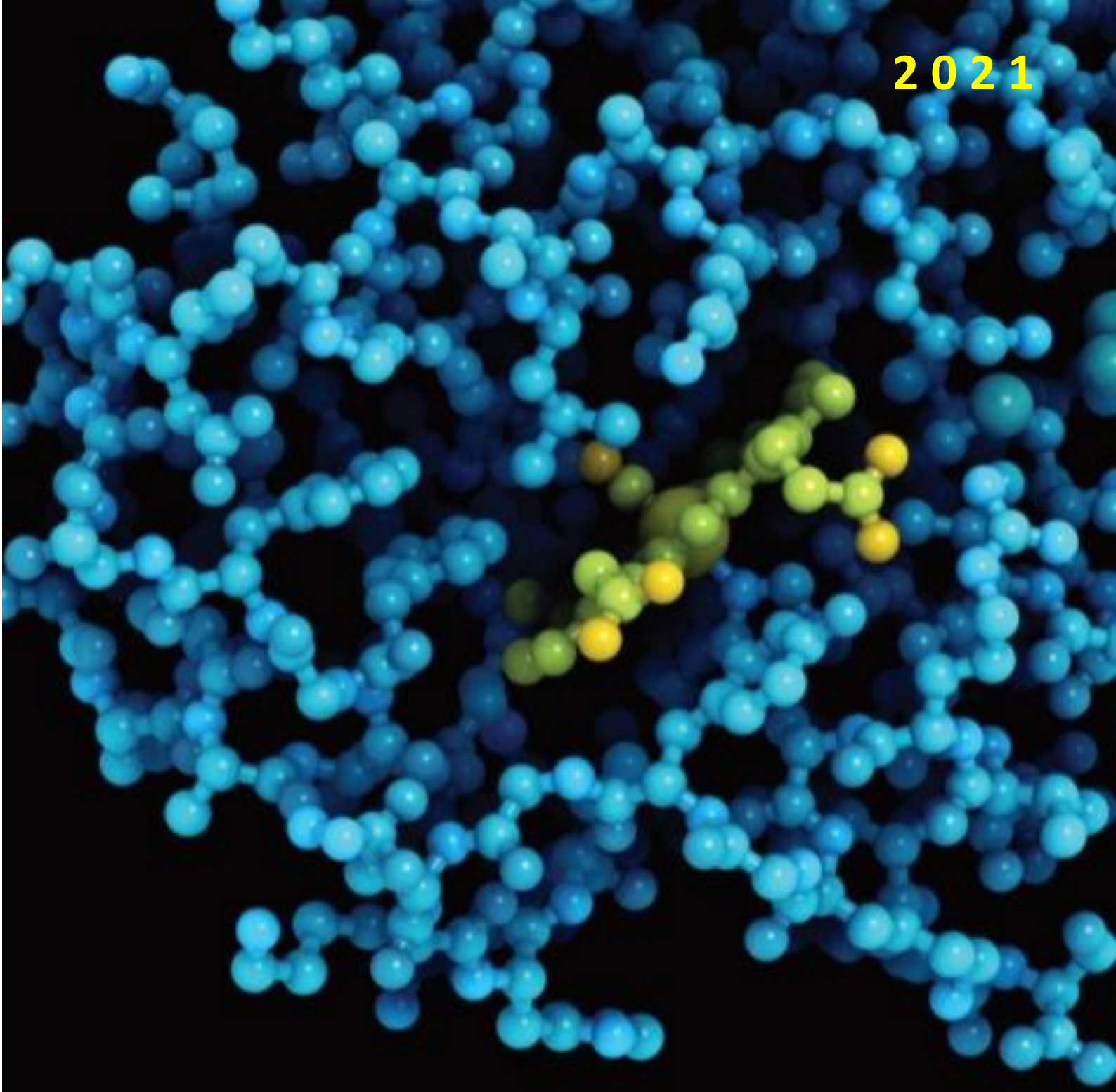

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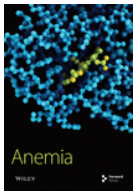

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Research Article

Reticulocyte Hemoglobin Equivalent: Diagnostic Performance in Assessment of Iron Deficiency in Patients with Hypothyroidism

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Introduction. Iron deficiency affects approximately 30% of the world population and is frequently encountered in hypothyroid patients. Early recognition and prompt treatment of iron deficiency in hypothyroid patients lead to a favorable outcome. The aim of this study is to prove the usefulness of reticulocyte hemoglobin equivalent (Ret-He) as a reliable and effective tool in diagnosis of iron deficiency in hypothyroid patients. *Materials and Methods*. 154 patients with hypothyroidism were included in the study. They were divided into 4 groups. Group 1 included 66 hypothyroid patients without iron deficiency. They were taken as controls. Group 2 included 66 hypothyroid patients with iron deficiency anemia (IDA). Group 3 included 12 hypothyroid patients with iron deficiency but without anemia (ID). Group 4 included 10 hypothyroid patients which had concomitant iron deficiency with anemia of chronic disorder (ACDC). Ret-He was measured by analyzing blood samples on System XN 350. Thyroid profile, serum ferritin, and biochemical data were measured by an automated analyzer. Statistical analysis was performed by using SPSS 23. *Results*. Ret-He was significantly lower with ($p < 0.001$) in group 2 (hypothyroid patients with IDA), group 3 (hypothyroid patients with ID), and in group 4 (hypothyroid patients with ACDC) as compared to controls in group 1 (hypothyroid patients without iron deficiency). After ROC analysis area under the curve (AUC) of Ret-He for hypothyroid patients with IDA was 0.96 at cutoff 28.5 pg with sensitivity of 93% and specificity of 90%. AUC of Ret-He in the hypothyroid group with ACDC was 0.99 at cutoff 30.8 pg with sensitivity of 90% and specificity of 90%. AUC of Ret-He in hypothyroid patients with ID was 0.97 at cutoff 31.7 pg with sensitivity of 91% and specificity of 70%. *Conclusion*. Ret-He is a reliable, rapid, and cost-effective tool for diagnosing iron deficiency in hypothyroid patients.

1. Introduction

Anemia is a global health problem affecting about one-third of population worldwide [1]. Iron deficiency anemia is one of the most common causes of anemia. Approximately half of all the cases of anemia throughout the world are due to iron deficiency. It affects all age groups, particularly children and women of childbearing age. It remains as one of the leading causes of morbidity and mortality. Iron deficiency may develop before development of frank anemia, and it may exist in combination with other anemias. Worldwide iron deficiency remains undiagnosed in most of the patients admitted with different illnesses in hospitals, especially before the development of frank iron deficiency anemia [2–5]. Iron deficiency is a state in which body is unable to meet its requirements due to unavailability of iron. It may be in the form of absolute iron deficiency, in which there is insufficient iron in body stores for adequately matching the demand by erythropoiesis in the bone marrow, whereas functional iron deficiency is a state in which the body contains sufficient or more than sufficient iron, but the iron cannot be mobilized from the stores in the reticuloendothelial system to meet the demand for the erythropoiesis [6]. The other important cause of anemia related to iron metabolism is immune activation causing anemia of chronic disorder [7]. Iron deficiency is commonly seen in hypothyroid patients. This can occur due to a variety of reasons. These reasons include decreased iron absorption from gut

because of decreased enzymes and acids, decreased levels of erythropoietin found in hypothyroid patients, and in females with menorrhagia due to hypothyroidism. Many hypothyroid patients are not screened for iron deficiency due to financial constraints of ordering multiple tests and cumbersome ways of diagnosing it particularly in the undeveloped countries. Timely recognition and treatment of iron deficiency in hypothyroid patients can lead to better outcomes with greater and improved response to treatment with thyroxine. Iron deficiency can manifest itself as frank iron deficiency anemia, latent iron deficiency, and iron deficiency in combination with anemia of chronic disorder in hypothyroid patients [8–13]. Diagnosis and investigation of iron deficiency requires many parameters which include serum ferritin, serum iron, total iron binding capacity, serum transferrin, and transferrin saturation [14]. Reticulocytes are immature RBCs that are released from the bone marrow. New parameters have been defined for reticulocyte analysis which measure hemoglobin content of reticulocytes and stages of their development. These parameters include immature reticulocyte fraction (IRF), reticulocyte hemoglobin equivalent (Ret-He), and reticulocyte hemoglobin content (CHr). Ret-He and CHr give information about mean content of hemoglobin in developing reticulocytes and are thus affected earlier in diminished hemoglobin production in iron deficiency and iron deficiency anemia as compared to other parameters. Ret-He can be used as an effective tool to detect iron deficiency in different states and anemias. It can be done on the same sample taken for complete blood count in EDTA anticoagulant. Instead of performing many biochemical tests for detecting iron deficient states, it can be used solely for the identification of iron deficiency. Performing many tests leads not only to increased cost for patients, but some of the tests used for detecting iron deficiency have a limited value in presence of infection and malignancy [15]. Ret-He can be used as a single, rapid, and cost-effective test for determining iron status [16–18]. Many studies have been conducted in the past establishing effectiveness of Ret-He in diagnosing iron deficiency in patients suffering from different disorders. To our knowledge, this is one of the first studies aiming to determine the effectiveness of Ret-He in evaluating iron deficiency with and without anemia in hypothyroid patients. This will lead to timely recognition of iron status in hypothyroid patients resulting in better and effective management and improved outcome in these patients.

2. Materials and Methods

This prospective study was conducted at Nuclear Medicine Oncology and Radiology Institute, Islamabad, over a period of 2 years from February 2019 till November 2020.The study protocol was approved according to Ethics Review Board of Nuclear Medicine Oncology and Radiotherapy Institute, Islamabad, on 29th January 2019 with ethical reference no. NORI-2(10)/88. One hundred and fifty-four adult patients with hypothyroidism (based on clinical diagnosis and laboratory confirmation) presenting to the outpatient department were randomly enrolled in the study. Hypothyroid patients with hemoglobinopathies and nutritional deficiency anemia other than iron deficiency were excluded from the study. As the study was conducted based on thyroid profile, CBC reports, and relevant biochemical investigations of patients with no direct contact with the patient, informed consent was not required. Waiver for informed consent was provided by the Ethical Committee and Review Board of Nuclear Medicine Oncology and Radiotherapy Institute, Islamabad. Hypothyroid patients were divided into four groups. Group 1 included 66 hypothyroid patients without iron deficiency. They were taken as controls. Group 2 included 66 hypothyroid patients with iron deficiency anemia (IDA). Group 3 included 12 hypothyroid patients with iron deficiency but without anemia (ID). Group 4 included 10 hypothyroid patients who had concomitant iron deficiency with anemia of chronic disorder (ACDC). The inclusion criteria for hypothyroidism, IDA (males and females), ID (males and females), ACDC (males and females), and control (males and females) are mentioned.

Hypothyroidism: serum TSH >4.0 mIU/L, T3 <3.1 pmol/L, and/or T4 <12 pmol/L.

IDA (female): Hb <7.45 mmol/L, serum ferritin <13 ug/L, TIBC >46.36 umol/L, serum iron <11 umol/L, and CRP <5 mg/L

IDA (male): Hb <8.7 mmol/L, serum ferritin <30 ug/L, TIBC >46.36 umol/L, serum iron <14 umol/L, and CRP <5 mg/L

ID (female): Hb >7.45 mmol/L, serum ferritin <13 ug/L, serum TIBC >46.36 umol/L, serum iron <11 umol/L, and CRP <5 mg/L

ID (male): Hb >8.07 mmol/L, serum ferritin <30 ug/L, serum TIBC >46.36 umol/L, serum iron <14 umol/L, and CRP <5 mg/L

ACDC (female): Hb <7.45 mmol/L, serum TIBC <46.36 umol/L, serum iron >11 umol/L, serum ferritin >13 ug/L, and CRP >5 mg/L.

ACDC (male): Hb <8.07 mmol/L, serum TIBC <46.36 umol/L, serum iron >11 umol/L, serum ferritin >30 ug/L, and CRP >5 mg/L.

Control (male): Hb> 8.07 mmol/L, serum ferritin >13 ug/L serum TSH >4.2 mIU/L, T3 <3.1 pmol/L, and/or T4 <12 pmol/L, CRP< 5 mg/L.

Control (female): Hb >7.45 nmol/L, serum ferritin >13 ug/L, serum TSH >4.2 mIU/L, T3 <3.1 pmol/L, T4 <12 pmol/L, and CRP <5 mg/L.

Thyroid profile was performed on COBAS E602 by using electrochemiluminescence technology. Biochemical parameters were measured using ROCHE COBAS 60000 by using immunoassay (electrochemiluminescence method). CBC parameters including Hb, MCV, RDWCV, RDWSD, and RDWSD were measured by using Sysmex XN 350, which used the cyanide-free SLS method for measuring hemoglobin and the impedance method with hydrodynamic focusing for measuring RBC parameters. Reticulocyte

hemoglobin equivalent was measured on the same sample by running Sysmex XN 350 on the retic mode by using the fluorescence flow cytometry method. All the data were analyzed by using SPSS Version 23. Descriptive analysis of the data was performed. Age and gender were statistically comparable between 4 groups. The Kolmogorov–Smirnov and Shapiro–Wilk test revealed no significant departure from normality for the variables. ROC curve was used to find area under curve (AUC) for Ret-He in IDA, ID, and ACDC in hypothyroid patients keeping serum ferritin and transferrin saturation levels <20% as gold standard.

3. Results

In group 1 (hypothyroid patients without iron deficiency taken as control), there were 28 males and 38 females with age range of 28–64 years. In group 2 (hypothyroid patients with IDA), there were 22 males and 44 females with age range of 18–61 years. In group 3 (hypothyroid patients with ID), there were 7 males and 5 females with age range of 43–60 years. In group 4 (hypothyroid patients with ACDC), there were 4 males and 6 females with age range of 43–63 years. After ROC analysis area under curve (AUC) of Ret-He for detecting IDA in hypothyroid patients was 0.96 at cutoff 28.5 pg with maximum specificity of 90% and sensitivity of 93%. AUC of Ret-He in hypothyroid patients with ACDC was 0.99 at cutoff 30.8 pg with sensitivity of 90% and specificity of 90%. AUC of Ret-He for diagnosing ID in hypothyroid patients was 0.97 at cutoff 31.7 pg with sensitivity of 91% and specificity of 70%. ROC curves for IDA, ID, and ACDC in hypothyroid patients have been compared as shown in Figure 1.

Hypothyroid patients in IDA, ID, and ACDC groups had significantly lower values of Ret-He than controls ($p < 0.001$). Mean values of Ret-He, Hb, ferritin, TSH, T3, and T4 in hypothyroid patients with IDA, ACDC, ID, and control are given in Table 1.

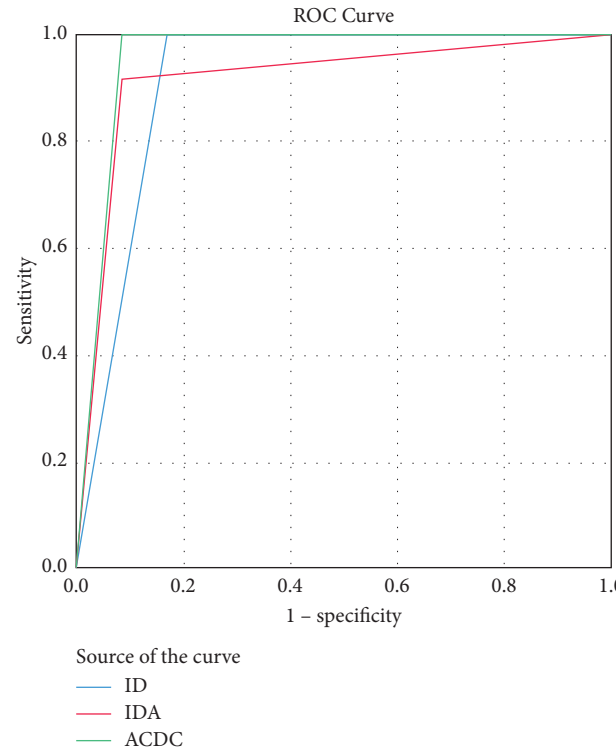

Figure 1: Comparison of ROC curve for IDA, ID, and ACDC in hypothyroid patients.

