruptions can lead to significant damage. Currently, three iron chelators are available for patients with iron overload (Porter, 2021a; El-Beshlawy *et al.*, 2024):

i. Deferoxamine (DFO): Administered via subcutaneous or intravenous injection.
ii. Deferiprone (DFP): Available as an oral tablet or solution.
iii. Deferasirox (DFX): Available as oral dispersible or film-coated tablets.

Iron chelation is typically initiated for patients who have received 10–20 blood transfusions or have a serum ferritin level exceeding 1000 ng/ml. DFX is approved for treatment in patients older than 2 years of age (Porter, 2021a; El-Beshlawy *et al.*, 2024). TIF (2021) recommends starting DFX at a dose of 14 mg/kg/day, with the usual maximum dose being 21 mg/kg/day, taken once daily as an oral dispersible tablet. If tolerated, or in case of heavy iron load, the dose can be increased up to 28 mg/kg/day. The national guideline of Nepal recommends a starting DFX dose of 20 mg/kg/d (MoH, 2017).

At KCH, monotherapy with DFX is the standard treatment. Chelation therapy begins when serum ferritin levels reach 1000 ng/ml, the child reaches 2 years of age, or after approximately 10 blood transfusions (Thakali, 2024). The prescribed daily dosage of DFX for children is 20-40 mg/kg/day.

DFX must be dissolved in a large glass of water or juice and taken on an empty stomach, 30 minutes before a meal. While administration with fatty food increases the bioavailability of the drug, taking the medication with a low-fat meal can be considered if fasting impedes adherence (Galanello *et al.*, 2008). Potential side effects of DFX include skin rash and gastrointestinal issues such as nausea, diarrhea, abdominal pain, and occasionally upper gastrointestinal ulceration. Additionally, the use of DFX may lead to zinc deficiency and should be monitored (Erdoğan *et al.*, 2013; Fung *et al.*, 2023).

2.4.2.2 Monitoring of iron level

Iron stores in the body are primarily in the form of ferritin, with small amounts secreted into the plasma. The concentration of plasma or serum ferritin is positively correlated with the total body iron stores in the absence of inflammation. Serum ferritin (SF) is a reliable and cost-effective indicator of body iron stores and is commonly used to evaluate iron overload in β-TM. A decreasing SF trend suggests a reduction in body iron burden, although the absence of such a trend does not definitively rule out a reduction (Taher *et al.*, 2018; TIF, 2021). Table 2.1 shows the advantages and disadvantages of serum ferritin for monitoring chelation therapy.

Table 2.1 Use of serum ferritin for monitoring chelation treatment

Advantages	Disadvantages
• Simple and can be assessed repeatedly	• Provides an indirect measure of iron burden
• Cost-effective	• Levels can be elevated due to inflammation
• Allows trend identification with repeated tests	• Cannot directly measure iron balance
• Long-term control correlates with outcomes	• Non-linear response to high iron loads
• Useful for adjusting dosage as iron levels decrease	• Absence of decrease doesn't necessarily indicate response • Relationship to iron load varies with chelator • Correlation to liver iron concentration (LIC) varies across different diseases

Adapted from: TIF (2021)

A target of maintaining a serum ferritin level of no more than 1000 ng/ml is recommended by TIF (2021), as it is associated with reduced morbidity and mortality. This level is also used to indicate the need for initiation of iron chelation therapy. While SF has limited ability to predict cardiac iron overload, it can reliably indicate cardiac and endocrine disease when levels exceed 2500 ng/ml (Taher *et al.*, 2021).

SF measurement is widely accessible and often the only feasible option in resource-limited settings. The TIF (2021) guidelines recommend assessing SF every three months to

facilitate patient follow-up and make informed treatment decisions. This regular monitoring ensures effective management of iron levels and allows for timely adjustments to therapy. At KCH, a similar protocol is followed, with SF levels being assessed every three months (Thakali, 2024).

2.4.3 Splenectomy

In β-TM, the production of hemoglobin β-chains is either absent or significantly reduced. A key pathophysiological feature of this condition is the increased destruction of red blood cells by the reticuloendothelial system, particularly the spleen, resulting in splenomegaly Historically, splenectomy has been used as an adjunct or alternative to transfusion therapy in β-TM patients. The primary therapeutic rationale for splenectomy has been to reduce the volume and frequency of blood transfusion, thereby reducing iron accumulation (Cappellini *et al.*, 2018; Daar and Taher, 2021).

However, splenectomy has become nearly obsolete due to advancements in safer blood transfusions and effective iron chelation therapies (Taher *et al.*, 2018). Current transfusion protocols and iron chelation have significantly reduced the incidence of splenomegaly and the necessity for splenectomy (Daar and Taher, 2021; Trompeter, 2021).

2.6 Complications in β-thalassemia major children

In non-transfused and sub-optimally transfused β-TM patients, the combination of ineffective erythropoiesis and anemia leads to a range of health complications. Conversely, those who receive adequate transfusions primarily suffer from secondary iron overload and its associated morbidities (El-Beshlawy *et al.*, 2024).

Iron buildup is extremely toxic to many tissues, leading to severe health complications. When iron levels in the plasma surpass the transport capacity of transferrin, excess iron begins to circulate as non-transferrin-bound iron (NTBI). NTBI is the primary cause of tissue damage in both hepatic and extrahepatic organs in cases of transfusional iron overload. Such iron accumulation can be detected in children as young as 2-6 years, leading to organ damage and dysfunction, particularly affecting the heart, liver, and endocrine glands. The adverse effects of iron overload include (Fung *et al.*, 2023):

i. Growth deficiency
ii. Delayed puberty and hypogonadism

iii. Hypothyroidism and hypoparathyroidism
iv. Impaired glucose tolerance and diabetes mellitus
v. Adrenal insufficiency
vi. Bone disease
vii. Liver complications
viii. Cardiac complications
ix. Nephrolithiasis

Beyond iron overload, factors such as chronic hypoxia due to anemia can potentiate iron toxicity in endocrine glands. Viral infections and individual genetic susceptibility also play a role in causing endocrine dysfunction (De Sanctis *et al.*, 2013; Porter, 2021b).

2.7 Nutritional status of β-thalassemia children

Patients with β-TM often face significant nutritional challenges that can lead to growth disturbances, poor weight gain, reduced body mass index (BMI), and altered body composition. The etiology of these issues is multifaceted, with chronic anemia, iron overload from regular blood transfusions, and adverse effects of chelation therapy being the primary contributors (Rathaur *et al.*, 2020; Soliman *et al.*, 2023).

2.7.1 Growth disturbance in β-thalassemia major children

Growth impairment, including both stature and weight, is one of the most common complications in children and adolescents with β-TM, despite advancements in treatment (Soliman *et al.*, 2009; De Sanctis *et al.*, 2013). This issue manifests variably, with some individuals experiencing normal growth while others face disruptions later in childhood (Skordis and Kyriakou, 2011).

The growth failure observed in children with β-TM has multifactorial pathogenesis and is influenced by numerous factors (Fig. 2.3). A primary contributor to growth impairment in β-TM patients is chronic anemia and excessive iron load from frequent blood transfusions. Iron deposition in the pituitary gland emerges as a significant factor, disrupting the growth hormone-insulin-like growth factor (GH-IGF-1) axis (Taher and Saliba, 2017). Moreover, zinc deficiency impacts growth, bone health, and the immune system (Arab-Zozani *et al.*, 2021; Soliman *et al.*, 2023).

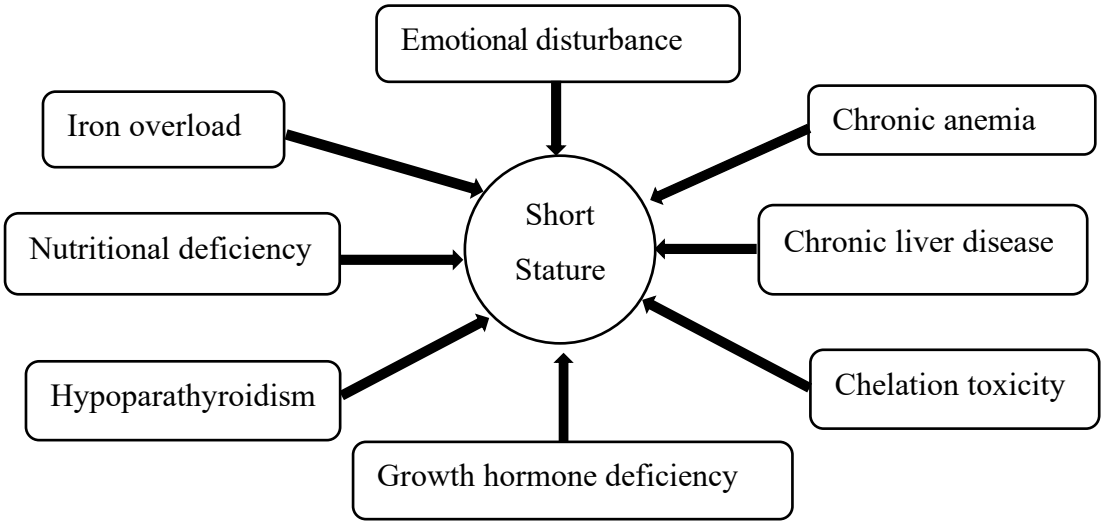
Hormonal imbalances, such as hypothyroidism, hypogonadism, disturbed calcium homeostasis, and growth hormone deficiency, exacerbate these issues. Furthermore, chronic liver disease, inadequate intake of essential macronutrients and micronutrients, including zinc, folic acid, vitamin D, vitamin E, and carotenoids, and psychological stress significantly contribute to the growth challenges faced by children with β-TM (Fung *et al.*, 2012; Soliman *et al.*, 2023). Moreover, studies have found that increased SF level, common in β-TM patients, positively correlates with body fat levels (Iwasaki *et al.*, 2005; Orta-Duarte *et al.*, 2020).

Growth disturbances in β-TM patients are recognized in three distinct phases according to age (Skordis and Kyriakou, 2011):

i. Phase 1: Early childhood growth issues are mainly due to hypoxia, anemia, ineffective erythropoiesis, and poor nutrition.

ii. Phase 2: In late childhood, growth retardation is primarily due to iron overload affecting the GH-IGF-1 axis and other endocrine complications.

iii. Phase 3: After ages 10-11, delayed or arrested puberty becomes a significant factor, preventing a normal growth spurt in adolescents.



Redrawn from: De Sanctis *et al.* (2021)

Fig 2.3 Etiology of growth impairment in β-TM patients

Malnutrition significantly contributes to growth impairment, particularly in poorer regions where inadequate nutrient intake is prevalent (Elalfy *et al.*, 2020). Moreover, breastfeeding practices, birthweight, and significantly impact children's growth patterns and quality of life (Baran *et al.*, 2019; Palaska *et al.*, 2024). Achieving optimal nutritional status is essential for realizing genetic growth potential, thereby making growth a critical metric for assessing overall nutritional status (Fung *et al.*, 2010).

2.7.1.1 Stunting in β-thalassemia major children

Stunting is defined as impaired growth and development manifested by low height-for-age, resulting from chronic or recurrent undernutrition (WHO, 2024). It is prevalent among thalassemic children and adolescents, with several studies highlighting this issue. Mirhosseini *et al.* (2013b) found that 41.4% of β-TM children and adolescents had short stature. Similar observations were reported by Pemde *et al.* (2011), who found 33% short stature among 154 Indian thalassemic children and adolescents, along with a high prevalence of impaired growth velocity. The incidence of short stature was consistent across multiple studies: Rathaur *et al.* (2020) reported 65.71%, Sharma and Bezboruah (2022) found 68%, Jana *et al.* (2016) noted 65.8%, Fadlyana *et al.* (2017) observed 62%, and Das and Majumdar (2019) identified 67% stunting among TDT patients.

De Sanctis *et al.* (2017) evaluated the prevalence rate of short stature among 3023 β-TM patients in 16 countries and reported that 53% were affected. A recent meta-analysis by Arab-Zozani *et al.* (2021) further confirmed that the prevalence of short stature is particularly pronounced in thalassemic patients older than seven years. These consistent findings underscore the growth disturbances in TDT patients, necessitating early intervention and comprehensive management strategies to address and mitigate stunting in affected children.

2.7.1.2 Undernutrition in β-thalassemia major children

Among the numerous complications β-TM patients face, undernutrition remains particularly prevalent during childhood and adolescence. Soliman *et al.* (2023) reviewed 22 studies on undernutrition in β-TM across 12 countries and found significant regional variation in its prevalence. This variability is largely attributable to differences in income levels and nutritional status of each country. Developing countries, such as India, Pakistan, Iran, and

Egypt, exhibited higher rates of undernutrition compared to high-income countries like Turkey, Greece, Canada, and the USA.

Several studies have reported the prevalence of underweight status among β-TM children based on Body Mass Index (BMI). Biswas *et al.* (2021) found that 48.2% of 328 β-TM children in India were underweight. Similarly, Sheikh *et al.* (2017) observed that 58.69% of 305 β-TM children in Pakistan were underweight. Even higher rates were reported by Rathaur *et al.* (2020) and Das and Majumdar (2019), with 77% and 70% of children being underweight, respectively.

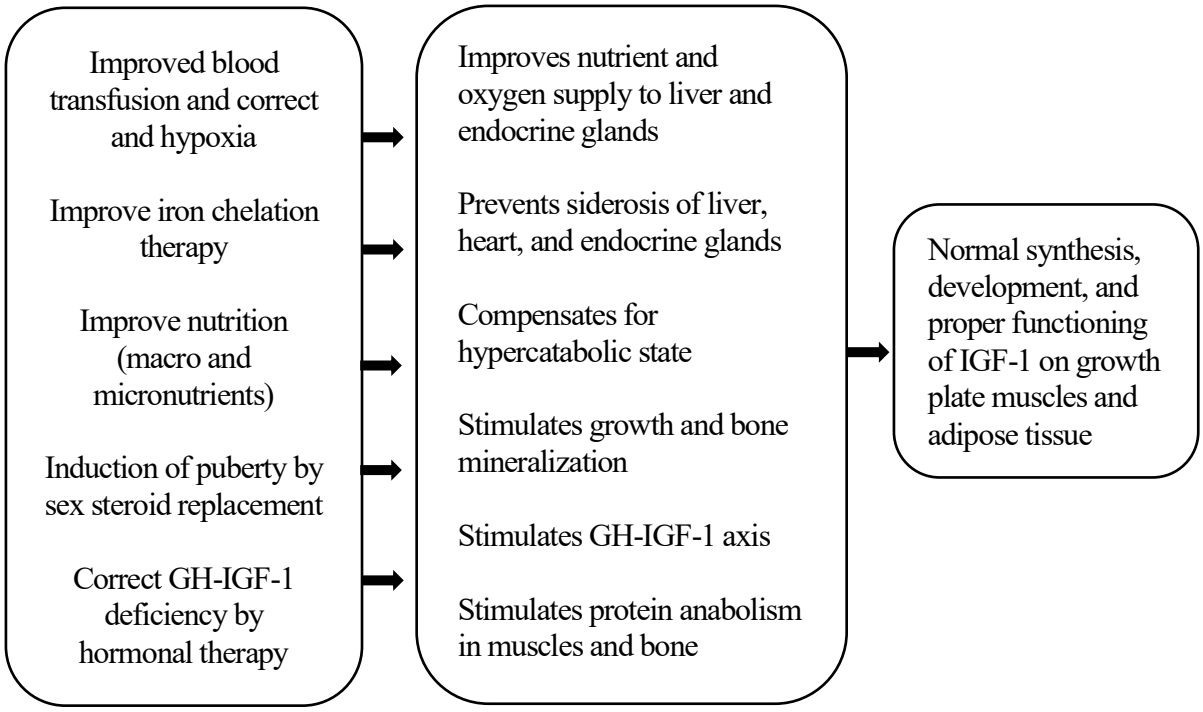
Moiz *et al.* (2018) also noted that 40% of β-TM patients were underweight. Underweight is defined as low weight-for-age, indicating that a child may be stunted, wasted, or both (WHO, 2024). Badiger and Baruah (2019) similarly observed 40% undernutrition among irregularly transfused thalassemic children.

2.7.1.3 Prevention and management of growth failure

The TIF (2021) guidelines and Nutrition Guide of Thalassemia (Fung *et al.*, 2023) recommend the following interventions to prevent and manage growth and stature deficits in β-TM:

i. Blood transfusions should be initiated early in life to enhance oxygenation and suppress endogenous ineffective erythropoiesis, which can lead to a hypermetabolic state and bone deformities affecting growth (Longo *et al.*, 2021). It is also important to maintain a pre-transfusion hemoglobin level greater than 90 g/l for effective management.
ii. Adequate caloric intake has been shown to significantly increase IGF-1 levels in thalassemic children, thereby partially correcting growth impairment (Soliman *et al.*, 2004) (Fig. 2.4)
iii. Maintaining adequate iron chelation to prevent endocrinopathies and other organ damage. Strict adherence to iron chelation protocols, guided by regular monitoring, aims to maintain serum ferritin levels below 1000 µg/l.
iv. Identifying and addressing common nutritional deficiencies, including zinc, vitamin D, vitamin A, and folate, is essential (Bulgurcu *et al.*, 2021; Goldberg *et al.*, 2022). Patients with proven deficiencies should receive zinc supplementation.

v. Early identification and treatment of hypothyroidism and abnormal glucose homeostasis.
vi. Providing psychological and social support to enhance adherence to treatment routines and minimize stress.



Redrawn from: Soliman *et al.* (2009)

Fig 2.4 Treatment and management of growth failure in transfusion-dependent thalassemia (GH = Growth hormone, IGF-1 = Insulin-like growth factor)

Growth is commonly used as a primary indicator of a child's overall nutritional status. Regular monitoring of height and weight is essential for detecting and addressing growth impairments in children with TDT at an early stage. (Fung *et al.*, 2023) recommend measuring height and weight monthly and quarterly respectively. Table 2.2 lists the anthropometric monitoring parameters for TDT patients. In KCH, while weight is measured during every visit, height is only assessed every six months. Moreover, BMI and growth velocity are not routinely measured (Thakali, 2024).

Additionally, annual growth screenings through endocrine assessments are recommended starting from the age of 9 years, or earlier if clinically indicated, to further monitor and address potential growth impairments in children with TDT (De Sanctis *et al.*, 2021).

Table 2.2 Recommended anthropometric monitoring guidelines for thalassemia patients

Anthropometry	Frequency
Weight (kg)	Monthly
Height (cm)	Quarterly till reaches adult height
BMI (kg/m^2)	Every 6 months
Head circumference	Every 6 months
Growth velocity (cm/year)	Every 6 - 12 months till reach adult height

Source: Fung *et al.* (2023)

2.7.2 Body fat in β-thalassemia major children

Body fat serves as a good indicator of health and nutritional status. The subcutaneous fat functions as an energy reserve in times of nutritional deficiency. Where there is a negative energy balance, fat reserves are depleted, leading to a decrease in both body fat and fat mass (Mandal *et al.*, 2011; Wickramasinghe, 2012). The influence of steroid hormones leads to more pronounced differences in body fat distribution between genders (Karastergiou *et al.*, 2012). Ethnic backgrounds also contribute to differences in body fat percentages, with studies showing that Asians typically exhibit higher body fat than Caucasians (WHO, 2000; Deurenberg *et al.*, 2002).

In a study involving 183 pediatric patients with thalassemia, it was found that female patients had higher body fat levels than male patients. After adjusting for age, sex, and ethnicity, the body fat percentage was significantly higher in TDT patients compared to those with NTDT patients (Fung *et al.*, 2010). Furthermore, another study observed a higher body fat percentage in female patients and in those who had undergone splenectomy compared to their non-splenectomized counterparts. An inverse relationship was observed between age and body fat percentage, suggesting a decrease in body fat as age increases. This research concluded that the reduction in body fat percentage in β-TM patients is likely due to inadequate energy and fat intake (Elalfy *et al.*, 2020).

Within the context of Nepal, there is limited research focusing on the body fat patterns of children. A notable study by Ghosh *et al.* (2009), which included 1988 Nepalese children, assessed body fat using skinfold thickness measurements of the triceps and subscapular. This study revealed low body fat levels across the cohort, which were linked to reduced energy intake potentially related to socio-economic challenges. Moreover, the study found that FFM

was substantially higher in Nepalese boys than in girls, further emphasizing notable disparities in body composition across various demographic groups.

2.7.3 Serum micronutrient profile in children with β-thalassemia major

In a comprehensive review of 97 studies, Goldberg *et al.* (2022) identified common micronutrient deficiencies in β-TM patients such as vitamins A, C, D, selenium, and zinc. These deficiencies were strongly associated with age and the degree of iron overload. Despite sufficient dietary intake, many patients with β-TM present low levels of these essential micronutrients, suggesting an increased physiological demand or excessive micronutrient loss (Soliman *et al.*, 2023).

Additionally, the pathological iron accumulation in β-TM can compromise the integrity of the intestinal lining, This compromise can lead to increased permeability, altered gut microflora, and consequently impaired nutrient absorption and disrupted energy metabolism, as detailed by Soliman *et al.* (2023). Such gastrointestinal (GI) disturbances further exacerbate the nutritional deficiencies in β-TM patients.

Table 2.3 Recommended laboratory investigation and monitoring for thalassemia patients

Nutrient	Thalassemia International Federation (TIF)	Kanti Children's Hospital (KCH)	Supplementation provided at KCH
Calcium	Serum and urinary calcium annually	Serum calcium every 6 months	Yes
Folate	Serum folate annually	Not monitored	Yes
Vitamin D	Serum 25-OHD every 6 months	Serum 25-OHD every 6 months	Yes
Zinc	Serum zinc every 6 months	Not monitored	No

Source: Fung *et al.* (2023)

Antioxidants like vitamins C and E, β carotene, copper, and zinc are known for their potential to mitigate oxidative stress and inflammation associated with iron toxicity (Walter *et al.*, 2006; Fibach and Dana, 2019). However, the therapeutic use of chelators affects the bioavailability of these nutrients. Chelation therapy, while essential, leads to unintended binding and removal of these essential minerals alongside the targeted free iron, thereby

diminishing their efficacy and contributing further to nutritional deficiencies (Goldberg *et al.*, 2018).

Vitamin D and zinc are most frequently found to be deficient in patients with thalassemia, and their supplementation offers considerable potential benefits (Goldberg *et al.*, 2022). Table 2.3 outlines the practices of KCH on the recommended laboratory investigation of micronutrients and monitoring in TDT patients. (More details in Appendix G)

2.7.3.1 Vitamin D

Vitamin D is essential for bone mineralization and calcium absorption, particularly during periods of rapid growth such as infancy and puberty. In β-TM patients, deficiencies in vitamin D, coupled with reduced physical activity, lead to severe skeletal complications like bone pain and increased risk of fractures. Moreover, low vitamin D levels are linked with decreased cardiac function, muscle weakness, glucose insensitivity, and congestive heart failure (Goldberg *et al.*, 2022; Fung *et al.*, 2023).

The high prevalence of vitamin D deficiency among β-TM patients can be attributed to several factors. Impaired 25-hydroxylation due to liver hemosiderosis, intestinal malabsorption, limited sun exposure, and impaired synthesis by the skin due to icterus are significant contributors. Goldberg *et al.* (2022) reported that hepatic iron concentrations inversely correlated with 25-hydroxyvitamin D (25-OHD) levels, and patients deficient in vitamin D also exhibited elevated serum ferritin levels. Notably, despite comparable UV light exposure, patients with thalassemia consistently showed lower circulating levels of 25-OHD compared to healthy controls.

Moreover, low vitamin D status is associated with poor growth indicators such as BMI, weight-for-age (WAZ), and skinfold thickness. These findings emphasize a negative correlation between insufficient vitamin D intake and various anthropometric measurements, suggesting that vitamin D deficiency may significantly contribute to the stunted physical development frequently observed in β-TM patients (Soliman *et al.*, 2023).

Management and monitoring of vitamin D levels

The serum 25-OHD level is the most reliable biomarker for assessing vitamin D status. Deficiency in vitamin D is defined as serum 25-OHD levels below 20 ng/ml, while insufficiency as levels between 20-30 ng/ml. To effectively manage these conditions, it is

recommended to maintain serum 25-OHD within the optimal range of 30–40 ng/ml. This target range not only promotes bone formation and cardiac health but also minimizes the risks associated with excessive vitamin D supplementation, such as hypercalciuria and nephrolithiasis. (TIF, 2021; Fung *et al.*, 2023).

In geographical areas with limited sunlight exposure, a minimum supplementation of 1000 IU of vitamin D per day is advised to prevent deficiency. For those patients demonstrating low serum 25-OHD levels, a more aggressive approach is suggested with an initial regimen of 50,000 IU of vitamin D weekly. This treatment continues until the serum 25-OHD level reaches the desired threshold of 30 ng/ml. Subsequently, a lower maintenance dosage should be given to maintain these levels. It is essential to monitor that serum level does not exceed 50 ng/ml to prevent potential toxicity (Goldberg *et al.*, 2022; Fung *et al.*, 2023).

The TIF (2021) recommends regular bi-annual assessments of vitamin D levels for thalassemia patients to prevent and effectively correct deficiencies. In KCH, a similar protocol is followed for the assessment of vitamin D levels (Thakali, 2024).

2.7.3.2 Calcium

Patients with thalassemia, irrespective of transfusion dependence, commonly experience low bone mass and hypercalciuria. Calcium homeostasis is further complicated by generally insufficient levels of 25-OH vitamin D among thalassemia patients (Vogiatzi *et al.*, 2009; Fung *et al.*, 2023).

Thiagarajan *et al.* (2019) conducted an intervention involving 29 children aged 2-12 years with thalassemia, administering 500 mg of calcium and 1000 IU of vitamin D daily for one year. The study reported an increase in bone mineral content, suggesting the potential benefits of such supplementation. However, the absence of a control group in this study limits the ability to definitively attribute improvements solely to the supplementation.

Additionally, hypercalciuria in thalassemia patients has been linked to renal tubular dysfunction, as documented by Quinn *et al.* (2011), and may also be associated with the use of DFX, a common iron chelator used in thalassemia treatment, as indicated by (Wong *et al.*, 2016).

Management and monitoring of calcium levels

In thalassemia patients, accurately assessing calcium status is challenging due to the body's strict regulation of serum calcium, typically maintained within a narrow range of 8.5 to 10.5 mg/dl. As a result, standard blood tests often fail to reflect the true nutritional calcium status. Significant fluctuations in serum calcium levels typically occur only with severe disruptions in calcium homeostasis (Fung *et al.*, 2023). Therefore, TIF (2021) recommends evaluating both serum and urinary calcium annually to provide a more comprehensive understanding of calcium metabolism.

Interestingly, TIF (2021) does not advocate for routine calcium supplementation to prevent nephrolithiasis. Instead, it recommends a diet rich in calcium such as milk, milk products, and oily fish. However, in KCH, calcium supplementation is given and monitoring is done every six months (Thakali, 2024).

2.7.3.3 Folate

Folate is essential for cell division and effective erythropoiesis, which underscores its importance in managing hemoglobinopathies. However, routine folate supplementation often lacks robust clinical evidence (Goldberg *et al.*, 2022). While β-TM patients on high transfusion regimens rarely develop folate deficiencies due to suppressed endogenous erythropoiesis, insufficient dietary intake can still impact folate levels (Fung *et al.*, 2012; Angastiniotis, 2021).

Recent research shows that folate levels in thalassemia patients generally match those in healthy individuals (Sherief *et al.*, 2014), although studies like Claster *et al.* (2009) suggest that serum folate may decrease with age in patients with heavy iron loads and dependence on transfusions.

Management and monitoring of folate levels

Serum folate is commonly used to evaluate folate status, with levels below 3 ng/ml indicating deficiency. However, serum folate primarily reflects recent dietary intake and may not accurately represent long-term folate status. Therefore, TIF (2021) recommends an annual assessment of serum folate levels.

To maintain optimal folate levels, TIF recommends a supplementation regime with either 1 mg/day or 5 mg/week of folic acid (Fung *et al.*, 2023), supported by increased consumption of folate-rich foods, as demonstrated by Agrawal *et al.* (2022).

At KCH, folate supplementation is prescribed at a standard dose of 2.5 mg/day for most patients, with a maximum dosage of 5 mg/day. For patients under two years of age, a lower dose of 1.25 mg/day is prescribed. Additionally, there is no practice of monitoring serum folate levels at KCH (Thakali, 2024).