4. Discussion

The prevalence of thyroid disorders is quite high in Pakistan, but most hypothyroid patients with iron deficiency are misdiagnosed. Ret-He has been employed for the identification of iron deficiency in patients with different diseases, but, to our knowledge, this is one of the first studies which has focused on determining the effectiveness of Ret-He in diagnosing iron deficiency in hypothyroid patients. The study shows that Ret-He can be used as an effective single marker of iron deficiency in hypothyroid patients in different states. It is cost-effective, easily measured, performed on the same sample taken for complete blood count, and can identify iron deficiency in different states [19, 20]. We calculated Ret-He in 66 hypothyroid patients without iron deficiency taken as controls and 66 hypothyroid patients with IDA. Values of Ret-He were significantly lower in hypothyroid patients having IDA as compared to controls. Uçar et al. [21] conducted a study in which the value of Ret-He was significantly lower in iron deficient groups as compared to control. Our study also showed the value of Ret-He was significantly low in groups with iron deficiency in combination with anemia of chronic disorder and latent iron deficiency. A study was conducted by Wirawan et al. [22] on concordance between Ret-He and CHr in diagnosing iron deficiency in chronic kidney disease patients. They found Ret-He at cutoff 29.2 pg had a sensitivity and specificity of 95.5% and 94% in assessing the target of iron supplementation in hemodialyzed patients. A study performed by Gelaw et al. [23] shows significance of Ret-He in diagnosing iron deficiency in IDA and functional iron deficiencies. We found at cutoff 28.5 pg Ret-He had a sensitivity of 93% and specificity of 90% in diagnosing IDA. Research performed by Chinudomwong et al. [24] had comparable results. Their study demonstrated that Ret-He at cutoff 30 pg had 96% sensitivity and 97% specificity in diagnosing IDA. A study performed by Khan et al. [25] revealed Ret-He at cutoff <27.6 pg with sensitivity and specificity of 93.3% and 83.3% in diagnosing iron deficient states. Ret-He holds a special significance in anemia of chronic disease combined with iron deficiency as serum ferritin levels which are widely used in diagnosing iron deficiency that are altered due to chronic inflammation. We evaluated the role of Ret-He in identifying iron deficiency in combination with anemia of chronic disorder. Ret-He at cutoff 30.8 pg had sensitivity of 90% and specificity of 90% for identifying iron deficiency coexisting with anemia of chronic disorder. In cases where iron deficiency was present before developments of frank anemia, Ret-He at cutoff 31.7 pg had 91% sensitivity and 70% specificity. A study conducted by Singh et al. [26] evaluated the role of Ret-He in

Table 1: Biochemical parameters and thyroid profile in hypothyroid patients with IDA, ACDC, ID, and control.

Variables	Hypothyroid patients without iron deficiency and anemia (control) $n = 66$	Hypothyroid patients with iron deficiency and anemia (IDA) $n = 66$	Hypothyroid patients with iron deficiency without anemia (ID) $n = 12$	Hypothyroid patients with anemia of chronic disorder combined with iron deficiency (ACDC) $n = 10$
Ret-He (pg)	33.0 ± 2.1	21.0 ± 1.3	28.2 ± 0.2	25 ± 1.5
Hb (nmol/L)	8.98 ± 0.81	6.19 ± 0.1	9.10 ± 0.75	6.37 ± 0.2
Ferritin (ug/L)	144 ± 23.62	10.1 ± 2.0	123 ± 20.5	260.46 ± 55.75
TSH (mIU/L)	34.71 ± 0.15	36.0 ± 1.8	39 ± 2.1	40 ± 2.8
T3 (pmol/L)	2.97 ± 0.21	1.98 ± 0.13	2.01 ± 0.16	2.89 ± 0.22
T4 (pmol/L)	11.01 ± 0.32	9.41 ± 0.21	9.785 ± 0.23	10.2 ± 0.29

The data are expressed as mean ± S.D.

rheumatologic patients. They found Ret-He of <24 pg in IDA, 24–26.5 pg highly sensitive and specific in iron deficiency combined with anemia of chronic disorder, and >26.5 pg in anemia of the chronic disease group. Toki et al. [18] conducted a study on 211 patients aged 14–91 years of age. They classified patients into four groups: IDA group, ID group, control group, and anemia without ID group. The median Ret-He values were 22.3 (15.1–35.6 pg), 29.7 pg (19.2–34.9 pg), 34 pg (25.9–38 pg), and 32.5 pg (19.1–46.3 pg) in the IDA, ID, control, and anemia without ID. We had mean Ret-He of 21.35 pg in IDA. Dalimunthe and Lubis [27] calculated receiver operating characteristic (ROC) curve for Ret-He in iron deficient patients which revealed area under the curve of 0.818 ($p < 0.0001$), which is slightly lower than our study. Our study showed area under curve of 0.93. Their cutoff for diagnosing iron deficiency anemia was slightly higher than our results. They used 31.65 pg as cutoff Ret-He with 81.5% sensitivity and 61.6% specificity as compared to our cutoff Ret-He at 28.5 pg.

4.1. Limitations of Study. Despite being an important study, which assesses the importance of Ret-He in diagnosing iron deficiency in different states in hypothyroid patients, this study has some limitations. The role of Ret-He in diagnosing iron deficiency in hypothyroid patients with different hemoglobinopathies needs to be investigated as the number of patients having hemoglobinopathies specifically thalassemia is quite high in our country. Additionally, the number of hypothyroid patients in the study having ID and ACDC, the parameters suggesting the efficacy of Ret-He in diagnosing these states in hypothyroid patients, is quite small, and larger studies need to be conducted for further establishing the effectiveness of Ret-He in diagnosing ID and ACDC in hypothyroid patients. A further longitudinal limitation of this study is that it lacks follow up of patients after starting iron therapy.

5. Conclusion

In conclusion, Ret-He is an effective, rapid, inexpensive, and reliable parameter with very high sensitivity and specificity for ruling out concomitant iron deficiency in hypothyroid patients.

Abbreviations

Ret-He: Reticulocyte hemoglobin equivalent
IDA: Iron deficiency anemia
ID: Latent iron deficiency
ACDC: Anemia of chronic disorder combined with iron deficiency.

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available due to hospital policy on maintaining confidentiality and privacy of patient information but are available from the corresponding author upon request.

Ethical Approval

"The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved." The study protocol was approved by the Ethics Committee and Institutional Review Board of Nuclear Medicine Oncology and Radiotherapy Institute Islamabad on 29th January 2019 with ethical reference no. NORI-2(10)/88. Ethical approval by Research Training and Monitoring Cell (RTMC) of Nuclear Medicine, Oncology and Radiotherapy Institute, was granted with vide reference no. RTMC 4/1-150-2021/NORI-ERC-10/1, dated July 2021.

Consent

Informed consent is not applicable as all the data were collected from laboratory investigations performed, with no direct involvement of patients. Waiver for informed consent was provided by Ethics Committee and Review board of Nuclear Medicine Oncology and Radiotherapy Institute, Islamabad.

Disclosure

Conference proceedings: Aslam W, Habib M, Mahmood H, Liaquat A, Aziz S, Fatima S, and Habib M. Reticulocyte Hemoglobin Equivalent: A Study on Comparison and Effectiveness in Assessment of Iron Deficiency: ISLH 2019

Abstracts, International Journal of Laboratory Hematology. Abstract Proceedings of the 32nd Annual Meeting of the International Society for Laboratory Hematology, Vancouver, Canada, 9–11 May 2019. Link for preprint: https://doi.org/10.1111/ijlh.13105. Link for abstract is presented in ISLH 2019. All authors have completed the ICMJE uniform disclosure form.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Wardah Aslam, Maryam Habib conceptualized, designed, and conceived the presented research and contributed towards smooth collection of data, getting IRB approval, and getting institutional support. Wardah Aslam, Maryam Habib, and Saeeda Aziz participated in acquiring data and enrollment of patients in the study. Wardah Aslam, Maryam Habib, and Madiha Habib collected and assembled data. Maryam Habib and Madiha Habib helped in formulation of final data, analytical calculations, and interpretations of results. Wardah Aslam, Maryam Habib, Saeeda Aziz, and Madiha Habib wrote the article. All authors provided critical feedback, helped in manuscript writing, and formulating final version of research.

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Review Article

Orofacial Manifestation and Dental Management of Sickle Cell Disease: A Scoping Review

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Aims. Sickle cell disease (SCD) is an upcoming global health problem with rapid progress in therapy especially since 2017. However, systematic reviews found no clinical trials on the dental treatment of sickle cell disease (SCD). This article aims to outline the oral features of the sickle disease and discuss oral management strategies that can serve as guidelines for dental professionals. *Material and Methods*. A comprehensive literature review was conducted using PubMed, Google Scholar, and Web of Science. The search strategies were developed to cover publications from January 2010 to March 2020. With the help of keywords, multiple abstracts were identified. These abstracts were further reviewed, which included the information about the SCD manifestation, particularly about the oral health features. Based on all these articles and clinical experience, a narrative review was constructed, which summarizes all the aspects of the oral manifestation in people with SCD. *Results*. The results of this study demonstrate that there is distinct evidence available, indicating the developmental enamel defect leading to hypoplasia and increasing susceptibility to dental caries. Another important result of this review found that people with SCD have a vaso-occlusive crisis in the microcirculation in the dental pulp leading to symptomatic and asymptomatic pulpal necrosis without any signs of odontogenic pathology in an apparently healthy tooth. The study also found that early detection, intervention, and prevention are crucial for improving oral health care, and involving a multidisciplinary approach plays an important role in managing people with SCD. *Conclusion*. Patients with sickle cell disease have chronic overall health problems. The hematological disorder becomes their main concern and impaired oral health becomes secondary, increasing the risk for dental caries at the most. This paper broadly describes the oral manifestations of SCD, additionally; this paper also provides recommendations for better dental management of patients with SCD. Patients with SCD are often misjudged and, due to lack of knowledge and guidelines, dental providers are not able to provide adequate care. This paper attempts to highlight the essential measures to provide better dental care.

1. Introduction

Sickle cell disease (SCD) is a group of disorders that cause red blood cells to become misshapen and break down prematurely. In SCD, there is an abnormality of the hemoglobin that carries oxygen to cells throughout the body [1]. The abnormal hemoglobin, known as hemoglobin S, has a lower functional capacity and causes multiple systemic complications. Hemoglobin S distorts the shape of the red blood cell into a sickle or crescent shape, giving the disease its name [1].

SCD is one of the most common inherited blood disorders in the United States. According to the Centers for Disease Control and Prevention (CDC), it is estimated that over 100,000 Americans have SCD [2]. Though the exact number of people living with SCD is unknown, it has been estimated that 1 of every 365 African American babies is born with SCD while 1 of every 13 has the sickle cell trait [2]. The U.S. incidence estimate for sickle cell trait (based on information provided by 13 states) was 73.1 cases per 1,000 black newborns, 3.1 cases per 1,000 white newborns, and 2.2

cases per 1,000 Asian or Pacific Islander newborns. The incidence estimate for Hispanic ethnicity (within 13 states) was 6.9 cases per 1,000 Hispanic newborns [2]. Combinedly almost 90% of the world's SCD population collectively lives in three countries, that is, Nigeria, India, and the Democratic Republic of Congo [3, 4]. According to the World Health Organization (WHO), it is estimated that approximately 5% of the entire world population caries trait genes for the hemoglobin disorders, out of which mainly, sickle cell disease and thalassemia are more prevalent [5]. It has been approximated that the number of children born with sickle cell disease is expected to grow by nearly 30% from 2010 to 2050 [6]. The great number of the newborn with the SCD occurs in lower- and middle-income countries and due to lack of the early diagnosis and treatment, most of the affected die in the first few years of life, with reported excess mortality reaching up to 92% [7].

The structure of normal adult hemoglobin (Hb-A) molecules contains four polypeptide chains, two alpha units and two beta units [8]. Each chain includes one heme group, which acts as a binding site for the oxygen molecule. Both chains have distinct sequences of amino acids, which fold up to form different three-dimensional structures. The four chains are bound together by noncovalent interactions.

In SCD, a point mutation changes glutamic acid to valine in the hemoglobin beta (β) chain. This type of abnormal hemoglobin is known as hemoglobin S (Hb-S), and it causes red blood cells to become stiff and abnormally shaped. Instead of having its normal, round disk shape, the red blood cell is distorted into crescent or sickle shape.

In SCD, the life span of red blood cells is severely diminished from the usual 90–120 days to about 10 days [8]. Due to atypical hemoglobin and their sickle shape, red blood cells break down prematurely in the spleen, causing fewer overall red blood cells and leading to sickle cell anemia and hyperbilirubinemia. Since hemoglobin in the RBC is the main molecule that delivers oxygen to all the cells throughout the body, sickle cell anemia results in multiple symptoms of oxygen deficit, including fatigue, irritability, dizziness, lightheadedness, tachycardia, and shortness of breath. Furthermore, the rapid breakdown of RBC, hemolytic anemia, may also cause yellowing of the eyes and skin, known as jaundice. Oral health consequences of hemolytic anemia are generalized paleness of the oral mucosa and pain due to vaso-occlusive crisis within the microcirculation of the dental pulp.

2. Materials and Methods

2.1. Selection Criteria. In this study, literature related to sickle cell disease and oral symptoms was reviewed. The literature describing SCD, oral manifestation, and dental management includes controlled clinical studies, retrospective studies, and experimental studies and review.

2.2. Search Strategy. The descriptive search including PubMed and Medline (1946–present), CINAHL, Cochrane Central Register of Controlled Trials, Embase, Web of Science, Google Scholar, the US National Institutes of Health Trials Registry, WHO Library, IndMED, LILACS, and African Index Medicus until October 2020, with no language filter. Additional dental organization websites were searched, including the American Dental Association, to identify articles and statistics that examined an association between the sickle cell disease and oral manifestations. Details of the search strategy are provided in Table 1.

2.3. Search Terms. The search terms are as follows: Sickle cell disease, Oral Health, and Dental Symptoms.

2.3.1. Inclusion Criteria. The literature included in this study was based on the following inclusion criteria:

(1) Studies discussing sickle cell disease and dental/oral symptoms
(2) Studies reporting sickle cell disease and oral manifestations
(3) Studies on sickle cell disease and dental management

2.3.2. Exclusion Criteria. The literature eligible for inclusion in this study was based on the following exclusion criteria:

(1) Literature discussing anemia
(2) Literature considering medical management
(3) Literature on language other than English

2.4. Data Collection and Analysis

2.4.1. Selection of Studies and Data Extraction. The articles were evaluated for their relevance based on the titles and abstracts. Further validity of the articles was done by obtaining the full text of the possible relevant studies that met the inclusion criteria. All the articles were reviewed by the reviewers. The studies assessed by MK and deemed eligible were checked by SA and AP for methodological quality and inclusion criteria. All disagreements were resolved verbally, with strict adherence to the predetermined inclusion criteria (refer to Figure 1).

3. Results

A narrative review was constructed reporting items with the question focused on "What the different oral manifestation observed in the patients with the SCD and how these patients are can better be managed." *Population (P):* patients with SCD; *intervention (I):* common dental procedures at a clinical setting; *control (C):* no treatment, healthy controls; and *outcome measure (O):* dental management approaches. The aim of this article is to outline the oral features of SCD and discuss oral management strategies that can serve as guidelines for dental professionals.

Table 1: Database search strategies.

Database	Keywords	Results
Google Scholar	"Sickle cell" ("Oral Health" OR "dental")	23,000
Embase	Population—Sickle cell Intervention—Oral Health Comparison—none Outcome—none	35
PubMed	"sickle cell anemia"/mj AND "health"/mj "anemia, sickle cell"[MeSH Terms] AND ("oral health" [MeSH Terms] OR "dental health services" [MeSH Terms])	69
PubMed	"anemia, sickle cell"[MeSH Terms] AND ("oral manifestations" [MeSH Terms]	13

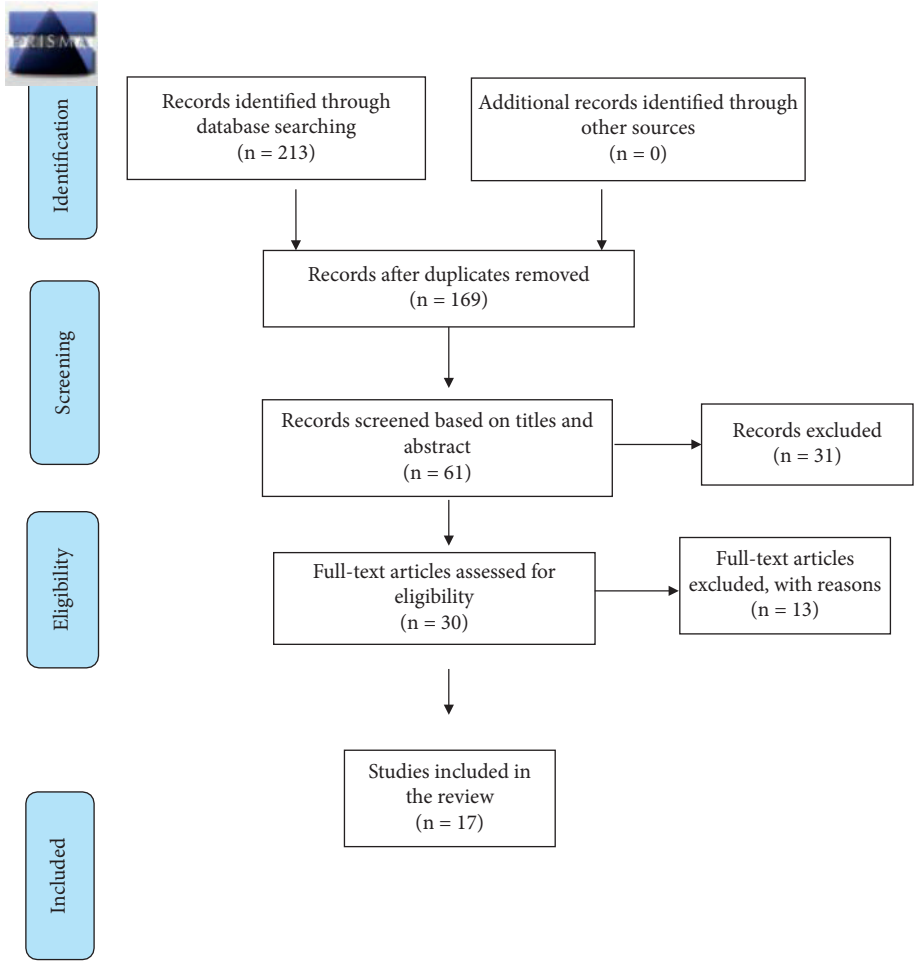

Figure 1: PRISMA 2009 flow diagram.