2.7.3.4 Zinc

Zinc is an essential mineral in thalassemia, which is often deficient due to factors like iron chelation therapy (Erdoğan *et al.*, 2013) and poor dietary intake or absorption (Fung *et al.*, 2012). Bekheirnia *et al.* (2004) and Mirhosseini *et al.* (2013a) found positive correlations between zinc levels and growth parameters such as height, weight, BMI, and body circumference among young TDT patients.

Moreover, a Cochrane review by Swe *et al.* (2013) suggests that zinc supplementation can significantly enhance growth in children with thalassemia. Beyond growth, zinc also plays crucial roles in bone health and metabolic functions, influencing bone formation through growth hormone and osteoblast activity (O'Connor *et al.*, 2020). Mousa *et al.* (2021) linked zinc deficiency to increased pancreatic dysfunction in TDT patients. Furthermore, recent studies by Matter *et al.* (2020) and Fung *et al.* (2020) demonstrated that zinc supplementation of 25–40 mg/day for 3 months improved insulin sensitivity and glucose homeostasis.

Management and monitoring of zinc levels

TIF (2021) advises that patients undergoing chelation therapy should have their serum zinc levels monitored every six months. For those diagnosed with a zinc deficiency, daily supplementation of 20-25 mg is recommended to effectively manage and correct the deficiency. However, at KCH, neither monitoring nor supplementation of zinc is currently practiced (Thakali, 2024).

2.7.4 Increased energy expenditure in β-thalassemia major children

Although specific studies assessing the thermic effect of food (TEF) in thalassemia are lacking, it is assumed that the contribution to total energy expenditure (TEE) from dietary

composition would be similar to that of the general population (Fung *et al.*, 2012). In thalassemia, elevated caloric needs often arise due to increased protein turnover, inflammation, and changes in cardiac or respiratory function (Soliman *et al.*, 2023). The energy and protein requirements for growth in children and adolescents must also be considered in their TEE. Typically, voluntary activity, which is the most variable component of TEE, is reported to be lower in individuals with thalassemia compared to the healthy population (Fung *et al.*, 2015).

2.8 Dietary pattern and nutrient intake of β-thalassemia major children

The determination of ideal nutritional requirements for thalassemia patients and effective methods to fulfill these needs remains an unresolved issue. (Soliman *et al.*, 2023). Nutritional deficiencies in these patients arise from various factors and vary between children and adults. Common factors across all age groups include cultural dietary restrictions, misinformation about food avoidance, and personal food intolerances. These, coupled with increased nutrient excretion and higher dietary needs, amplify the risk of nutritional inadequacies (Fung *et al.*, 2023) (Table 2.4).

The primary cause of these nutritional deficiencies often lies in inadequate dietary intake, which can be influenced by a range of psychological and physiological factors including stress, depression, adrenal insufficiency, fatigue, physical inactivity, and cultural influences (Nanas *et al.*, 2009; Fung *et al.*, 2012; Fung *et al.*, 2015; Huang *et al.*, 2015). Severe anemia can also impair appetite, altering hunger and taste preferences, and leading to decreased food intake. Moreover, side effects like nausea from oral iron chelators often result in missed meals and food intolerance (Fung *et al.*, 2023). These issues are compounded by increased energy demands due to hypermetabolism associated with active bone marrow, tachycardia, and elevated respiratory rates, as well as gastrointestinal disturbances that can induce anorexia and reduce food digestion and absorption (Soliman et al., 2023).

A study by Fung *et al.* (2012) found that adult thalassemia patients consumed calories sufficient to match their energy expenditure. However, their diets lacked adequate nutrient density, resulting in deficiencies in essential micronutrients and trace elements. Nutritional inadequacy extends to younger patients as well, as demonstrated by Elalfy *et al.* (2020), who studied 200 TDT pediatric patients. Their findings indicated that these children consumed 22% fewer calories than healthy controls, with lower intake levels of protein, carbohydrates,

calcium, and phosphorus. These dietary deficiencies were correlated with stunted physical growth, with TDT patients being significantly shorter and lighter than their healthy counterparts. Moreover, in energy-deprived states, dietary protein is predominantly catabolized for energy production, despite not being the primary energy source under normal conditions (Carbone *et al.*, 2019).

Table 2.4 Summary of factors that may lead to nutritional deficiencies in patients with thalassemia

Factors	Description
Dietary habits and nutrient intake	• Inadequate or inappropriate intake due to feeding behavior issues in toddlers and young children • Poor appetite due to anemia, adrenal insufficiency, zinc deficiency, or inactivity • Inactivity and inadequate fluid intake may lead to chronic constipation, lowering appetite and dietary intake • Insufficient nutrient density (foods high in calories yet limited nutritional value) • Avoidance of foods rich in heme-iron leading to limited protein and zinc intake; avoidance of nonheme-iron leading to limited intake of key vitamins and phytonutrients • Nausea, cramping abdominal pain from the use of oral chelators leading to missed meals, specific food intolerances, and decreased food consumption
Energy expenditure and nutrient losses	• Increased mineral losses due to nonspecific chelation effects • Essential trace mineral sequestration (Zn, Cu) in the liver due to iron overload • Increased non-transferrin-bound iron leading to increased oxidative stress and antioxidant consumption • Ineffective erythropoiesis and increased cardiac output leading to elevated energy expenditure

Source: Fung *et al.* (2023)

Despite the limited research, some small-scale nutritional intervention studies have shown promising results in linking nutritional deficiencies to growth issues in thalassemia. For example, Soliman *et al.* (2004) placed 15 thalassemia children on a high-calorie diet (130–150% of the estimated requirement) for two months, resulting in significant increases in BMI, skinfold thickness, mid-arm muscle circumference, and IGF-1 levels. Similarly, an earlier study on thalassemia toddlers reported improvements in weight gain and levels of plasma zinc, alpha-tocopherol, and IGF-1. Although these increases did not lead to increases in linear growth, the positive changes in IGF-1 suggest that addressing nutritional deficiencies could partially correct growth impairments in young thalassemia patients. (Fuchs *et al.*, 1996; Fuchs *et al.*, 1997).

2.8.1 Iron absorption from the diet in β-thalassemia major patients

In β-TM patients, managing iron overload is a significant concern. Under normal conditions, the intestines absorb approximately 1-2 mg of iron daily (Porter, 2021a). However, classic studies have shown that iron absorption can increase to nearly 20% in individuals with severe anemia, as observed in thalassemia patients (de Alarcon *et al.*, 1979). This increased absorption inhibits the synthesis of the hepatic hormone hepcidin, caused by ineffective erythropoiesis and chronic hypoxia, which subsequently enhances intestinal iron uptake and release from macrophages (Porter, 2021b; Taher *et al.*, 2021).

As a standard care measure, β-TM patients are typically advised to adhere to a low-iron diet. This dietary recommendation includes avoiding iron-rich foods such as red meats, organ meats, and iron-rich cereals. Despite these guidelines, it is essential to recognize that dietary iron absorption is minimal compared to the substantial iron load received through blood transfusions (Fung *et al.*, 2012). For example, one unit of packed red blood cells contains approximately 200 mg of iron, vastly exceeding the amount from dietary sources (For example: ~2 mg of iron from 100 g of goat meat (Longvah *et al.*, 2017)).

The effectiveness of dietary iron reduction in TDT remains a topic of debate. While a low-iron diet might offer some benefits, it can also negatively impact the quality of life for patients. Limited food choices can result in nutritional imbalances, social isolation, and increased stress. Additionally, such dietary restrictions might create a false sense of security, potentially leading to decreased adherence to essential chelation therapy (Fung *et al.*, 2012).

Avoiding iron in the diet often leads to reduced zinc intake, a nutrient shown to be beneficial for immune status, bone health, and growth in thalassemia patients (Fung *et al.*, 2023).

The 2024 nutrition guide in thalassemia by TIF recommends that for patients receiving transfusions and regular chelation treatment, with iron levels closely monitored, the amount of iron absorbed from the diet is negligible. Therefore, strict avoidance of iron-rich foods should not be a primary focus (Fung and Yardumian, 2024).

2.9 Nutrition guidelines and recommendations for β-thalassemia major patients

Previously, the clinical approach to managing β-TM largely focused on blood transfusions, iron chelation therapy, and the management of associated complications. Over the last decade, however, there has been a shift towards enhancing the quality of life, including the integration of nutritional support. Nutrition significantly impacts the overall health of individuals with β-TM but generally does not necessitate a specialized or restrictive diet.

Recently, the TIF has developed extensive guidelines based on both empirical research and the practical experiences of medical professionals dedicated to thalassemia care. These guidelines provide crucial nutritional recommendations to support the well-being of patients with β-TM (Fung *et al.*, 2023; Fung and Yardumian, 2024). The key nutritional recommendations include:

i. Integrate a comprehensive nutritional assessment into the individualized patient care plan annually, performed by a qualified nutrition professional. This assessment should include anthropometric measurements, nutritional biochemistries, and the use of clinical and dietary evaluation tools.
ii. Careful monitoring of the balance between caloric intake and energy expenditure, particularly during periods of rapid growth in children, to ensure energy needs are met adequately.
iii. Ensure sufficient intake of high-quality protein, especially for patients on vegetarian or vegan diets.
iv. Address hydration needs to prevent complications like fatigue and constipation, considering factors such as age, gender, activity level, and environmental conditions.
v. Encourage consumption of a high-fiber diet and restrict intake of sugary beverages. Promote daily consumption of fruits, vegetables, and whole grains.

vi. Include dietary sources rich in vitamin E and folate, such as nuts, vegetable oils, dark green leafy vegetables, and legumes.
vii. Routine supplementation of nutrients other than folate, vitamin D, and zinc is not recommended unless specific deficiencies are identified. However, administering a daily multivitamin/mineral supplement that does not contain iron may be beneficial to the overall health of patients.
viii. Prioritize dietary sources of calcium like milk and milk products, tofu over supplements to reduce the risk of conditions like nephrolithiasis.
ix. For NTDT patients, dietary iron restriction may be considered to manage iron levels, though it should not be a primary focus for TDT patients, where iron overload from transfusions significantly outweighs dietary iron absorption.
x. Drinking tea with meals can help reduce iron absorption in NTDT patients and is recommended. However, this is not advised for children who would benefit more from nutrient-rich alternatives like milk.

2.10 Socio-economic burden in children with thalassemia

The 2023 Global Thalassemia Review underscores the profound socio-economic impact of thalassemia in the Southeast Asia region. Although access to healthcare services was relatively easier for 49.80% of patients, a significant 73.64% had financial burden of essential care and testing. Moreover, one in five respondents faced difficulties traveling to treatment centers, a challenge exacerbated by the associated high costs. Additionally, more than 74% of patients reported losing over 11 days per year from education or work due to their treatment needs, highlighting the disruptive nature of thalassemia in daily life (Angastiniotis *et al.*, 2023)**.**

In India, a detailed study revealed that annual treatment costs for thalassemia patients vary significantly across different age groups, ranging from INR 41,515 (US$ 629) to INR 151,836 (US$ 2,300.50), with an average expense of INR 74,948 (US$ 1,135.57). Notably, over half (51.4%) of these expenditures are allocated to medications, primarily for iron chelation therapy (Moirangthem and Phadke, 2018). These costs are consistent with findings from Thailand and Italy, drawing attention to the substantial financial demands of such treatments (Scalone *et al.*, 2008; Riewpaiboon *et al.*, 2010). The chronic nature of β-TM necessitates sustained medical interventions and imposes significant burdens on patients, their families, and healthcare systems.

2.11 β-thalassemia in Nepal

β-thalassemia is a widely recognized hemoglobin disorder in Nepal, yet comprehensive national prevalence data and accurate statistics remain unpublished by the Nepalese government (Eleftheriou and Angastiniotis, 2022; Lama *et al.*, 2022). This lack of precise data hinders a full understanding of the overall disease burden. Recent estimates from the Global Thalassemia Report indicate that approximately 4% of the population in Nepal are β-thalassemia carriers, with an estimated 600 individuals affected by the condition (Table 2.5).

Table 2.5 Epidemiology of β-thalassemia syndromes in Nepal

Thalassemia patients	Prevalence
β-thalassemia carriers	4%
Expected HbE/β-thalassemia births/1000	0.88
Estimated β-thalassemia patients	600
Expected NTDT patients	NA

HbE = Hemoglobin E, NTDT = Non-transfusion dependent thalassemia

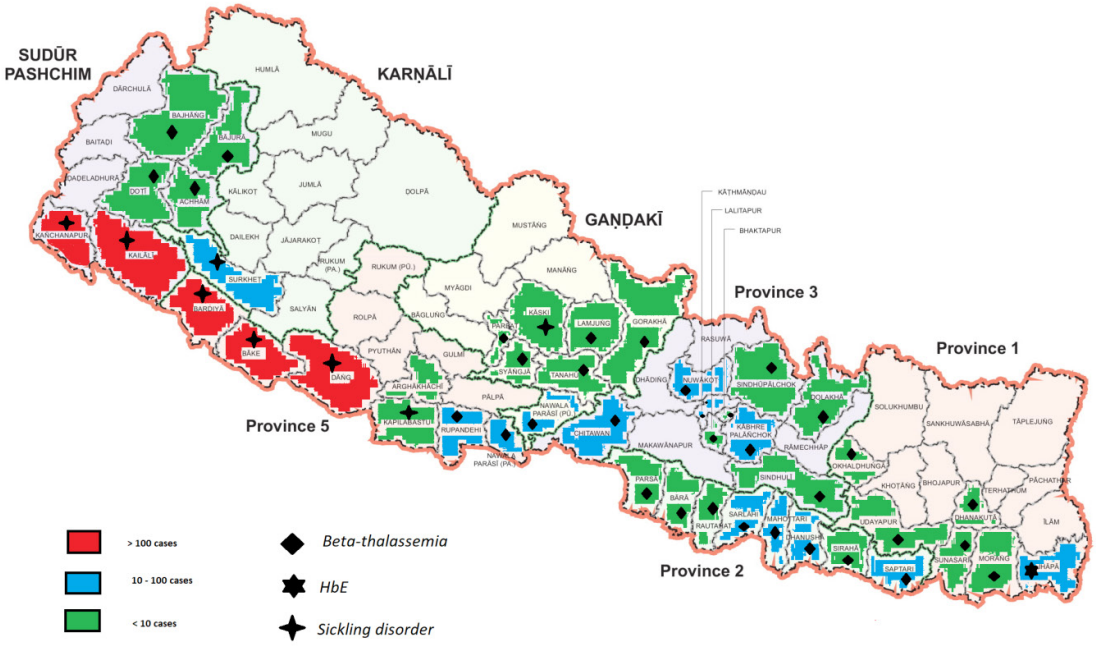
Source: Angastiniotis *et al.* (2023)

The Nepal Thalassemia Society (NTS), a member of TIF, plays a crucial role in maintaining a registry of thalassemia patients, providing a valuable database (Sharma Poudyal *et al.*, 2023). As of 2021, the NTS has registered 730 patients across the country. However, this data likely underrepresents the true incidence, with many cases going unreported or undiagnosed (Angastiniotis *et al.*, 2023).

β-thalassemia is widespread spread across Nepal but is predominantly found in the Terai region (Shrestha *et al.*, 2020). Significant prevalence has been observed among various ethnic groups, including the *Brahmin*, *Chhetri*, *Rai/Limbu*, *Newar*, and *Tamang* communities (Lama *et al.*, 2022), and *Tharu* communities (Jha and Jha, 2014). Fig. 2.5 illustrates the map of Nepal showing the distribution of hemoglobinopathy cases and common types.

Most β-thalassemia patients receive treatment at the KCH (for those up to 14 years) and Bir Hospital (for those above 14 years) in Kathmandu, where all basic care is funded by the government (Upreti, 2023). Since 2016, the Government of Nepal (GoN), through the

Ministry of Health (MoH), has extended financial aid to economically disadvantaged individuals with thalassemia (MoH, 2017). In 2019, the Finance Ministry allocated 20 million rupees to fund free treatment for thalassemia patients in Kathmandu, including free blood transfusions, routine tests, and iron-chelating agents. However, these benefits have not reached all patients nationwide. Furthermore, the lack of a prevention program leaves the status of patients outside the capital city uncertain (Angastiniotis *et al.*, 2023).



Source: Shrestha *et al.* (2020)

Fig. 2.5 Map of Nepal showing the total number of hemoglobinopathy cases and common hemoglobinopathy types

The management of β-thalassemia in Nepal presents significant challenges due to resource limitations. The condition, characterized by chronic anemia and reliance on regular blood transfusions, not only poses medical and nutritional complexities but also places a substantial financial burden on affected families and the healthcare system. The estimated annual cost of thalassemia treatment is around 70,000 NPR (approximately US $533) for children and 2,50,000 NPR (about US $1906) for adults, covering expenses for blood transfusions, travel to transfusion centers, and iron chelation therapy. Despite cultural openness to screening and testing, Nepal faces numerous obstacles, such as limited

awareness, insufficient funding, and diagnostic complexities, leading to inadequate screening practices for β-thalassemia (Sharma Poudyal *et al.*, 2023).

The nutritional aspect of thalassemia management in Nepal is particularly challenging. A study by Sharma Poudyal *et al.* (2023) revealed that 93% of β-TM patients registered with the NTS suffer from stunted growth. This is largely due to a combination of factors, including limited resources, traditional dietary habits, and economic constraints. Compounding the issue is the lack of comprehensive nutritional guidelines tailored to the requirements of β-thalassemia patients. The existing guidelines issued by the Ministry of Health in 2017, intended for thalassemia and sickle cell disease management, include dietary recommendations advocating a low-iron diet for transfusion-dependent β-TM (MoH, 2017). However, this approach is rooted in outdated conceptions of the disorder and its optimal management (Fung *et al.*, 2023; Fung and Yardumian, 2024).

In an effort to support patients, the NTS, in collaboration with the TIF, provides educational resources to thalassemia patients in Nepal. Each registered patient receives a Nepali translation of the book "About Thalassemia", which offers comprehensive information about the condition and its treatment options (Eleftheriou and Rayamajhi, 2007). This initiative aims to empower patients and their families with knowledge about the disorder.

Despite Nepal's location within the world's thalassemia belt, there is a significant lack of comprehensive information on various aspects of thalassemia, including its epidemiology, clinical course, mortality, complications, and treatment outcomes.

2.12 Assessment of nutritional status

Nutritional assessment is a methodical approach to gathering, confirming, and analyzing data required to identify nutrition-related problems, the related etiologies, the magnitude of the problem, as well as the symptoms and indicators displayed by the nutrition-related problem. The purpose of the nutrition assessment is to establish individual nutritional needs, document baseline nutritional parameters, identify nutritional risk factors and specific nutritional deficits, and identify medical, psychosocial, and socioeconomic factors that may affect the recommendation and administration of nutrition support therapy (Hickson *et al.*, 2018). Assessment of nutritional status can be done using the following two methods:

1. The direct method of nutritional survey, which works with people and assesses the objective criteria. The direct method can be summarized as ABCD steps (Nieman, 2019): (i) anthropometric method, (ii) biochemical method, (iii) clinical examination, and (iv) dietary evaluation method

2. The indirect method of nutritional survey, which makes use of community health indicators that take dietary factors into account. The indirect methods of the nutritional survey are (Jelliffe, 1966):

i. Ecological variables including agricultural crop production, food balance sheet, health, and educational services.
ii. Socioeconomic factors such as family size, occupation, per capita income, population density, education, customs, and social habits.
iii. Vital health statistics particularly infant (under 5) mortality and morbidity related to protein-energy malnutrition, school-age child stunting and wasting, anemia, goiter, diarrhea, measles, and parasitic infestation

2.12.1 Anthropometric method of nutritional assessment

Anthropometry is the most widely applicable, affordable, and non-invasive approach for determining the size, proportions, and composition of the human body (WHO, 1995). The anthropometric measures vary with age, sex, race, and level of nutrition. Anthropometric measurements provide a practical method to assess an individual's nutritional status and, indirectly, their body composition. Due to their simplicity, affordability, and accessibility, these indicators are used in clinical practice. Additionally, these measurements can provide information on past nutritional histories, which cannot be obtained with the same confidence using other assessment techniques. In some cases, they can detect moderate and severe degrees of malnutrition, but the methods cannot be used to identify specific nutrient deficiency states (Gibson, 2005).

The most used anthropometric indicators are listed as follows:

2.12.1.1 Height for age

Height-for-age (H/A) is a measure of linear cumulative growth that evaluates long-term nutritional deficiencies and/or chronic or recurrent illnesses relative to age and sex. It is more commonly employed as a population indicator than for tracking individual growth (WHO,

2006). The term "length-for-age" refers to children under 2 years old; "H/A" refers to children over 2 years old (Cogill, 2003; Cashin and Lesley, 2018). Table 2.6 classifies stunting according to H/A and expressed as a z-score. In the present study, WHO Anthro software was used to calculate z-scores for H/A, which were then interpreted using the WHO standard reference population to assess the nutritional status of the study participants.

Table 2.6 Classification of height-for-age index expressed as z-score

Indicator	z-score
Severe stunting	< -3
Moderate stunting	- ≥ -3 to < -2
Normal	≥ -2

Source: (WHO, 2006, 2007)

2.12.1.2 Weight for age

Weight-for-age (W/A) reflects the relationship between body mass, age, and sex. It is frequently used to track growth and measure how the severity of malnutrition has changed over time (WHO, 2006). Table 2.7 classifies underweight according to W/A and expressed as a z-score. In the present study, WHO Anthro software was used to calculate z-scores for W/A, which were then interpreted using the WHO standard reference population to assess the nutritional status of the study participants.

Table 2.7 Classification of weight-for-age index expressed as z-score

Indicator	z-score
Severe underweight	< -3
Moderate underweight	- ≥ -3 to < -2
Normal	≥ -2

Source: (WHO, 2006, 2007)

In children aged 10 years and older, using BMI-for-age and H/A in combination provides a more accurate reflection of nutritional status compared to W/A alone. W/A can be misleading as it does not differentiate whether the weight is in proportion to the child's height or indicative of their actual body mass. This differentiation becomes especially critical during periods of puberty and growth spurts, where children might appear to be overweight when they are just taller (de Onis *et al.*, 2007).

2.12.1.3 Mid-upper arm circumference

The mid-upper arm circumference (MUAC) is a widely used method for assessing nutritional status in children and adolescents. It measures the diameter of the upper arm, evaluating both fat stores and muscle mass. MUAC is particularly valuable when it's challenging to gather information on age, height, and weight, making it the suggested nutritional status indicator in such cases (WHO and UNICEF, 2009).

MUAC is typically measured using a non-stretchable Shakir tape. The measurement is taken on the left arm for right-handed individuals (or the non-dominant arm for left-handed people), at the midpoint between the tip of the shoulder (acromion process) and the tip of the elbow (olecranon process) (UNICEF; Nieman, 2019).

Table 2.8 MUAC cut-off points for children and adolescents

Age	Severely acute malnutrition	Moderate acute malnutrition	Normal
1 to 4 years	< 115 mm	≥ 115 to < 125 mm	≥ 125 mm
5 to 9 years	< 135 mm	≥ 135 to < 145 mm	≥ 145 mm
10 to 14 years	< 160 mm	≥ 160 to < 185 mm	≥ 185 mm

Source: (WHO, 2004); WHO and UNICEF (2009)

This method is preferred for its simplicity and ease of measurement compared to other indicators. The cut-off points for MUAC interpretation are generally not sex-specific and cover broad age categories, such as 5 to 9 years, 10 to 14 years, and 15 to 17 years (Cashin and Lesley, 2018).

2.12.1.4 Body mass index for age

Body mass index (BMI), also known as the Quetelet index, is a measure used to define overweight and thinness. BMI is the ratio of weight (in kg)/ recumbent length or standing height (in m^2) (Cogill, 2003). BMI-for-age (BMI/A) is used to indicate both thinness and overweight/obesity in children and adolescents and is interpreted according to age and sex.

However, it is important to acknowledge that BMI alone may not accurately reflect body fat, as it does not distinguish between fat mass (FM) and fat-free mass (FFM) (Holmes and Racette, 2021; Więch *et al.*, 2021). Table 2.9 classifies BMI/A expressed in z-score.

Table 2.9 Classification of BMI-for-age index expressed as z-score

Indicator		z-score
1 to 4 years	5 to 19 years	
Severely wasting	Severe thinness	< -3
Moderately wasting	Moderately thinness	- ≥ -3 to < -2
Normal	Normal	≥ -2 to < +1
Possible risk of overweight	Overweight	> +1 to ≤ +2
Overweight	Obesity	> +2 to ≤+3
Obesity		> +3

Source: (WHO, 2006, 2007)

2.12.1.5 Skin fold thickness

Skin fold thickness (SFT) measurement is a reliable and non-invasive method for estimating body fat percentage of all age groups, which is a key indicator of nutritional status. This method involves measuring the thickness of subcutaneous fat at specific body sites using calipers, typically at the triceps, biceps, subscapular, and suprailiac areas (Rodríguez *et al.*, 2005). Subcutaneous fat serves as an energy reserve and reflects the nutritional status of an individual (Wickramasinghe, 2012).

SFT values can be used to estimate total body fat using predictive mathematical formulas (Peterson *et al.*, 2003). This approach is particularly useful in assessing the nutritional status in cases where direct measurement of body fat is not feasible. Moreover, SFT measurements have been validated with gold-standard methods like dual-energy X-ray absorptiometry (DEXA) (Schmelzle and Fusch, 2002). Its application is widespread in both clinical and research environments, offering a means to track body composition changes over time and evaluate the impact of nutritional interventions.

Accurate measurement of skinfolds necessitates precise site selection and adherence to the standard protocol and predictive equations In North America, researchers typically perform skinfold measurements on the right side of the body, whereas European researchers commonly use the left side. The triceps and subscapular skinfolds are the most commonly measured sites due to their accessibility and are prominently featured in large studies like the National Health and Nutrition Examination Survey (NHANES).

Various prediction equations have been developed for estimating body fat, tailored to specific populations or generalized across different demographics. However, the validity of these equations depends on the similarity between the sample population and the reference population used in their development. Predominantly, these equations are derived from data on populations of white Caucasian or Black African descent from Western Europe or North America (Nieman, 2019). There are only a few validated equations for South Asian populations, which typically focus on adults (Wickramasinghe *et al.*, 2008). Moreover, it is well documented that ethnic variations influence body fat percentages, with Asians having higher body fat compared to Caucasians (Deurenberg *et al.*, 2002).

Table 2.10 Body fat percentage equation for 5 to 15 years old children

Index	Equation
Fat mass	(0.68 × age) + (0.246 × SFT-triceps in mm) + (0.383 × SFT-subscapular in mm) – (1.61 × Sex Code) – 3.45
% Fat mass	(–0.28 × Age) + (0.49 × SFT-triceps in mm) + (0.34 × SFT-subscapular in mm) – (7.97 × Sex Code) + 26.8

Sex code: Male = 1, Female = 0, SFT = Skinfold thickness

Source: Wickramasinghe *et al.* (2008)

Wickramasinghe *et al.* (2008) developed and statistically validated SFT equations to estimate FM and FM percentages in Sri Lankan children aged 5 to 15 years. These equations used the D_2O (Deuterium Oxide) dilution method as a reference and utilized measurements from two specific sites: the triceps and subscapular areas. The equation accounts for sex and age differences, for estimating SFT is outlined in Table 2.10.

Table 2.11 Body fat percentage equation for 1 to 5 years old children

Sex	Equation
Boys	5.304 + (0.269 × SFT-triceps in mm) + (0.50 × SFT-subscapular in mm) + (0.685 × MUAC) - (0.063 × Age in months)
Girls	7.017 - (0.053 × SFT-triceps in mm) + (0.201 × SFT-subscapular in mm) + (0.765 × MUAC) + (0.052 × Age in months)

SFT = Skinfold thickness, MUAC = Mid-upper arm circumference

Source: Shaikh and Mahalanabis (2004)

Similarly, Shaikh and Mahalanabis (2004) developed SFT equations for preschool children aged 1 to 5 years, using Bioelectrical Impedance Analysis (BIA) as the reference method (Table 2.11). The accuracy of BIA was further validated against the D_2O dilution method. These sex and age-specific equations incorporate measurements from the triceps and subscapular skinfolds and MUAC. The study supports that these equations provide accurate estimations of body fat for South Asian children.

2.12.2 Biochemical method of nutritional assessment

In nutritional assessment, biochemical methods include analyzing nutrients or their metabolites in blood, feces, or urine. This analysis provides valuable insights into the nutrient levels, helping to identify deficiencies, toxicities, and potential underlying causes. Each nutrient has its own unique metabolism, primarily regulated by the intestines or kidneys. These organs adjust the absorption and re-absorption of nutrients at the distal tubule in the kidney to maintain homeostasis despite fluctuations in dietary intake. Exhaustion of these mechanisms may result in an increased risk for nutritional deficiency (Nieman, 2019; Fung *et al.*, 2023). For example, high levels of ferritin in the blood can indicate iron overload, while low levels suggest iron deficiency, which may lead to anemia.

2.12.3 Dietary method of nutritional assessment

Dietary methods generally involve surveys measuring the quantity of the individual foods and beverages consumed during the course of one to several days or assessing the pattern of food use during the previous several months. Food and nutrient intakes can be estimated by quantitative and qualitative methods. A qualitative study gives information on the types of foods consumed, food preparation methods, food preferences, cultural influences, and attitudes toward foods whereas a quantitative survey provides data on the quantity of various foods consumed by individuals and/or populations. Each method has benefits and limitations. Therefore, it is advised that, depending on the type and purpose of information needed, a combination of both techniques be employed (CFNI, 2004).

2.12.3.1 Individual dietary survey

This method is used to obtain information on the past and present food and nutrient intakes of an individual (Hartog, 2006). Some of the methods are described as:

2.9.1.1. 24-hour diet recall

The 24-hour diet recall is a method used to estimate food and liquid intake over the preceding 24 hours. This approach is helpful for comparing dietary recommendations with nutrient intakes. However, its primary limitation is that it may not accurately represent normal consumption patterns due to day-to-day eating variability. The recall process should also consider factors such as fasting, feasting, festivals, and recent changes in appetite (CFNI, 2004; Nieman, 2019).

2.9.1.2 Food frequency questionnaire

Food frequency questionnaires (FFQ) are used to assess nutrient intake by determining how frequently an individual consumes a specific set of foods known to be major sources of nutrients or particular dietary components. The FFQ is designed to measure habitual dietary intakes over extended periods ranging from weeks to months or even years. The advantage of this approach is that it captures long-term dietary patterns, which are considered more relevant than short-term dietary data. However, the limitation of this method is that it could not offer information on precise quantities or portion sizes of foods consumed (CFNI, 2004; Nieman, 2019).

2.12.3.2 Dietary diversity score

The dietary diversity score (DDS) is a qualitative measure of food consumption developed by the Food and Agriculture Organization (FAO) to assess the variety of food groups consumed within a specific reference period, reflecting the nutritional quality and adequacy of a diet. The DDS evaluates the consumption of various food groups such as grains, legumes, vegetables, fruits, meats, and dairy products.

The utility of the DDS in nutritional studies is underscored by its simplicity and ease of implementation, which does not require an in-depth understanding of the nutrient composition of foods or precise nutrient intake measurements. According to Kennedy *et al.* (2010), a higher DDS is indicative of a more varied diet, which is often associated with an increased likelihood of meeting essential nutrient requirements.

2.9.2.1 Minimum dietary diversity for women

The minimum dietary diversity for women (MDD-W) is a dichotomous indicator specifically developed to assess dietary diversity among women of reproductive age. This

indicator, jointly developed by the FAO and FANTA (2016), serves as a micronutrient adequacy marker, reflecting the quality of the diet. Developed as a micronutrient adequacy indicator, MDD-W reflects the quality of women's diets in terms of micronutrient intake. This indicator has been applied broadly in various studies, not only to understand dietary patterns among women but also among younger populations and males, demonstrating its effectiveness in predicting nutritional adequacy across different demographic groups (Wiafe *et al.*, 2023; Gómez *et al.*, 2024; Hanley-Cook *et al.*, 2024).

MDD-W measurement can be conducted using two primary methods: the list-based method and the open recall approach. The latter involves a non-quantitative 24-hour recall, during which researchers assist participants in recalling all foods and beverages consumed the previous day and night. This process includes a series of standard questions designed to ensure a comprehensive dietary recall. The MDD-W assesses ten specific food groups, which are (FAO and FANTA, 2016; FAO, 2021):

i. Grains, white roots, tubers, and plantains
ii. Pulses (beans, peas, and lentils)
iii. Nuts and seeds
iv. Milk and milk products
v. Meat, poultry, and fish
vi. Eggs
vii. Dark green leafy vegetables
viii. Other vitamin A-rich fruits and vegetables
ix. Other vegetables
x. Other fruits

A score on the MDD-W ranges from 0 to 10, where a score of five or higher indicates consumption from at least five different food groups. This score is strongly associated with an increased likelihood of meeting adequate micronutrient needs. Typically, reaching this score means that the diet included various essential food categories, such as at least one type of animal-source food, pulses or nuts/seeds, and selections from at least two different groups of fruits and vegetables (Kennedy *et al.*, 2010; FAO, 2021).

2.9.2.2 Infant and young child feeding minimum dietary diversity

The infant and young child feeding minimum dietary diversity (IYCF-MDD) is an indicator for assessing micronutrient adequacy among children aged 6 to 23 months. Aligned with IYCF practices, MDD is identified as one of the eight core indicators for optimal early child feeding. It evaluates whether children have consumed at least five out of eight specified food groups within the last 24 hours. A significant revision in 2017 expanded the food groups from seven to eight, notably including breast milk to emphasize its vital nutritional role.

This revision adjusted the dietary adequacy threshold to require intake from five of these eight groups, correcting previous biases that favored formula-fed infants by including infant formula but excluding breast milk (WHO and UNICEF, 2017b, 2017a). Dietary diversity is linked to improved growth and reduced micronutrient deficiencies, which are vital for healthy physical and cognitive development in young children.

The IYCF-MDD is measured through two main methods: the list-based method and the open recall approach. These methods facilitate straightforward data collection, tabulation, and interpretation, simply requiring caregivers to verify whether their child consumed items from the specified groups in the last 24 hours. The eight food groups considered in this assessment are (WHO and UNICEF, 2021):

i. Breast milk
ii. Grains, roots, tubers and plantains
iii. Pulses (beans, peas, lentils), nuts and seeds
iv. Dairy products (milk, infant formula, yogurt, cheese)
v. Flesh foods (meat, fish, poultry, organ meats)
vi. Eggs
vii. Vitamin-A rich fruits and vegetables
viii. Other fruits and vegetables

2.12.3.3 Use of dietary reference intakes

Dietary reference intakes (DRIs) provide a comprehensive set of nutrient reference values used to plan and assess the dietary intake of healthy individuals. These references include key standards such as the estimated average requirement (EAR) and the recommended dietary allowance (RDA) (IOM, 2006; ICMR-NIN, 2020).

Table 2.12 Summary of EAR of nutrients for 1 to 15 years old children

Age group (years)	Sex	Energy (Kcal/kg/d)	*Protein (g/kg/d)	Calcium (mg/d)	Iron (mg/d)	Vitamin D(IU/d)	Folate (µg/d)	Zinc (mg/d)
1 to 3		83	0.79	400	6		97	2.8
4 to 6		74	0.70	450	8		111	3.7
7 to 9		67	0.75	500	10		142	4.9
10 to 12	M	64	0.75	650	12	400	180	7.0
	F	57	0.73		16		186	7.1
13 to 15	M	57	0.73	800	15		238	11.9
	F	49	0.70		17		204	10.7

*For people consuming cereal-based diets with low-quality protein, the protein requirements are 1g/kg per day. Source: ICMR-NIN (2024)

The EAR is defined as the average daily nutrient intake estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group (IOM, 2006). It is primarily used to assess nutrient adequacy at the population level, facilitated by the cut-point method, which compares individual nutrient intakes against the EAR thresholds (Fung *et al.*, 2012; ICMR-NIN, 2020). This approach helps in estimating nutrient inadequacy prevalence within populations. While the EAR offers a framework for assessing insufficient nutrient intake in individuals, it is not designed to prescribe daily nutrient targets (IOM, 2006; Nieman, 2019). The EAR of 1 to 15 years old children is presented in Table 2.12.

Table 2.13 Summary of RDA of nutrients for 1 to 15 years old children

Age group (years)	Sex	*Protein (g/kg/d)	Calcium (mg/d)	Iron (mg/d)	Vitamin D (IU/d)	Folate (µg/d)	Zinc (mg/d)
1 to 3		0.97	500	8		120	3.3
4 to 6		0.87	550	11		135	4.5
7 to 9		0.92	650	15		170	5.9
10 to 12	M	0.91	850	16	600	220	8.5
	F	0.90		28		225	8.5
13 to 15	M	0.89	1000	22		285	14.3
	F	0.87		30		245	12.8

*For people consuming a cereal-based diet with low-quality protein, the protein requirements are 1g/kg per day. Source: ICMR-NIN (2024)

Instead, the RDA, set at two standard deviations above the EAR to meet the needs of 97-98% of healthy individuals, specifies the daily intake needs of most individuals in a demographic group, thus minimizing the risk of nutrient deficiencies (IOM, 2006). However, is often incorrectly interpreted as a recommended intake rather than a minimum requirement (Carbone and Pasiakos, 2019). The RDA of 1 to 15 years old children is presented in Table 2.13.

These misapplications are compounded by limitations in the methodologies used to derive DRIs for protein, primarily nitrogen balance studies, which tend to overestimate intake and underestimate excretion, potentially leading to underestimated nutrient requirements (Carbone and Pasiakos, 2019).

Part III

Materials and methods

3.1 Study area

The study was conducted in Kanti Children's Hospital (KCH), located in Maharajgunj, Kathmandu, Nepal. KCH is the only government referral-level children's hospital in Nepal that provides medical care to children up to 14 years of age. The hospital's thalassemia unit serves as a primary care center for children with beta-thalassemia major (β-TM) in the region, where all basic care is funded by the government.

3.2 Study population

The study population consisted of pediatric patients aged 1 to 14 years who were diagnosed with β-TM, registered with the Nepal Thalassemia Society (NTS), and receiving regular blood transfusions at the thalassemia unit of KCH from March 1 to April 30, 2024.

3.3 Selection criteria

The study participants were selected based on the following inclusion and exclusion criteria:

i. Inclusion criteria:
   - Children diagnosed with β-TM and undergoing regular blood transfusions.
   - Children aged between 1 and 14 years.
   - Children registered with the NTS.

ii. Exclusion criteria:
   - Children diagnosed with other variants of thalassemia.
   - Children not undergoing regular blood transfusions.
   - Children with major complications associated with β-TM, defined as multiple co-morbidities or those requiring urgent medical intervention.
   - Children who do not wish to participate in the survey.

3.4 Research design

A cross-sectional, hospital-based, descriptive study was conducted to assess the nutritional status and associated factors among transfusion-dependent pediatric patients with β-TM at KCH. The data collection process consisted of four distinct parts:

i. Structured questionnaire

ii. Anthropometric assessment
iii. Biochemical measurements, and
iv. Dietary assessment

The study was carried out using the census method, which involved selecting every subject who met the study criteria during the data collection period from March 1 to April 30, 2024. In the context of this research, this meant enrolling all pediatric patients aged 1 to 14 years with β-TM who visited KCH for regular blood transfusions during the specified timeframe. The thalassemia unit at KCH has only six beds available. Consequently, a maximum of six β-TM children could attend the thalassemia unit daily, based on their follow-up schedule.

3.5 Study population size

A total of 112 thalassemic patients aged between 1 to 14 years, registered in the thalassemia unit and NTS, and receiving regular blood transfusions at KCH, were identified through an inquiry with the in-charge nurse of the thalassemia unit. Considering the inclusion and exclusion criteria, the eligible sample size for this study, which targeted patients with transfusion-dependent β-TM, was determined to be 105.

Due to the thalassemia unit's capacity, patient follow-up scheduling, and geographical constraints, the average number of patients available for interviews each day was limited to three. Over 36 data collection days, spanning nearly two months, 92 study participants were enrolled in the study.

3.6 Research instruments

The following equipment was used in the survey (For more details, see Appendix E)

i. A digital weighing machine (seca) with a capacity of 250 kg and having the least count of 0.1 kg was used.
ii. Stadiometer (ShorrBoard®) with a maximum height capacity of 206 cm and a precision of 0.1cm.
iii. Shakir tape for measuring mid-upper arm circumference (MUAC).
iv. Holtain Caliper for measuring skin fold thickness.
v. Kobo Toolbox, a digital data collection platform, as a medium for collecting information using structured and pretested sets of questionnaires.

vi. Standardized measuring utensils of 130 ml glass, 15 ml, and 5 ml spoons for determining approximate portion size.

3.7 Study variables

3.7.1 Dependent variables

The dependent variables of the study are presented in Table 3.1:

Table 3.1 Dependent variables of the study

Variables	Description
Stunting	Defined as height-for-age below -2 SD from the WHO reference median value (WHO, 2006, 2007).
Underweight	Defined weight-for-age below -2 SD from the WHO reference median value (WHO, 2006, 2007).
Wasting/ Thinness*	Defined as BMI-for-age below -2 SD from the WHO reference median value. Classified as wasting for children below 5 years and thinness for children above 5 years (WHO, 2006, 2007).
MUAC	Cut-off values for different age groups were used to define MUAC values as normal and moderate acute malnutrition (WHO, 2004); WHO and UNICEF (2009).
Body fat %	Calculated using skinfold measurements and two equations: Shaikh and Mahalanabis (2004) for children below 5 years of age and Wickramasinghe *et al.* (2008) for children above 5 years of age.

*For ages less than 5 years, "wasting" is used to describe BMI/A; for ages more than 5 years, "thinness" is used.

3.7.2 Independent variables

The independent variables under study were:

1. Socio-economic and demographic variables: Ethnicity, religion, occupation, education of father and mother, head of household, family size, annual income, and migration status.
2. Child characteristics: Age, sex, birth order, breastfeeding status, age gap with elder child, birth weight, morbidity status, and education status.

3. Child care practices: Breastfeeding after birth, initiation time, colostrum feeding, breastfeeding frequency and duration, exclusive breastfeeding, vaccination information, etc.
4. Thalassemia-related information: Disease history, total number, and frequency of transfusions, iron chelation status, drug dosage, usage of oral supplements, and caretaker's knowledge related to nutritional management of thalassemia.
5. Biochemical factors: Pre-transfusion hemoglobin level, serum ferritin, serum vitamin D, and serum calcium determined through patient's hospital records.
6. Eating behavior: Vegetarian/non-vegetarian, source of food, number of meals, food avoidance and its reason, skipping meals, etc.
7. Dietary intake: 24-hour dietary recall, food frequency, dietary diversity score, macronutrient distribution, adequacy of nutrient intake assessed through interview.

3.8 Pretesting

Pretesting of the questionnaire and anthropometric instruments was conducted on a small sample of children within the target age groups to assess their efficiency and feasibility. Due to the limited sample size of the thalassemia patients, the pretesting was performed on the non-thalassemic pediatric population. The questionnaire was initially developed in a paper survey format. Later, it was digitized and integrated into the Kobo Toolbox to minimize errors and optimize the data collection process.

Based on the findings of the pre-testing study, confusing, potentially misleading, and misinterpreted questions were eliminated from the questionnaire. Additionally, serum calcium and vitamin D levels were included as additional biochemical parameters. The revised questionnaire was then submitted to the IRC of KCH for approval.

3.9 Validity and reliability of the study tools

All anthropometric instruments employed in this study were certified and calibrated for research-grade standards. As an additional measure to guarantee precision, the weighing balance used in the study was cross-validated against a calibrated weighing balance from the hospital ward. For accurate dietary data collection in the 24-hour recall, standardized measuring utensils (glasses and spoons) were used to ensure precise portion size estimation. Before actual data collection, the reliability of the questionnaire was established through

pretesting focusing on the completeness of information collected, consistency of question interpretation across respondents, and clarity of language and structure.

3.10 Data collection techniques

This study used a combination of primary and secondary data collection methods. Primary data were collected through structured questionnaires, anthropometric measurements, and dietary assessments. Interviews were conducted with the children and their caretakers to complete the questionnaires (See more in Appendix H). Secondary data were obtained from hospital records and key informants, including executive members of NTS and the nurse in charge of the thalassemia unit. The data collection process encompassed the following components:

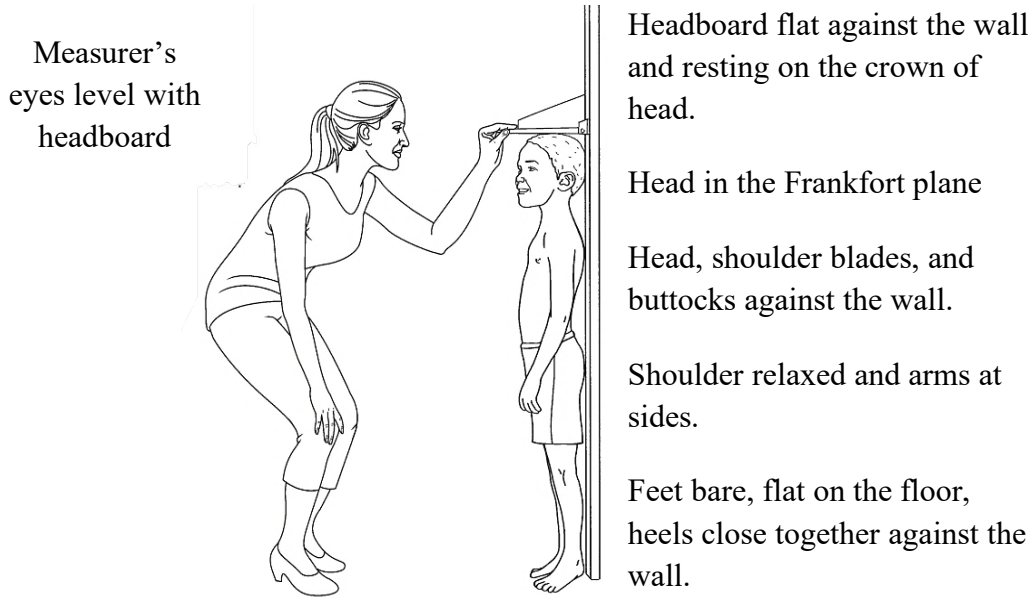
i. Socio-demographic information: Respondents were asked about their age, ethnicity, religion, parental occupation, education, family size, family income, etc.

ii. Anthropometric assessment: Anthropometric measurements were conducted using standardized procedures (WHO, 2008; Nieman, 2019):

- Weight: Body weight was measured to the nearest 0.1 kg using a digital scale. Subjects were weighed after emptying their bladder and removing shoes and heavy clothing. The scale was placed on a hard, flat surface and zeroed before each measurement. Subjects were instructed to stand unassisted in the center of the scale, looking straight ahead while remaining relaxed but still.

- Height: Height was measured to the nearest 0.1 cm using a stadiometer. Subjects stood barefoot on a horizontal platform with heels together and arms hanging loosely. The head was aligned with the Frankfurt plane, with buttocks and shoulder blades in contact with the stadiometer's vertical surface. Subjects were instructed to inhale deeply and stand tall to aid in spinal straightening while keeping shoulders relaxed.

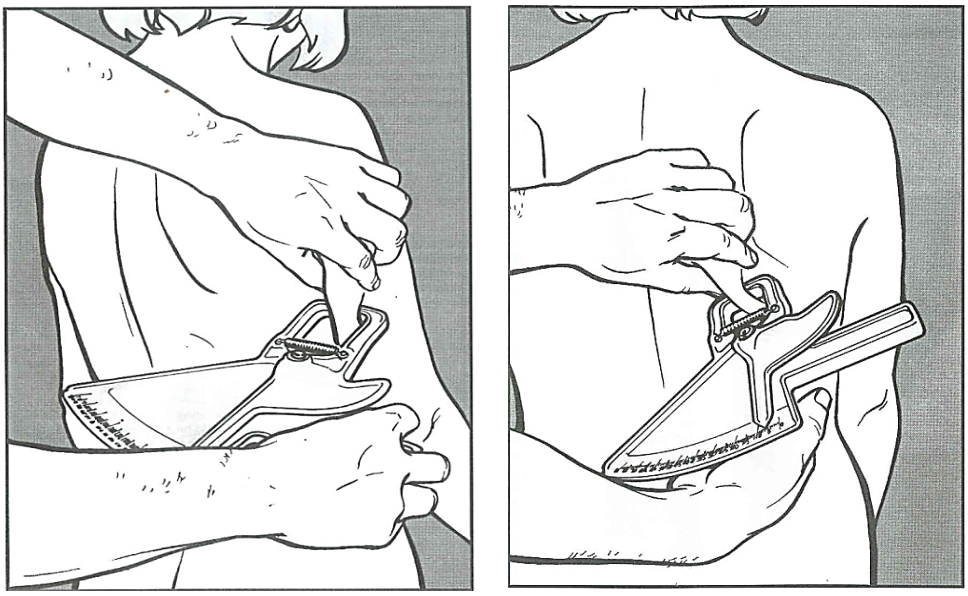
  The movable headboard was lowered to touch the crown of the head, and the measurement was taken with the examiner's eyes level with the headboard to avoid parallax error (Fig. 3.1). When readings fell between two values, the lower reading was recorded.



Source: Nieman (2019)

Fig. 3.1 Body position when measuring stature

- Skin fold measurements: Skinfold thickness was assessed using a Holtain caliper at two sites: (i) triceps and (ii) subscapular, as illustrated in Fig. 3.2.



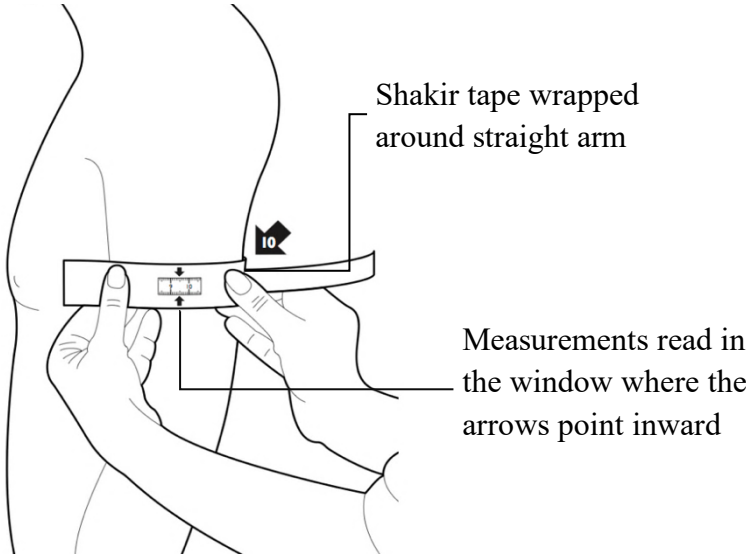
(a) (b)

Source: Donoghue (2009)

Fig 3.2 Measurement techniques for measuring triceps skinfold (a) and subscapular skinfold (b) using a caliper.

The triceps skinfold was measured on the back of the arm, halfway between the acromion process of the scapula and the olecranon process of the ulna, while the subscapular skinfold was measured 1 cm below the inferior angle of the scapula, with the skinfold oriented at a 45º angle, running downward and laterally (Fig. 3.2). The skinfold was firmly grasped approximately 1 cm above the measurement site and pulled away from the underlying muscle using the thumb and index finger. The caliper was then applied at a right angle to the skinfold, with the dial facing upward for ease of reading. Measurements were taken to the nearest 0.1 mm, approximately 4 seconds after releasing the caliper's lever arm (Nieman, 2019).

- MUAC: MUAC was measured using a stretch-resistant Shakir tape. Subjects removed clothing from their dominant arm and bent it 90 degrees with the palm facing upward. The midpoint between the acromion process (shoulder joint) and the olecranon process (elbow) was marked. The measurement was taken with the arm relaxed and hanging by the side (Fig. 3.3)



Source: Nutriverse (2018)

Fig. 3.3 Arm position when measuring MUAC

iii. Dietary assessment

- 24-hour recall method: A 24-hour dietary recall was conducted to quantify food and drink consumption over the previous day. Participants were asked to recount their intake chronologically, from the first meal of the morning to the last before bedtime. Factors such as fasting, feasting, and festivals were considered, and recent changes

in appetite were noted. Standardized utensils were used to estimate portion sizes accurately.

- Food frequency questionnaire (FFQ): The FFQ was used to gather data on the types and frequency of foods consumed by respondents. Subjects were asked to categorize their intake of specific foods as “never/rarely/less than once per month", "once per week", "3-4 times per week", or "daily." This information was used to assess typical dietary patterns based on the frequency of intake of specified foods over a period of time.

- Dietary diversity score (DDS): Age-specific DDS were calculated to measure the variety and nutrient adequacy of the diet, based on the 24-hour dietary recall data. For children above 2 years, the 10-food group score using the Minimum Dietary Diversity for Women (MDD-W) by FAO (2021) was used. For children below 2 years, the 8-food group score using the Infant and Young Child Feeding Minimum Dietary Diversity (IYCF-MDD) by WHO and UNICEF (2021) was used.

iv. Biochemical and clinical assessment: Relevant clinical and biochemical data were recorded from the hospital data. Medical histories were obtained with particular emphasis on transfusion history, iron chelation therapy, drug dosage, and medical history of iron overload complications such as endocrinopathies.

Biochemical data collected included serum ferritin, serum vitamin D, serum calcium, and pre-transfusion hemoglobin levels. Additionally, information on nutritional supplements and their dosages was recorded.

3.11 Data analysis

The data collected through Kobo Toolbox were exported to Microsoft Excel 2021 for initial data cleaning and organization. The exported datasets were manually coded, entered into the database, and thoroughly examined for missing values and consistency.

Anthropometric data analysis:

For anthropometric data analysis, the study participants were categorized into three age groups: (i) 1 to 4 years, (ii) 5 to 9 years, and (iii) 10 to 14 years. This classification facilitated precise data analysis and interpretation, aligning with WHO growth assessment tools. WHO

Anthro software (version 3.2.2) was used for children aged 1 to 4 years, while WHO AnthroPlus software (version 1.0.4) was employed for those aged 5 to 14 years. These tools calculated z-scores for weight-for-age (W/A), height-for-age (H/A), and BMI-for-age (BMI/A). The calculated z-scores were then interpreted using the WHO standard reference population to assess the nutritional status of the study participants. Additionally, this age stratification allowed for the application of age-specific MUAC cut-off values, which vary across these age ranges.

Dietary data analysis:

Dietary data obtained from the 24-hour recall were analyzed using food composition tables provided by the DFTQC (2017) and Indian ICMR Longvah *et al.* (2017) to determine the nutrient content and macronutrient distribution of the consumed foods. The adequacy of calorie and protein intake among the study participants was assessed using the Estimated Average Requirement (EAR) values set by the ICMR-NIN (2024). The FFQ data were further categorized into three groups based on the frequency of food consumption. Foods consumed daily or 3-4 times per week were classified as "regular", those consumed once a week were considered "frequent", and consumed once a month or less were categorized as "rare". DDS was considered adequate when the score was more than and equal to five and inadequate when the score was less than five.

Statistical analysis:

Qualitative data were coded and transcribed by assigning labels to various categories. The cleaned and coded data were then transferred to IBM® Statistical Package for Social Sciences (SPSS®) version 26.0 for statistical analysis. The study used both descriptive and inferential statistical techniques. Descriptive statistics included the calculation of frequencies and percentages for categorical variables and the computation of measures of central tendency (mean, median) and dispersion (standard deviation).

For normally distributed quantitative variables, Pearson correlation was used to examine relationships between variables, while independent samples t-test and one-way analysis of variance (ANOVA) were used to compare means across categorical variables with two and more than two categories, respectively. For ANOVA, post-hoc comparisons were conducted using Tukey's test. For non-normally distributed non-parametric variables, Spearman correlation, Mann-Whitney U test, and Kruskal-Wallis test were utilized. When Kruskal-

Wallis tests were significant, post-hoc pairwise comparisons were performed using Bonferroni correction. To compare age- and sex-specific dietary intake to corresponding EAR values, a paired-sample t-test was conducted. All statistical analyses were performed using a 95% confidence interval, with a p-value of less than 0.05 considered statistically significant.