3.1. Summary of the Intraoral and Dental Manifestations

3.1.1. Oral Mucosa and Tongue. The most common intraoral manifestation of SCD is mucosal pallor and jaundice. This is caused by premature breakdown of RBCs in the spleen and the low number of available RBCs in the blood vessels leading to hemolytic anemia and hyperbilirubinemia [9, 10]. Due to the low blood oxygen, the color of the skin turns pale. This also can be observed in the intraoral buccal and labial mucosa, as well as the gingiva [9].

3.1.2. Enamel and Dentin. There have been conflicting research results about the effect of SCD on teeth. A microradiography study of the dental tissues in SCD patients revealed diffused hypomineralized zones in tooth enamel. The study also found unusual inclusions in the lumens of the dentinal tubules and pulp chambers were found to contain denticle-like calcified bodies [11]. Many studies have reported enamel hypoplasia, dentin hypoplasia, and delayed tooth eruption in SCD patients [12]. There has been no distinct, evidence-based research demonstrating an

association between SCD and greater risk of caries. However, there are several studies indicating that developmental enamel defects such as hypoplasia are postulated to have increased susceptibility to dental caries [12–14]. Defective enamel sites (hypoplasia or hypocalcification) may provide a suitable local environment for adhesion and colonization of cariogenic bacteria, and bacteria may be retained at the base of defects in contact with exposed dentin, enabling dental caries to develop more rapidly [14, 15]. Some studies have reported that patients with SCD are less susceptible to early childhood caries. Fakuda et al. concluded that that long-term use of penicillin prophylaxis in SCD patients may prevent the acquisition of Mutans Streptococci, resulting in significantly lower caries rates in this population. This benefit occurs only during active administration of the drug, however, and only delays the acquisition of Mutans Streptococci [16].

3.1.3. Dental Pulp. The primary reason people with SCD visit dental providers is extreme pain and sensitivity. One way to explain this pain is due to caries approaching the pulp, resulting in inflammation of the pulp, a condition known as pulpitis. Another effect of SCD on the dental pulp is vaso-occlusive crisis, when obstruction of the microcirculation in the pulp produces symptomatic and asymptomatic pulpal necrosis without any signs of odontogenic pathology in an apparently healthy tooth [17–19].

Furthermore, arbitrated blood supply may cause blood clots within the blood vessel, commonly known as blood thrombosis, which can result in calcified pulp stones in the pulp chamber [10, 11].

3.1.4. Mental Nerve Neuropathy: "Numb Chin Syndrome". Vasculo-occlusion (VOC) in the maxillofacial region can also occur in the narrow canals of major nerves supplying the maxilla and the mandible causing loss of sensation and neuropathy. Because they pass through the narrow foramina and boney canals, the mental nerve and inferior alveolar nerve are the two major nerves that are vulnerable to VOCs. This infarction of the blood supply to the nerves can cause loss of sensation and persistent anesthesia to the lower lip and chin, which can last up to 24 months.

The first to describe mental nerve neuropathy as a result of SCD was Konotey-Ahulu in 1980. He found that 4% of patients had moderate-to-severe pain in the mandible during a sickle cell crisis, with many developing burning sensations and numbness in the lower lip along the path of the mental nerve. That recovery of sensation could take months [19]. Another case report was of a 40-year-old black man who described that his right mandibular first premolar, canine, and incisors "felt like wooden blocks." A needle prick test was performed, and it was determined that the patient had profound anesthesia in the regions supplied by the mental nerve. A radiograph of the right mental nerve showed a 2×1 cm ovoid radiolucency, which was deciphered to be decreased trabeculation or an acute bony infarct of the mandible as a result of his sickle cell crisis. This lesion in the mandible was similar to other lesions found in the patient's pelvis and both right and left femoral heads. The pain eventually disappeared, although there was still numbness in the lower lip. The patient was followed for 12 months, with no changes in radiographic findings of radiolucency and loss of sensation in the lower lip [10, 20].

3.1.5. Alveolar Bone and Radiographic Manifestation. Radiographic features in SCD have multiple causes. First and foremost is bone marrow hypertrophy and erythroblastic hyperplasia due to increased numbers of sickle cells and their premature destruction, causing low numbers of RBCs. Consequences of this are changes in the trabecular pattern of the bone, including loss of fine trabeculae and formation of large bone marrow spaces [9, 10, 12]. Thus, dental radiographs may appear to have distended medullary spaces and diminished trabeculation. There may be thinning of the cortical plate, and the inferior border of mandible may appear irregular and dissipated on the radiographic [10].

Another important feature of the SCD patient is developmental enamel hypomineralization and hypoplasia, which can affect enamel translucency and may be seen radiographically.

Maxillary sinus opacification may be observed in patients with SCD due to bone marrow hyperplasia of the maxillary sinus [10, 21–23].

There are many challenges when working with patients with SCD. We have reviewed these challenges in more detail in Table 2.

3.2. General Recommendations for Oral Health Management in Dental Practice

3.2.1. Early Intervention. Patients with SCD are often seen in the emergency department due to severe pain. Likewise, they frequently present to dental clinics for emergency appointments rather than preventive care. Thus, by the time they come to a dental office, their oral health is quite deteriorated. Preventive dental therapy is ideal for sickle cell disease patient. The goal of the pediatric dentist is to improve and maintain excellent oral health in order to decrease the possibility of various oral infections [24]. Treatment should never be initiated during a crisis unless it is inevitable as in emergency situations.

Hence, it is important to establish routine dental [1] visits and comprehensive care from the beginning. It is crucial that patients are educated about good oral hygiene and encouraged to have periodic oral health screening and prophy at least every 6 months [25]. Individuals with SCD should be encouraged by their medical providers to seek regular dental care.

It is critical to maintain a multidisciplinary and collaborative approach to health care management, including the primary care physician, hematologist, and dentist to ensure that the patient is receiving a well-planned comprehensive treatment [24]. This is essential to ensure the patient is comfortable with their healthcare team so their condition can be managed before it worsens into sickle cell crisis.

Table 2: Challenges in oral management for individuals with sickle cell disease.

Oral health care	(i) Patients with SCD have chronic overall health problems; their hematological disorder becomes their main priority and oral health becomes secondary, increasing their risk for dental caries. (ii) Patients with SCD are often seen only for emergency appointments when they have severe mouth pain; thus, most of their dental disease is diagnosed during this visit. (iii) Lack of regular dental visits and comprehensive care deteriorates their condition significantly, which further demoralizes and demotivates the patient to see a dentist for regular preventive care.	(i) Preventive dental therapy is the best approach for SCD patients (Rada et al., 1987). (ii) Excellent oral health can reduce the possibility of oral diseases. (iii) Incorporate home fluoride treatment (Rouse and Hays, 1979) (iv) Incorporate routine dental check-ups (Rouse and Hays, 1979).
Complaint of pain "without any cause"	(i) Patients with SCD often presented clinically with facial and dental pain without an obvious etiology. This makes it difficult for the provider to properly diagnose the reason for that pain [1, 2]. (ii) Patients are often perceived by health care practitioners as "drug seekers," which results in delayed effective pain relief often resulting in under treatment that can prolong suffering and result in repeat emergency visits.	(i) Dentist should perform thorough medical history. (ii) Pale mucosa, delayed eruption of teeth, hypoplasia of teeth, and radiographic changes are common oral signs in SCD patients (Cox and Soni, 1984). (iii) Consult a physician before treating the SCD patient. (iv) Use acetaminophen for pain as salicylates causes acidosis. (v) Regular use of narcotics to alleviate pain should be avoided to prevent drug addiction.
Severe anxiety for dental procedures	SCD patients have severe anxiety towards the oral care. This is mainly due to the severe pain that is experienced on the facial region including maxillary and mandibular bone. Due to this unresolved pain, patients restrict their visit dentist since they are very nervous and uneasy with the overall dental experience.	(i) Oral sedation helped to decrease preoperative anxiety level (Malamed, 1985). (ii) Cullen (1982) proposed chloral hydrate or Valium as a premedication for anxiety. (iii) Dental appointment can be scheduled during morning time for a short visit (Primley et al., 1982).
Infections	People with SCD have an increased risk of developing certain infections including pneumonia, blood stream infections, meningitis, and bone infections. Early in life, sickled cells can clog blood vessels in the spleen, leading to damage and increased susceptibility to infection.	(i) Antibiotic therapy is recommended for infections and all efforts should be incorporated to prevent acidosis and dehydration (DeBaun and Galadanci) [3].

Education and spreading awareness of the importance of daily oral health care, as well as encouragement of patients to maintain regular dental check-ups and dental cleanings, is essential. Thus, conducting oral health promotions and screening programs for individuals with SCD is of utmost importance [24].

3.2.2. Strategies to Manage Dental Anxiety. The physical, emotional, and social disabilities from life-long medical and dental issues reinforces dental anxiety over painful procedures, such as tooth extractions, and contributes to avoidance of dental visits [26, 27]. Dental anxiety can be multifactorial and proper evaluation is crucial to identify root causes [26, 28]. Recognizing the etiology and severity of anxiety can help the dental provider better formulate a plan to ensure increased compliance with recommendations.

Evaluation of anxiety can be performed during the initial appointment. Providers can ask patients about their feelings regarding procedures, anesthesia, and sounds along with past dental experience. This can help to inform providers of the patient's level of anxiety prior to dental procedures, so measures can be taken to alleviate their anxiety and make the visit as comfortable as possible [24, 29].

It is important to communicate with the patient regarding the optimal time for their appointment. In general, short morning appointments are recommended.

Pharmacological pain management methods are advised for the mildly anxious patient, which can be achieved by the use of anxiolytics and sedatives, such as midazolam or diazepam [24, 25]. Use of nitrous oxide gas, alone or in combination with a sedative, is also found to be an effective approach for management of dental anxiety in mild-to-moderate cases [30].

In the case of the highly anxious patient requiring extensive multiple dental or surgical procedures, general anesthesia is the most recommended approach [24].

Nonpharmacological pain management strategies include the use of relaxation strategies such as imagery, deep breathing, and distraction. In addition, finding ways to improve the comfort of the environment (e.g., playing music) is another way to help patients feel more relaxed and thus reduce their pain [30].

3.2.3. Restorative Management. Many factors contribute to caries prevalence in SCD, including salivary buffering capacity, salivary flow, improper oral hygiene, systemic conditions, socioeconomic status, and medications [31]. It is important that more proactive measures and a strategic approach are taken to prevent caries and disease spread.

(a) Caries diagnosis: early detection of caries is the key to prevention. Thus, regular visits to a dentist, at least every 6 months, are recommended for early detection and prevention of dental caries [32].

(b) Oral hygiene: patient and community education to increase awareness of appropriate oral care is of utmost importance. This includes an emphasis on removing dental plaque daily by brushing twice a day, daily flossing, and use of oral rinses. It is important that the correct brushing techniques are explained and demonstrated for the most effective and efficient results [33].

(c) Protective methods: regular use of fluoride containing products such as toothpaste, oral mouthwash, fluoride varnish, and calcium phosphate agents can help prevent caries and reverse the oral microflora environment. Pit and fissure sealants, antibacterial, and antimicrobial are other important protective agents [24].

(d) Diet consultation: high sugar dietary content is a common and well-known etiology of dental caries. Informing patients about the relationship between diet and oral health and helping them reduce sugar content is a helpful way to prevent dental caries [34]. Use of sugar substitute products such as xylitol, which has anticariogenic properties, should be introduced. Consultation with a dietician can help patients understand their individual nutritional needs to determine the appropriate amount and frequency of sugar intake [35]. In addition to the amount of sugar intake, the frequency with which teeth are exposed to sugary substances contributes to poor oral health and reducing such frequency is crucial in preventing dental caries.

(e) Proactive dental caries treatment: in addition to early diagnosis of dental caries, early intervention is imperative to maintain good oral health. Dental caries can progress aggressively; thus, direct and indirect restorative procedures should be completed in a timely manner to prevent further deterioration of the dentition [34].

3.2.4. Dental Implants. Dental implants are a widely acceptable procedure to replace single or multiple missing teeth. Dental implants are not contraindicated in sickle cell patients; however, it is very crucial to understand this disorder and its clinical physiology to avoid any complications. Due to various clinical manifestations of sickle cell disease, such as osteonecrosis of bone where the blood supply to the jaw is compromised due to clotting in the blood vessels, can cause failure of dental implants [36]. Nevertheless, with meticulous understanding of the nature of the disease, severity of the condition and previous response to procedures can help to plan successful surgery with minimal postoperative complications [37]. Additionally, complete blood count (CBC) and radiographs should be done as a part of the treatment plan. Depending on the CBC results, the patient may need a blood transfusion before or after the surgery to reduce the sickle cell concentration in the blood. In the case where patient may need a blood transfusion, implant surgery should be carried out in the hospital-based setting, with collaborative participation of hematologist and primary care physician [29]. The application of the immediate installation technique has the advantage of achieving satisfactory results with a high success rate. The use of this technique reduces the number of surgical interventions and shortens the time between tooth extraction and permanent installation of the prosthesis, eventually avoiding the process of bone resorption, thereby leading to the preservation of alveolar ridge in terms of proportion, size, and width [38].

3.2.5. Orthodontic Management. Apart from the other orodental manifestations, certain cephalometric changes are characteristic in SCD patients [39]. Orthodontic treatments for the sickle cell disease patient are strictly elective as these patients may have malocclusions or skeletal abnormalities, so their correction can improvise the child's self-esteem.

Some of the common malocclusion features in SCD, including incisal crowding, overjet, open bite, and posterior open bite, are distinctive [40]. Additionally, inclination towards a class II molar relationship, delayed tooth eruption, and increased crowding in the lower anterior region is prominent in children with SCD [41]. It is highly suggested that patients with SCD get orthodontic treatment at the earliest appropriate opportunity to avert problems associated with malocclusions and avoid other complications later in life. Timely orthodontic treatment can help improve quality of life [36]. Orthodontic treatment basically moves teeth through remodeled bone or changes growth patterns by repositioning the lower jaw. However, the disease process of sickle cell disease may compromise the outcome of the planned treatment (van Venrooy and Proffit, 1985) and therefore, treatment ought to be monitored closely, especially during a crisis. Also, orthodontic appliances should be designed with great caution to prevent irritation of soft tissues.

3.2.6. Infection Management. Patients with SCD are at higher risk than the general population of infection, including dental infection [24]. There are several factors contributing to this. Contributing risk factors for various dental and periodontal infections include daily smoking, older age, and lack of daily dental flossing. One of the best ways to prevent dental infection is the early detection and elimination of periodontal and dental sources of infection. Dental infection can impact systematic health through various pathways [42]. Dental infection may also trigger or aggravate sickle cell crises. Thus, all oral infections must be treated aggressively at the local and systematic levels. They

must be treated with suitable antimicrobial agents such as antibiotics and rinses [43]. In the case of the severe infection, hospitalization is recommended for administration of intravenous antibiotics, fluids, pain control, and monitoring. Prophylactic antibiotic is recommended for invasive and extensive surgical procedures to prevent systemic infections, vaso-occlusive crisis, and osteomyelitis [12].

However, during sickle cell crisis only acute infections or trauma should be treated, delaying elective procedures until the crisis is resolved.