3.12 Ethical consideration

This research was conducted with the approval of the IRC of KCH (Reference number: 1398) and with permission from the Department of Nutrition and Dietetics, Central Campus of Technology. The study adhered to strict ethical standards, ensuring the confidentiality of all information gathered during the research process. (See Appendix F for more details)

3.13 Informed consent

Before study enrollment, a comprehensive informed consent process was conducted following IRC guidelines. Written informed consent was obtained from parents or legal guardians of all participants, and assent was obtained from children who were above 7 years of age. The process included providing participants and their guardians with a detailed patient information sheet (PIS), which outlined the study's purpose, procedures, significance, and measures to ensure confidentiality. Participants were informed of their right to withdraw from the study at any time without prejudice to their ongoing medical care. All consent and assent documents were approved by the IRC of KCH prior to the initiation of the study. (More details in Appendix A, B, C)

Part IV

Results and discussion

The cross-sectional survey assessed the nutritional status and associated factors of transfusion-dependent β-thalassemia major (β-TM) pediatric patients aged 1 to 14 years visiting Kanti Children's Hospital (KCH) for regular blood transfusions. Data were collected using questionnaires, anthropometric assessments, biochemical evaluations, and dietary assessments. Anthropometric data were analyzed using WHO software and dietary data using food composition tables and dietary intake references. Descriptive characteristics, correlations, and group differences were analyzed using IBM® SPSS. The key findings are discussed in the following sections:

4.1 Socio-economic and demographic characteristics

The information on socio-economic and demographic characteristics is shown below:

4.1.1 Age and sex distribution

The study participants were categorized into three age groups: (i) 1 to 4 years, (ii) 5 to 9 years, and (iii) 10 to 14 years. The majority of the participants (52.2%) were in the 5 to 9 years age group, followed by 27.2% in the 10 to 14 years group, and 20.7% in the 1 to 4 years group. The mean age of the study participants was 7.46 ± 3.519 years. Out of 92 participants, 65.2% were male and 34.8% were female, as detailed in Table 4.1.

Table 4.1 Age and sex distribution of the study participants (n=92)

Variable	Frequency	Percentage
Age		
1 to 4 years	19	20.7
5 to 9 years	48	52.2
10 to 14 years	25	27.2
Sex		
Female	32	34.8
Male	60	65.2

4.1.2 Ethnicity and religion distribution

As illustrated in Fig. 4.1 the religion of the study participants, the majority (84.8%) were Hindu. Muslims constituted the second largest religious group at 6.5%, followed by Buddhists at 5.4%, Christians at 2.2%, and other religions at 1.1%.

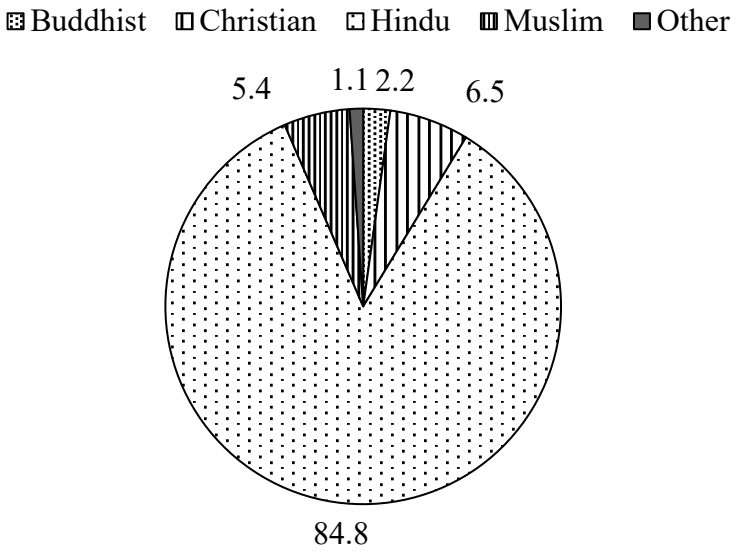


Fig. 4.1 Religion of the study participants (n=92)

The Janajati ethnic group, which includes *Rai*, *Newar*, *Magar*, and *Tamang* communities, constituted the largest proportion (34.8%) of the study participants, followed by *Tharu* (16.3%), and *Brahmin/Chhetri* (14.1%) as presented in Table 4.2. This observation aligned with previous reports of higher β-thalassemia prevalence in these communities (Jha and Jha, 2014; Lama *et al.*, 2022).

Table 4.2 Ethnicity of the study participants (n=92)

Variable	Frequency	Percentage
Ethnicity		
Brahmin/Chhetri	13	14.1
Janajati	32	34.8
Dalit	11	12.0
Madhesi	10	10.9
Tharu	15	16.3
Others	11	12.0

4.1.3 Birthplace and migration

Half (50%) of the study participants were born in Bagmati Province, while 21.7%, 10.9%, 6.5%, 3.3%, and 1.1% were born in Madhesh, Lumbini, Gandaki, Koshi, and Karnali Provinces, respectively, as shown in Fig. 4.2.

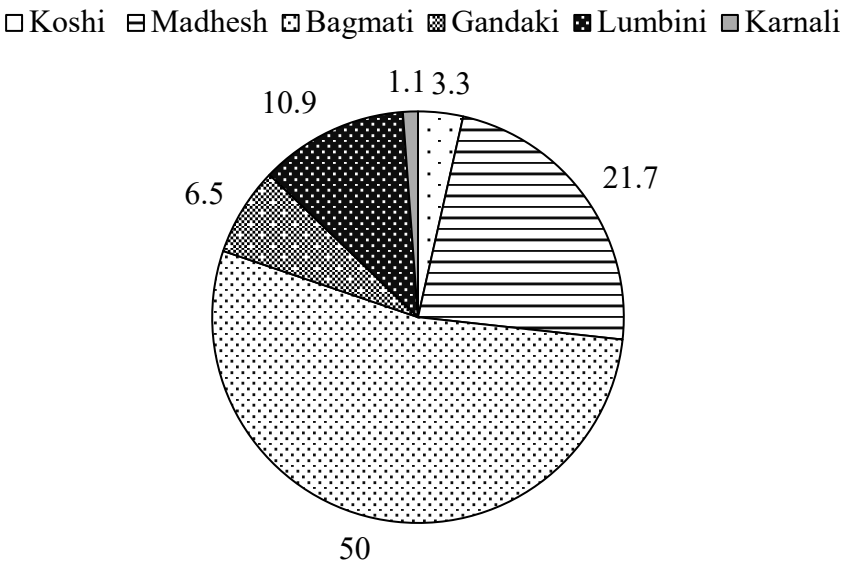


Fig. 4.2 Birth province of the study participants

As shown in Table 4.3, the study found that 39.1% of the participants migrated to Kathmandu valley. Among them, 88.9% primarily migrated to access health services for thalassemia, whereas 11.1% mentioned other reasons for their migration.

Table 4.3 Reason for migration of the study participants

Variable	Frequency	Percentage
Migration to Kathmandu (n=92)		
Yes	36	39.1
No	56	60.9
Reason of migration (n=36)		
Health services	32	88.9
Other	4	11.1

4.1.4 Educational status

The study showed that while the majority of the participants (88.1%) were currently enrolled in school, a significant proportion (11.9%) had either never attended or discontinued their

education, as shown in Table 4.4. However, regular school attendance was a challenge for many of these participants, with 85.2% missing up to a week of classes and 14.8% being absent for more than seven days every month. The primary reason for these absences was reported to be travel to the hospital for blood transfusions, which also resulted in lost working days for accompanying guardians. These factors contribute to hidden costs, such as lost wages and the impact on the child's education (Moirangthem and Phadke, 2018). The findings align with the challenges and socio-economic burdens faced by children with thalassemia and their families in the Southeast Asia region (Angastiniotis *et al.*, 2023).

Table 4.4 Educational status of the study participants (n=92)

Variable	Frequency	Percentage
School (n=92)		
Currently attending	81	88.1
Used to/ Never attended	11	11.9
Absent from school (n=81)		
≤ 7 days	69	85.2
> 7 days	12	14.8

4.1.5 Socio-economic factors

In terms of the head of the household, fathers were the heads of most households (59.9%), while other relatives, including grandfathers, uncles, and grandmothers led 28.2% of households. Only 12.0% of households were headed by mothers. The family size of the participants was nearly evenly distributed, with 57.6% having less than 5 members and 42.4% having 5 or more members in their family. Regarding the family type, 62.0% of the participants belonged to nuclear families, while 38.0% were from joint families The annual income of the family was less than 1 lakh for the majority (62.0%) of the participants, while only 2.2% had an annual income of more than 3 lakhs, as presented in Table 4.5.

The educational status of the study participants' parents (Fig. 4.3) presents a mixed landscape. While illiteracy was relatively low, with only 13% of mothers and 5.4% of fathers reported as illiterate, a considerable proportion had not received formal education, with 14.1% of mothers and 17.4% of fathers having informal education. However, the majority of parents had attained some level of formal education, with the secondary level being the most common among mothers (37%) and fathers (30.4%). Although university-level

education had the lowest representation, it was still notable, with 6.5% of mothers and 10.9% of fathers having attained this highest level of educational qualification.

Table 4.5 Distribution of socio-economic factors of the study participants (n=92)

Variable	Frequency	Percentage
Head of the household		
Father	55	59.8
Mother	11	12.0
Other	26	28.2
Family size		
< 5 members	53	57.6
≥ 5 members	39	42.4
Family type		
Joint	35	38.0
Nuclear	57	62.0
Annual income of the family		
Less than 1 lakh	57	62.0
Between 1 to 3 lakhs	33	35.9
More than 3 lakhs	2	2.2

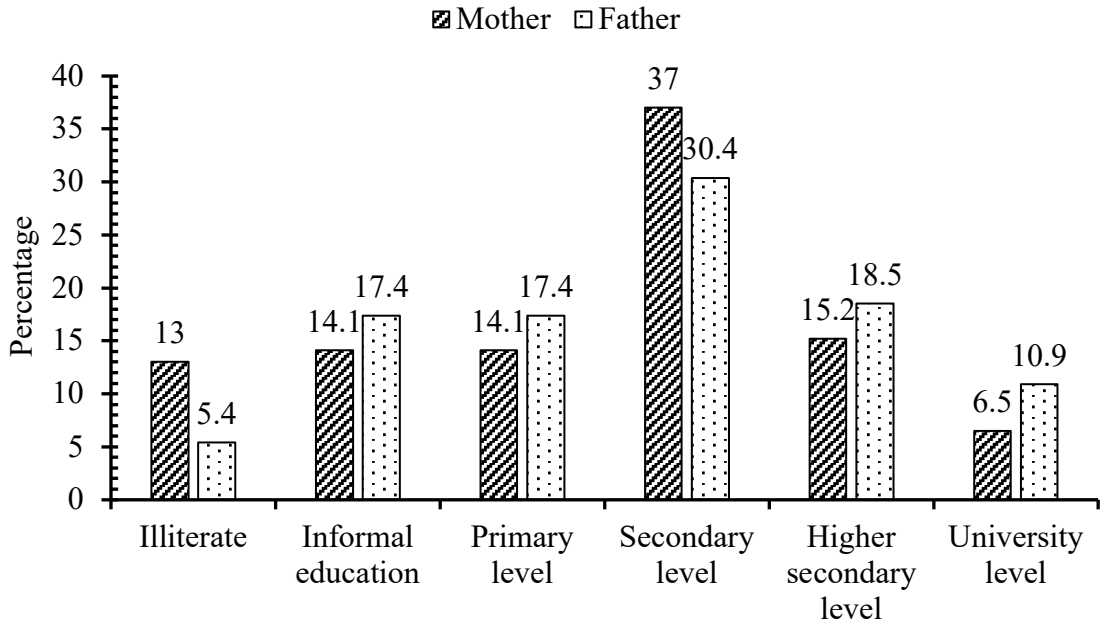


Fig. 4.3 Parental education status of the study participants

The data on parental occupations (Fig. 4.4) highlight the different roles of mothers and fathers within the study participants. The majority of mothers (66.3%) were dedicated to homemaking, underlining the traditional gender roles in family structures. However, fathers were more likely to be engaged in paid work outside the home, with 66.3% involved in employment such as service jobs, government jobs, or business. Notably, 18.5% of fathers were engaged in foreign employment.

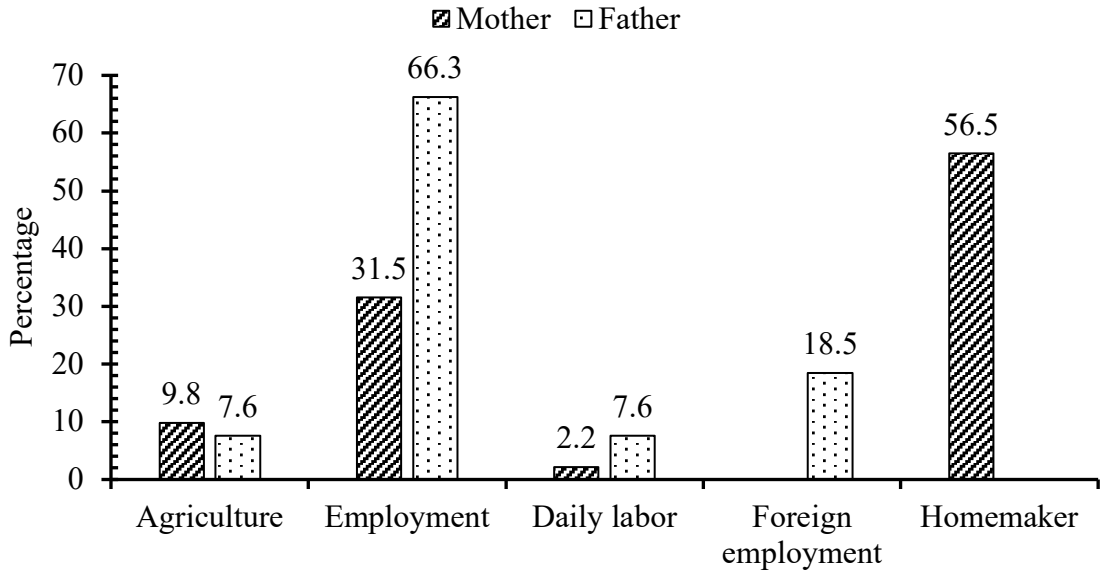


Fig. 4.4 Parental occupation of the study participants

4.2 Child characteristics and child caring practices

The information on child characteristics and child caring practices is shown below:

4.2.1 Birth characteristics

Table 4.6 Birth characteristics of the study participants (n=92)

Variable	Frequency	Percentage
Birth weight		
< 2500 g	22	23.9
≥ 2500 g	70	76.1
Birth order		
First	49	53.3
Second	36	39.1
Third/Fourth	7	7.6

Out of 92 study participants, 76.1% had a birth weight of 2500 g or more, while 23.9% had a birth weight of less than 2500 g. Among the participants, 53.3% were the eldest children in their families, 39.1% were second-born, and 7.6% were either third or fourth-born, as shown in Table 4.6.

4.2.2 Breastfeeding practices

Table 4.7 presents the breastfeeding practices among 92 participants, revealing that 93.5% were breastfed soon after birth. Within this group, the majority (75.0%) were breastfed within the first hour of life, while smaller percentages began breastfeeding within eight hours (6.5%), within 24 h (2.2%), or after 24 h (9.8%) of birth. Colostrum intake was reported in 90.2% of the study participants. These findings reflect strong adherence to WHO recommendations regarding the early initiation of breastfeeding and colostrum intake, which are known to provide significant health benefits to newborns (WHO and UNICEF, 2021).

Despite these high rates of early breastfeeding, only 67.4% of mothers reported exclusive breastfeeding for the recommended first six months. The primary reasons for non-exclusive breastfeeding included insufficient milk production (76.7%), medical complications (13.3%), and work obligations (10.0%). The duration of breastfeeding varied among the participants, with 67.9% being breastfed beyond two years and 32.1% for less than two years. This finding aligns with the WHO's recommendation for continued breastfeeding up to two years of age or beyond, alongside the introduction of appropriate complementary foods, to support the nutritional needs of growing children (WHO and UNICEF, 2021). The prolonged duration of breastfeeding observed in this study may also reflect cultural preferences and traditions that support extended breastfeeding.

Among 12% of those who were still being breastfed at the time of the survey, there was considerable variability in feeding frequency. Nearly half (45.5%) were breastfed less than 8 times per day, while 36.4% were fed between 8 and 12 times, and 18.2% were breastfed more than 12 times daily. This variation in feeding frequency is consistent with the natural dietary transition from exclusive reliance on breast milk to the gradual introduction of solid foods. This progression aligns with WHO guidelines, which recommend adapting feeding practices as children grow to meet their changing nutritional demands (WHO and UNICEF, 2021).

Table 4.7 Breastfeeding practices of the study participants (n=92)

Variable	Frequency	Percentage
Breastfeeding after birth (n=92)		
Yes	86	93.5
No	6	6.5
Initiation of breastfeeding (n= 86)		
Within 1 hour	69	75.0
Within 8 hours	6	6.5
Within 24 hours	2	2.2
After 24 hours	9	9.8
Colostrum feeding (n=92)		
Yes	83	90.2
No	9	9.8
Exclusive breastfeeding (n= 92)		
Yes	62	67.4
No	30	32.6
Current breastfeeding status (n=92)		
Still breastfeeding	11	12.0
Stopped breastfeeding	81	88.0
Age at which breastfeeding was stopped (n=81)		
Before 2 years of age	26	32.1
After 2 years of age	55	67.9
Breastfeeding frequency (n=11)		
Less than 8 times a day	5	45.5
About 8-12 times a day	4	36.4
More than 12 times a day	2	18.2

4.2.3 Child caring practices

The child-caring practices of the study participants are presented in Table 4.8. The majority of the children (96.7%) had completed the necessary vaccinations, though a small fraction faced barriers due to illness or hospital stays (2.2%) and a lack of knowledge (1.1%). Additionally, 90.0% of the participants reported receiving vitamin A supplementation and deworming tablets. Regarding growth monitoring, more than half of the respondents (55.4%)

participated in these programs, while 37.0% did not participate, and 7.6% were unaware that such programs existed. For healthcare during child illnesses, pharmacies or medical halls were the most commonly used facilities (62.0%), followed by hospitals or private clinics (32.5%). Only a few participants relied on health posts (3.3%) or traditional healers (2.2%).

Baalvita, a micronutrient powder, was used by only 29.3% of the children, with 34.8% of caregivers not familiar with the product. The relatively low uptake of *Baalvita* was primarily attributed to its iron content, as patients with thalassemia in Nepal are often advised to limit iron intake (MoH, 2017).

Table 4.8 Child caring practices of the study participants (n=92)

Variable	Frequency	Percentage
Completed vaccination of the child		
Yes	89	96.7
Due to illness/hospital stay	2	2.2
Lack of knowledge	1	1.1
Intake of vitamin A and deworming tablet		
No	2	9.9
Yes	20	90.9
Participation in growth monitoring		
Never heard of it	7	7.6
No	34	37.0
Yes	51	55.4
Intake of *Baalvita*		
Never heard of it	32	34.8
No	33	35.9
Yes	27	29.3
Usual place of treatment during illness		
Health post	3	3.3
Hospital/ Private clinics	30	32.5
Pharmacy/ Medical Hall	57	62.0
Traditional healers	2	2.2

4.3 Thalassemia-related factors

The information related to the disease characteristics, treatment practices, biochemical levels, family history, and healthcare services is shown below:

4.3.1 Diagnosis and blood transfusion practices

The majority (70.6%) of the study participants were diagnosed with thalassemia before the age of 6 months, with the remaining 29.3% diagnosed after 6 months. The mean age of diagnosis of the participants was 6.859 ± 7.602 months. In terms of treatment initiation, 68.5% of the participants started receiving blood transfusions before reaching 6 months of age, while 31.5% began their transfusions later, as presented in Table 4.9.

Table 4.9 Diagnosis and blood transfusion practices of the study participants (n=92)

Variable	Frequency	Percentage
Age of diagnosis		
Before 6 months of age	65	70.6
After 6 months of age	27	29.3
Age of first blood transfusion		
Before 6 months of age	63	68.5
After 6 months of age	29	31.5
Blood transfusion frequency		
Once a month	36	39.1
Twice a month	48	52.2
Thrice a month	5	5.4
Irregular	3	3.3
Total transfusion received last year		
Less than 12 times	3	3.3
12- 24 times	84	91.3
More than 24 times	5	5.4

The frequency of blood transfusions varied among the participants: 39.1% received transfusions once a month, 52.2% twice a month, 5.4% three times a month, and 3.3% followed an irregular schedule. Most participants (91.3%) received between 12 and 24 transfusions in the last year, while 3.3% received fewer than 12 and 5.4% more than 24.

Early diagnosis is important for the effective management of β-TM. In this study, a substantial proportion of participants were diagnosed early, facilitating the timely initiation of blood transfusions. The predominant transfusion interval was bi-monthly, aligning with standard practices in thalassemia management that aim to maintain adequate hemoglobin levels and prevent severe complications, such as growth retardation and bone deformities (Trompeter, 2021).

4.3.2 Practices of chelation therapy

The present study found that a high proportion of participants (90.2%) had initiated iron chelation therapy, while only 9.8% had not yet started the treatment (Table 4.10). The median age at which chelation therapy was started among the study participants was 5.0 years, with an interquartile range (IQR) of 4.25 years.

At KCH, Deferasirox (DFX), under the brand name *Desifer*, is used as the standard monotherapy for chelation. The mean dosage of *Desifer* administered to the participants was found to be 34.956 ± 5.909 mg/kg body weight per day.

Table 4.10 Practices of chelation therapy in the study participants (n=92)

Variable	Frequency	Percentage
Chelation therapy		
Not started	9	9.8
Started	83	90.2

The Thalassemia International Federation (TIF) recommends starting DFX at a dose of 14 mg/kg/day, with the usual maximum dose being 21 mg/kg/day (TIF, 2021). If tolerated, or in case of heavy iron load, the dose can be increased up to 28 mg/kg/day. Meanwhile, the national guideline of Nepal recommends a starting DFX dose of 20 mg/kg/d (MoH, 2017). The slightly higher mean dosage of *Desifer* observed in this study compared to the TIF and national guidelines may be attributed to the severity of iron overload in the study population or the clinical judgment of the treating physicians at KCH.

4.3.3 Splenectomy and presence of co-morbidities

As shown in Table 4.11, out of 92 participants, only 4.3% had undergone a splenectomy, while the vast majority (95.7%) had not. Regarding the presence of co-morbidities, 89.1%

of participants did not report any additional health issues. However, a few participants had hepatomegaly (3.3%), splenomegaly (5.4%), and dental problems (2.2%).

Table 4.11 Splenectomy and presence of co-morbidities in the study participants (n=92)

Variable	Frequency	Percentage
Undergone splenectomy		
No	88	95.7
Yes	4	4.3
Presence of co-morbidities		
No	82	89.1
Hepatomegaly	3	3.3
Splenomegaly	5	5.4
Dental problem	2	2.2

In patients with β-TM, the spleen and liver can become enlarged due to the persistent processing of abnormal red blood cells (Cappellini *et al.*, 2018). The low incidence of splenectomy in this study may be due to advancements in transfusion and chelation therapies.

Current guidelines recommend maintaining hemoglobin levels between 90-105 g/l through frequent transfusions, which has been effective in reducing the incidence of splenomegaly and the need for splenectomy (TIF, 2021).

4.3.4 Family history of thalassemia

Most (60.9%) participants confirmed a family history of β-thalassemia, while 39.1% had not undergone thalassemia testing to verify their family history, as shown in Table 4.12. Among the 56 participants who provided details about their family relations, 34.8% reported that both parents were affected, 14.1% indicated that both parents and a sibling were affected, 5.5% had one affected parent, and 6.5% had an affected sibling.

Regarding specific types of thalassemia, 39.1% of participants were identified with β-thalassemia minor/trait, 18.5% were β-thalassemia carriers, and 3.3% had β-TM, indicating a range of disease severity within the study group.

Table 4.12 Family history of thalassemia in the study participants

Variable	Frequency	Percentage
Presence of thalassemia in the family (n=92)		
Not done test	36	39.1
Yes	56	60.9
Relationship with the participant (n=56)		
Both father and mother	32	34.8
Both parents and sibling	13	14.1
Father or Mother	5	5.5
Sibling	6	6.5
Type of thalassemia (n=56)		
β-thalassemia carrier	17	18.5
β-thalassemia trait/minor	36	39.1
β-thalassemia major	3	3.3

4.3.5 Use of oral supplements

The majority of participants (91.3%) were prescribed less than 600 mg of calcium per day. Vitamin D intake followed a similar pattern, with 95.6% of participants recommended less than 600 IU daily. Notably, 42.2% of the participants practiced intermittent supplementation gaps, either taking supplements every alternate day or following a regimen of two weeks on, followed by two weeks off, for both calcium and vitamin D. Regarding folic acid, 65.2% of the participants had a daily intake of 2.5 mg, 33.7%, consumed 5.0 mg daily, while only 1.1% took a higher dose of 7.5 mg per day (Table 4.13)

In KCH, thalassemia patients received calcium and vitamin D_3 (cholecalciferol) in suspension form, while folic acid was provided in tablet form. Doctors recommended an intermittent supplementation strategy for calcium and vitamin D to prevent excessive nutrient accumulation, a concern due to the altered renal and hepatic functions in thalassemia patients. However, a significant counseling gap was evident, as over half of the patients were unaware of and non-adherent to this regimen. TIF nutritional guidelines advocate for dietary calcium sources over supplements to minimize nephrolithiasis risk and recommend 1 mg/day or 5 mg/week of folic acid for optimal folate status (Fung *et al.*, 2023). The observed 7.5 mg

of folic acid intake in some patients likely resulted from miscommunication, given that KCH protocol typically limits the prescription to a 5 mg daily maximum (Thakali, 2024).

Table 4.13 Distribution of use of oral supplements in the study participants

Variable	Frequency	Percentage
Calcium intake per day (n=92)		
No intake	2	2.2
Less than 600 mg per day	84	91.3
More than 600 mg per day	6	6.5
Vitamin D intake per day (n=92)		
No intake	2	2.2
Less than 600 IU per day	88	95.6
More than 600 IU per day	2	2.2
Gap in the intake of calcium and vitamin D supplements (n=90)		
No	52	57.8
Yes	38	42.2
Folic acid intake per day (n=92)		
2.5 mg per day	60	65.2
5.0 mg per day	31	33.7
7.5 mg per day	1	1.1

4.3.6 Biochemical levels

Table 4.14 presents the distribution of biochemical levels among the study participants. The pre-transfusion hemoglobin levels (9.610 ± 1.066 g/dl) were found to be less than 9.5 g/dl in 40.2% of participants, between 9.5 to 10.5 g/dl in 43.5%, and above 10.5 g/dl in 16.3% of the cases. Regarding serum ferritin (SF) levels (maximum: 17009 ng/ml; minimum: 194 ng/ml), more than half of the participants (58.7%) had seriously elevated SF levels. Only 10.9% of the participants had serum ferritin levels within the target range.

Conversely, the serum vitamin D levels (30.747 ± 11.356 ng/ml) showed more favorable results. A majority of 52.2% of the participants had sufficient vitamin D levels. However, 30.4% of participants had insufficient levels, and 17.4% were found to have deficit levels.

The majority of participants (90.2%) had serum calcium levels (9.402 ± 0.679 mg/dl) between 8.5-10.5 mg/dl, which falls within the normal range.

Table 4.14 Distribution of biochemical levels in the study participants (n=92)

Variable	Frequency	Percentage
Pre-transfusion hemoglobin level (g/dl)		
< 9.5	37	40.2
9.5 - 10.5	40	43.5
> 10.5	15	16.3
Serum ferritin (ng/ml)		
Target level (< 1000)	10	10.9
Moderately elevated (1000 - 2500)	28	30.4
Seriously elevated (> 2500)	54	58.7
Serum vitamin D (ng/ml)		
Sufficient (≥ 30)	48	52.2
Insufficient (20 - 29)	28	30.4
Deficit (< 20)	16	17.4
Serum calcium (mg/dl)		
< 8.5	8	8.7
8.5 - 10.5	83	90.2
> 10.5	1	1.1

Reference level reproduced from TIF (2021)

The study findings indicate that a substantial portion of participants may not be meeting the TIF (2021) recommended pre-transfusion hemoglobin levels between 9.5 and 10.5 g/dl, potentially increasing the risk of chronic hypoxia and associated complications. Iron overload was prevalent, with 58.7% of participants exhibiting seriously elevated serum ferritin (SF) levels exceeding 2500 ng/ml, a threshold associated with increased cardiac and endocrine disease risk in thalassemia patients (Taher *et al.*, 2021). This observation emphasizes the need for more effective iron chelation therapy to manage iron overload in these participants.