4. Conclusion

This paper broadly describes the oral manifestations of SCD and provides recommendations for better management and understanding of the underlying etiology of such complications. Patients with SCD are often misjudged and, due to lack of knowledge and guidelines, dental providers are not able to provide adequate care. This paper attempts to highlight the essential measures to provide better dental care. It is important that a collaborative approach is adopted with the help of hematologist, dentist, and primary care physician. Early detection, intervention, and prevention are important for improving oral health care in patients with SCD. More research is encouraged to provide evidence for which treatment modalities are most effective.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Correlations between Iron Load and CD4 in Adult Transfusion-Dependent Beta Thalassemia

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Background. Thalassemia is a hereditary disease, and severe anemia is the main phenotype of major thalassemia. Furthermore, the most important method in the management of this disease is red blood cell transfusion. Regular transfusions administered 1 or 2 times every month improve prognosis and survival. However, there is higher risk of infections and iron overload, especially in transfusion-dependent thalassemia (TDT). Infections are the second leading cause of death in adult TDT, after heart failure. Higher risk of infection is also influenced by multiple blood transfusions which causes alteration in immune response due to alloimmunization, transfusion-related infections, and iron overload. Meanwhile, iron overload in TDT alters both innate and specific immune responses. Furthermore, previous studies have shown the correlation between ferritin with CD4, but this has not been carried out in Indonesia. Therefore, this study aims to determine the correlations between iron overload (serum ferritin and transferrin saturation) and specific immune cells (CD4). *Methods*. This is a cross-sectional study, and a total number of 64 subjects were examined consecutively. Chest X-ray and blood sera were obtained. The total number of subjects was 64. The seromarkers HBsAg, anti-HCV, and anti-HIV were tested using the ELISA method. Serum ferritin and transferrin saturation was tested using ECLIA, and lymphocyte subsets were analyzed using flowcytometry. Meanwhile, the correlation between variables was determined using Spearman's test. *Results*. The results showed that 4.9% subjects were HBsAg positive, 10.7% were anti-HCV positive, and none were anti-HIV positive. There were 4 subjects with lung tuberculosis based on the 41 chest X-ray. Meanwhile, there was a weak negative and insignificant correlation between serum ferritin with CD4 ($p = 0.75$; $r = -0.04$) and a weak positive and insignificant correlation between transferrin saturation with CD4 ($p = 0.133$; $r = 0.19$). *Conclusion*. There were no correlations between iron overload (ferritin) and cellular immunity (CD4) in adult transfusion-dependent thalassemia.

1. Background

Thalassemia is a hereditary disorder on formation of alpha and beta globin chains in erythrocytes. This leads to structural abnormalities of the erythrocytes which causes anemia. Furthermore, anemia in thalassemia patients ranges from mild or moderate (thalassemia minor and intermedia) to severe (thalassemia major). Estimation has shown that there are 7% of thalassemia patients in the world population while the disease spreads mainly in the tropic and subtropic countries.[1]. In Indonesia, especially in the Cipto Mangunkusumo National Referral Hospital (RSCM) in Jakarta, there are approximately 1570 thalassemia patients undergoing regular blood transfusions.[2].

Patients with thalassemia major who suffer from anemia would require regular red blood cell transfusions to maintain growth and development. Moreover, thalassemia is a hereditary disorder; hence, patients need regular transfusions throughout lifetime. Adult thalassemia patients are classified as transfusion-dependent thalassemia (TDT), which

requires regular transfusions of red blood cells once or twice every month and non-transfusion-dependent thalassemia (NTDT).[1].

Hypertransfusion extends the life expectancy and improve the life quality of patients, especially in TDT. However, it increases the risk of infection (transfusion-related infection) and also leads to iron overload. Meanwhile, both infection and iron overload are considered as risk factors for severe infections in patients with thalassemia.

Infection is the second leading cause of death after heart failure in TDT patients in Cipto Mangunkusumo Hospital (RSCM) [2]. Assadov, et al. [3] stated that patients with thalassemia possess a decrease in the specific or nonspecific immune response. Furthermore, a decrease in the number of cluster of differentiation 4 (CD4) T lymphocytes would be observed at the cellular immunity level. This is supposedly caused by multiple transfusions due to a charge of excess iron. A decreased immune response in patients with thalassemia is one of the risk factors for infection in thalassemia. In a previous study on beta thalassemia major, Kadam et al. [4] stated that the CD4 subset was lower compared to normal population. Moreover, the primary haemochromatosis study in Porto [5] showed a positive correlation between iron load and CD4/CD8 ratio, and a negative correlation between iron load and CD8. However, the mechanism of decreasing immune response in thalassemia is still ambiguous. Excessive iron load is one of the main factors which play a role through the oxidative stress pathway [5].

2. Methods

This is a cross-sectional study conducted in the Kiara Outpatient Thalassemia Unit, RSCM, in October 2016. Samples were collected consecutively from adult patients with thalassemia who went to the clinic and received blood transfusions every month. The exclusion criteria were patient refusal or if the patient had undergone splenectomy. This study was approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia, with clearance number 837/UN2.F1/ETIK/2016. Appropriate consent was obtained from all subjects.

The medical history and physical and laboratory examination of peripheral blood from the subject venous blood were taken at the Laboratory of Clinical Pathology, RSCM. This includes a complete peripheral blood test, hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (anti-HCV), anti-human immunodeficiency virus (anti-HIV), serum ferritin, transferrin saturation, high-sensitivity C Reactive Protein (hsCRP), and CD4 and CD8 cells. A complete blood count was performed using Sysmex® XN-1000 from Sysmex™ Corporation, and assay for CRP was carried out using Roche™ Cobas® c311. Furthermore, Seromarkers HBsAg, anti-HCV, and anti-HIV tests were performed by the ELISA method using Abbott™ Architect® i1000/i2000 devices. The serum ferritin and transferrin saturation tests were also performed by the ECLIA method using Architect® C4000/8000 by Abbott™, and flowcytometry was conducted using FACS Calibur® by Becton Dickinson™ company.

The chest x-ray examination was performed in the RSCM radiology department. Furthermore, data were recorded on the study worksheet and processed into a scatter diagram while the correlation was tested using Spearman correlation.

3. Results

Out of the 78 transfusion-dependent beta thalassemia adult patients recruited, 14 were excluded because 12 patients had undergone splenectomy and 2 refused to participate. Therefore, 64 subjects agreed to participate in the blood test. However, only 41 subjects underwent chest x-ray examinations.

There were 33 female (52%) and 31 male subjects (48%) with ages ranging from 18 to 37 years, with a median of 22 years of age (Table 1). In this study, there were 27 subjects (42.2%) with β-thalassemia major and 37 subjects (57.8%) with hemoglobin E (HbE) thalassemia.

Chronic infectious diseases documented include tuberculosis (TBC), HIV, and chronic hepatitis. At chest X-ray examination, there were 4 subjects (9.7%) out of 41 screened subjects with pulmonary tuberculosis while one subject received anti-tuberculosis drug therapy. The patients with hepatitis disease consisted of 8 people (12.5%) out of 64 patients. Moreover, 1 patient had hepatitis B (1.6%) positive, 5 had hepatitis C (7.8%), and 2 were with hepatitis B and C (3.1%). There were no results of patients with a history of human immunodeficiency virus (HIV) disease. The data for serum ferritin and transferrin saturation are shown in Table 2.

3.1. Correlation between Serum Ferritin with CD4 Count in Adult Transfusion-Dependent Beta Thalassemia Patients. Spearman's correlation analysis was conducted using the results of the r value = −0.04 with a significance level of 0.75 (Figure 1, Table 3)

In Table 2, a correlation (r) value of −0.04, with a significance value of 0.75 ($p > 0.05$), was obtained. Therefore, there is no correlation between serum ferritin with CD4.

3.2. Correlation between Transferrin Saturation with CD4 Count in Adult TDT Patients. Analysis of Spearman's correlation analysis was performed using the correlation r value of 0.19 with a significance level of 0.133 (Figure 2, Table 4).

Based on Table 3, r correlation values of 0.19 showed a weak positive correlation. The p value of 0.133 ($p > 0.05$) showed that there was no significant correlation between transferrin saturation and CD4.

4. Discussion

The results showed a weak positive, and there was no significant correlation between serum ferritin with CD4. Similarly, iron load markers showed transferrin saturation with CD4, and the results showed an insignificant and a weak negative correlation. The correlation between excess iron load with CD4/CD8 ratio as established by Porto et al. [5] who

Table 1: Baseline characteristics of study subjects.

Variables	Gender		$N = 64$
	Male ($n = 31$ [48.4%])	Female ($n = 33$ [51.6%])	
Age (years)			
Median (range)	22 (18, 37)	21 (19, 33)	22 (18, 37)
Type of thalassemia			
Thalassemia β major	14 (45.2)	13 (39.4)	27 (42.2)
Thalassemia β HbE	17 (54.8)	20 (60.6)	37 (57.8)
Chronic infectious diseases			
HIV	0	0	0
Hepatitis B	0	1 (3.0)	1 (1.6)
Hepatitis C	5 (16.1)	0	5 (7.8)
Hepatitis B and C	0	2 (6.1)	2 (3.1)
TBC ($N = 41$)	2 (6.5)	2 (6.1)	4 (9.8)
First transfusion			
Less than 6 years	24 (77.4)	26 (78.8)	50 (78.1)
More than 6 years	7 (22.6)	7 (21.2)	14 (21.9)
Number of transfusions			
More than 1 time per month	21 (67.7)	24 (72.7)	45 (70.3)
One month	10 (32.3)	9 (27.3)	19 (29.7)
Iron-chelating drug			
Mono	26 (83.9)	30 (90.9)	56 (87.5)
Combination	5 (16.1)	3 (9.1)	8 (12.5)
Regularity of taking medicine			
Not a routine	24 (77.4)	26 (78.8)	50 (78.1)
Routine	7 (22.6)	7 (21.2)	14 (21.9)
Enlargement of the spleen (splenomegaly)			
Normal	4 (12.9)	3 (9.1)	7 (10.9)
Splenomegaly	27 (87.1)	30 (90.9)	57 (89.1)
Facies Cooley			
No	16 (51.6)	18 (54.5)	34 (53.1)
Yes	15 (48.4)	15 (45.5)	30 (46.9)
Hemoglobin (g/dL)* The mean (SB)	8.2 (1.3)	7.8 (1.1)	8.01 (1.2)
Leukocyte (10^3/uL) Median (range)	4.9 (2.3, 9.5)	4.7 (1.7, 11.6)	4.9 (1.7, 11.6)
Neutrophils Median (range)	2820 (920, 5460)	2770 (730, 7160)	2795 (730, 7160)
Platelets (10^3/uL) Median (range)	135 (41, 331)	130 (52, 366)	131 (41, 366)
Hs CRP (mg/L) Median (range)	2.0 (0.4, 9.5)	1.6 (0.6, 8.9)	1.8 (0.4, 9.5)

Table 2: Iron status.

Variables	Median (interquartile range)
Ferritin (ng/mL)	4,595.00 (3,233.25)
Transferrin saturation (%)	91 (16)

stated that, in a hereditary haemochromatosis population, excess iron load is not due to transfusion. However, the difference in results is due to variation in the population which is the charge of excess iron due to hypertransfusion in subjects. Meanwhile, more factors contributed to iron overload and immune deficiency in our patients compared to haemochromatosis patients in Porto et al.'s [5] study.

A previous study with similar aims by Amrita et al. [6] recruited 36 TDT patients and showed correlation between ferritin serum and CD4 counts [6], but this study had different results. This is due to larger sample size (64 vs. 36) and greater genetic diversity in subjects. In addition, the study by Amrita et al. was conducted at a regional referral center, while this study was conducted in a national referral center. Therefore, this study had a greater genetic diversity due to subjects referred from nationwide thalassemia centers.

The results of this study are in accordance with a previous study by Arseno et al. [7] in pediatric TDT patients which had no correlation between ferritin levels and CD4 cell count [7]. Meanwhile, Arseno et al. stated that the absolute number of CD4 count has no significant role in TDT. However, immune dysfunction in children with TDT is caused by defect in the T-lymphocyte function rather than by the decrease of absolute T-cell count [7].

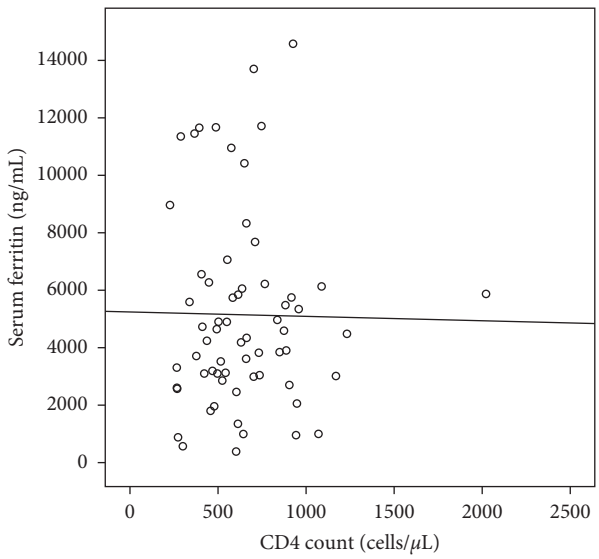

Figure 1: Scatter diagram correlation between serum ferritin with CD4.

Table 3: Correlation between serum ferritin with CD4 count in adult TDT patients.

Variables	*r*	*p (Spearman's)*
Serum ferritin with CD4	−0.04	0.75

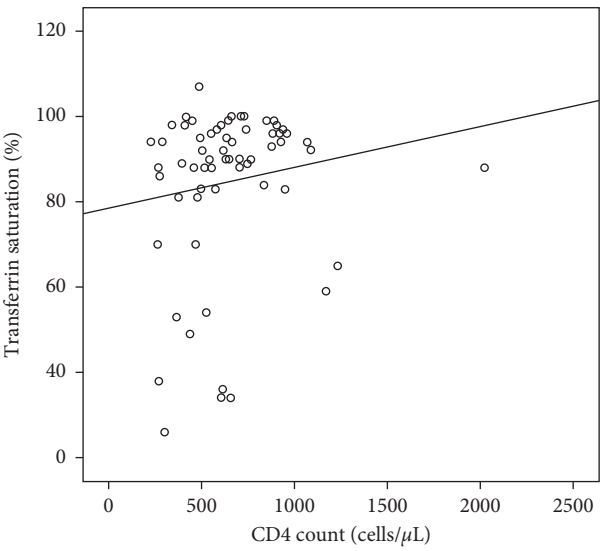

Figure 2: Scatter diagram of the correlation between transferrin saturation with CD4.

Meanwhile, the correlation between iron load and cellular immunity was studied by Hagag et al. [8] which describes a significant correlation between serum ferritin with CD4 and CD8. The study population consisted of non-splenectomized children with serum ferritin >1000 ng/mL and was compared to a normal population. However, other studies show differences in the humoral immune response where quantitative measurements are not included. Serum ferritin and transferrin saturation were used as the iron load marker in this study. Ferritin is an intracellular-extracellular iron-binding protein which refers to the iron-containing molecules while the term apoferritin is used for the iron-free molecules [9]. Transferrin saturation is obtained from the calculation of serum iron to total iron-binding capacity ratio, and the results showed that both had insignificant data. Although there were some differences, it is not yet concluded that the saturation transferrin provides better monitoring on hematopoiesis. Furthermore, the charge of excess iron is best monitored by measuring redox-active iron such as non-transferrin-bound iron (NTBI) and labile plasma iron (LPI) in plasma and labile iron pools (LIP) in the cellular cytoplasm.

Table 4: Correlation between transferrin saturation with a CD4 cell count in adult patients with transfusion-dependent beta thalassemia.

Variables	*r*	*p (Spearman's)*
Transferrin saturation with CD4 count	0.19	0.133

Ferritin is an acute phase reactant; therefore, acute or chronic infection interfered with the results. However, in this study, the subjects had normal median hsCRP levels. Therefore, infection can be excluded, and the increase in serum ferritin is considered to be solely caused by iron overload.

The factors that caused insignificant correlation in our study were high levels of serum ferritin, iron chelation, and vitamin *E* treatments. Although average ferritin was high in subjects, the effects of iron chelation occurred in a short period of time. Meanwhile, previous studies stated that administration of iron chelation poses an effect on CD8 and directly reduces inflammation [10, 11]. Oxidants decrease immune response; therefore, administration of vitamin *E* has a role in improving cellular immune response [12]. The subject examined had both vitamin *E* and iron chelation as routine drug treatment for thalassemia.

One of the factors to be considered is the large percentage of patients with splenomegaly (89.1%). The spleen plays an important role in the host defense against invading pathogens [13]. A previous study by Chu et al. [14] in cirrhotic patients with splenomegaly showed that there is an impairment of cellular immune function and T-lymphocytes subset while the number of CD3+, CD4+, and CD8+ increases significantly [14]. In addition, a previous study in thalassemia patients showed that splenectomized patients had higher absolute lymphocyte subset count [15].

The relationship between iron chelation agents and CD4 count involves a complex mechanism. Iron is important for the survival of organisms including pathogens [16]. However, iron overload hinders optimal immune response. Adequate iron chelation is essential to defend the host against a pathogenic microorganism [17]. All chelating drugs when used appropriately are sufficient to prevent bacterial growth. Meanwhile, Kontoghiorghes [17] stated that deferiprone has the highest long-term antimicrobial effect. In addition, a previous study by Del Vecchio et al. [18] showed that deferiprone normalizes the CD4/CD8 ratio

after treatment. Hence, this study showed that the iron chelating regiment varied and large percentage of patients were unable to achieve optimal chelation target due to budget restriction in medications. Further studies are required to determine the role of specific iron chelating therapy in modulating the host immune response. Based on the results, complex interplaying factors changed the immunological response of patients with TDT.

The limitations of this study are the cross-sectional study design and the conditions of patients were different from one another which affected the results. Furthermore, variations in spleen size and iron chelating regiment also influenced the results.

5. Conclusions

There was no significant correlation between serum ferritin and transferrin saturation with CD4 in adults with transfusion-dependent beta thalassemia.

Data Availability

Additional data can be requested by contacting the corresponding author through the e-mail address provided.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Human Acquired Aplastic Anemia Patients' Bone-Marrow-Derived Mesenchymal Stem Cells Are Not Influenced by Hematopoietic Compartment and Maintain Stemness and Immune Properties

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Background and Objective. Acquired aplastic anemia (aAA) is a bone marrow failure disorder characterized by pancytopenia and bone marrow aplasia. Bone marrow Mesenchymal Stem Cells (BM-MSCs) are an important component of BM microenvironment, associated with hematopoietic and immune homeostasis. Any alterations in BM microenvironment can disrupt the normal functioning and it needs to be assessed. *Methods*. In the current study, we investigated the morphological differences, proliferation capacity, population doubling time (PDT), surface marker profiling, trilineage differentiation potential, and immunosuppressive ability of BM Mesenchymal Stem Cells (BM-MSCs) from untreated aAA patients and in the same number of age- and gender-matched controls. *Results*. We observed similar morphology, proliferation capacity, phenotype, trilineage differentiation potential, and immunomodulatory properties of BM-MSCs in aAA patients and control subjects. *Conclusion*. Our results confirm that the basic and immunosuppressive properties of BM-MSCs from aAA patients do not differ from normal BM-MSCs. Our data suggest that BM-MSCs from aAA patients might not be involved in disease pathogenesis. However, owing to a smaller number of samples, it is not conclusive, and future studies with more exhaustive investigation at transcriptome level are warranted.

1. Introduction

The characteristic features of acquired aplastic anemia (aAA) are peripheral blood (PB) pancytopenia and bone marrow (BM) hypocellularity. It is believed that autoreactive T cells destroy hematopoiesis in aAA [1]. Apart from this, there are qualitative and quantitative defects of stem cells in BM of aAA patients [2]. BM stroma consists of different cell populations of hematopoietic and nonhematopoietic stem cells [3]. The nonhematopoietic progenitor cells are called bone marrow mesenchymal stem cells (BM-MSCs) [4]. MSCs are self-renewing and can differentiate into multilineages such as adipocytes, chondrocytes, and osteocytes [5]. MSCs affect hematopoietic stem cells and immune cells including T cells by cytokine secretion and direct cell-to-cell interaction [6–8].

The underlying pathogenetic mechanism of acquired aplastic anemia involves inefficient hematopoiesis and abnormal immune responses. Hence, alterations in bone-marrow-derived mesenchymal stem cells could primarily or secondarily lead to acquired aplastic anemia. Features of altered BM microenvironment have been described in acquired aplastic anemia [5, 9]. Previous studies on BM-MSCs in aAA have shown equivocal outcomes, such as abnormal morphology [10, 11], lower population doubling time, and poor proliferation and differentiation capacity of aAA BM-

MSCs [10, 12–17], whereas others have found no differences in aAA BM-MSCs as compared to normal BM-MSCs [18–20]. Thus, the exact picture of BM microenvironment and its role in the disease need investigation.

To understand the difference between the characteristics of acquired aplastic anemia BM-MSCs and normal healthy BM-MSCs, the following study was designed. In this study, we have evaluated the morphology, proliferation capacity, population doubling time, surface marker profiling, and differentiation potential of BM-MSCs from aAA patients compared to normal BM-MSCs. We have also evaluated the immunomodulatory potential of these MSCs as it is one of the important mechanisms by which MSCs show their repairable and regenerative potential.

2. Materials and Methods

Five untreated aAA patients diagnosed as per the standard international criteria (Camitta et al.) [21] were enrolled from the Department of Hematology, All India Institute of Medical Sciences (AIIMS), New Delhi, India. The patients were stratified into nonsevere, severe, and very severe aAA. Signed informed consent was taken from all the study subjects. 1 ml of bone marrow sample was collected from all the patients undergoing the routine medical test procedure. This study was approved by the Institutional Ethics Committee of AIIMS, New Delhi, India (Ref: IEC/T-353/30/08/13).

2.1. Isolation and Expansion of Bone Marrow Mesenchymal Stem Cells. MSCs were isolated and cultured as described previously [22, 23]. Unmanipulated bone marrow was seeded in 60 mm culture dish (BD, USA) in complete growth media containing 1X Dulbecco's Modified Eagle Medium-Low Glucose (DMEM-LG) (Life Technologies, USA) media with 10% Fetal Bovine Serum (FBS) (HyClone, USA), 1% Penicillin (100U/ml) + Streptomycin (100 μg/ml) (Life Technologies, USA). The cells were incubated in a humidified atmosphere at 37°C with 5% CO_2. Medium was changed every 3 days until the cell confluency reached 80%. Adherent cells were then passaged with 0.25% trypsin-EDTA (Invitrogen, Gibco) and reseeded at 1×10^4 cell/cm^2.

2.2. Trilineage Differentiation. Mesenchymal Stem Cells are multipotent cells which can be differentiated into osteocytes, adipocytes, and chondrocytes. Therefore, the healthy and aAA MSCs were characterized for their trilineage differentiation potential as per the previous established protocols of our laboratory [24].

2.3. Population Doubling Time (PDT). MSCs for each sample ($N = 3$) were seeded at a density of 50×10^3 cells per 35 mm petri dish (Becton Dickinson, USA). After 72 hrs, MSCs were enumerated and assessed for viability using Trypan Blue dye exclusion (Life Technologies, USA) assay. The PDT was obtained by the following formula [24]:

$$\mathrm{PDT} = T - T_{\mathrm{o}}\,\mathrm{Log}\,2\,(\mathrm{Log}\,N - \mathrm{Log}N_o), \quad (1)$$

where T is the time of harvesting, T_{o} is the time of seeding, N is the number of cells harvested, and N_{o} is the number of cells seeded.

2.4. Measurement of Metabolic Activity by MTT Assay. Proliferation rate of hMSCs ($N = 3$) was performed at days 1, 3, 5, 7, and 14 and measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, USA) assay. The technique was performed as per the previous established protocol [12].

2.5. Immunophenotyping. At passage 3, cells were characterized using monoclonal antibodies specific for CD105-APC, CD73-PE, CD29-FITC, CD90-PerCp-Cy5.5, HLA-ABC-APC, HLA-DR-FITC, and CD34/45-PE/FITC (BD Pharmingen, France). Acquisition and data analysis were performed using flow cytometry BD-LSR-II (BD Biosciences, France) and FACS Diva Software Version 6.2.

2.6. Immunofluorescence. Immunofluorescence studies were performed for the detection of intracellular Vimentin protein in the MSC samples. Trypsinised cells were seeded on coverslips kept in a 35 mm dish (BD Biosciences, USA), and MSC expansion media were added and incubated at 37°C, 5% CO_2. Once confluency reached within 40–50%, the existing media from the dish were removed and cells were washed with PBS. Cells were fixed with 1 ml of 4% paraformaldehyde for 20 mins at 4°C. Permeabilization and blocking was done with 0.05% Tween-20 followed by 2% BSA in PBS for 30 mins at room temperature. MSCs were incubated with the Mouse monoclonal vimentin (Abcam, Cambridge, USA) primary antibody (1/100) for overnight at 4°C. Next day, after washing with PBS, cells were incubated with the secondary fluorescent antibody (1/500) for 40 mins at room temperature (RT). To visualise nuclei, slides were stained with diluted 1/4000 DAPI (Life Technologies, USA) for 3 mins at RT followed by thorough washing of the cells with PBS. The acquisition and imaging of the cells were performed using Floid Microscopy (Life Technologies).

2.7. Immunosuppressive Ability of MSCs: One-Way Mixed Lymphocyte Reaction. The study was approved by the Institutional Ethics Committee (IEC) (Ref no. IECPG-345/07.09.2017 (RT-6/29.11.2017)). All the samples were obtained after taking the donor's informed consent. Human Peripheral Blood Mononuclear Cells (PBMCs) were isolated by Ficoll-Paque (Axis-Shield; Oslo, Norway) density gradient centrifugation from blood donated by healthy volunteers. Phytohemagglutinin A (PHA (Sigma, USA); 35 μg/mL) was used to stimulate the activation of human peripheral blood mononuclear cells (PBMCs) before coculture [25]. For coculture experiments, hMSCs were treated with Mitomycin C (Sigma, USA); 15 μg/ml and cocultured (1×10^4 cells/well) with PHA activated hPBMCs (5×10^4 cells/well) in 1 : 5 ratio in RPMI-1640 medium

(Gibco, USA) containing 10% FBS for 3 days in 96-well plates (Costar, USA). Proliferation of hPBMCs was assessed by MTS assay. The 200 μl cell culture supernatant containing hPBMCs was collected in prelabelled 0.6 ml Eppendorf tube, and 20 μl of MTS reagent (Promega, USA) was added in each tube, followed by incubation for 3 h at 37°C and 5% CO_2. Afterwards, the tubes were centrifuged at 300g for 5 mins. 200 μl of supernatant was collected from each tube and transferred into the fresh 96-well plate. The absorbance was taken at 490 nm using ELISA reader (BioTek, Germany). Lastly, % decrease was calculated by calculating the difference between positive control (activated PBMCs) and test group (MLR). Then, we divided the decrease by the positive control and multiplied the answer by 100.

3. Statistical Analysis

Data analysis was performed using Prism 5 (GraphPad) and Excel (Microsoft). A statistically significant difference among groups was determined by t-test or one-way analysis of variance (ANOVA). The cutoff value of significance (P value) was 0.05. Results were expressed as mean ± SD.

4. Results

4.1. Morphological Analysis and Immunophenotypic Profile of aAA-MSCs and Control-MSCs. A total of 10 subjects, five aAA donors and five controls, were enrolled in the study. Clinical data of these donors are outlined in Table 1. The average age was 20.4 years. All BM aspirates were obtained at the time of diagnosis before therapy was started.

MSCs of aAA and control group shared a similar spindle-shaped morphology *in vitro* (Figures 1(a) and 1(b)). Both revealed a consistent immunophenotypic profile which was negative for CD34/CD45. HLA-DR was positive for CD105, CD90, CD29, CD73, and HLA-ABC (Figures 2(a) and 2(b)). No significant difference was noted in the expression of any single surface marker between the two study groups.

4.2. Growth Kinetics of aAA-BM-MSCs and Normal-BM-MSCs. To determine the growth kinetics of aAA-MSCs and normal MSCs, proliferation was assessed from day 1 to day 14 by MTT assay (Figure 3(a)). We used cells from passages 3 to 5 in order to avoid hematopoietic stem cell contamination. This also prevents adulteration of senescent or differentiating MSCs in later passages [19]. aAA group showed similar expansion rate of proliferation as compared to control group. To further confirm the growth kinetics, another set of cells was passaged to assess the population doubling time (PDT), which was 31 ± 1.5 hrs for aAA MSC and 30 ± 2.10 hrs for control group MSCs (Figure 3(b)).

4.3. Differentiation Potential of aAA-MSCs and Normal-MSCs. MSCs from patients and normal donors were exposed to differentiation media (Figure 4). In osteogenic conditions, aAA-MSCs could not differentiate into osteocytes as robustly as control MSCs. This was evident by lower mineralization and less intense alizarin red staining. Under adipogenic environment, aAA-MSCs had fewer lipid containing cells, while in control-MSCs, there were larger fat droplets in a single adipocyte. Oil red "O" staining was used for the identification of neutral fat vacuoles. However, the overall osteogenic and adipogenic characteristics of aAA-MSCs and control MSCs did not show a significant difference. In chondrogenic differentiation media, aAA-MSCs and control MSCs showed similar ability to differentiate into chondrocytes. However, the characteristics of normal MSCs and aAA-MSCs did not show significant differences.

4.4. Immunosuppressive Ability of aAA-BM and Normal BM-MSCs. The observed results of our experiments suggested that aAA-BM and normal BM-MSCs were able to suppress the proliferation of PBMNCs in coculture experiments. At an MSC:PBMNC ratio of 1:5, the inhibition of proliferation caused by the MSCs was quite significant. However, the calculated percentage of immune suppression by aAA-MSCs and normal MSCs showed that it was similar in both sources of BM-MSCs (Figure 5). The current study states comparable results of MLR experiments, with the percentage decrease of immune cell proliferation being 66.70%, 65.06% and 64.46% for aAA-BM-MSCs in each individual patient, whereas normal BM-MSCs showed 61.13%, 72.93%, and 64.20% of suppression (Table 2).The differences in the immunomodulatory properties of the three samples of aAA-BM and normal-BM have been suggested to be primarily due to quantitative aspects of the suppression, which are likely to be related to the respective metabolic activities of the tissue source. Our data indicate that aAA-BM are similar to normal BM-MSCs in their functional immunological property and retain their ability to suppress allogenic immune cells.

5. Discussion

Mesenchymal Stem Cells (MSCs) are multipotent cells that may be isolated from the bone marrow (BM), adipose tissue, dental pulp, umbilical cord blood, or umbilical cord and they are known to contribute to the organization and functioning of the hematopoietic niche. Endothelial cells are known source of MSCs in BM niche and regulate hematopoietic stem cell (HSCs) proliferation and differentiation. However, according to the International Society for cellular therapy, the cells should be able to adhere to plastic surface, express surface markers (positive for CD105, CD90, CD73, and CD29 and negative for CD34, CD14, CD45, and HLA-DR) [26]. MSCs are capable of differentiating into fibroblast, osteoblasts, adipocytes, and chondroblasts. Hence, in the present study, MSCs from aAA patients and healthy donors were compared for cellular morphology, surface marker profiling, population doubling time, and trilineage differentiation potential.

We found spindle-shaped typical morphology of MSCs in aAA and healthy donors. Additionally, our aAA and healthy BM-MSCs expressed vimentin protein. Vimentin protein plays a vital role in cellular stability and is generally expressed in normal mesenchymal cells [25]. Similarly,

Table 1: Characteristics of acquired aplastic anemia patients.