Regarding vitamin D status, 52.2% of participants had sufficient serum 25-hydroxyvitamin D (25-OHD) levels, aligning with the optimal range of 30-40 ng/ml

recommended for promoting bone formation and cardiac health while minimizing risks associated with excessive supplementation (TIF, 2021). Serum calcium levels were maintained, with 90.2% of participants falling within the normal range of 8.5 to 10.5 mg/dl, consistent with the body's strict regulation of calcium homeostasis in thalassemia patients (TIF, 2021; Fung *et al.*, 2023).

4.3.7 Thalassemia healthcare services

As illustrated in Table 4.15, most participants (85.9%) were introduced to the NTS through the healthcare team, while 14.1% learned through other means. Regarding nutrition counseling, doctors and nurses were the primary sources of guidance for most participants (92.4%), with only a small fraction (5.4%) receiving counseling directly from the NTS.

Table 4.15 Distribution of thalassemia healthcare services in the study participants (n=92)

Variable	Frequency	Percentage
Introduction to Nepal Thalassemia Society (NTS)		
Healthcare team	79	85.9
Others	13	14.1
Received nutrition counseling		
Doctor/Nurse	85	92.4
NTS	5	5.4
Not received	2	2.2
Read/heard about the nutritional management of thalassemia		
No	44	47.8
Book	8	8.7
Pamphlet	38	41.3
Social media	2	2.2

However, a notable gap in the dissemination of nutritional management information was identified. Nearly half of the participants (47.8%) reported no exposure to information about nutritional management for thalassemia. Among those who did access such information, pamphlets were the predominant source (41.3%), followed by books (8.7%) and social media (2.2%). These pamphlets were displayed in the thalassemia unit of KCH, as detailed in

Appendix D. The NTS provides a comprehensive book on thalassemia in Nepali to registered patients. This resource offers detailed information about the condition, its treatment, and dietary recommendations, including specific advice on iron avoidance for β-TM patients (Eleftheriou and Rayamajhi, 2007). Despite this significant effort in patient education, the study found that only a small percentage of participants (8.7%) reported books as their primary source of information.

4.3.8 Caregiver's knowledge of nutrition and thalassemia

Table 4.16 presents the caregiver's knowledge of nutrition and thalassemia among the study participants. Over half of the caregivers (55.4%) demonstrated familiarity with malnutrition, and a substantial proportion (91.3%) recognized the risk of malnutrition in thalassemia patients.

Notably, 88% were aware of increased nutritional requirements associated with the condition. Regarding dietary practices, most caregivers (77.2%) did not adhere to any special diet, and only a small portion provided milk and milk products (18.4%) or black tea (4.4%). Notably, 93.5% of caregivers seriously avoided iron-rich foods, citing concerns about iron overload. This aligns with the finding that 87% of them knew about the complications of iron overload in thalassemia patients.

The high proportion of caregivers avoiding iron-rich foods reflects adherence to existing guidelines in Nepal, including those practiced at KCH, which recommends a low-iron diet for transfusion-dependent β-TM (MoH, 2017). These guidelines include avoiding red meat, organ meats, dark green leafy vegetables, and fruits such as watermelon, pomegranate, beans, and chocolate (See more in Appendix D). However, recent TIF nutrition guidelines (Fung *et al.*, 2023; Fung and Yardumian, 2024) suggest strict avoidance may be unnecessary for patients receiving regular transfusions and chelation therapy, as dietary iron absorption is negligible compared to transfusional iron load.

While a low-iron diet may have some benefits, it can negatively impact quality of life, create a false sense of security, potentially reduce chelation therapy adherence, and lead to reduced intake of beneficial nutrients like zinc (Fung *et al.*, 2012; Fung *et al.*, 2023).

Table 4.16 Caregiver's knowledge of nutrition and thalassemia of the study participants

Variable	Frequency	Percentage
Knowledge of malnutrition (n=92)		
No	41	44.5
Yes	51	55.4
Cause of malnutrition (n=51)		
Low food intake	51	100.0
Knowledge about the risk of malnutrition in β-TM (n=92)		
No	8	8.7
Yes	84	91.3
Knowledge about increased nutritional requirements in β-TM (n=92)		
No	11	12.0
Yes	81	88.0
Special diet for thalassemia (n=92)		
No	71	77.2
Black tea	4	4.4
Milk and milk products	17	18.4
Give special diet to child (n=21)		
No	2	2.2
Yes	19	20.7
Avoidance of iron-rich foods (n=92)		
No	6	6.5
Yes	86	93.5
Reason for avoidance of iron-rich foods (n=86)		
Iron overload	86	100.0
Knowledge of iron overload complications (n=92)		
No	12	13.0
Yes	80	87.0

The caregivers' awareness of increased nutritional requirements in β-TM patients is consistent with current scientific understanding. Research has established that β-TM patients experience elevated energy expenditure due to various physiological factors, including increased protein turnover and chronic inflammation (Soliman *et al.*, 2023). The practice of providing black tea indicates some awareness of dietary strategies to manage iron absorption. Black tea contains tannins, which can inhibit iron absorption. However, instead of tea, TIF recommends offering more nutritionally dense alternatives, particularly milk, to pediatric patients (Fung *et al.*, 2023).

4.4 Household food and cooking characteristics

Table 4.17 Household food and cooking characteristics of the study participants

Variable	Frequency	Percentage
Source of food and produces (n=92)		
Donation	3	3.3
Purchase from the market	75	81.5
Subsistence farming	14	15.2
Cooking oil (n=92)		
Vegetable oil	92	100.0
Use of iodized salt (n=92)		
Yes	92	100.0
Source of drinking water (n=92)		
Jar water	34	37.0
Tap	46	50.0
Tube well	12	13.0
Water purification (n=92)		
No	56	60.9
Yes	36	39.1
Method of water purification (n=36)		
Boiling	12	13.0
Boiling and filtration	2	2.2
Filtration	22	23.9

The majority of participants (81.5%) purchased food and produce from the market, while 15.2% relied on subsistence farming, and only 3.3% received food through donations. In this context, donation refers to food that the participants received from various organizations, charities, or individuals without having to pay for it. All participants reported using vegetable oil for cooking and iodized salt in their meals, as shown in Table 4.17.

Regarding the source of drinking water, half of the participants (50%) used tap water, 37% used jar water, and 13% relied on tube wells. Among the participants who purified their water, 23.9% used filtration as their primary method, 13% boiled their water, and 2.2% used a combination of boiling and filtration.

4.5 Dietary intake

4.5.1 Eating behavior

Table 4.18 Eating behavior in the study participants

Variable	Frequency	Percentage
Vegetarian (n=92)		
No	88	95.6
Yes	5	5.4
Reason for being vegetarian (n=5)		
Food preference	2	2.2
Religion	3	3.3
Food allergy (n=92)		
No	89	96.7
Yes	3	3.3
School meal (n=81)		
Take from home	37	40.2
School canteen	44	47.8
Water intake in a day (n=92)		
< 500 ml	45	48.9
500 ml to 1 l	37	40.2
> 1 l	10	10.9

Table 4.18 shows that the majority of study participants (95.6%) were non-vegetarians, with only 5.4% following a vegetarian diet. Most participants (96.7%) reported no food allergies, while 3.3% had milk and citric acid-containing food allergies. Regarding school meals, 40.2% of participants took meals from home, and 47.8% ate at the school canteen.

Daily water intake varied among participants, with 48.9% consuming less than 500 ml, 40.2% consuming 500 ml to 1 l, and 10.9% consuming more than 1 l per day. Adequate hydration is crucial for β-TM patients to prevent complications such as fatigue and constipation (Fung *et al.*, 2023).

4.5.2 Food consumption pattern

The food frequency questionnaire (FFQ) was used to analyze the dietary practices of the study population. The consumption of food was considered "regular" if consumed daily or 3-4 times a week, "frequent" when consumed once a week, and "rare" when consumed once a month or less.

The data presented in Table 4.19 shows the food consumption patterns among study participants. Rice was the predominant food item, with 100% of participants reporting regular consumption. Lentils were also prominent in the dietary profile, regularly consumed by 95.7% of participants. This aligns with the fact that rice and lentils are staples in the Nepalese diet. While rice dominated, other grains such as maize, barley, and millet were rarely consumed. Interestingly, caregivers perceived these grains as rich sources of iron, potentially influencing their limited inclusion in participants' diets.

Animal-based protein sources exhibited lower regular consumption frequencies, with poultry at 38.0%, eggs at 50.0%, and fish at 3.3%. Red meat was rarely consumed by 92.4% of participants, aligning with guidelines recommending a low-iron diet for transfusion-dependent β-TM patients (MoH, 2017). Beans, pulses, and soy products were rarely consumed (57.6%, and 81.5% respectively), as were green leafy vegetables (39.1% rare consumption). This pattern corresponds with recommendations to limit iron-rich foods. Other vegetables were moderately consumed.

The majority of participants rarely consumed fruits and nuts, potentially due to both dietary restrictions and economic constraints. Tea was regularly consumed by 63% of participants, likely due to its iron-binding properties. However, this may not be optimal for

pediatric patients (Fung *et al.*, 2023). While 67.4% reported regular milk intake, many consumed milk tea rather than whole milk. Other dairy products were consumed less frequently.

Table 4.19 Distribution of food consumption frequency of the study participants

Variables	Food items	Frequency (%)		
		Regular	Frequent	Rare
Cereals	Rice	92 (100)	-	-
	Whole wheat	29 (31.5)	24 (26.1)	39 (43.4)
	Maize, barley, millet	9 (9.8)	9 (9.8)	74 (80.4)
Legumes and pulses	Lentils	88 (95.7)	3 (3.3)	1 (1.1)
	Beans and pulses	6 (6.5)	33 (35.9)	53 (57.6)
	Soy and soy products	6 (6.5)	11 (12.0)	75 (81.5)
Fruits and vegetables	Green leafy vegetables	37 (40.2)	19 (20.7)	36 (39.1)
	Other vegetables	66 (71.7)	14 (15.2)	12 (13.0)
	Fruits	6 (6.5)	9 (9.8)	77 (83.7)
Milk and milk products	Milk	62 (67.4)	3 (3.3)	27 (29.3)
	Curd, buttermilk	16 (17.4)	18 (19.6)	58 (63.0)
	Paneer	1 (1.1)	7 (7.6)	84 (91.3)
Egg, meat, and fish	Poultry	35 (38.0)	38 (41.3)	19 (20.7)
	Fish	3 (3.3)	15 (16.3)	74 (80.4)
	Red meat	2 (2.2)	5 (5.4)	85 (92.4)
	Egg	46 (50.0)	26 (28.3)	20 (21.7)
Others	Nuts and seeds	5 (5.4)	1 (1.1)	86 (93.5)
	Tea	58 (63.0)	9 (9.8)	25 (27.2)
	Sugar and confectionery	44 (47.8)	19 (20.7)	29 (31.5)
	Sugary drinks	20 (21.7)	13 (14.1)	59 (64.1)
	Carbonated drinks	3 (3.3)	8 (8.7)	81 (88.0)
	Processed ready-to-serve foods	65 (70.7)	12 (13.0)	15 (16.3)
	Restaurant/fast foods	11 (12.0)	33 (35.9)	48 (52.2)

Alarmingly, 70.7% of participants regularly consumed processed ready-to-serve foods, with biscuits and noodles being daily snacks for most. The most common snacks reported

were milk tea and biscuits in the study participants. This high intake of processed foods, coupled with the low consumption of diverse whole foods, raises concerns about overall diet quality and nutrient intake.

4.5.3 Dietary diversity score

In the present study, for children above 2 years, the 10-food group score using the Minimum Dietary Diversity for Women (MDD-W) by FAO (2021) was used. For children below 2 years, the 8-food group score using the Infant and Young Child Feeding Minimum Dietary Diversity (IYCF-MDD) by WHO and UNICEF (2021) was used.

The mean dietary diversity score (DDS) was found to be 4.230 ± 1.070 in the present study. A score of 5 or higher indicates a more diverse and potentially more nutritious diet, while a score below 5 suggests limited dietary variety, which may be associated with nutritional inadequacies (FAO, 2021; WHO and UNICEF, 2021). As shown in Table 4.20 in the 1 to 4 years age group, 57.9% exhibited inadequate dietary diversity, while 42.1% achieved adequate scores.

Table 4.20 Distribution of dietary diversity score of the study participants (n=92)

Age group	n	Frequency (%)	
		Inadequate (< 5)	Adequate (≥ 5)
1 to 4 years	19	11 (57.9)	8 (42.1)
5 to 9 years	48	35 (72.9)	13 (27.1)
10 to 14 years	25	9 (36.0)	16 (64.0)
Total	92	55 (59.8)	37 (40.2)

The 5 to 9 years age group demonstrated the highest prevalence of inadequate dietary diversity, with 72.9% falling below the threshold for adequacy. In contrast, the 10 to 14 years age group showed the most favorable outcomes, with 64.0% attaining adequate DDS.

4.5.4 Dietary intake in the preceding day

Dietary data collected through 24-hour recall interviews were analyzed using food composition tables from the DFTQC (2017) and Indian ICMR (Longvah *et al.*, 2017) to determine the nutrient content and macronutrient distribution of the consumed foods. The age group was classified according to the ICMR-NIN (2020). The analysis revealed

consistent patterns in macronutrient distribution across all age groups, as shown in Table 4.21. Carbohydrates emerged as the primary energy source, contributing 50-68% of total caloric intake. Fat provided approximately 16-38% of calories, while protein accounted for 8-15% of the total energy intake.

Age-related trends in macronutrient consumption were observed, with protein intake, peaking in the 13 to 14 years age group. Conversely, fat consumption appeared to decrease with age, from ~28% in the youngest group to ~22% in the oldest. Carbohydrate intake remained relatively constant across age groups but showed a subtle increase in older age groups.

Table 4.21 Macronutrient distribution of the study participants (n=92)

Age group (in years)	n	Sex	% Nutrient from total calorie intake (Mean ± SD)		
			% Protein	% Fat	% Carbohydrates
1 to 3	17		10.178 ± 1.974	28.252 ± 9.617	56.765 ± 7.557
4 to 6	19		11.249 ± 2.399	26.188 ± 6.084	55.419 ± 6.292
7 to 9	32		10.701 ± 3.619	24.717 ± 6.870	58.575 ± 7.053
10 to 12	11	M	11.977 ± 3.619	24.193 ± 7.519	59.539 ± 8.968
	5	F	10.952 ± 1.809	24.571 ± 4.820	58.519 ± 7.792
13 to 14	4	M	11.8442 ± 1.041	22.475 ± 5.517	60.214 ± 7.943
	4	F	11.572 ± 1.732	21.173 ± 5.151	59.829 ± 4.122
Total	92		10.971 ± 2.352	25.312 ± 7.208	57.827 ± 7.169

Age group reproduced from ICMR-NIN (2020)

Gender-based disparities in macronutrient intake were minimal, with males showing marginally higher carbohydrate and protein intake compared to females, while fat consumption exhibited negligible gender-based variation.

4.5.5 Nutrient intake in various age groups

The nutrient content and macronutrient distribution of foods consumed by study participants were analyzed based on data collected through 24-hour dietary recall interviews. To evaluate the adequacy of calorie and protein intake, these values were compared against the Estimated Average Requirement (EAR) standards set by the ICMR-NIN (2024).

i. 1 to 3 years

Table 4.22 presents nutrient adequacy data for study participants aged 1 to 3 years, comparing their actual intake to the EAR for calories and protein. The mean daily caloric intake (934.452 ± 298.538 Kcal/d) was not significantly different ($p>0.05$) than the EAR (969.635 ± 146.644 Kcal/d suggesting that caloric intake in this age group aligns with recommendations. In contrast, protein intake (23.797 ± 8.677 g/d) substantially exceeded ($p<0.001$) the EAR (9.229 ± 1.396 g/d), indicating that protein consumption in this age group was higher than estimated needs.

Table 4.22 Nutrient adequacy in the 1 to 3 years age group (n=17)

Variables	Mean ± SD	
	Calorie (Kcal/d)	Protein (g/d)
Intake	934.452 ± 298.538	23.797 ± 8.677
EAR	969.635 ± 146.644	9.229 ± 1.396
% EAR	96.147 ± 27.527	257.662 ± 85.868
p-value*	0.584	<0.001

*p-values were derived from paired t-tests comparing intake to EAR

ii. 4 to 6 years

The mean daily caloric intake (1143.141 ± 286.882 Kcal/d) was significantly different ($p>0.05$) than the EAR (1326.937 ± 177.447 Kcal/d), suggesting that participants in this age group consumed fewer calories than recommended. In contrast, protein intake (31.853 ± 10.194 g/d) significantly exceeded ($p<0.001$) the EAR (12.552 ± 1.676 g/d), indicating that protein consumption in this age group was higher than estimated needs, as shown in Table 4.23.

Table 4.23 Nutrient adequacy in the 4 to 6 years age group (n=19)

Variables	Mean ± SD	
	Calorie (Kcal/d)	Protein (g/d)
Intake	1143.141 ± 286.882	31.853 ± 10.194
EAR	1326.937 ± 177.447	12.552 ± 1.676
% EAR	87.009 ± 22.039	254.81 ± 80.229
p-value*	0.019	<0.001

*p-values were derived from paired t-tests comparing intake to EAR

iii. 7 to 9 years

The nutrient adequacy data for study participants aged 7 to 9 years revealed significant differences between actual consumption and EAR for both calories and protein, as shown in Table 4.24. Mean daily caloric intake (1248.959 ± 322.176 Kcal/d) was statistically significant ($p< 0.001$) than the EAR (1537.441 ± 231.982 Kcal/d), indicating a consistent pattern of calorie underconsumption in this age group. In contrast, protein intake (32.925 ± 9.448 g/d) significantly exceeded ($p<0.001$) the EAR (17.2102 ± 2.597 g/d), suggesting that protein consumption in this age group was higher than estimated needs.

Table 4.24 Nutrient adequacy in the 7 to 9 years age group (n=32)

Variables	Mean ± SD	
	Calorie (Kcal/d)	Protein (g/d)
Intake	1248.959 ± 322.176	32.925 ± 9.448
EAR	1537.441 ± 231.982	17.2102 ± 2.597
% EAR	82.464 ± 22.230	195.506 ± 62.543
p-value*	<0.001	<0.001

*p-values were derived from paired t-tests comparing intake to EAR

iv. 10 to 12 years

Table 4.25 presents nutrient adequacy data for study participants aged 10 to 12 years.

Table 4.25 Nutrient adequacy in the 10 to 12 years age group (n=16)

Sex	Variables	Mean ± SD	
		Calorie (Kcal/d)	Protein (g/d)
Male (n=11)	Intake	1593.817 ± 392.181	49.182 ± 23.802
	EAR	1789.672 ± 243.231	20.983 ± 2.745
	% EAR	90.365 ± 25.463	238.157 ± 121.409
	p-value*	0.195	0.003
Female (n=5)	Intake	1255.360 ± 151.462	34.600 ± 7.995
	EAR	1669.340 ± 304.444	20.956 ± 3.515
	% EAR	76.324 ± 9.863	165.893 ± 33.022
	p-value*	0.016	0.013

*p-values were derived from paired t-tests comparing intake to EAR

Male participants showed a higher mean caloric intake (1593.817 ± 392.181 Kcal/d) compared to their female counterparts (1255.360 ± 151.462 Kcal/d). However, both groups had low-calorie intake relative to their respective EARs, with males achieving 90.365% and females 76.324% of recommended levels. Protein consumption patterns showed differences, with males significantly exceeding their EAR (238.157% ± 121.409), while females showed a moderate excess (165.893% ± 33.022). These differences were statistically significant ($p<0.05$) for both males and females.

v. 13 to 14 years

Table 4.26 shows the nutrient adequacy data for study participants aged 13 to 14 years. The data displayed notable patterns in calorie and protein intake relative to their EAR. Male participants showed higher average caloric consumption (1445.775 ± 614.166 Kcal/d) than females (1178.850 ± 502.623 Kcal/d).

Notably, both groups failed to meet their EAR for calories. Males achieved only 70.453% of their EAR, while females reached 67.323%. The caloric deficit was statistically significant for females ($p<0.05$) but not for males ($p>0.05$). In contrast to caloric intake, protein consumption exceeded requirements for both sexes. Males consumed 162.956% ± 62.418 of their protein EAR, while females reached 139.379% ± 49.416. However, these protein excesses were not statistically significant ($p>0.05$) for both males and females.

Table 4.26 Nutrient adequacy in the 13 to 14 years age group (n=8)

Sex	Variables	Mean ± SD	
		Calorie (Kcal/d)	Protein (g/d)
Male (n=4)	Intake	1445.775 ± 614.166	42.250 ± 15.403
	EAR	2073.660 ± 204.398	26.193 ± 2.592
	% EAR	70.453 ± 30.996	162.956 ± 62.418
	p-value*	0.157	0.136
Female (n=4)	Intake	1178.850 ± 502.623	35.250 ± 19.798
	EAR	1711.325 ± 396.837	24.448 ± 15.669
	% EAR	67.323 ±14.332	139.379 ± 49.416
	p-value*	0.018	0.256

*p-values were derived from paired t-tests comparing intake to EAR

The nutrient adequacy data for all the age groups in the study population revealed a concerning pattern of caloric deficiency coupled with excess protein intake. Although the mean calorie intake across age groups generally fell below the EAR, there was significant variability when considering standard deviations, with calorie intake ranging from 39% to 124% of EAR across all age groups. Similarly, protein intake consistently exceeded the EAR across all age groups, ranging from approximately 90% to 360% of requirements.

The benefits of high-protein diets in maintaining muscle mass and overall protein balance in the body diminish when calorie intake falls below 40% of daily energy requirements (Carbone and Pasiakos, 2019). In conditions of significant calorie deficit, a metabolic shift occurs whereby dietary protein is predominantly catabolized for energy production, despite not being the primary energy source under normal physiological conditions (Carbone *et al.*, 2019). This metabolic adaptation compromises the potential benefits associated with high protein intake, such as muscle protein synthesis and growth, particularly crucial during periods of rapid growth of the study participants, aged 1 to 14 years (Fung *et al.*, 2023).

4.6 Nutritional status

4.6.1 Prevalence of malnutrition

Anthropometric indices are the major tools for the assessment of nutritional status of children and adolescents. The indices obtained were weight-for-age (underweight), height-for-age (stunting), BMI-for-age (wasting/thinness, overweight, and obesity), mid-upper arm circumference (MUAC), and body fat percentage to assess the prevalence of nutritional status of the study participants.

Fig. 4.5 presents the prevalence of malnutrition among 92 study participants. Stunting was the most prevalent condition, with 19.6% with moderate stunting, followed by severe stunting at 7.6%. Underweight conditions were also notable, with moderate underweight and moderate thinness observed in 6.5% and 5.4% of participants, respectively. Severe underweight was less common but still present in 2.2% of the sample. Interestingly, overweight status was identified in 2.2% of participants, equal to the prevalence of severe underweight.

The study findings of 27.2% stunting are notable, yet appear lower than rates reported in several other studies of β-TM children and adolescents. For instance, Mirhosseini *et al.*

(2013b) found that 41.4% of β-TM children and adolescents had short stature, while Pemde *et al.* (2011) 33% short stature among 154 Indian thalassemic children and adolescents. Even higher rates were observed by Rathaur *et al.* (2020), Sharma and Bezboruah (2022), Jana *et al.* (2016), Fadlyana *et al.* (2017), and Das and Majumdar (2019) who reported stunting prevalence ranging from 62% to 68% among TDT patients.

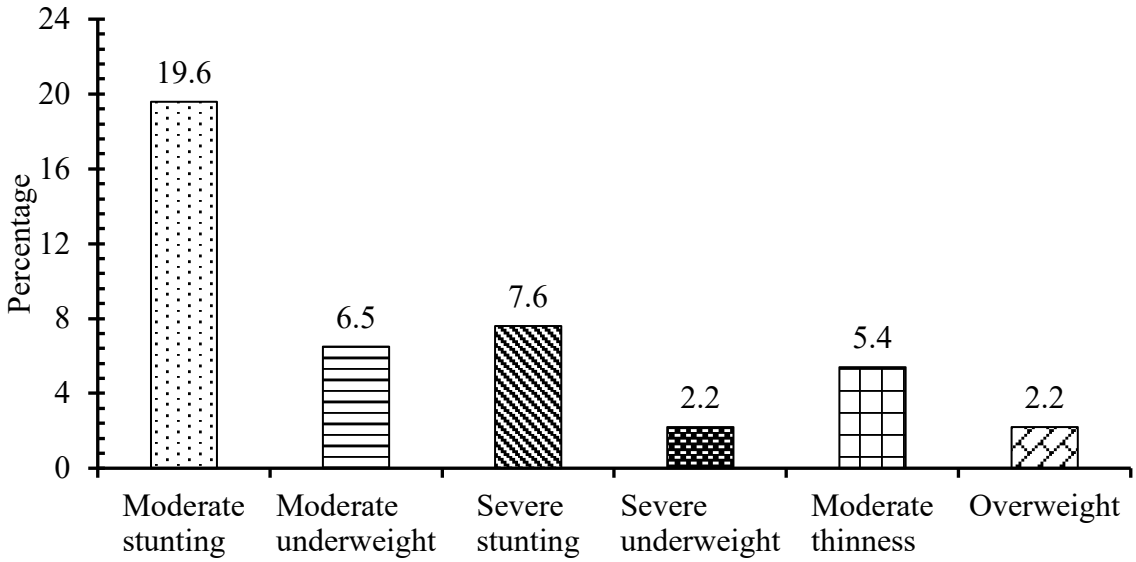


Fig. 4.5 Prevalence of malnutrition among the study participants (n=92)

In a recent study in Nepal, Sharma Poudyal *et al.* (2023) found that 93% of β-TM patients registered with the NTS were affected by stunted growth. The lower prevalence of stunting in the present study may suggest improved management strategies or potentially different demographic characteristics compared to these other studies.

Regarding underweight status, this study found considerably lower rates than reported in other studies. Biswas *et al.* (2021) found that 48.2% of 328 β-TM children in India were underweight while Sheikh *et al.* (2017) observed 58.69% underweight status among 305 β-TM children in Pakistan were underweight. Even higher rates were reported by Rathaur *et al.* (2020) and Das and Majumdar (2019), at 77% and 70% respectively.

4.6.2 Nutritional status according to sex

The nutritional status assessment showed notable sex-based differences across various indicators as presented in Table 4.27.

Table 4.27 Distribution of nutritional status according to sex in the study participants (n=92)

Indicators	Classification (Interpretation)	Sex (%)		
		Female (n=32)	Male (n=60)	Total (n=92)
BMI/A	≥ -2 to < +1 (Normal)	30 (93.8)	55 (91.7)	85 (92.4)
	- ≥ -3 to < -2 (Moderate wasting/thinness*)	2 (6.3)	3 (5.0)	5 (5.4)
	> +1 (Overweight)	0 (0.0)	2 (3.3)	2 (2.2)
H/A	≥ -2 (Normal)	23 (71.9)	44 (73.3)	67 (72.8)
	- ≥ -3 to < -2 (Moderate stunting)	8 (25.0)	10 (16.7)	18 (19.6)
	< -3 (Severe stunting)	1 (3.1)	6 (10.0)	7 (7.6)
W/A	≥ -2 (Normal)	22 (68.8)	39 (65.0)	61 (66.3)
	- ≥ -3 to < -2 (Moderate underweight)	2 (6.3)	4 (6.7)	6 (6.5)
	< -3 (Severe underweight)	0 (0.0)	2 (3.3)	2 (2.2)
	Not applicable**	8 (25.0)	15 (25.0)	23 (25.0)
MUAC#	Normal	27 (84.4)	50 (83.3)	77 (83.7)
	Moderate acute malnutrition	5 (15.6)	10 (16.7)	15 (16.3)
Body fat %	< 20%	2 (6.3)	12 (20.0)	14 (15.2)
	20 to 30%	9 (28.1)	48 (80.0)	57 (62.0)
	> 30%	21 (65.6)	0 (0.0)	21 (22.8)

*For ages less than 5 years, "wasting" is used to describe BMI/A; for ages more than 5 years, "thinness" is used.