S. no.	Age (in years)	Sex	Haemoglobin (g/l)	Total leukocyte count (10^9/l)	Platelet count (10^9/l)	Absolute neutrophil count (10^9/l)	Disease severity
1	24	M	54	2.5	59	0.5	Nonsevere AA
2	19	M	53	2.3	19	0.14	Very severe AA
3	19	F	100	2.22	38	1	Nonsevere AA
4	19	F	50	4.5	55	0.54	Nonsevere AA
5	21	M	54	3.37	13	0.2	Very severe AA

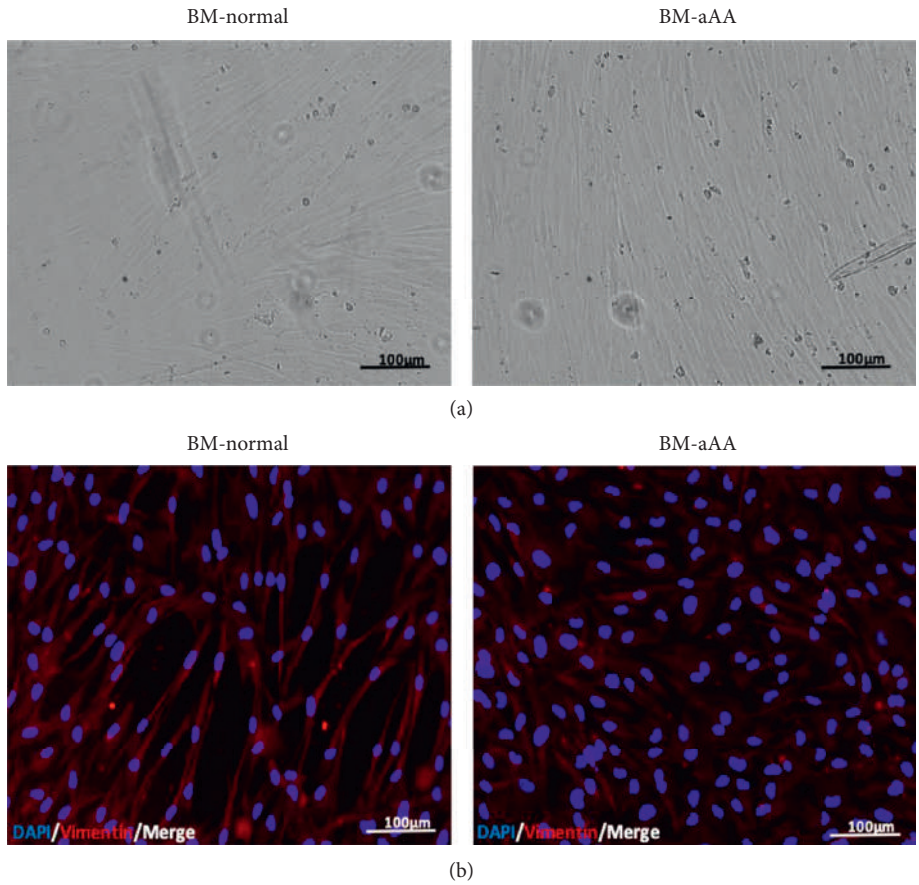

Figure 1: Representative images for BM-MSCs. (a) A micrograph to show typical spindle-shaped morphology of BM-MSCs from normal donor versus aplastic anemia. (b) An immunofluorescence image for vimentin expression level of vimentin in BM-normal vs. BM-aAA.

many studies have reported similar spindle-shaped and fibroblastic morphology of aAA-MSC [13, 19, 27–29]. On the contrary, in some studies, aAA-MSCs were irregularly shaped and swollen [10, 30].Morphology of aAA-MSCs also varied from healthy controls in cultures infected with short hairpin containing lentiviruses [11]. The morphological variation indicates that there might be other responsible factors such as *in vivo* defect or different *in vitro* conditions.

aAA and healthy control MSCs were then characterized using cell surface marker profiling. MSCs were identified based on the minimal criteria of the International Society of Cellular Therapy (ISCT) for human MSCs [26]. The MSCs were negative for CD34/CD45 and HLA-DR and positive for CD105, CD90, CD29, CD73, and HLA-ABC.

Michelozzi and colleagues reported no difference in population doubling time of aAA-BM and normal-BM [29], whereas others have shown lower population doubling time and decreased proliferation in aAA BM-MSCs [13, 19, 27]. In a study, aberrantly expressed genes associated with cellular proliferation, differentiation, and apoptosis from aAA- BM-MSCs were implicated in lower proliferation [30]. Low level of fibroblastic growth factor (FGF2) was also shown to affect functions and growth of aAA-MSCs [28]. However, when we compared the aAA-BM and normal-BM, we did not find any ambiguities in proliferation and population doubling time. It has been observed that the qualities of the growth surface and expansion media can greatly affect cell behavior [14]. This

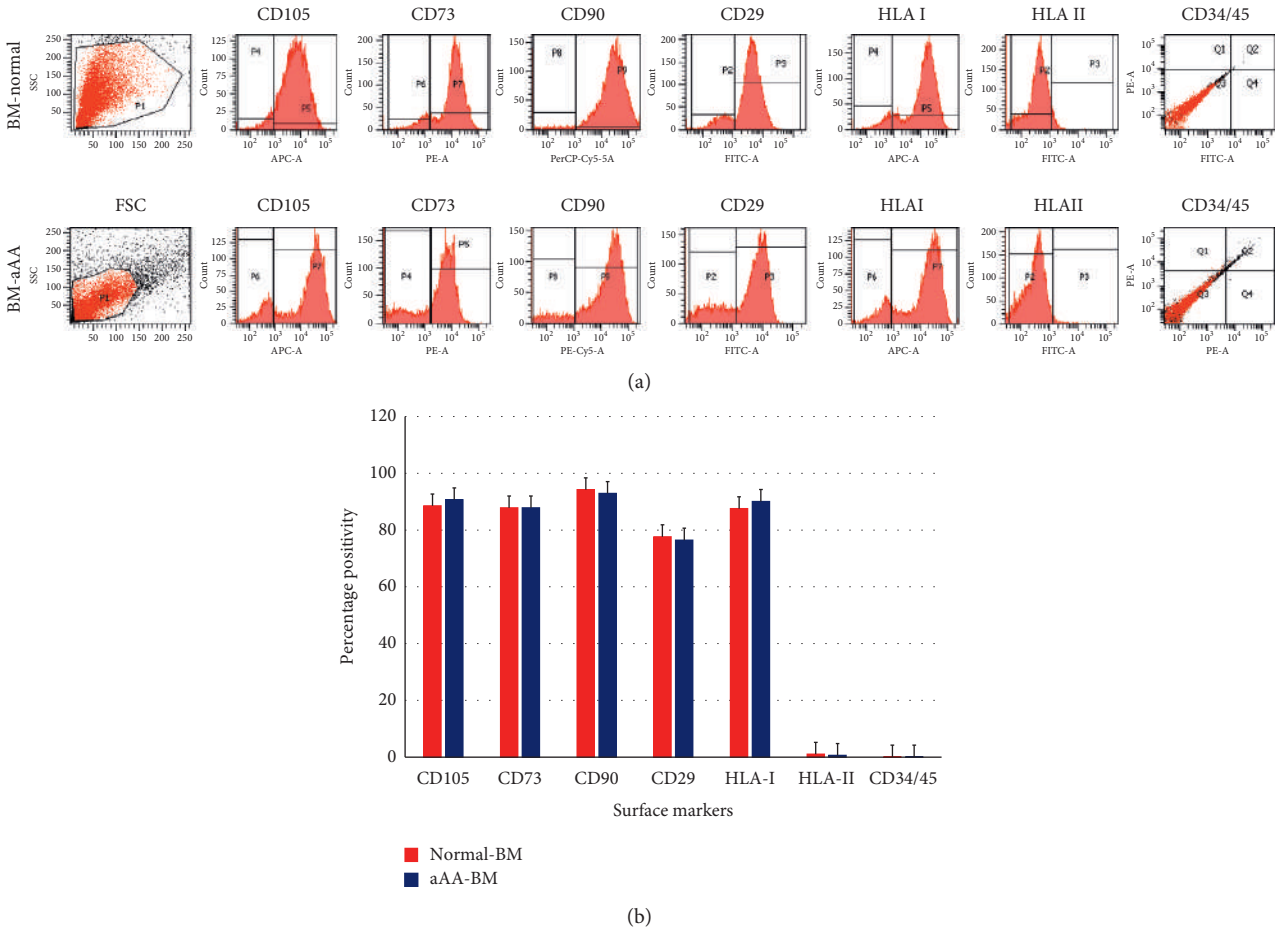

FIGURE 2: Immunophenotypic analysis of mesenchymal stem cells from BM-aAA and BM-normal. (a) Representative histograms for a panel of surface marker profiling. (b) A cumulative graph comparing the surface marker expression in normal MSCs and MSCs from aplastic anemia.

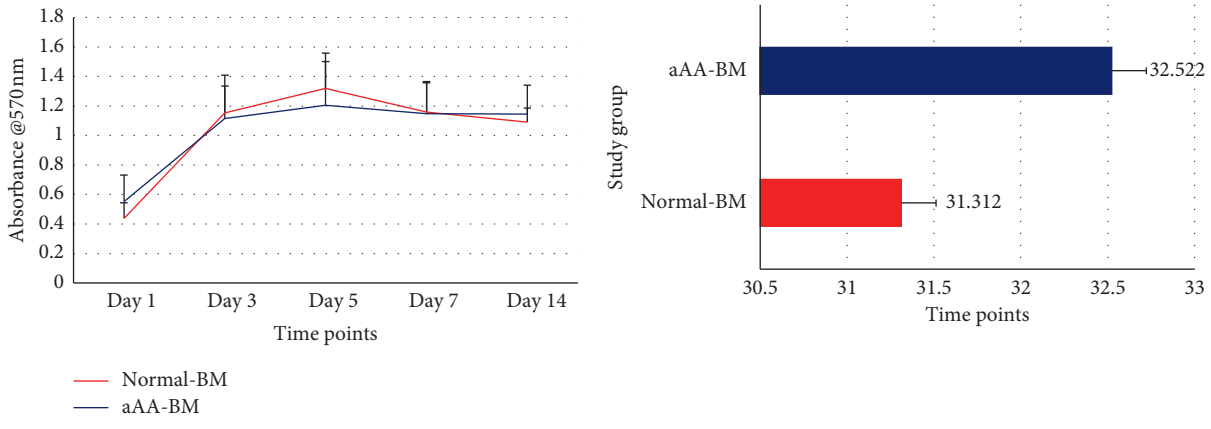

FIGURE 3: The representative data show the comparison of the proliferative index of aAA-BM and Normal BM. (a) A line graph representing the average metabolic activity of normal BM and aAA-BM ($n = 5$ for each group) at different time points. (b) A bar graph representing the comparison of cumulative PD time at passage 3, shown as mean and 95% confidence interval. $N = 5$.

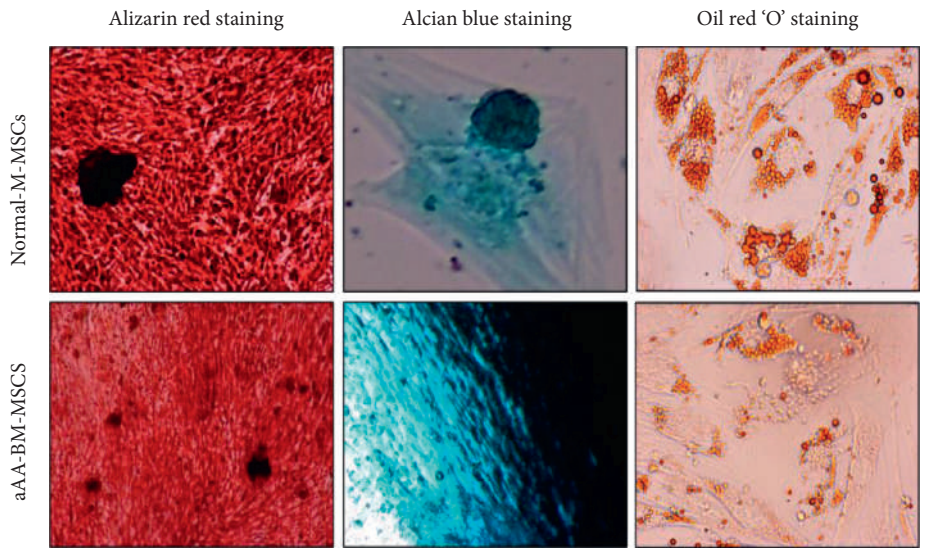

Figure 4: Representative images for trilineage differentiation of BM-MSCs from normal and aAA patients. Alizarin red staining represents the mineralization and signifies the osteogenic differentiation. Alcian blue staining represents the chondrocytes differentiation whereas oil red "O" staining represents the differentiation to adipocytes. Upper panel: normal BM-MSCs. Lower panel: aAA BM-MSCs.

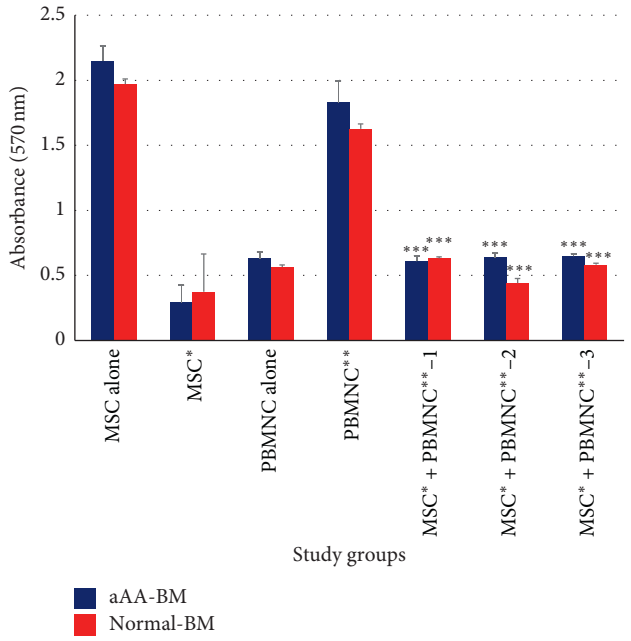

Figure 5: Bar graph representation of one-way mixed lymphocytes reaction (MLR) after establishing cocultures of PBMCs, normal BM, PBMCs, and aAA-BM ($n = 3$). PBMNC** corresponds to PHA stimulated PBMNCs versus MSC* + PBMNC** (coculture) from all three donors. P value <0.005 versus PBMNC** with MSCs* + PBMNC**1,2,3.

Table 2: Tabular representation of the comparative one-way mixed lymphocyte reaction data for the three MSC donors from normal and aAA patients, displaying the result as % decrease of immune cells P value <0.005.

Study groups	OD	% decrease-aAA-BM	OD	% decrease- Normal-BM
PBMNC**	1.829	—	1.626	—
MSC*+PBMNC**-1	0.608	66.70	0.632	61.13
MSC*+PBMNC**-2	0.639	65.06	0.44	72.93
MSC*+PBMNC**-3	0.65	64.46	0.582	64.20

could be the reason for variable growth capacities of MSCs in different studies.

Trilineage differentiation is a hallmark characteristic for identifying healthy and functionally active MSCs. Trilineage differentiation is comprised of osteocytes, adipocytes, and chondrocytes differentiation. Li et al. have reported easier adipogenic differentiation, while it was difficult to induce osteogenic differentiation in aAA-MSCs. Likewise, superior adipocyte differentiation was detected in aAA-MSCs, but osteocyte differentiation was same as controls or was repressed [15, 30]. Other studies have found impaired or decreased adipocytes and osteogenic differentiation in aAA-MSCs than controls [11, 16, 19, 27]. Therefore, it is possible that interaction between adipogenesis and osteogenesis might influence hematopoiesis. Thus, the stress induced by the imbalance of adipocytes and osteocytes might also impact the hematopoietic stem cells in these cases [1], whereas some studies did not find any difference between the differentiation potential of aAA and control MSCs [17, 20, 28, 29]. In accordance with the above reported studies, we observed that aAA patient and healthy MSCs effectively differentiated into osteocytes, adipocytes, and chondrocytes. Although the differentiation into osteocytes and adipocytes in aAA patients was lesser than healthy controls, it was not significant.

The disparities in various studies can be attributed to the variations in patient population and the heterogeneity of acquired aplastic anemia. Overall, a plethora of studies have reported contradicting observations, where aAA-MSCs have been shown to behave like MSCs from healthy donors while others have shown functionally impaired MSCs [13, 15–17, 19, 20, 27–31].

In the present study, at basic parameters, these MSCs show similar trend in terms of cell morphology, proliferation, population doubling time, and trilineage differentiation potential. Apart from these, MSCs are unique in terms of their immune response during inflammation via immunomodulatory factors and release of growth factors, chemokine and anti-inflammatory cytokines [32].

It is already established that MSCs help in tissue repair and prevention of graft-versus-host disease [33]. Altogether, studying and comparing immunomodulatory property of aAA-MSCs and normal MSCs is an essential aspect to understand the functionality of the aAA-MSCs. Xu et al. and Bueno et al. have shown comparable functional immunomodulatory properties of aAA-MSCs. Shipounova et al. observed the differences in the immunomodulatory properties of aAA-BM and normal-BM. Recently, Huo et al. have also found abnormal immunoregulation of aAA MSCs. This could be primarily due to quantitative aspects of the suppression, which are likely to be related to the respective metabolic activities of the tissue source.

In the present study, the immunosuppressive potential of both the aAA and normal MSCs was maintained. Although minute differences were observed, the immunosuppressive ability of MSCs from aAA patients was not equivalent to those obtained from healthy donors.

BM-MSCs have been used in multiple studies and are also being evaluated at clinical level. However, obtaining bone marrow samples from healthy donor is not an easy task. Bone marrow aspiration is a painful process. There are a lot of ethical concerns with respect to bone marrow aspiration from healthy donors for MSCs isolation and expansion. In a routine scenario, bone marrow is obtained from patients undergoing aspiration as a part of routine treatment. During this aspiration, a small amount of bone marrow is required for MSCs isolation. However, there are certain queries related to the MSCs isolated from these patients.

In aAA condition, their will be a defective hematopoiesis which eventually leads to empty bone marrow [34]. The process of hematopoiesis is closely connected with mesenchymal stromal microenvironment of the bone marrow tissue [35]. Consequently, any flaws in bone marrow mesenchymal stromal cells can affect hematopoiesis. There is an urgent need to study MSCs from both donors (healthy versus aAA patient) in great depth both at transcriptional and translational level.

In conclusion, we have shown that BM-MSCs obtained from aAA patients and controls share almost the same morphological, phenotypical, growth, and differential properties; However, they differ in their immunosuppressive properties. Our results suggest that mesenchymal stem cells might not be involved in the pathogenesis of acquired aplastic anemia. Despite being not conclusive owing to the small number of samples, this study can be used as a base for further investigations. With future in-depth analysis and preclinical studies, much more therapeutic potential of MSCs can be explored. The multifaceted pathophysiology of acquired aplastic anemia is responsible for differences in results across various studies. The crosstalk between various components of bone marrow and disease pathogenesis is a fascinating area of research. Therefore, further investigations on stromal components in bone marrow of acquired aplastic anemia patients would be necessary for better understanding of the mechanism of this disease.

Data Availability

All data generated and analysed during this study are included within the article. The data are also available from the corresponding author upon request.

Disclosure

VS and SR have equal authorship.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

VS and SR contributed equally in this manuscript conceptualization and writing. SR, ST, and SG contributed in basic stem cell characterization studies. RS maintained the cell culture. SM and SR analysed the data and conceived the experiments. SM and TS contributed in writing and critical evaluation of the manuscript.

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Research Article

Iron Overload in Transfusion-Dependent Indonesian Thalassemic Patients

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Thalassemia is a genetic disease caused by disruption of globin chain synthesis leading to severe anemia and thus regular blood transfusion is necessary. However, there have been known transfusions-related consequences, including iron overload and multi-organ damage. The aims of this study were to evaluate liver and cardiac function in youth and adult transfusion-dependent Indonesian thalassemic patients and to assess its correlation with serum ferritin levels, as well as T2*magnetic resonance imaging (MRI). Transfusion-dependent thalassemic (TDT) outpatients ($n = 66$; mean age, 21.5 ± 7.2 years) were carried out for the complete assessment consisting of blood test including liver enzyme and serum ferritin, followed by electrocardiography (ECG) and echocardiography. Subjects were also divided by serum ferritin levels into three groups: < 2500 ng/mL, 2500–5000 ng/mL, and >5000 ng/mL. Additionally, subgroup analysis in patients with T2* MRI assessment was conducted. In terms of age of first blood transfusion, subjects with ferritin >5000 ng/mL were the youngest among others. The alanine aminotransferase (ALT) levels in group with serum ferritin >5000 ng/mL were significantly higher than those of the group with serum ferritin <2500 ng/mL. Additionally, youth and adult TDT patients whose serum ferritin >5000 ng/mL had significantly lower tricuspid annular plane systolic excursion (TAPSE) when compared with those who had serum ferritin <2500 ng/mL. Similarly, TAPSE in patients with moderate cardiac siderosis based on cardiac T2* MRI was significantly lower than those without cardiac siderosis. There was significant, but only moderate correlation between serum ferritin and cardiac T2* MRI. Based on these findings, it is important to routinely monitor iron accumulation-related complications, including liver and cardiac damage in youth and adult TDT patients.

1. Introduction

Thalassemia is a hereditary disorder caused by disruption of globin chain synthesis leading to reduced level of hemoglobin [1, 2]. Regular blood transfusion is the main treatment of thalassemia, which restores the important function of hemoglobin as oxygen carrier throughout the body tissues. However, multiple blood transfusions can result in iron overload, which furthermore interfere with the metabolism and lead to tissue and organ damage. Many studies have shown that use of iron-chelating agent resulted in an improved survival in transfusion-dependent thalassemic (TDT) patients. Therefore, regular blood transfusion and iron chelation therapy should be combined adequately [3, 4].

Excess of iron is deposited in vital organs such as heart, liver, spleen, and endocrine organs [5]. Iron overload causes most of the mortality and morbidity associated with thalassemia [6, 7]. Several studies have reported complications found in patients with chronic transfusion, including endocrine dysfunction, hypothyroidism, hyperparathyroidism, hypogonadism, cardiomyopathy, arrhythmia, progressive liver failure, and abnormal kidney function [8, 9]. Centers for Disease Control and Prevention (CDC) reported that complications occurred in adult patients with median age of 31.3 years, with an average age of 4.5 ± 8.2 years at the start of transfusion, and with a median duration of transfusion 18.5 ± 12.3 years [10].

The liver is one of the very susceptible organs commonly affected by iron toxicity. Additionally, cardiac toxicity is the most severe and life-threatening complication of iron overload. Various methods of complications assessment, including serum ferritin level, echocardiography, non-transferrin-bound iron, cardiac T2* magnetic resonance imaging (MRI), heart rate variability, liver biopsy, and myocardial biopsy, have been used for early detection of iron overload in thalassemia patients. However, controversial evidence and limitations of their use in clinical practice have been reported. Although T2*MRI is the recommended non-invasive tool for quantifying iron accumulation, the low cost and widespread availability of serum ferritin make its use indispensable [5]. Studies evaluated iron overload in Indonesian thalassemic patients were mostly performed in children [11] or without organ function assessment [12]. There are still limited studies on the relationship of iron overload and liver, as well as cardiac damage, especially in Indonesian youth and adult TDT patients. Therefore, the objectives of this study were to evaluate liver and cardiac function in Indonesian youth and adult TDT patients and to assess its correlation with serum ferritin levels and T2*MRI.

2. Methods

2.1. Subjects. Youth and adult TDT patients who regularly visited the outpatient clinics at Department of Internal Medicine, Division of Hematology and Medical Oncology, Hasan Sadikin General Hospital, Bandung, for the past 5 years were invited to join this study. Non-probability convenience sampling was used to recruit the patients. The inclusion criteria were as follows: patients who (1) were older than 14 years old, (2) have received transfusion for at least 2 years, (3) were judged capable of completing the survey physically, and cognitively and (4) were capable of understanding spoken and written Indonesian language. Patients who were on acute and/or severe infection determined by history taking and physical examination were excluded because it will interfere with the results of serum ferritin levels [13]. From June to October 2017, upon obtaining written informed consent, each patient with TDT completed questionnaire and blood examination, as well as cardiac function assessment. This study protocol was approved by the Ethics Committee of Faculty of Medicine, Universitas Padjadjaran, Bandung (institutional review board approval number 662/UN6.C10/PN/2017) and conducted in accordance with the Declaration of Helsinki.

Diagnosis of thalassemia was based on the clinical history, molecular diagnosis, and laboratory confirmation. Patients' data were retrieved from medical record and questionnaire. Demographic variables consisting of age, sex, occupation, and marital status were recorded. Questionnaire was used to obtain more personal information of the patients' disease, including age at the start of first transfusion and the interval between blood transfusions. Furthermore, the patients' blood samples were drawn and cardiac examination, including electrocardiography (ECG) and echocardiography, was performed. T2*MRI was conducted in Cipto Mangunkusumo National Public Hospital, Jakarta. Therefore, subgroup of patients was randomly selected especially for those who were willing to be checked for MRI in Jakarta.

2.2. Blood Examination. Hematological parameters were determined by 6 mL of blood, which was collected through venipuncture from each individual using a disposable syringe under sterile conditions, performed by trained laboratory technician. Automated hematology analyzer (Sysmex XN-®Series™ Hematology Analyzers) was used to analyze parameters, including pre-transfusion hemoglobin. Serum ferritin level was also measured by electrochemiluminescence immunoassay (ECLIA) method using ADVIA Centaur XPT Immunoassay System Siemens Healthineers. Although serum ferritin has limitation to predict cardiac iron overload, it can reliably predict siderosis when it is >2500 ng/mL [13]. Sayed et al. [14] previously defined the iron overload severity by classified the serum ferritin levels into three groups: <2500 ng/mL, 2500–5000 ng/mL, and >5000 ng/mL. Similarly, Eghbali et al. [15] also divided the serum ferritin levels into the same 3 groups.

The levels of liver enzymes, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were used to indicate liver function, as injury to acute or chronic liver eventually results in increase in both serum concentrations. To investigate the enzymes level, the tube containing blood sample was put on room temperature and then

centrifuged for 15 minutes at 3,000 rpm for further analysis. The assessment of the enzymes was carried out by using Automated Hematology Analyzer Dimension® EXL™ 200.

2.3. Cardiovascular Function Test. ECG and echocardiography to examine cardiovascular function were performed seven days after blood transfusion. Therefore, cardiac alterations found in the assessment could be assumed caused by iron overload and not by anemia. Standard 9-lead ECG was used and the cardiologist analyzed the result. In addition, complete resting two-dimensional and tissue Doppler echocardiography were performed to the patients in the left lateral decubitus position by the same experienced echo cardiographer, by using general electric vivid S6 cardiovascular ultrasound system equipped with 1.5–3.6 MHz Cardiac Section Probe M4S-RS. The diagnostic criteria were according to American Society of Echocardiography [16]. The examinations included left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), and posterior wall (PW) thickness, all expressed in millimeters. From apical four-chamber view, left ventricular end-systolic volume (LVESV), left ventricular end-diastolic volumes (LVEDV), and left ventricular stroke volume (LVSV) were assessed. Left ventricular ejection fraction (LVEF) and fractional shortening (FS) were measured to see left ventricular function. Left ventricular filling was evaluated by the tissue Doppler echocardiography, from the apical four-chamber view, velocities in early (E) and late (A) diastole were recorded, and also E/A ratio and deceleration time (DT) of E-wave were calculated. Mean values of early (E') myocardial velocities were calculated to find the E/E' ratio. Furthermore, the left atrial maximum volume index (LAVI) and mean pulmonary artery (PA) pressure were calculated.

2.4. T2 MRI Assessment.* The T2* MRI assessments of subgroup of patients were conducted at the Department of Radiology, Cipto Mangunkusumo National Public Hospital, Jakarta. All cardiac and liver T2* images were obtained at 1.5 Tesla MR scanner (Siemens Avanto Germany). Cardiac T2* time was obtained from the region of interest at ventricular septum to avoid artifact and liver T2* time was obtained from 10 mm slice through the center of the liver and analyzed via computer software [11]. Cardiac T2* value was considered as mild (15–20 ms), moderate (10–15 ms), and severe (<10 ms). Liver T2* value was considered as mild (3.8–11.4 ms), moderate (1.8–3.8 ms), and severe (<1.8 ms) [17].

2.5. Statistical Analysis. Data were presented as means ± standard deviation or median (interquartile range) for continuous variables and percentages for categorical variables. Variables were assessed for normality by Kolmogorov-Smirnov or Shapiro-Wilk test. Discrete variables were compared with the chi-square test (Kolmogorov-Smirnov test when appropriate) and continuous variables with one-way analysis of variance (ANOVA) and multiple comparisons with Tukey post hoc test (Kruskal-Wallis test when appropriate). Differences between two groups were analyzed using Student's t-test or Mann–Whitney U test when appropriate. Correlation coefficients for liver and cardiac function and serum ferritin, as well as T2* MRI, were obtained using Pearson product-moment correlations or Spearman's rho correlation coefficient test if appropriate. A p value < 0.05 was considered significant and all tests were two-tailed. Data were analyzed using SPSS 24.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Patient Characteristics. Patient characteristics for the total group are given in Table 1. The total group consisted of 25 men and 41 women (mean age, 21.5 ± 7.2 years). The age range in this study was 15–53 years. The mean age of the first blood transfusion was 60.1 ± 86.8 months with the mean transfusion interval 5.6 ± 6.3 weeks. Deferasirox was the most commonly used iron-chelating agent (46.4%), followed by deferiprone (41.1%). The mean hemoglobin before blood transfusion was 7.2 ± 1.7 g/dL.

The mean serum ferritin was 4414.5 ± 3165.2 ng/mL. Based on previous study [14, 15], the characteristics among three groups of serum ferritin levels (<2500 ng/mL, 2500–5000 ng/mL, and >5000 ng/mL) were compared, as shown in Table 2. Significant differences were observed in age of the first blood transfusion ($p \leq 0.01$), but not in other characteristics. With respect to the patients with different iron-chelating agents, there were no significant differences in number of patients among the three groups.

3.2. Liver Function Test. The results of liver function test stratified by serum ferritin levels are demonstrated in Table 2. The mean of AST and ALT in those with serum ferritin >5000 ng/mL tended to be the highest among the three groups ($p \leq 0.1$ and $p \leq 0.1$, respectively). In two groups comparison, ALT levels in group with serum ferritin >5000 ng/mL were significantly higher than those of the group with serum ferritin <2500 ng/mL (60.9 ± 43.6 ng/mL vs 37.5 ± 19.3 ng/mL, $p < 0.05$). By using T2* MRI, subgroup of patients was analyzed to examine the relationship of liver function in the subgroup. Although not reaching statistical significance, liver enzymes including AST and ALT seem to be higher in those with liver siderosis, as shown in Table 3.

In terms of correlation analysis, as shown in Figure 1, AST was positively correlated with serum ferritin ($r = 0.463$, $p < 0.05$). In addition, there was significantly strong and positive correlation between ALT and serum ferritin ($r = 0.526$, $p \leq 0.01$) in youth and adult TDT patients. Figure 2 shows that there was no correlation between serum ferritin and liver T2* MRI in subgroup analysis.

3.3. Cardiac Function Test. Table 2 lists comparison results of echocardiography parameters and ECG among the three groups. Regarding echocardiography parameters, only left ventricular posterior wall diastolic diameter (LVPWDD, 7.7 ± 1.1 mm) and tricuspid annular plane systolic excursion (TAPSE, 21.1 ± 2.9 mm) were significantly lower among three

Table 1: Patient baseline characteristics for the total sample and stratified by serum ferritin level classification.

	Serum ferritin (ng/mL)				
	<2500 ($n = 22$)	2500–5000 ($n = 20$)	>5000 ($n = 24$)	Total ($n = 66$)	p value
Demographic					
Age, years (mean ± SD)	22.6 ± 8.9	22.6 ± 8.0	19.6 ± 3.7	21.5 ± 7.2	0.275
Sex, n (%)					0.592
Male	10 (45.5)	6 (30.0)	9 (37.5)	25 (37.9)	
Female	12 (54.5)	14 (70.0)	15 (62.5)	41 (62.1)	
Clinical factors					
Age of first transfusion, months (mean ± SD)	109.2 ± 119.8	50.4 ± 61.5	23.2 ± 35.7*	60.1 ± 86.8	0.003
Transfusion interval, weeks (mean ± SD)	6.7 ± 10.3	6.0 ± 3.1	4.3 ± 1.9	5.6 ± 6.3	0.421
Type of chelation therapy, n (%)					
Deferoxamine monotherapy	1 (5.3)	2 (11.1)	1 (5.3)	4 (7.1)	0.261
Deferiprone monotherapy	11 (57.9)	7 (38.9)	5 (26.3)	23 (41.1)	
Deferasirox monotherapy	7 (36.8)	7 (38.9)	12 (63.2)	26 (46.4)	
Deferoxamine and deferasirox	0 (0.0)	2 (11.1)	1 (5.3)	3 (5.4)	
Pre-transfusion Hb, g/dL (mean ± SD)	6.9 ± 1.2	7.3 ± 1.4	7.4 ± 2.3	7.2 ± 1.7	

Hb: hemoglobin, *$p < 0.01$ compared with serum ferritin <2500.

Table 2: Liver enzyme and cardiac function parameters stratified by serum ferritin level classification.