**W/A is not applicable for children over 10 years old as it does not distinguish between height and body mass, potentially misclassifying tall children during pubertal growth.

#The cut-off values for MUAC vary according to the age group.

BMI-for-age (BMI/A) data revealed that the majority of participants (92.4%) fell within the normal range, with minimal sex-based variations. Moderate thinness was slightly more prevalent in females (6.3%) than males (5%), while overweight status was observed exclusively in males (3.3%).

Height-for-age (H/A) measurements indicated a higher prevalence of stunting among males. Although a similar proportion of females (71.9%) and males (73.3%) exhibited normal H/A, the distribution of stunting severity differed. Moderate stunting affected a higher percentage of females (25.0%) compared to males (16.7%), whereas severe stunting was more common in males (10.0%) than females (3.1%).

Weight-for-age (W/A) analysis demonstrated that a higher percentage of females (68.8%) were within the normal range compared to males (65.0%). Moderate underweight status was slightly more prevalent in males (6.7%) than females (6.3%), and severe underweight was observed only in males (3.3%). The prevalence of moderate acute malnutrition (MAM) was similar between sexes.

Body fat percentage exhibited the most pronounced sex-based disparities. A substantial majority (65.6%) of female participants had body fat exceeding 30%, while no males fell into this category. Conversely, 80.0% of males had body fat percentages between 20-30%, compared to only 28.1% of females. Furthermore, 20.0% of males had less than 20% body fat, versus 6.3% of females. The mean body fat percentage for all study participants was found to be 24.35 ± 4.938.

These observed sex differences in body fat distribution align with established physiological variations between males and females because of the influence of steroid hormones (Karastergiou *et al.*, 2012). Similar differences were observed by Fung *et al.* (2010) in a study involving 183 pediatric patients with thalassemia, where female patients had higher body fat levels than male patients. The relatively low proportion of participants with body fat percentages below 20% (6.3% of females and 20% of males) may indicate adequate energy reserves, given that subcutaneous fat serves as an energy reserve during periods of nutritional deficiency (Mandal *et al.*, 2011; Wickramasinghe, 2012).

4.6.3 Nutritional status according to various age groups

The nutritional status assessment showed notable sex-based differences across various indicators as presented in Table 4.28. BMI/A showed a clear age-related decline in normal status, from 100% in the 1 to 4 years group to 84% in the 10 to 14 years group. Correspondingly, the prevalence of moderate thinness increased with age, reaching 16% in the oldest group. Similarly, H/A showed a marked decrease in normal prevalence with age,

from 89.5% in the youngest group to 56% in the oldest. Severe stunting displayed a non-linear pattern across age groups (10.5%, 4.2%, and 12% respectively). These trends in nutritional status across age groups in the study population align with the growth disturbance phases described by (Skordis and Kyriakou, 2011) for β-TM patients, potentially reflecting the transition from early childhood growth issues to the compounded effects of iron overload and endocrine dysfunction in later years.

Table 4.28 Distribution of nutritional status according to age groups in the study participants

Indicators	Classification	Frequency (%)			
		1 to 4 years (n=19)	5 to 9 years (n=48)	10 to 14 years (n=25)	Total (n=92)
BMI/A	≥ -2 to < +1	19 (100.0)	45 (93.8)	21 (84.0)	85 (92.4)
	- ≥ -3 to < -2	0 (0.0)	1 (2.1)	4 (16.0)	5 (5.4)
	> +1	0 (0.0)	2 (4.2)	0 (0.0)	2 (2.2)
H/A	≥ -2	17 (89.5)	36 (75.0)	14 (56.0)	67 (72.8)
	- ≥ -3 to < -2	0 (0.0)	10 (20.8)	8 (32.0)	19 (19.6)
	< -3	2 (10.5)	2 (4.2)	3 (12.0)	7 (7.6)
W/A	≥ -2	18 (94.7)	41 (85.4)	2 (8.0)	61 (66.3)
	- ≥ -3 to < -2	1 (5.3)	5 (10.4)	0 (0.0)	6 (6.5)
	< -3	0 (0.0)	2 (4.2)	0 (0.0)	2 (2.2)
	Not applicable*	0 (0.0)	0 (0.0)	23 (92.0)	23 (25.0)
MUAC#	Normal	19 (100.0)	43 (89.6)	15 (60.0)	77 (83.7)
	MAM	0 (0.0)	5 (10.4)	10 (40.0)	15 (16.3)
Body fat %	< 20%	14 (73.7)	0 (0.0)	0 (0.0)	14 (15.2)
	20 to 30%	5 (26.3)	34 (70.8)	18 (72.0)	57 (62.0)
	> 30%	0 (0.0)	14 (29.2)	7 (28.0)	21 (22.8)

*W/A is not applicable for children over 10 years old as it does not distinguish between height and body mass, potentially misclassifying tall children during pubertal growth.

#The cut-off values for MUAC vary according to the age group.

W/A normal prevalence was highest in the youngest group (94.7%), decreasing to 85.4% in 5 to 9 years old. However, this indicator is not applicable for 92% of 10 to 14 years old, as it does not distinguish between height and body mass in older children (de Onis *et al.*, 2007). MUAC showed a substantial increase in MAM with age, from 0% in the youngest group to 40% in the oldest, potentially reflecting cumulative nutritional challenges.

Body fat distribution shifted notably, with 73.7% of the 1 to 4 years group having body fat less than 20%, while the majority of older children (70.8% of 5 to 9 years and 72% of 10 to 14 years) had body fat percentages between 20-30%. This shift may indicate endocrine complications affecting body composition in older children with β-TM (Skordis and Kyriakou, 2011; Soliman *et al.*, 2023).

The overall distribution of nutritional status according to age groups in the study population showed an increasing prevalence of stunting and thinness with age, coupled with the shift in body fat distribution, as illustrated in Table 4.28. This trend underscores the cumulative impact of growth disturbances in β-TM, likely resulting from a combination of hypoxia, iron overload, and endocrine complications that become more pronounced in late childhood and adolescence (De Sanctis *et al.*, 2021; Soliman *et al.*, 2023).

4.6.4 Nutritional status with reference to WHO standard

For the analysis of nutritional status using WHO standards, the study participants were categorized into below five (1 to 4 years) and above five (5 to 9 years and 10 to 14 years). The analysis was performed using WHO Anthro and WHO AnthroPlus respectively.

4.6.4.1 Nutritional status of 1 to 4 years age group with reference to WHO standard

1. BMI-for-age

Fig. 4.6 indicates the BMI/A curve of the 1 to 4 years age group. The study participants had a slightly lower median for BMI/A compared to WHO standards, with the center of their distribution shifted left by about -0.340 SD. This suggests a low prevalence of wasting in this study's participants compared to global standards. The present study showed a 100% normal BMI/A in this age group.

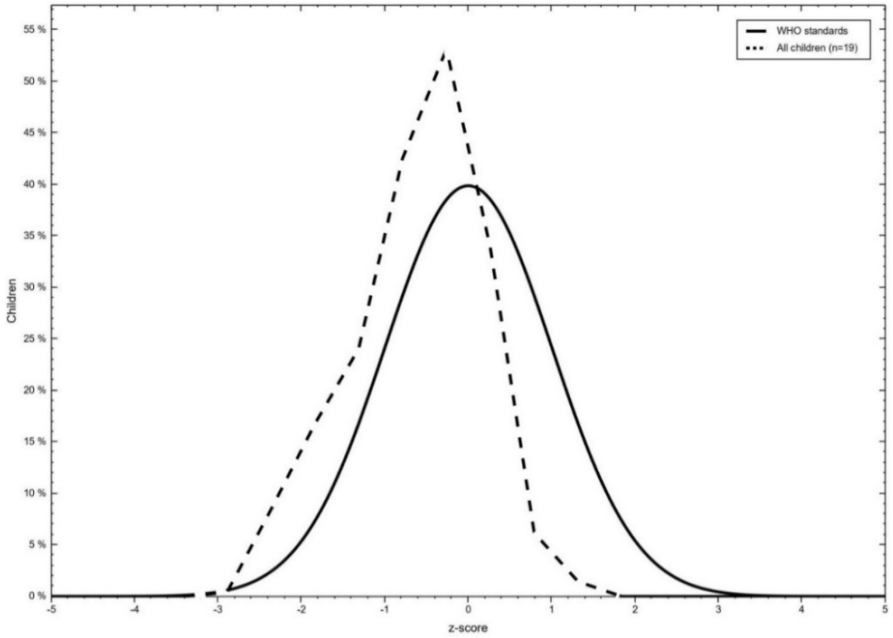


Fig. 4.6 BMI-for-age curve with reference to WHO standard (n=19)

2. Height-for-age

The H/A distribution for the 1 to 4 years age group showed a tendency toward lower heights compared to WHO standards, with a median z-score of -0.940. The leftward shift of the curve of H/A aligns with the negative median z-score, as shown in Fig. 4.7. However, 89.5% of the children maintained normal H/A status, falling within acceptable growth parameters. The distribution is skewed by 10.5% of participants experiencing severe stunting.

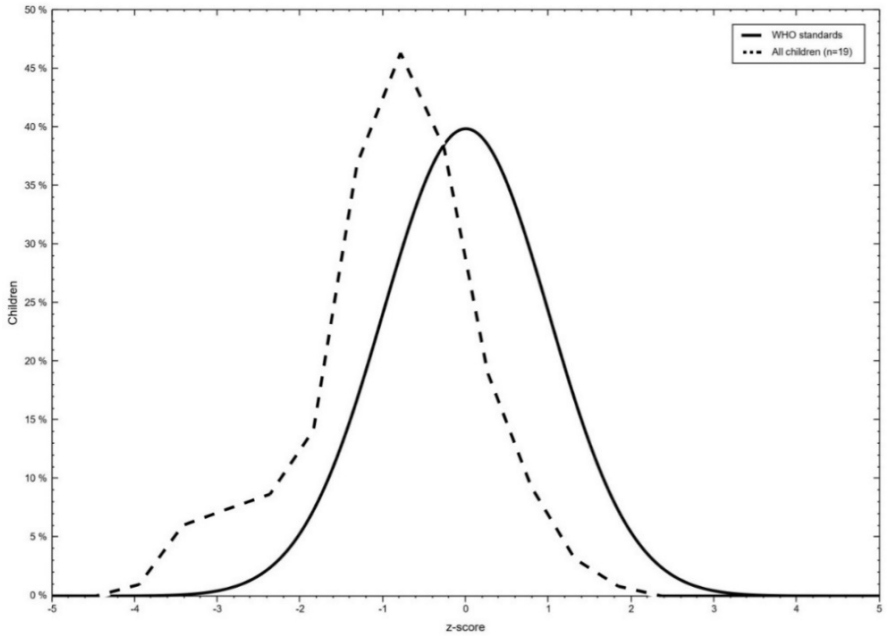


Fig. 4.7 Height-for-age curve with reference to WHO standard (n=19)

3. Weight-for-age

The W/A distribution for the 1 to 4 years age group showed a notable shift toward lower weights compared to WHO standards, with a median z-score of -0.830. The graph is shifted to the left of the WHO standards curve, as illustrated in Fig. 4.8. This leftward shift suggests that even participants not classified as underweight are generally lighter than global averages for their age. The prevalence of wasting was 5.6% in this age group.

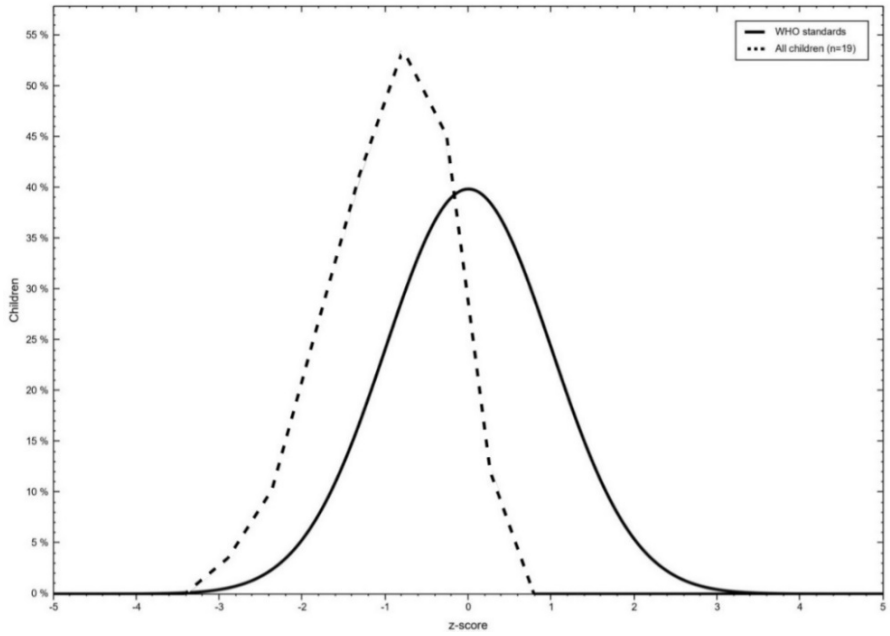


Fig. 4.8 Weight-for-age curve with reference to WHO standard (n=19)

4.6.4.2 Nutritional status of 5 to 14 years age group with reference to WHO standard

1. BMI-for-age

The BMI/A distribution for the 5-14 years age group showed a slight shift toward lower BMI values compared to WHO standards, with a median z-score of -0.520. The graph is shifted slightly to the left of the WHO standards curve, as shown in Fig. 4.9.

This slight leftward shift suggests that while participants are generally within normal ranges, they tend to have slightly lower BMI values than global averages for their age. Only 2.1% of 5 to 9 years old and 16.0% of 10 to 14 years old had moderate thinness.

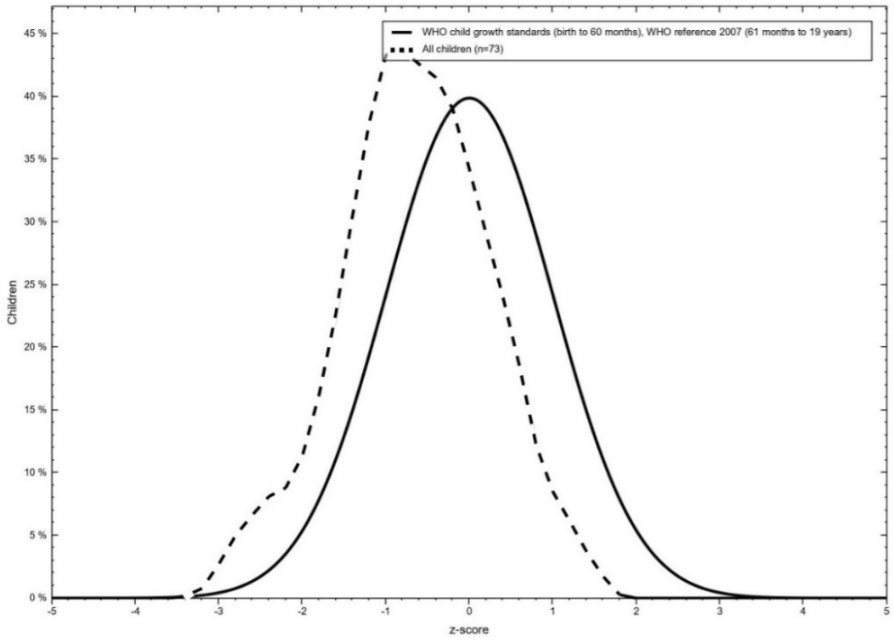


Fig. 4.9 BMI-for-age curve with reference to WHO standard (n=73)

2. Height-for-age

The H/A distribution for the study population showed a substantial deviation from WHO standards, with a median z-score of -1.490. Fig. 4.10 illustrates this deviation, showing the H/A curve markedly shifted to the left of the WHO reference curve. This significant leftward shift indicates that participants were generally shorter than global averages for their age, signifying a high prevalence of stunting in the population.

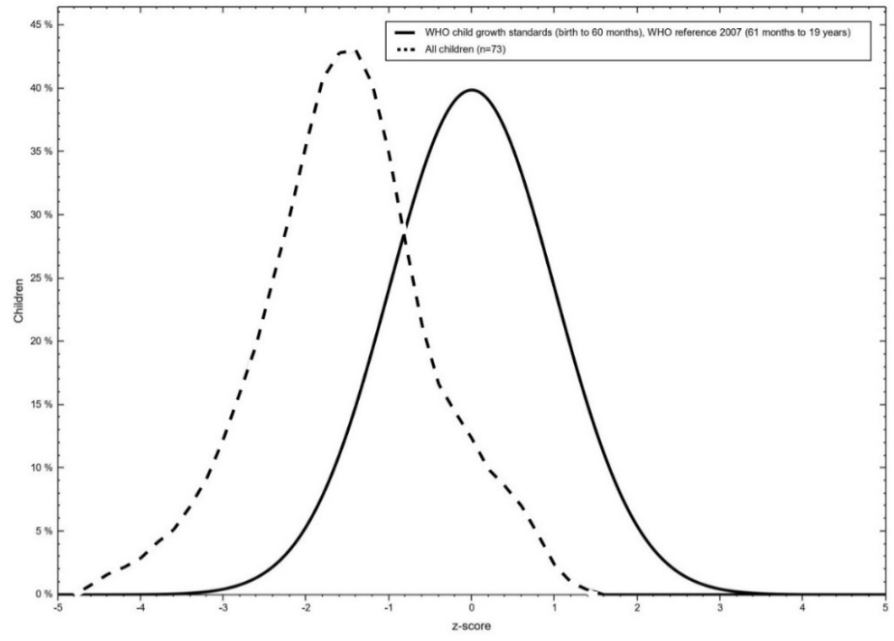


Fig. 4.10 Height-for-age curve with reference to WHO standard (n=73)

The present study showed that the severity and prevalence of stunting increased with age. 25% of participants aged 5 to 9 years and 44% of those aged 10 to 14 years were stunted. Within these age groups, 4.2% and 12% respectively were severely stunted. This progressive increase in stunting prevalence across age groups suggests the cumulative impact of growth disturbances in β-TM. A study by Pemde *et al.* (2011) also observed this trend with stunting more prominent during early adolescence (10 to 14 years of age).

The etiology is likely multifactorial, resulting from a combination of chronic hypoxia due to anemia, iron overload from repeated transfusions, and endocrine complications. These factors appear to become more pronounced in late childhood and adolescence, potentially preventing normal growth spurts and leading to the observed pattern of increasing growth impairment with age (Skordis and Kyriakou, 2011; Arab-Zozani *et al.*, 2021; Soliman *et al.*, 2023).

3. Weight-for-age

The W/A distribution for the study population showed a noticeable shift toward lower weights compared to WHO standards, with a median z-score of -1.235. The graph for W/A is shifted to the left of the WHO standards curve suggesting that participants were generally lighter than global averages for their age, as presented in Fig. 4.11.

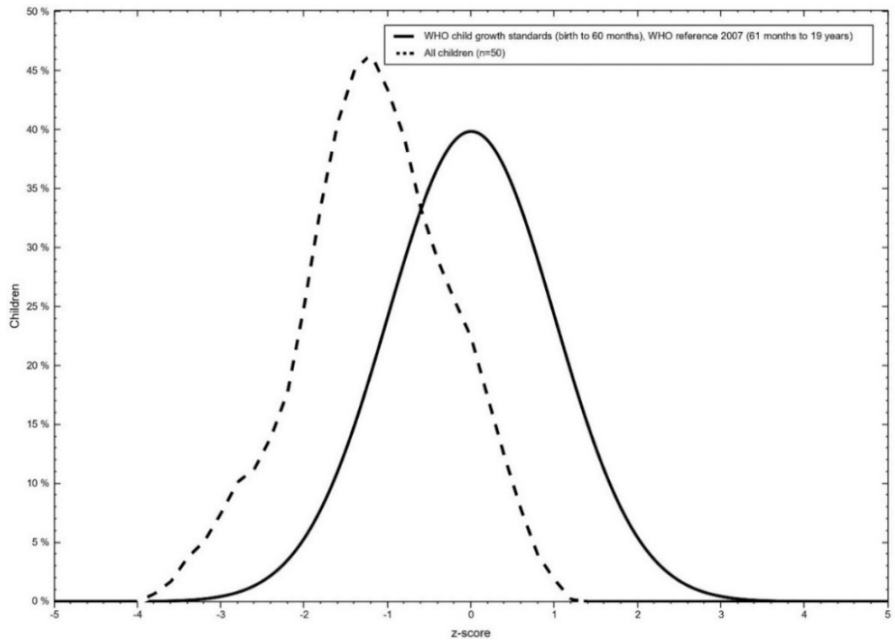


Fig. 4.11 Weight-for-age curve with reference to WHO standard (n=50)

The study data showed that among participants aged 5 to 9 years, 14.6% were underweight. However, W/A is not applicable for children above 10 years as it cannot distinguish between height and body mass, potentially misclassifying tall children during pubertal growth (de Onis *et al.*, 2007).

4.6.5 Nutritional status according to body fat percentage

Body fat percentage increased with age, with the most substantial change observed between the 1 to 4 and 5 to 9 years age groups. A consistent sexual dimorphism was evident, with females exhibiting higher body fat percentages than males across all age categories. This sex-based difference became more pronounced in older age groups, as shown in Table 4.29.

The findings from this study align with previous research on South Asian pediatric populations, consistently demonstrating gender differences in body fat percentage (Shaikh and Mahalanabis, 2004; Wickramasinghe *et al.*, 2008; Ghosh *et al.*, 2009).

Table 4.29 Distribution of body fat percentage in the study participants (n=92)

Age group	n	Body fat % (Mean ± SD)		
		Male	Female	Total
1 to 4 years	19	18.155 ± 1.340	20.994 ± 1.228	19.052 ± 1.859
5 to 9 years	49	22.607 ±1.379	31.455 ± 1.580	25.721 ± 4.512
10 to 14 years	25	22.528 ± 1.677	31.392 ± 2.105	25.717 ± 4.687

A clear age-related increase in body fat was observed in all studies, particularly pronounced in females. This trend was most evident in the Nepalese data, where the body fat percentage of girls increased from 11.79% at age 6 to 12.89% at age 10 while it remained stable (8.94% and 8.63% respectively) for boys (Ghosh *et al.*, 2009).

While methodological variations, such as different formulas for deriving body fat percentage from skinfold thickness (SFT), may contribute to some discrepancies in reported values, all studies, including the present one, consistently used triceps and subscapular SFT measurements.

4.7 Analysis of nutritional status and associated factors

4.7.1 Correlations between anthropometric parameters and associated factors

Table 4.30 presents correlation coefficients between various factors and anthropometric parameters of the study, focusing on factors affecting the nutritional status of the study participants.

W/A showed weak positive correlation ($p<0.05$) with DDS. This finding aligns with studies by Soliman *et al.* (2004) and (Fuchs *et al.*, 1996; Fuchs *et al.*, 1997), indicating that improved nutritional intake can lead to better growth outcomes. This suggests that a varied diet may be beneficial for weight maintenance in thalassemia patients, possibly due to a more comprehensive nutrient profile.

Table 4.30 Correlations between anthropometric parameters and associated factors

Factors	Correlation coefficient	p-value
Height-for-age		
Total age of chelation	-0.275[#]	0.013
Family size	-0.216	0.039
Weight-for-age		
Dietary diversity score	0.296	0.014
Body fat percentage		
Total age of chelation	0.284[#]	0.045
Serum ferritin	0.278	0.007
Energy	0.234	0.025
Protein	0.244	0.019
Desifer dose	0.385	<0.001
Mid-upper arm circumference		
Total age of chelation	0.654	<0.001
Desifer dose	0.681	<0.001
Energy	0.320	0.002
Protein	0.299	0.004
Carbohydrate	0.297	0.004

Data are expressed as Spearman correlation coefficient unless indicated as (#), which represents Pearson correlation coefficient

Several factors showed weak positive correlations ($p<0.05$) with body fat percentage, including total age of chelation, serum ferritin, energy, protein intake, and *Desifer* dose. Similarly, energy, protein, and carbohydrate intake demonstrated weak positive correlations ($p<0.05$) with MUAC. These results align with previous research by Mirhosseini *et al.* (2013b), who reported significant positive correlations between anthropometric parameters and energy intake. Despite their modest strength, these correlations underscore the importance of adequate macronutrient intake for maintaining healthy body composition in thalassemia patients.

The most statistically significant correlation ($p<0.001$) with body fat percentage was observed with the *Desifer* dose. MUAC showed a significant ($p<0.001$) moderate positive correlation with *Desifer* dose and total chelation age. These relationships suggest that patients receiving higher doses of *Desifer* and longer duration of chelation therapy tend to have higher body fat percentages and larger MUAC measurements. This association may be attributed to the severity of iron overload in these patients, which could independently affect nutritional status and body composition (Soliman *et al.*, 2023). The observed positive correlation between serum ferritin and body fat percentage aligns with findings by Orta-Duarte *et al.* (2020).

H/A demonstrated weak negative correlations ($p<0.05$) with the total age of chelation, indicating a slight tendency for H/A to decrease with increased duration of chelation therapy. This finding is particularly relevant given that more than half of the study participants (58.7%) exhibited seriously elevated serum ferritin levels, despite timely initiation of chelation therapy. These results, attributable to iron overload, are consistent with studies by Raghuwanshi *et al.* (2020) and De Sanctis *et al.* (2013), which identified iron overload as a key factor in growth impairment. Arab-Zozani *et al.* (2021) similarly reported that patients with higher iron concentrations tended to be shorter in stature.

Family size also showed a weak negative correlation ($p<0.05$) with H/A, which can be interpreted in the context of socioeconomic factors. Given that most participants had an annual family income below 1 lakh, larger families with limited resources might experience challenges in providing adequate nutrition to each child, potentially impacting growth. This socioeconomic influence is further supported by the observed association between MUAC and annual income, as discussed in section 4.7.4.

4.7.2 Group comparisons of anthropometric parameters and associated factors

Table 4.31 presents the results of group comparison tests between various factors and anthropometric parameters of the study participants. For BMI/A, significant differences ($p<0.05$) were observed based on exclusive breastfeeding status and birth weight. Notably, infants who were exclusively breastfed exhibited less negative BMI/A z-scores compared to those who were not.

Similarly, infants with birth weights ≥ 2500g showed less negative BMI/A z-scores than those with lower birth weights. These findings align with Palaska *et al.* (2024), who reported a statistically significant relationship between breastfeeding duration and children's BMI z-scores, and Baran *et al.* (2019), who found a significant positive association between birth weight and BMI centile.

Body fat percentage showed highly significant differences ($p<0.001$) based on sex and chelation status. Females exhibited substantially higher body fat percentage compared to males, consistent with observations by Fung *et al.* (2010) in pediatric populations. Interestingly, participants who had initiated chelation therapy showed higher body fat percentages than those who had not. This finding may be related to the relationship between serum ferritin levels and body fat, as reported by Orta-Duarte *et al.* (2020), given that chelation therapy is typically initiated when serum ferritin levels exceed 1000 ng/ml (TIF, 2021).

MUAC measurements differed significantly ($p<0.001$) based on chelation therapy status and school attendance. Participants who had started chelation therapy displayed larger MUAC values compared to those who had not. Both body fat percentage and MUAC showed significant associations ($p<0.001$) with school attendance. School-attending children demonstrated notably higher mean body fat percentages and median MUAC values compared to their non-attending counterparts.

These observed may primarily be attributed to the age disparity between these groups, rather than school attendance itself. This explanation aligns with previously discussed findings in sections 4.7.3 and 4.7.4, which detailed significant associations between age groups and both MUAC and body fat percentages.

Table 4.31 Group comparisons of anthropometric parameters and associated factors

Factors	n	Mean/Median	p-value
BMI-for-age			
Exclusive breastfeeding			
No	30	-0.906 ± 0.862	0.025
Yes	62	-0.499 ± 0.777	
Birth weight			
< 2500 g	22	-1.003 ± 0.803	0.014
≥ 2500 g	70	-0.515 ± 0.800	
Body fat percentage			
Sex			
Female	32	29.476 ± 4.444	<0.001
Male	60	21.621 ± 2.330	
Chelation therapy			
Not started	9	20.070 ± 2.050	<0.001
Started	83	24.818 ± 4.942	
School attending			
Yes	81	24.917 ± 4.947	<0.001
No	11	20.205 ± 2.146	
Mid-upper arm circumference			
Chelation therapy[#]			
Not started	9	14.30 (0.65)	<0.001
Started	83	17.20 (2.20)	
School attending[#]			
Yes	81	17.20 (2.10)	<0.001
No	11	14.30 (1.10)	

Data are expressed as mean ± SD where independent t-tests were used unless specified as (#), which are presented as median (interquartile range) where the Mann-Whitney test was used

4.7.3 Variance analysis between anthropometric parameters and associated factors

Analysis of variance (ANOVA) revealed significant differences across multiple variables in the study participants (Table 4.32). BMI/A was statistically associated ($p<0.05$) with age

group, and fruit intake, while H/A showed significant differences ($p<0.05$) across age groups, transfusion frequency, and milk intake.

Table 4.32 Analysis of variance between BMI/A, H/A, and associated factors

Factors	n	Mean ± SD	p-value
BMI-for-age			
Age group			
1 to 4 years	19	-0.507 ± 0.732^{ab}	
5 to 9 years	48	-0.470 ± 0.793^{a}	0.015
10 to 14 years	25	-1.034 ± 0.840^{b}	
Fruit intake			
Regular	6	-0.583 ± 0.469	
Frequent	9	-0.120 ± 0.825	0.021
Rare	77	-0.736 ± 0.811	
Height-for-age			
Age group			
1 to 4 years	19	-1.021 ± 0.976^{a}	
5 to 9 years	48	-1.339 ± 0.966^{a}	0.003
10 to 14 years	25	-1.982 ± 0.929^{b}	
Transfusion frequency			
Once a month	36	-1.047 ± 0.940^{a}	
Twice a month	48	-1.768 ± 1.001^{b}	0.011
Thrice a month	5	-1.222 ± 0.961^{ab}	
Irregular	3	-1.533 ± 0.336^{ab}	
Milk intake			
Regular	62	-1.272 ± 1.050^{a}	
Frequent	3	-2.170 ± 1.058^{a}	0.043
Rare	27	-1.773 ± 0.811^{a}	

Mean in the same column bearing the same superscript letter(s) are not significantly different ($p>0.05$)

Age-related differences were particularly notable in the study findings. Post-hoc tests indicated that children aged 10 to 14 years had significantly lower BMI/A compared to those aged 5 to 9 years. Moreover, H/A scores decreased significantly with increasing age, with

the 10 to 14 years group showing the lowest scores. These results suggest that growth disturbance may become more pronounced as children with thalassemia age, which in turn affects BMI/A due to its relation with height. These findings align with previous research on growth patterns in children with thalassemia. Iron overload particularly affects the growth hormone axis in children aged 5 to 9 years, with effects becoming more visible in the peripubertal years (10 to 12 years). This phenomenon can be attributed to iron deposition in endocrine glands, leading to hormonal imbalances that impact growth, especially during puberty (Skordis and Kyriakou, 2011).

Transfusion frequency was also found to significantly affect growth. Participants receiving transfusions twice a month had significantly lower H/A scores compared to those receiving transfusions once a month. This observation supports the proposition that increased transfusion frequency, while necessary for managing anemia, may exacerbate iron overload and its negative effects on growth (Rathaur *et al.*, 2020). These findings were consistent with those of Raghuwanshi *et al.* (2020) and De Sanctis *et al.* (2013), who identified iron overload as a key factor in growth impairment.

Regular milk intake was associated with better H/A outcomes compared to both frequent and rare intakes. Similarly, rare fruit intake was associated with lower BMI/A compared to frequent intake. These dietary observations are particularly relevant given the common occurrence of low bone mass and hypercalciuria in patients with thalassemia. In light of these challenges, TIF (2021) recommends a diet rich in calcium, including milk and milk products. The study findings on the positive impact of regular milk consumption align well with these recommendations.

The analysis of body fat percentage across different age groups revealed significant variations ($p<0.001$), reflecting developmental changes in body composition throughout childhood, as shown in Table 4.33. Participants aged 1 to 4 years exhibited the lowest mean body fat percentage, which was significantly lower than older groups. Notably, the 5 to 9 years and 10 to 14 years groups demonstrated similar, elevated body fat percentages, with no significant difference between them. This pattern aligns with established literature on pediatric body composition, particularly in South Asian populations, where age-related changes in body fat distribution have been consistently documented (Shaikh and Mahalanabis, 2004; Ghosh *et al.*, 2009).

Table 4.33 Analysis of variance between body fat percentage and associated factors

Factors	n	Mean ± SD	p-value
Body fat percentage			
Age group			
1 to 4 years	19	19.052 ± 1.859^{a}	
5 to 9 years	48	25.741 ± 4.512^{b}	<0.001
10 to 14 years	25	25.719 ± 4.687^{b}	
Maize, barley, millet intake			
Regular	9	25.644 ± 4.762^{ab}	
Frequent	9	28.455 ± 3.668^{a}	0.016
Rare	74	23.698 ± 4.871^{b}	
Red meat intake			
Regular	2	20.509 ± 2.258^{a}	
Frequent	5	30.218 ± 4.445^{b}	0.013
Rare	85	24.099 ± 4.791^{a}	
Tea intake			
Regular	58	25.475 ± 5.392^{a}	
Frequent	9	21.685 ± 2.073^{ab}	0.014
Rare	25	22.712 ± 3.668^{b}	

Mean in the same column bearing the same superscript letter(s) are not significantly different ($p>0.05$)

Dietary factors were found to play a substantial role in body fat percentage variability. The consumption frequency of maize, barley, and millet demonstrated a significant association ($p<0.05$) with body fat percentage. Frequent intake of these grains was significantly associated with the highest body fat percentage, significantly exceeding that of rare consumers. This finding suggests a potential link between high consumption of these grains and increased adiposity. Similarly, red meat consumption exhibited a significant association ($p<0.05$) with body fat percentage. Participants reporting frequent red meat intake displayed the highest body fat percentage, significantly surpassing both regular and rare consumers. This might be possibly due to the higher caloric and fat content of red meat (DFTQC, 2017). Moreover, the portion size of the red meat and consumption of overall calorie can affect the body fat percentage.

While the present study adhered to TIF guidelines for transfusion and chelation therapy, it's important to note that nutritional challenges persist even with optimal treatment. Soliman *et al.* (2004) observed that patients with β-TM receiving regular transfusion therapy and optimal iron chelation still exhibited compromised nutritional status. This compromised state was characterized by hypermetabolism and reduced insulin-like growth factor I (IGF-1) synthesis. Importantly, their research showed that introducing a high-caloric diet could lead to growth improvement and increased IGF-1 production, suggesting that growth impairment in these patients is correctable through targeted nutritional intervention to some extent (Soliman *et al.*, 2004).

4.7.4 Comparison of associated factors according to MUAC

The factors associated with MUAC are presented in Table 4.34. Age group showed a significant association ($p<0.001$) with MUAC, with median MUAC increasing consistently across age groups. This trend aligns with expected growth patterns in childhood and early adolescence and was similarly observed with body fat percentages. Naturally, the arm circumference of a child increases with age. The significant differences detected between all age categories show a consistent growth trajectory among the study participants, as previously explained in section 4.7.3.

Transfusion frequency exhibited a significant relationship ($p<0.001$) with MUAC, where frequent transfusions correlated with higher MUAC values compared to monthly or irregular transfusions. This pattern was further supported by the significant association ($p<0.05$) between last year's transfusion frequency and MUAC measurements. These findings align with research by Biswas *et al.* (2021), which indicates that maintenance of optimal hemoglobin levels promotes growth and mitigates anemia-related complications, thereby reducing the likelihood of malnutrition.

Socioeconomic status, as indicated by annual income, showed a significant association ($p<0.05$) with MUAC. Higher-income was associated with larger MUAC values, potentially due to enhanced access to nutrition and healthcare resources among more affluent families.

Table 4.34 Comparison of associated factors according to MUAC

Factors	n	Median (IQR)	p-value
Mid-upper arm circumference			
Age group			
1 to 4 years	19	14.60 (1.80)[a]	
5 to 9 years	48	17.05 (1.72)[b]	<0.001
10 to 14 years	25	18.50 (3.30)[c]	
Annual income			
< 1 lakh	57	16.20 (3.25)[a]	
1 to 3 lakhs	33	17.50 (1.80)[b]	0.023
> 3 lakhs	2	17.35[ab]	
Last year transfusion frequency			
< 12 times	3	14.60[a]	
12 – 24 times	84	17.10 (2.20)[ab]	0.045
> 24 times	5	17.80 (3.35)[b]	
Transfusion frequency			
Once a month	36	16.20 (2.95)[a]	
Twice a month	48	17.55 (2.00)[b]	<0.001
Thrice a month	5	17.70 (2.20)[ab]	
Irregular	3	16.1[ab]	

Median in the same column bearing the same superscript letter(s) is not significantly different (p>0.05)

Part V

Conclusions and recommendations

5.1 Conclusions

The study assessed the nutritional status and associated factors among pediatric patients with transfusion-dependent β-thalassemia major (β-TM) visiting Kanti Children's Hospital in Nepal. The following conclusions can be drawn from the results and discussion presented:

1. Stunting and underweight were prevalent among pediatric patients with β-TM, indicating compromised nutritional status. The prevalence of growth impairment increased with age.

2. Dietary intake analysis revealed inadequate caloric consumption compared to age- and sex-specific requirements. However, protein intake consistently exceeded estimated needs across all age groups.

3. A significant proportion of patients had suboptimal dietary diversity scores, suggesting limited variety in food consumption patterns.

4. The majority of participants avoided iron-rich foods, and the intake of ready-to-eat processed foods was high.

5. Body fat percentage showed notable sex-based differences, with females exhibiting substantially higher values than males. Body fat percentage and mid-upper arm circumference were positively associated with age, chelation therapy status, and serum ferritin levels.

6. Biochemical parameters, particularly serum ferritin levels, indicated a high prevalence of iron overload among participants, despite regular chelation therapy.

7. Socioeconomic factors, such as annual income and family size, influenced the nutritional status of study participants. Larger family size was negatively associated with height-for-age scores.

8. Thalassemia-related factors, including chelation status, total duration of chelation therapy, Deferasirox dose, frequency of blood transfusions, and serum ferritin level,

were associated with growth impairment and altered body composition in the study participants.

9. Caregivers' knowledge regarding the nutritional management of β-TM was limited, with a significant proportion adhering to outdated dietary recommendations, such as strict iron avoidance, which may compromise overall nutritional adequacy.

5.2 Recommendations

Based on the findings of this study, the following recommendations are proposed:

1. Current dietary guidelines for β-TM patients in Nepal may need revision, particularly regarding avoidance of iron-rich foods, in light of recent international recommendations.

2. A multidisciplinary approach should be established to thalassemia management, involving dietitians to address the nutritional needs of these patients.

3. A national registry for thalassemia patients should be established to facilitate larger-scale studies and improve understanding of disease patterns and treatment outcomes across Nepal.

Part VI

Summary

β-thalassemia major (β-TM) is a severe inherited blood disorder requiring regular blood transfusions for survival. In Nepal's resource-limited healthcare setting, β-TM poses significant medical and nutritional challenges, impacting the overall health and quality of life of the patients. This study aimed to assess the nutritional status and associated factors among transfusion-dependent pediatric β-TM patients visiting Kanti Children's Hospital in Nepal. A cross-sectional, hospital-based, descriptive study was conducted on 92 β-TM patients aged 1 to 14 years receiving regular blood transfusions. Data were collected using structured questionnaires, anthropometric assessments (height, weight, mid-upper arm circumference, skinfold thickness), biochemical evaluations (serum ferritin, hemoglobin, vitamin D, calcium), and dietary assessments (24-hour dietary recall, food frequency questionnaire, dietary diversity score). Anthropometric data were analyzed using WHO Anthro and WHO AnthroPlus software, dietary data using food composition tables and dietary intake references, and statistical analyses were performed using SPSS.

The prevalence of stunting and underweight was 27.2% (moderate: 19.6%, severe: 7.6%) and 8.7% (moderate: 6.5%, severe: 2.2%), respectively. Stunting prevalence increased significantly with age ($p<0.05$). Caloric intake was inadequate compared to age- and sex-specific requirements, while protein intake exceeded estimated needs. The mean dietary diversity score was suboptimal (4.230 ± 1.070). Body fat percentage showed significant sex-based differences and associations with age, chelation therapy ($p<0.001$), and total age of chelation ($p<0.05$). Despite regular chelation therapy, 58.7% of participants had seriously elevated serum ferritin levels (>2500 ng/ml). Socioeconomic status, thalassemia-related factors (age at diagnosis, transfusion frequency), and dietary practices were significantly associated with nutritional status ($p<0.05$). Caregiver knowledge regarding nutritional management was limited, with a significant proportion adhering to outdated dietary recommendations.

Pediatric β-TM patients in Nepal have compromised nutritional status, influenced by disease-related factors, dietary inadequacies, and socioeconomic characteristics. The high prevalence of stunting, suboptimal dietary diversity, and iron overload underscore the need for comprehensive nutritional assessment and management as an integral part of β-TM care in this setting.

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Appendixes

Appendix A: Consent form

मञ्जुरीनामा पत्र

नमस्कार! मेरो नाम आरजु पलिखे हो। म केन्द्रिय प्रविधि क्याम्पस, धरानमा पोषण तथा आहार बिज्ञान, आठौं शत्रमा अध्ययनरत विध्यार्थी हुँ। यस संकायको आठौं शत्रको पाठ्यक्रम अन्तर्गत म सोधपत्र गरिरहेको छु। मेरो सोधकार्यको विषय, "**Nutritional Status and Associated Factors in Transfusion-Dependent Beta-Thalassemia Major Pediatric Patients visiting Kanti Children's Hospital**" रहेको छ। यस सर्वेक्षणले बिटा थ्यालेसेमिया मेजर भएका बालबालिकाहरुको पोषण आवश्यकताहरू बुझ्न र व्यवस्थापन गर्ने अन्तरलाई सम्बोधन गर्ने लक्ष्य राखेको छ र यस जानकारी उनीहरुको स्वास्थ्य परिणाम र जीवनको गुणस्तर सुधार गर्न महत्त्वपूर्ण हुनेछ।

त्यसैले यस सर्वेक्षणमा म तपाईंलाई सहभागी हुन अनुरोध गर्दछु जसमा म तपाईंलाई तपाईंको बच्चाको खानपिन बारे प्रश्न सोध्नेछु। म तपाईंको बच्चाको तौल, उचाइ, र छालाको बाक्लोपन पनि लिनेछु। अन्तरवार्ता लगभग १५ मिनेटको लागि हुनेछ। तपाईंलाई सोधिने प्रश्नहरुको सही वा गलत उत्तर भन्ने हुदैँन। तपाईंका जुनसुकै उत्तरहरु यस सर्वेक्षणको लागि महत्वपूर्ण तथा उपयोगी हुनेछन्।

यस अध्ययनमा तपाईको सहभागीता स्वैच्छिक हुनेछ। तपाईंले जस्तोसुकै निणर्य लिएपनि म त्यसलाई स्वीकार्ने छु। यो अध्ययनले तपाईंको बच्चालाई कुनै हानी पुऱ्याउने छैन। यस अन्तर्वार्ता पुरा गर्न तपाईंको समय र सहयोग वाहेक अन्य कुनै पनि शूल्क लाग्नेछैन।

तपाईंले अहिले सहभागी हुने निणर्य लिएपनि पछि आफनो विचार परिवर्तन गर्न सक्नुहुनेछ। यदि तपाईं यो अन्त्रतवार्ता अन्त्य गर्न चाहानुहुन्छ भने कुनै पनि समयमा मलाई भन्नुहोला। यदि तपाईंलाई कुनै प्रश्नको उत्तर दिन मन लागेन भने भन्नु होला म अर्को प्रश्नमा जानेछु। म अनुगमनलागि मात्र तपाईंको नाम र ठेगाना रेकर्ड गर्नेछु। तपाईंले प्रदान गर्नुभएको नाम र ठेगाना सहित सबै जवाफहरू गोप्य रहनेछन्। तपाईंले दिनु भएका सूचनाहरु सर्वेक्षणमा सहभागी अन्य उत्तरदाताहरुले दिनु भएका सूचनाहरुसगैँ विश्लेषण गरिनेछ।

तपाईंलाई यस सर्वेक्षणबाट प्रत्यक्ष लाभ नभएता पनि तपाईंले दिनुभएका जानकारीहरुले बिटा थ्यालेसेमिया मेजर भएका बालबालिकाहरुको पोषण सुधारमा ठूलो योगदान दिनेछ।
यस अध्ययनमा सहभागी हुनका लागि तपाईंको मञ्जुरीनामा आवश्यक छ। कुनै प्रश्नहरु छन् भने कृपया सोध्नुहोला। तपाईं यस अन्तरवार्ताका लागि मञ्जुर हुनुहुन्छ ?

१. छु _____
२. छैन _____

अभिभावकको नाम:	अन्तर्वार्ताकर्ताको नाम:
मिति:	मिति:
सहि:	सहि:

धन्यवाद ।

Appendix B: Assent form

मन्जुरीनामा पत्र

नमस्कार!

मेरो नाम आरजु पालिखे हो, म केहि अध्ययन गर्दैछु जसले तपाइँ जस्तै नियमित रगत चढाउने बिटा थ्यालेसेमिया मेजर भएका बालबालिकाको पोषण अवस्था बुझ्न मद्दत गर्दछ। यस अध्ययनले पोषण आवश्यकताहरू बुझ्न र व्यवस्थापन गर्ने अन्तरलाई सम्बोधन गर्ने लक्ष्य राखेको छ, जुन तपाईं जस्ता बालबालिकाको स्वास्थ्य परिणाम र जीवनको गुणस्तर सुधार गर्न महत्त्वपूर्ण हुनेछ।

म तपाईंलाई यस अध्ययनको जानकारी दिनेछु र तपाईंलाई यस सोधकार्यमा सहभागी बनाउन चाहन्छु। तपाइँ भाग लिन चाहनुहुन्छ वा चाहनुहुन्न भन्ने छनौट गर्न सक्नुहुन्छ। मैले यो अनुसन्धानलाई तपाईंको अभिभावक(हरू)सँग छलफल गरेको छु र उहाँहरूलाई थाहा छ कि मैले तपाईंको सहमतिको लागि पनि सोधिरहेको छु। यदि तपाइँ अध्ययनमा भाग लिन चाहनुहुन्छ भने, तपाइँको अभिभावक(हरू) पनि सहमत हुनुपर्दछ। तर यदि तपाइँ सोधकार्यमा भाग लिन चाहनुहुन्न भने, तपाइँको अभिभावक(हरू) सहमत भएतापनि तपाइँ सहभागी नहुँदा हुनेछ।

"यस सोधकार्यमा सहभागी हनु भन्दा पहिले नै मलाई यस सोधकार्यको बारेमा सम्पूर्ण जानकारी गरिएको छ र म आफ्नो स्वेच्छाले यस अध्ययनमा सहभागी हुँदैछु। म सोधिने प्रश्नहरूको जवाफ दिनेछु र म मेरो शरीरको मापन गर्न अनुमति दिनेछु। मेरो इच्छा नभएमा, म कुनै पनि समयमा यस सोधकार्यका लागि दिएको मन्जुरीनामा फिर्ता लिन पाउनेछु भन्ने बिषयमा पनि म जानकार छु" ।

यस अध्ययनमा सहभागी हुनका लागि तपाईंको मञ्जुरीनामा आवश्यक छ। कुनै प्रश्नहरु छन् भने कृपया सोध्नुहोला। तपाईंले नबुझेका केही शब्दहरू हुन सक्छन् वा तपाईंले चासो राख्नुभएमा म थप व्याख्या गर्नेछु। तपाईं यस अन्तरवार्ताका लागि मञ्जुर हुनुहुन्छ ?

१. छु _____

२. छैन _____

सहभागीको नाम: अन्तर्वार्ताकर्ताको नाम:

मिति: मिति:

सहि: सहि:

धन्यवाद ।

Appendix C: Patient information sheet

बिरामी जानकारी पत्र

नमस्कार! मेरो नाम आरजु पलिखे हो। म केन्द्रिय प्रविधि क्याम्पस, धरानमा पोषण तथा आहार बिज्ञान, आठौं शत्रमा अध्ययनरत विध्यार्थी हुँ। यस संकायको आठौं शत्रको पाठ्यक्रम अन्तर्गत म सोधपत्र गरिरहेको छुँ। मेरो सोधकार्यको विषय, **"Nutritional Status and Associated Factors in Transfusion-Dependent Beta-Thalassemia Major Pediatric Patients visiting Kanti Children's Hospital"** रहेको छ। यस सर्वेक्षणले बिटा थ्यालेसेमिया मेजर भएका बालबालिकाहरुको पोषण आवश्यकताहरू बुझ्न र व्यवस्थापन गर्ने अन्तरलाई सम्बोधन गर्ने लक्ष्य राखेको छ र यस जानकारी उनीहरुको स्वास्थ्य परिणाम र जीवनको गुणस्तर सुधार गर्न महत्त्वपूर्ण हुनेछ।

त्यसैले यस सर्वेक्षणमा म तपाईंलाई सहभागी हुन अनुरोध गर्दछु जसमा म तपाईंलाई तपाईंको बच्चाको खानपिन बारे प्रश्न सोध्नेछुँ। म तपाईंको बच्चाको तौल, उचाइ, र छालाको बाक्लोपन पनि लिनेछुँ। यो सबै प्रकृया स्वास्थ्य मापदण्ड अनुसार हुनेछ।

अन्तरवार्ता लगभग १५ मिनेटको लागि हुनेछ। तपाईंलाई सोधिने प्रश्नहरुको सही वा गलत उत्तर भन्ने हुदैँन । तपाईंका जुनसुकै उत्तरहरु हाम्रा लागि महत्वपूर्ण तथा उपयोगी हुनेछन्।यस अध्ययनमा तपाईको सहभागीता स्वैच्छिक हुनेछ। तपाईंले जस्तोसुकै निणर्य लिएपनि म त्यसलाई स्वीकार्ने छुँ। यो अध्ययनले तपाईंको बच्चालाई कुनै हानी पुऱ्याउने छैन। यस अन्तर्वार्ता पुरा गर्न तपाईंको समय र सहयोग वाहेक अन्य कुनै पनि शूल्क लाग्नेछैन।

तपाईंले अहिले सहभागी हुने निणर्य लिएपनि पछि आफनो विचार परिवर्तन गर्न सक्नुहुनेछ । यदि तपाईं यो अन्त्रतवार्ता अन्त्य गर्न चाहानुहुन्छ भने कुनै पनि समयमा मलाई भन्नुहोला । यदि तपाईंलाई कुनै प्रश्नको उत्तर दिन मन लागेन भने भन्नु होला म अर्को प्रश्नमा जानेछुँ। कुनै पनि प्रश्न भए सोध्नुहोला।

यस अध्ययन बारे थप जानकारी चाहिएमा वा अध्ययनमा मानव अधिकार हननका घटना बारे अपिल गर्नु परेमा अनुसन्धानकर्ताहरु वा यस अस्पतालको स्वास्थ्य अनुसन्धान परिषदमा सम्पर्क गर्न सक्नुहुनेछ ।

मुख्य अनुसन्धानकर्ता: आरजु पलिखे	फोन न: ९८६२३६२१२५
पर्यवेक्ष्यक: देवेन्द्र भट्टराई	फोन न: ९८४२७७१०९७
कान्ति बाल अस्पताल स्वास्थ्य अनुसन्धान परिषद	फोन न: ९७७-१४५११५५०

तपाईंले दिनु भएका सूचनाहरु सर्वेक्षणमा सहभागी अन्य उत्तरदाताहरुले दिनु भएका सूचनाहरुसगैँ विश्लेषण गरिनेछ र बिटा थ्यालेसेमिया मेजर भएका बालबालिकाहरुका स्वास्थ्य तथा पोषण सम्बन्धि सुधार गर्न भविष्यमा पनि यी सूचनाहरु प्रगोग गर्न सकिनेछ ।

धन्यवाद!!!

Appendix D: Dietary guidelines for thalassemia patients at Kanti Children's Hospital

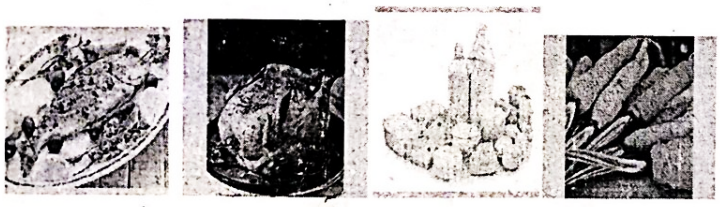
Diet for Thalassemia Patients / थालेसिमिया भएका बिरामीले के खाने के नखाने

- Iron (फलाम) कम भएको खान खाने,
- फलामको भाडामा खाना नापकाउने

फलाम बढी हुने खानाहरु

- रातो मासु - खसी/ बाख्रा/ सुंगूर/ समुन्द्री खाना
- कलेजो/ मुटु जस्ता अंग का मासुहरु
- धेरै हरियो सागपातहरु
- फलफुलहरुमा- खर्भुजा/ खजुर/ किसमिस/ मटरकोसा / तोफु/ ब्रोकौली / सिमि / गेडागुडी
- फलाम मिस्रित खाना वा भिटामिनहरु
- चकलेट

फलाम कम हुने खानाहरु



- माछा/ कुखुराको मासु
- गाजर/ मकै/ जौ/
- चिया/ कफी/ दुध/ दहि/ पनिर/ डेरी को खाना
- प्रतेक दिन एक गिलास दुध पिउने बानी बसालौं

थालेसेमिया युनिट ,कान्ति बाल अस्पताल

Appendix E: Research instruments

Source: seca

seca 876 Weighing Balance

Source: ShorrBoard®

Infant/Child/Adult ShorrBoard® ICA Stadiometer

Source: Holtain

Holtain skinfold caliper

Source: UNICEF

Shakir/ MUAC tape

150 ml standardized glass

5 ml and 15 ml standardized spoons

Appendix F: Ethical approval letter

नेपाल सरकार
स्वास्थ्य तथा जनसंख्या मन्त्रालय
कान्ति वाल अस्पताल
महाराजगञ्ज, काठमाण्डौ
Government of Nepal
Ministry of Health and Population
KANTI CHILDREN'S HOSPITAL
Maharajgunj, Kathmandu
Ethical Review Committee

Inquiry: 01-4511550, 4514798
Office: 01-4513398
Fax: 01-4427449
P.O.Box: 2664
Email: kantikch@gmail.com

Ref. No.: 1398

Date: 23 Feb, 2024

To,

Ms.Arju Palikhey
Principal Investigator
Tribhuvan University
Institute of Science and Technology

Subject: "Nutritional status and associated factors in transfusion dependent beta thalassemia major pediatric patients visiting Kanti Children's Hospital."

Dear Palikhey,

With reference to above mentioned proposal, the protocol and related documents were discussed in IRC on 04 Falgun, 2080 (16 Feb 2024) at 12.00 PM. Following members of the IRC and special invitee attended the meeting:

1. Dr. Bina Prajapati Manandhar	Chairperson
2. Dr. Sanjit Kumar Shrestha	Member
3. Dr. Anupama Thapa Basnet	Member
4. Dr.Sadikshya Shah Malla	Member
5. Mr. Santosh Ghimire	Member
6. Mrs. Puspa Kumari Ghimire	Member
7. Dr.Moni Subedi	Member
8. Dr Madhusudhan Kayastha	Member
9. Dr. Jagat Jeevan Ghimire	Member-Secretary

Advisor Committee

1. Mrs.Sangita Regmi	Acting Head of Nursing Department

Special invitee

1.Mrs.Pushpa Kumati Ghimire	Hospital Nursing Inspector

नेपाल सरकार
स्वास्थ्य तथा जनसंख्या मन्त्रालय
कान्ति वाल अस्पताल
महाराजगञ्ज, काठमाण्डौ
Government of Nepal
Ministry of Health and Population
KANTI CHILDREN'S HOSPITAL
Maharajgunj, Kathmandu
Ethical Review Committee

Inquiry: 01-4511550, 4514798
Office: 01-4513398
Fax: 01-4427449
P.O.Box: 2664
Email: kantikch@gmail.com

Ref.No :1398

The protocol has been ***approved*** from the ethical board with effective from 11Falgun 2080 (23 Feb. 2024).subject to the following condition:

a) The approval is valid for the study period of the conduct of the study according to the protocol .It is the responsibility of principal investigator to ensure it.

b) No significant change in the protocol should be made and implemented without prior permission from IRC. Any planned changes should be approved by IRC.

c) It was voted by all members attending the meeting.

d) Principal investigator responsible to ensure timely monitoring of the study by reviewer as per SOP of IRC.

e) Principal investigator should furnish 6 months study progress report and should complete the completion report by 6 months of completion of study. Completion report should be mailed to IRC Kanti children Hospital.

f) Extended review from IRC is to be done if the study duration exceeds a period of 1 year.

With Best regards

.....
Dr. Jagat Jeevan Ghimire
Member Secretary

Appendix G: Recommended routine clinical and laboratory testing for transfusion-dependent thalassemia patients

GENERAL TIMETABLE FOR CLINICAL AND LABORATORY INVESTIGATION			
ROUTINE TESTS	Clinical Testing	Laboratory Testing	Imaging Testing
Children and Adolescents			
3-Monthly	6-Monthly	12-Monthly	As Clinically Indicated
Height	Growth Velocity	Bone Deformity	
Weight	Tanner Stage (Pubertal Development, from 10 years)	Bone Age (for the first 10 years of life)	
Adults			
3-Monthly	6-Monthly	12-Monthly	As Clinically Indicated
Serum Ferritin	Zinc Levels	Audiology	Holter ECG
Transferrin Saturation	Magnesium Levels	Ophthalmology	Right Cardiac Catheterisation (if Elevated TRV)
Transaminases	Vitamin D Levels	Dental	Liver Biopsy (Optional)
γ-GT, ALP, LDH	Cholesterol / Triglycerides	ECG	Duplex Abdominal Ultrasonography
Bilirubin (t,d)	Urinalysis	Ergospirometry (if clinically indicated)	MRI of Hypothalamic - Pituitary Region
Total Protein		Echocardiography	MRI Pancreas
Albumin		MRI Cardiac T2*	Thyroid Ultrasound
Blood Urea		MRI Liver Iron (LIC)	PQCT
Creatinin		Abdominal Ultrasound (every 6 ms if liver disease)	Lateral Spinal X-Ray for Spinal Fractures
Uric Acid		DEXA Scan	Direct Coomb's Test
Electrolytes		Fibroscan (6ms if liver disease)	C-Reactive Protein
Calcium Ionised		HAV Serology (unless vaccinated)	Serum Folate
Phosphate		HBV Serology/PCR (unless vaccinated)	Creatinine Clearance
Fasting Sugar		HCV Serology/PCR	PT, PTT, Fibrinogen (if Active Disease)
Creatine Kinase		HIV Serology	Alpha Fetoprotein
		OGTT	Troponins
		Thyroid Function (>9yrs)	Nitrouretic Peptides
		Parathormone (>16yrs)	Fructosamine (if Diabetes)
		LH-ICMA (>12-14yrs)	Fasting Plasma Insulin
		FSH (12-14yrs)	GH-Secretion
		Estradiol/Testosterone (12-14yrs)	Osteocalcin
		IGF-1, IGF BP-3	Test for Bone Formation e.g. Bone Specific ALP
		Cortisol + after ACTH Stimulation	Test for Bone Resorption e.g. RANKL

Source: TIF (2021)

Appendix H. Survey questionnaire

Date of interview:	Code no:
Name of the child:	Age:
Date of birth (dd/mm/yyyy):	Sex:

Section A: Socio-demographic information

1. Informant	☐ Mother ☐ Father ☐ Other _____
2. Place of birth	☐ Nepal _____ ☐ Outside of Nepal
3. Current place of residence	
4. Reason for migration	☐ Health service ☐ Employment ☐ Education ☐ Other _____
5. Caste/Ethnicity	☐ Brahmin ☐ Chhetri ☐ Magar ☐ Tharu ☐ Tamang ☐ Newar ☐ Limbu ☐ Yadav ☐ Rai ☐ Other _____
6. Religion	☐ Hindu ☐ Buddhist ☐ Muslim ☐ Kirat ☐ Christian ☐ Other_____
7. Education status	☐ Currently attending ☐ Never attended ☐ Used to attend
7a. Current grade	
7b. Absent from school	_____ days in a month
8. Head of the household	☐ Mother ☐ Father ☐ Other _____
9. Family size	Total number of family members _____
10. Family type	☐ Joint ☐ Nuclear
11. Major source of income	☐ Agriculture ☐ Employment ☐ Business ☐ Government work ☐ Service work ☐ Other _____
12. Father's education	☐ Less than primary ☐ Primary ☐ Secondary ☐ Higher secondary ☐ University ☐ Informal education ☐ Illiterate
13. Mother's education	☐ Less than primary ☐ Primary ☐ Secondary ☐ Higher secondary ☐ University ☐ Informal education ☐ Illiterate
14. Father's occupation	☐ Agriculture ☐ Daily labor ☐ Service work ☐ Government work ☐ Remittance ☐ Business ☐ Other _____
15. Mother's occupation	☐ Agriculture ☐ Daily labor ☐ Service work ☐ Government work ☐ Remittance ☐ Business ☐ Housewife ☐ Other _____
16. Annual family income	☐ Less than 1 lakh ☐ Between 1-3 lakhs ☐ More than 3 lakhs

Section B: Anthropometric and birth information

Height (cm)	Weight (kg)	MUAC (cm)	SFT (mm)
			TSFT: SSFT:

1. Present childbirth order	☐ 1st ☐ 2nd ☐ 3rd ☐ 4th ☐ 5th
1a. If not first, the age difference between the present child and the earlier child	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ Other _____
2. Birth weight of the child	☐ < 2.5 kg ☐ 2.5 kg ☐ > 2.5 kg

Section C: Thalassemia-related information

1. Age of diagnosis (years)	
2. Age of first transfusion (years)	
3. Transfusion frequency (months)	☐ Once ☐ Twice ☐ Thrice ☐ Irregular ☐ Other _____
4. No. of blood transfusions received last year	
5. Age of start of chelation therapy (years)	☐ _____ years ☐ Not started
5a. Name of drug used for chelation therapy	
5b. Dose of drug	
6. Presence of endocrinopathy	☐ Yes ☐ No
6a. If yes, mention	
7. Presence of co-morbidities	☐ Yes ☐ No
7a. If yes, mention	☐ Heart complication ☐ Liver disorder ☐ Other _____
8. Undergone splenectomy	☐ Yes, when _____ ☐ No
9. Family history of thalassemia	☐ Yes ☐ No
9a. If yes, mention the type and relation	
10. Use of oral supplement	☐ No ☐ Yes, mention _____
10a. Gap in the supplement intake	☐ Yes ☐ No
10b. Dosage of supplement	

Section D: Laboratory findings

1. Last pre-transfusion hemoglobin level (g/dl):	3. Serum calcium (mg/dl):
2. Serum ferritin level (ng/ml):	4. Vitamin D (ng/ml):

Section E: Child caring practices

1. Did you breastfeed your child after birth?	☐ Yes ☐ No
1a. If yes, when did you initiate?	☐ Within 1 hr ☐ Within 8 hrs ☐ Within 24 hrs ☐ After 24 hrs
2. Did you exclusively breastfeed for the first six months?	☐ Yes ☐ No
2a. If no, can you share the reason?	☐ Insufficient milk production ☐ Medical issues ☐ Other _____
3. Are you still breastfeeding your child?	☐ Yes ☐ No
3a. If no, when did you stop? (age)	
3b. If yes, frequency of breastfeeding	
4. Did you feed colostrum milk to your child?	☐ Yes ☐ No
5. Have you gotten your child vaccinated according to schedule?	☐ Yes ☐ No
5a. If no, why did you miss it?	
6. Did your child have vitamin A and deworming tablets in the last six months?	☐ Yes ☐ No
7. Have you given Baal vita to your child?	☐ Yes ☐ No ☐ Never heard about it
8. Did your child attend a growth monitoring session in any health facility?	☐ Yes ☐ No ☐ Never heard about it
9. Has the child suffered from any illness in the past 7 days?	☐ No ☐ Yes, mention _____
9a. If yes, did you seek treatment?	☐ No ☐ Yes, mention _____
11. At times of illness, where do you usually take your child for treatment?	☐ Hospital ☐ Health post ☐ Traditional practitioner ☐ Private clinic ☐ Pharmacy ☐ Other _____

Section F: Food behavior and cooking habits

1. Is your child vegetarian?	☐ Yes ☐ No ☐ Other _____
1a. If yes, what is the reason?	☐ Religion ☐ Food preference ☐ Health reasons ☐ Other _____
2. How many times does your child consume food in a day?	_____ main meals _____ snacks
3. On a typical day, does your child skip any meal?	☐ Yes ☐ No
3a. If yes, which meal does he/she skip?	☐ Breakfast ☐ Lunch ☐ Day snacks ☐ Dinner
3b. If yes, how often does he/she skip?	☐ 1-2 times/week ☐ 3-4 times/week ☐ Every day ☐ Occasionally
3c. What is the main reason for skipping?	☐ Food deficient ☐ Time constraints ☐ No time for meal preparation ☐ Other_____
4. What does your child usually consume for school tiffin? (Only for school-going children)	☐ Home meal ☐ School canteen ☐ Packaged ready to eat food ☐ Other _____
5. Does your child have any food allergy?	☐ No ☐ Yes, mention _____
6. How much water does your child drink in a day?	_____ liters
7. Do you use iodized salt for cooking?	☐ Yes ☐ No
8. Which oil do you usually use for cooking?	☐ Vegetable oil ☐ Animal fat ☐ Combined ☐ Other
9. What is the main source of drinking water at your home?	☐ Tap water ☐ Well ☐ Jar water
9a. Is your drinking water purified?	☐ No ☐ Yes, method _____
10. What is the main source of food for your family?	☐ Subsistence farming ☐ Purchase from market ☐ Donation ☐ Other _____

Section G: Caretaker's knowledge

1. Do you know about malnutrition?	☐ Yes ☐ No
1a. If yes, what causes malnutrition?	☐ Low food intake ☐ Supernatural reasons ☐ Illness ☐ Other _____
2. Do you know that β-thalassemia major patients are at risk of being malnourished?	☐ Yes ☐ No
3. Do you know that there is an increased nutritional requirement in β-thalassemia major patients?	☐ Yes ☐ No
4. Do you think there is a special diet for β-thalassemia major?	☐ Yes ☐ No
4a. If yes, mention the diet	
4b. If yes, do you give your child a special diet?	☐ Yes ☐ No
5. Do you think iron-rich foods should be avoided in β-thalassemia major patients?	☐ Yes ☐ No
5b. If yes, mention the reason	
6. Do you know about iron overload?	☐ Yes ☐ No
7. Do you know that iron overload can cause various health complications?	☐ Yes ☐ No
8. Do you know about the Nepal Thalassemia Society?	☐ Yes ☐ No
8a. If yes, how did you find out?	☐ Healthcare team ☐ Relatives ☐ Blood bank ☐ Other _____
9. Have you ever received nutritional counseling for the management of β-thalassemia major patients?	☐ Yes ☐ No
9a. If yes, from whom did you receive it?	☐ Dietitian ☐ Doctor ☐ Nurse ☐ Other _____
10. Have you ever read/heard about the nutritional management of β-thalassemia major patients?	☐ Yes ☐ No
10a. If yes, where did you read/hear?	☐ Book ☐ Pamphlet ☐ social media ☐ Television ☐ Other _____

Section H: 24-hour dietary recall

Time of consumption	Place of consumption	Food/Beverage	Amount	Description	Cooking method

Was this food intake usual for your child? ☐ Y ☐ N If no, how was it unusual?	Was your child ill yesterday? ☐ Y ☐ N If yes, did it affect your child's appetite? ☐ Y ☐ N If yes, did it: ☐ Increase ☐ Decrease
Was yesterday a feast or festival? ☐ Y ☐ N	
Was yesterday a fasting day? ☐ Y ☐ N	**Note**

Section I a: Dietary diversity score (6-23 months)

S.N.	No	Food categories	Description	Consumed	Score
1.	A.	Breastmilk			
2.	B.	Grains	Bread, biscuits, noodles, rice, jaulo, beaten rice, maize, wheat, millet or porridge made from these		
	C.	White roots and tubers	Potatoes, white yams, white sweet potato, cassava or other foods made from roots		
3.	D.	Pulses	Beans, peas, or lentils or any foods made from these		
	E.	Nuts	Peanuts, cashew, walnut, sesame seeds, flaxseeds, or any foods made from these		
4.	F.	Milk	Milk		
	G.	Milk products	Yoghurt, paneer, buttermilk, cheese, or other food made from milk		
	H.	Infant formulas	Lactogen, Similac		
5.	I.	Organ meats	Liver, kidney, heart, gizzard, intestine, blood, or other organ meats		
	J.	Other meats	Chicken, goat, buff, pork, lamb, duck		
	K.	Fish	Big/small fresh or dried fishes		
6.	L.	Eggs	Chicken, duck, quail, etc.		
7.	M.	Vitamin A-rich vegetables, tubers	Pumpkin, carrots, squash, sweet potatoes that are orange inside		
	N.	Vitamin A-rich fruits	Ripe mango, papaya, jackfruit, persimmon		
8.	O.	Other vegetables	Tomato, cucumber, eggplant, cauliflower, radish, long bean, cabbage, mushroom, bottle gourd, bitter gourd, zucchini, etc.		
	P.	Other fruits	Oranges, bananas, coconut flesh, apple, watermelon, pineapple, grapes, guava, etc.		
Total score					

Section I b: Dietary diversity score (Above 2 years)

S.N.	No	Food categories	Description	Consumed	Score
1.	A.	Foods made from grains	Rice, roti, bread, puffed rice, maize, rice flakes, noodles, millet, porridge, wheat, buckwheat, or other foods made from grains		
	B.	White roots, tuber, or other starchy foods	Potatoes, white yams, white sweet potato, cassava or other foods made from roots		
2.	C.	Pulses	Beans, peas, or lentils or any foods made from these		
3.	D.	Nuts and seeds	Peanuts, cashew, walnut, sesame seeds, flaxseeds, or any foods made from these		
4.	E.	Milk	Milk		
	F.	Milk products	Yoghurt, paneer, buttermilk, cheese, or other food made from milk		
5	G.	Organ meat	Liver, kidney, heart, gizzard, intestine, blood, or other organ meats		
	H.	Other meats	Chicken, goat, buff, pork, lamb, duck		
	I.	Fish	Big/small fresh or dried fishes		
6.	J.	Eggs	Chicken, duck, quail, etc.		
7.	K.	Dark green leafy vegetables	Spinach, amaranth leaves, mustard leaves, pumpkin leaves, colocasia leaves, etc.		
8.	M.	Other vitamin A-rich vegetables, tubers	Pumpkin, carrots, squash, sweet potatoes that are orange inside		
	N.	Other vitamin A-rich fruits	Ripe mango, papaya, jackfruit, persimmon		
9.	O.	Other vegetables	Tomato, cucumber, eggplant, cauliflower, radish, long bean, cabbage, mushroom, bottle gourd, bitter gourd, zucchini, etc.		
10.	P.	Other fruits	Oranges, bananas, coconut flesh, apple, watermelon, pineapple, grapes, guava, etc.		
Total score					

Section J: Food frequency questionnaire

Food Item	Daily	3-4 times per week	Once a week	Rarely (once in a month or less)
Rice				
Wheat				
Maize/ Barley/ Millet				
Lentils				
Beans/ Peas/ Pulses				
Soy and soy products				
Green leafy vegetables				
Other vegetables				
Fruits				
Milk				
Yoghurt				
Paneer				
Egg				
Poultry				
Red meat				
Fish				
Nuts and seeds				
Sugar and confectionery				
Tea				
Sugary drinks				
Carbonated drinks				
Processed ready-to-serve foods				
Restaurant/ Fast foods				

Appendix I: Color plates

Plate 1 Measuring height of the subject

Plate 2 Measuring weight of the subject

Plate 3 Measuring triceps skin fold thickness

Plate 4 Measuring subscapular skin fold thickness

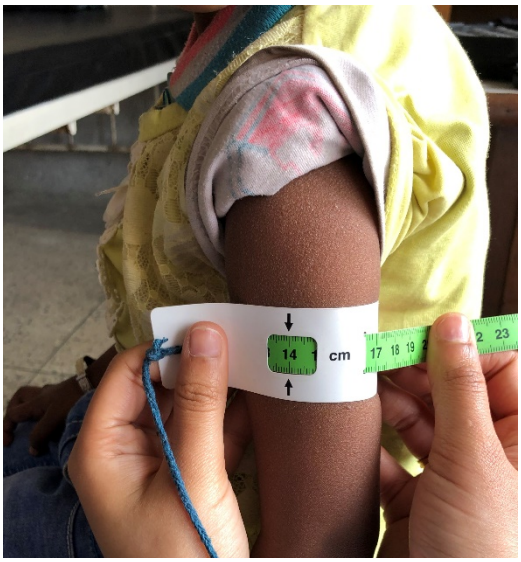
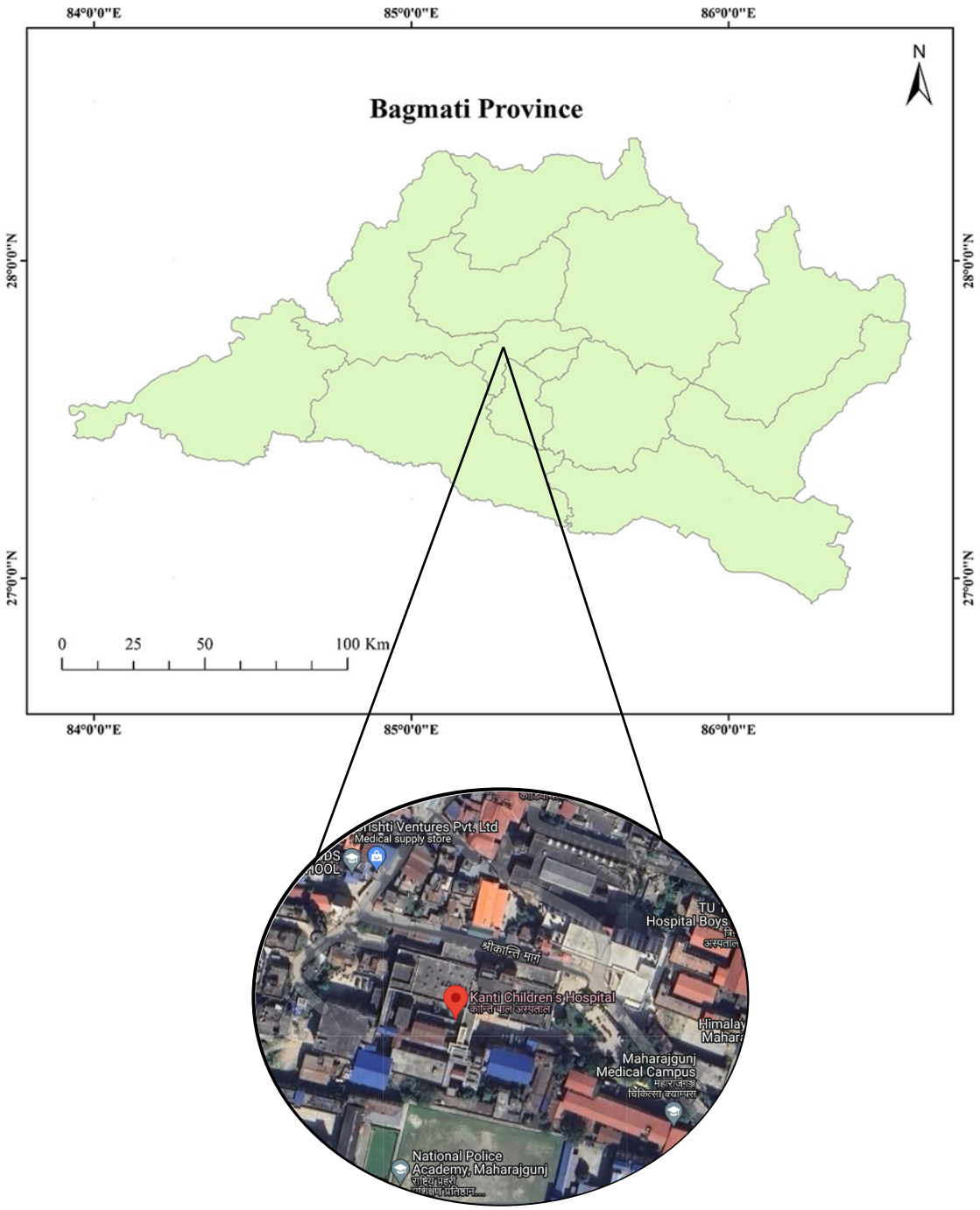


Plate 5 Measuring mid-upper arm circumference

Plate 6 Interview with the subject's guardian

Plate 7 Thalassemia unit of Kanti Children's Hospital

Plate 8 Thalassemia unit of Kanti Children's Hospital



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Source: Belbasesuraj (2020)

Plate 9 Study site: Kanti Children's Hospital, Maharajgunj, Bagmati Province, Nepal (Indicated by red balloon)

Appendix J: Independent sample t-test

Factors	n	Mean ± SD	t	df	p-value
BMI-for-age					
Exclusive breastfeeding					
Yes	30	-0.9057 ± 0. 8621	-2.494	90	0.025
No	62	-0.4985 ± 0. 7768			
Birth weight					
< 2500 g	22	-1.003 ± 0.803	-2.494	90	0.014
≥ 2500 g	70	-0.515 ± 0.800			
Body fat percentage					
Sex					
Female	32	29.476 ± 4.444	9.344	40.317	<0.001
Male	60	21.621 ± 2.330			
Chelation therapy					
Not started	9	20.070 ± 2.050	-5.442	20.463	<0.001
Started	83	24.818 ± 4.942			
School attending					
Yes	81	24.917 ± 4.947	5.549	27.831	<0.001
No	11	20.205 ± 2.146			

Appendix K: Mann-Whitney test

Factors	n	Mean rank	U	Z	p-value
Chelation therapy					
Not started	9	12.39	66.500	-4.036	<0.001
Started	83	50.20			
School attending					
Yes	81	50.15	149.500	-3.563	<0.001
No	11	19.59			

Appendix L: Analysis of variance test

Factors		SS	df	MS	F	p-value
BMI-for-age						
Age group	Between groups	5.596	2	2.798	4.438	0.015
	Within groups	56.119	89	0.631		
	Total	61.716	91			
Fruit intake	Between groups	5.162	2	2.581	4.062	0.021
	Within groups	56.554	89	0.635		
	Total	61.716	91			
Height-for-age						
Age group	Between groups	11.184	2	5.592	6.086	0.003
	Within groups	81.780	89	0.919		
	Total	92.965	91			
Transfusion frequency	Between groups	10.972	3	3.657	3.925	0.011
	Within groups	81.992	88	0.932		
	Total	92.965	91			
Milk intake	Between groups	6.336	2	3.168	3.255	0.043
	Within groups	86.628	89	0.973		
	Total	92.965	91			
Body fat percentage						
Age group	Between groups	673.028	2	336.514	19.372	<0.001
	Within groups	1546.016	89	17.371		
	Total	2219.004	91			
Maize, barley, millet intake	Between groups	198.226	2	99.113	4.365	0.016
	Within groups	2020.818	89	22.706		
	Total	2219.004	91			
Red meat intake	Between groups	207.023	2	103.511	4.579	0.013
	Within groups	2012.021	89	22.607		
	Total	2219.004	91			
Tea intake	Between groups	204.456	2	102.228	4.516	0.014
	Within groups	2014.589	89	22.636		
	Total	2219.044	91			

Appendix M: Post hoc analysis using Tukey test

Comparison		Mean difference	Standard error	P_{tukey}
BMI-for-age				
Age group	**Age group**			
1 to 4 years	5 to 9 years	-0.03695	0.21523	0.984
5 to 9 years	10 to 14 years	0.56398	0.19585	0.014
10 to 14 years	1 to 4 years	-0.52703	0.24168	0.080
Fruit intake	**Fruit intake**			
Regular	Frequent	0.06167	0.42013	0.988
Frequent	Rare	-0.61571	0.28081	0.078
Rare	Regular	-0.67738	0.33787	0.117
Height-for-age				
Age group	**Age group**			
1 to 4 years	5 to 9 years	0.31927	0.25982	0.439
5 to 9 years	10 to 14 years	0.64301	0.23643	0.021
10 to 14 years	1 to 4 years	-0.96227	0.29175	0.004
Transfusion frequency	**Transfusion frequency**			
Once a month	Twice a month	0.72069	0.21282	0.006
Once a month	Thrice a month	0.17478	0.46068	0.981
Twice a month	Thrice a month	-0.54592	0.45361	0.626
Twice a month	Irregular	-0.23458	0.57445	0.977
Thrice a month	Irregular	0.31133	0.70493	0.971
Irregular	Once a month	-0.48611	0.58005	0.836
Milk intake	**Milk intake**			
Regular	Frequent	0.89774	0.58322	0.278
Frequent	Rare	-0.39667	0.60042	0.787
Rare	Regular	-0.50108	0.22748	0.076

Appendix N: Post hoc analysis using Tukey test

Comparison		Mean difference	Standard error	P_{tukey}
Body fat percentage				
Age group	**Age group**			
1 to 4 years	5 to 9 years	-6.68881	1.12967	<0.001
5 to 9 years	10 to 14 years	0.02155	1.02798	1.000
10 to 14 years	1 to 4 years	6.66727	1.26850	<0.001
Maize/Barley	**Maize/Barley**			
Millet intake	**Millet intake**			
Regular	Frequent	-2.81042	2.24627	0.427
Frequent	Rare	4.75728	1.68217	0.016
Rare	Regular	-1.94686	1.68217	0.016
Red meat intake	**Red meat intake**			
Regular	Frequent	-9.70840	3.97805	0.044
Frequent	Rare	6.11911	2.18800	0.017
Rare	Regular	3.58929	3.40139	0.544
Tea intake	**Tea intake**			
Regular	Frequent	3.79016	1.70451	0.073
Frequent	Rare	-1.02659	1.84947	0.844
Rare	Regular	-2.76357	1.13829	0.045

Appendix O: Kruskal-Wallis test of MUAC

Factors		n	Mean rank	H	df	p-value
Age group	1 to 4 years	19	17.87	43.318	2	<0.001
	5 to 9 years	48	45.00			
	10 to 14 years	25	71.14			
Annual income	< 1 lakh	57	40.54	7.507	2	0.023
	1 to 3 lakhs	33	56.44			
	> 3 lakhs	2	52.25			
Transfusion frequency	Once a month	36	31.56	20.282	3	<0.001
	Twice a month	48	56.54			
	Thrice a month	5	62.90			
	Irregular	3	37.83			
Last year transfusion frequency	< 12 times	3	19.17	6.220	2	0.045
	12 to 24 times	84	46.23			
	> 24 times	5	67.40			
Wheat intake	Regular	29	34.55	8.494	2	0.014
	Frequent	24	52.40			
	Rare	39	51.76			

Appendix P: Post hoc test using Bonferroni correction

Comparison		Mean difference	Standard error	p-value
Age group	**Age group**			
1 to 4 years	5 to 9 years	-27.132	7.235	0.001
1 to 4 years	10 to 14 years	-53.272	8.124	<0.001
5 to 9 years	10 to 14 years	-26.140	6.583	<0.001
Annual income	**Annual income**			
< 1 lakh	> 3 lakhs	-11.706	19.202	1.000
< 1 lakh	1 to 3 lakhs	-15.896	5.839	0.019
> 3 lakhs	1 to 3 lakhs	4.189	19.438	1.000
Transfusion frequency	**Transfusion frequency**			
Once a month	Irregular	-6.278	16.040	1.000
Once a month	Twice a month	-24.986	5.885	<0.001
Once a month	Thrice a month	-31.344	12.739	0.083
Irregular	Twice a month	18.708	15.885	1.000
Irregular	Thrice a month	25.067	19.493	1.000
Twice a month	Thrice a month	-6.358	12.543	1.000
Last year transfusion frequency	**Last year transfusion frequency**			
< 12 times	12 to 24 times	-27.065	15.683	0.253
< 12 times	> 24 times	-48.233	19.493	0.040
12 to 24 times	> 24 times	-21.168	12.287	0.255
Wheat intake	**Wheat intake**			
Regular	Rare	-17.205	6.545	0.026
Regular	Frequent	-17.844	7.366	0.046
Rare	Frequent	0.639	6.925	1.000