	Serum ferritin (ng/mL)			
	<2500 ($n = 22$)	2500–5000 ($n = 20$)	>5000 ($n = 24$)	p value
Liver enzymes, ng/mL (mean ± SD)				
AST	44.8 ± 21.9	54.6 ± 2.0	65.3 ± 35.9	0.066
ALT	37.5 ± 19.3	50.5 ± 25.5	60.9 ± 43.6*	0.051
Cardiac function parameters (mean ± SD)				
Echocardiography				
LVPWDD (mm)	9.0 ± 1.9	8.8 ± 1.5	7.7 ± 1.1*	0.017
LVPWSD (mm)	13.6 ± 1.8	14.8 ± 4.5	13.5 ± 2.7	0.312
LVEDV (mL)	145.5 ± 14.3	96.0 ± 21.5	88.8 ± 19.0	0.066
LVESV (mL)	36.8 ± 11.5	32.8 ± 9.7	31.5 ± 10.7	0.221
LVSV (mL)	66.4 ± 14.2	63.1 ± 13.3	57.3 ± 10.2*	0.054
LVEF (%)	64.7 ± 4.8	66.3 ± 4.5	65.2 ± 5.2	0.552
LVFS (%)	35.4 ± 3.9	36.6 ± 3.3	35.6 ± 3.9	0.580
LV diastolic function E/A ratio (m/s)	1.6 ± 0.4	1.6 ± 0.3	1.6 ± 0.3	0.886
LV diastolic function DT (ms)	168.0 ± 43.3	155.5 ± 27.2	154.0 ± 25.6	0.304
LV diastolic function E/E′	9.7 ± 2.7	13.5 ± 18.5	12.4 ± 13.1	0.636
LV diastolic function IVRT (ms)	70.5 ± 8.9	71.3 ± 5.1	64.5 ± 17.2	0.262
LV diastolic function LAVI (mL/m^2)	33.9 ± 17.3	28.2 ± 5.4	27.3 ± 6.8	0.130
Mean PA pressure (mmHg)	18.6 ± 10.1	18.6 ± 8.3	20.2 ± 8.5	0.799
TAPSE (mm)	24.0 ± 3.8	23.4 ± 2.8	21.1 ± 2.9*	0.011
Electrocardiography, n (%)				0.958
Normal	11 (52.4)	8 (44.4)	10 (41.7)	
Tachycardia	2 (9.5)	0 (0.0)	4 (16.7)	
T-wave inversion	3 (14.3)	10 (55.6)	7 (29.2)	
Left ventricular hypertrophy	2 (9.5)	0 (0.0)	2 (8.3)	
RBBB	2 (9.5)	0 (0.0)	0 (0.0)	
Arrhythmia	1 (4.8)	0 (0.0)	0 (0.0)	
Supraventricular extra systole	0 (0.0)	0 (0.0)	1 (4.2)	

*$p < 0.05$ compared with serum ferritin <2500, AST: aspartate aminotransferase; ALT: alanine aminotransferase; LVPWDD: left ventricular posterior wall diastolic diameter; LVPWSD: left ventricular posterior wall systolic diameter; LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volume; LVSV: left ventricular stroke volume; LVEF: left ventricular ejection fraction; LVFS: left ventricular fractional shortening; LV: left ventricle; DT: deceleration time; IVRT: isovolumic (or isovolumetric) relaxation time; LAVI: left atrial volume index; PA: pulmonary artery; TAPSE: tricuspid annular plane systolic excursion; RBBB: right bundle branch block.

groups ($p < 0.05$). In two comparisons, youth and adult TDT patients whose serum ferritin >5000 ng/mL had significantly lower LVPWDD (7.7 ± 1.1 mm vs 9.0 ± 1.9 mm, $p < 0.05$), left ventricular stroke volume (LVSV, 57.3 ± 10.2 mL vs 66.4 ± 14.2 mL, $p < 0.05$), and TAPSE (21.1 ± 2.9 mm vs 24.0 ± 3.8 mm, $p < 0.05$) compared with those with serum ferritin <2500 ng/mL. In line with this, subgroup analysis presented in Table 4 shows the relationships between presence of cardiac siderosis based on T2* MRI and cardiac function. TAPSE in patients with moderate cardiac siderosis was

Table 3: Liver function stratified by presence of liver siderosis based on T2* MRI.

	T2* ≥ 1.8 ($n = 4$)	T2* < 1.8 ($n = 20$)	p value
Age (years), median (IQR)	23.5 (18.5, 28.5)	20.0 (18.0, 21.75)	0.185
Serum ferritin (ng/mL), median (IQR)	1850.45 (563.1, 10363.4)	4008.0 (2815.8, 5598.5)	0.245
Liver enzymes (ng/mL), median (IQR)			
AST	60.0 (40.0, 80.8)	39.0 (33.3, 80.0)	0.509
ALT	55.0 (26.0, 65.3)	41.5 (29.8, 65.5)	0.877

IQR: interquartile range, AST: aspartate aminotransferase; ALT: alanine aminotransferase.

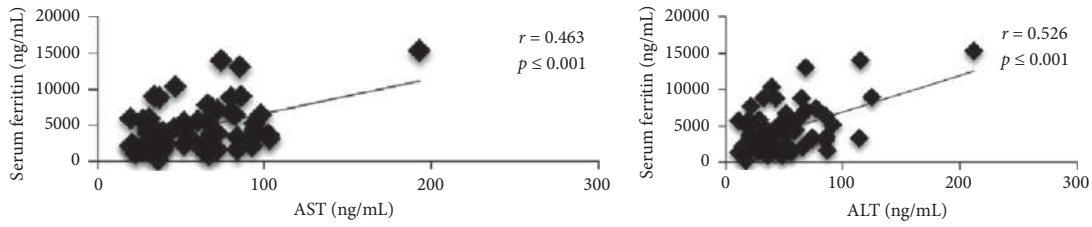

Figure 1: Correlation between serum ferritin and liver enzyme. (a) Correlation between serum ferritin and aspartate aminotransferase (AST). (b) Correlation between serum ferritin and alanine aminotransferase (ALT).

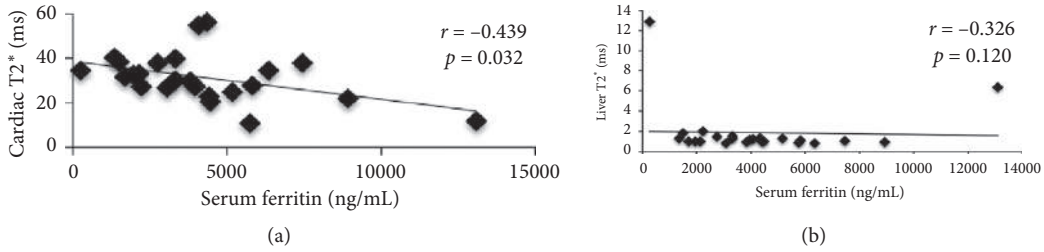

Figure 2: Correlation between serum ferritin and T2* MRI. (a) Correlation between serum ferritin and cardiac T2*. (b) Correlation between serum ferritin and liver T2*.

significantly lower than those without cardiac siderosis (17.0 ± 2.8 mm vs 23.8 ± 3.6 mm, $p < 0.05$). There were some tendencies regarding the relationships of LVPWDD, LVSV, and LVFS among patients with and without moderate cardiac siderosis ($p \leq 0.1$).

With regard to ECG, there were no significant differences in ECG results among the three groups. Most of youth and adult TDT patients have normal ECG findings. T-wave inversion (31.7%) was the most common ECG abnormality, followed by tachycardia (9.5%) and left ventricular hypertrophy (6.3%). After being stratified by presence of cardiac siderosis based on T2* MRI findings, there was significant difference on ECG changes in TDT patients as shown in Table 4. On the other hand, there was significant, but moderate correlation between serum ferritin and cardiac T2* MRI as shown in Figure 2.

4. Discussion

Serum ferritin remains an inexpensive and easily available tool for assessment of iron overload regardless of its limitation, but T2* MRI still becomes a recommended strategy to evaluate iron overload [13, 18]. Prior works have demonstrated some correlation between liver or cardiac function tests and serum ferritin or T2* MRI [11, 19–23]. However, most of those studies included pediatric to young adult thalassemic patients. In this study, we tested the extent to which serum ferritin levels as well as T2* MRI correlated with liver and cardiac function among youth and adult TDT patients.

In this study, age of the first blood transfusion was found significantly associated with serum ferritin levels. The earlier the age at the start of blood transfusion, the higher the serum ferritin levels. Mishra and Tiwari [24] reported that serum ferritin level can also be increased due to frequency of blood transfusion and the age of the patient. Serum ferritin is commonly used to monitor patients with transfusion iron overload as it often is used to adjust or switch chelation regimens. Appropriate chelation therapy has been shown to reduce liver and myocardial iron overload [18].

In our study, most of patients were given deferasirox followed by deferiprone as their iron chelation therapy. It is known that deferasirox and deferiprone offer an important treatment option for people with thalassemia and secondary iron overload [25–28]. Deferoxamine was administered only in small number of patients due to its negative impact on patient's quality of life, such as discomfort and time-consuming in its administration. Combination of two chelation

Table 4: Cardiac function stratified by presence of cardiac siderosis based on T2* MRI.

	None (T2* > 20 ms, $n = 22$)	Moderate (T2* 10–15 ms, $n = 2$)	p value
Age, years (mean ± SD)	20.9 ± 4.0	22.5 ± 2.1	0.247
Serum ferritin, ng/mL (mean ± SD)	3732.9 ± 2096.8	9413.0 ± 5188.7	0.060
Cardiac T2* (ms)	33.3 ± 9.2	11.2 ± 0.7	0.022
Echocardiography			
LVPWDD (mm)	8.7 ± 1.5	7.2 ± 0.0	0.059
LVSV (mL)	64.7 ± 11.3	48.5 ± 6.4	0.067
LVEF (%)	66.2 ± 4.7	60.0 ± 2.8	0.058
LVFS (%)	36.5 ± 3.5	31.5 ± 2.1	0.050
LV diastolic function E/A ratio (m/s)	1.7 ± 0.3	1.5 ± 0.4	0.531
LV diastolic function DT (ms)	159.7 ± 43.3	165.0 ± 14.1	0.497
LV diastolic function E/E′	15.9 ± 21.3	12.2 ± 4.1	0.347
LV diastolic function IVRT (ms)	64.7 ± 17.6	74.0 ± 0.0	0.348
LV diastolic function LAVI (mL/m^2)	29.3 ± 6.6	28.1 ± 3.0	0.870
Mean PA pressure, mmHg	17.7 ± 8.5	27.0 ± 11.3	0.154
TAPSE, mm	23.8 ± 3.6	17.0 ± 2.8	0.040
Electrocardiography, n (%)			0.037
Normal	12 (60.0)	0 (0.0)	
Tachycardia	0 (0.0)	1 (50.0)	
T-wave inversion	6 (30.0)	0 (0.0)	
Left ventricular hypertrophy	0 (0.0)	1 (50.0)	
RBBB	1 (5.0)	0 (0.0)	
Arrhythmia	1 (5.0)	0 (0.0)	

LVPWDD: left ventricular posterior wall diastolic diameter; LVPWSD: left ventricular posterior wall systolic diameter; LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volume; LVSV: left ventricular stroke volume; LVEF: left ventricular ejection fraction; LVFS: left ventricular fractional shortening; LV: left ventricle; DT: deceleration time; IVRT: isovolumic (or isovolumetric) relaxation time; LAVI: left atrial volume index; PA: pulmonary artery; TAPSE: tricuspid annular plane systolic excursion; RBBB: right bundle branch block.

agents was the smallest number because of patient's limitation and problem with insurance coverage. Similar to our study, deferasirox is more often used as an oral iron-chelator followed by deferiprone in Europe. Although information on compliance was not systematically collected in our study, the lack of compliance to iron chelation therapy possibly explains high levels of serum ferritin in several patients [29].

Our study demonstrated higher AST and ALT levels occurred with increased serum ferritin concentration. In line with this, Al-Moshary et al. [19] have discovered similar correlation in children to young adult thalassemic patients. In addition, a study conducted in Bangladesh has reported that the higher levels of serum AST and ALT in beta-thalassemia patients indicate an abnormal muscle and liver function [30]. These finding were also found in Jordan; a positive correlation between serum ALT and AST concentrations and serum ferritin levels in beta-thalassemia patients compared to controls was found [31]. Previous studies conducted in children and adolescences showed correlation between liver T2*MRI and serum ferritin, as well as liver enzymes [11, 17]. However, in this study, only similar trends were found although not reaching statistical significance, probably because of the small sample sizes, which affect the power of analysis.

Cardiac dysfunction secondary to iron overload in thalassemia patients may start early in life although clinical signs are not observed in most patients [32]. Regarding cardiac iron concentration, the T2* MRI inspection remains the best way to detect cardiac hemosiderosis [33]. In this study, the correlation between cardiac T2* MRI and serum ferritin was not strong. Similarly, other studies also found weak or no correlation between cardiac T2* and serum ferritin [11, 17]. It indicated the importance of cardiac T2* MRI to be routinely screened to detect cardiac overload.

ECG abnormalities reported in thalassemia patients have been documented in previous studies [34–37]. The most common abnormalities presented in this study were in accordance to Ramazzotti et al. [37], which reported T-wave inversion as the most common ECG changes. These findings suggested that repolarization were affected by myocardial iron deposit. The changes in repolarization are consistent with impairment of delayed rectifier potassium channels observed in animal models of iron overload [35]. In addition, presence of T-wave inversion had been shown to be related to higher risk for severe cardiac events [38].

In this study, LVPWDD had relationship with serum ferritin levels. It may imply that diastolic dysfunction may occur with the increased of serum ferritin, reflecting an alteration in diastolic property most probably caused by iron accumulation in the heart. Based on natural history, diastolic dysfunction generally appears before systolic dysfunction [36, 39]. Similarly, Sayed et al. [14] reported that diastolic functions were significantly impaired in patients with serum ferritin >5000 ng/mL. Diastolic dysfunction secondary to iron overload can be explained by the initial phase of the structural heart alterations that iron can affect all cardiac structures including papillary muscles, conduction system, and pericardium [40]. The cardiac abnormalities may also be related to poor compliance of iron-chelator [41].

In terms of ventricular systolic function evaluation, decreased LVSV in patients with higher serum ferritin levels suggested some degree of left ventricular dysfunction. In study by Rodrigues et al. [40], left ventricular dysfunction

was indicated by the lower value of the percentage of systolic posterior wall thickening. Another interesting finding in our study is that TAPSE was significantly correlated with serum ferritin levels and cardiac T2* MRI. It may indicate that iron deposition in myocardium in patients with cardiac siderosis could affect right heart function [21].

There were some limitations in this study. This was a cross-sectional study, which does not allow for the inference of cause and effect relationship. Furthermore, a greater number of subjects are necessary in any further study. Although T2* MRI is the best way to quantify iron accumulation, the low cost and widespread availability of serum ferritin make its use necessary [5].

In conclusion, our results indicated that serum ferritin level and T2* MRI value in youth and adult TDT patients have relationships with liver and cardiac function. It is necessary to re-evaluate the chelation therapy in patients with higher serum ferritin levels, including the compliance to chelation therapy. In addition, access of T2* MRI should be provided in area with high prevalence of TDT. Based on these findings, it is important to routinely monitor any possible complications, including liver and cardiac damage in youth and adult patients with TDT. Early detection and therefore timely treatment of such complications could improve the quality of life of these patients.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

This study protocol was approved by the Ethics Committee of Faculty of Medicine, Universitas Padjadjaran, Bandung (institutional review board approval number 662/UN6.C10/PN/2017), and conducted in accordance with the Declaration of Helsinki.

Conflicts of Interest

The authors have no financial conflicts of interest to disclose concerning the manuscript.

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Research Article

Microcytic and Malarial Anaemia Prevalence in Urban Children ≤15 Years in the Mount Cameroon Area: A Cross-Sectional Study on Risk Factors

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Background. Anaemia, a common nutritional deficiency, is a public health problem in the Mount Cameroon area. This study determined the prevalence and possible risk factors of microcytic and malarial anaemia in children less than ≤15 years residing in the Buea and Limbe municipalities in the Mount Cameroon area. *Methods*. A total of 566 children were clinically examined in a cross-sectional study from December 2018 to August 2019 for anaemia and malaria parasites. Blood samples collected were used in evaluating full blood count with the aid of an automated haemoanalyser, and malaria parasite was confirmed by microscopy. Anaemia was defined based on WHO standards while microcytic anaemia and malarial anaemia were defined as microcytosis + anaemia and malaria + anaemia, respectively. Factors that showed significance in the bivariate analysis were entered into a multinomial logistic regression to determine risk factors for microcytic and malarial anaemia. *Results*. The overall prevalence for anaemia, microcytosis, microcytic anaemia, and malarial anaemia was, respectively, 68.7%, 48.9%, 36.9%, and 19.6% with microcytic anaemia representing 53.7% of all anaemic cases. Risk factors for microcytic anaemia included child age of 1–5 years ($P = 0.007$), forest ethnicity ($P = 0.019$), parents being farmers ($P = 0.038$) or jobless ($P = 0.009$), and having moderate malaria parasitaemia ($P = 0.048$) while those for malarial anaemia were child age of 6–10 years ($P = 0.008$), parents' age of 26–35 years ($P = 0.049$), parents being jobless ($P = 0.023$), and consuming plantains 3-4 times ($P = 0.024$) a week. *Conclusion*. Microcytic anaemia is getting to be a severe public health concern while malarial anaemia is a mild public health issue in children residing in urban areas of Mount Cameroon. Parents' occupation was directly linked to all anaemia forms; hence, any intervention to curb anaemia should consider aspects that will raise the socioeconomic status of the population.

1. Introduction

Anaemia is still a public health problem in developing countries [1] across all age groups [2]. It is a condition where blood haemoglobin concentration is lower than the normal for a person's age, gender, and environment [3, 4]. Notably pregnant women, and children because of rapid development and impact on cognitive development, are most at risk of anaemia. The adverse health consequences and impact are felt at both the individual and societal levels [5–8]. Globally, anaemia prevalence stands at around 2 billion with over 800 million anaemic children and women; Africa and Asia bear the brunt with an estimated 85% prevalence [5]. In Cameroon, as in the Mount Cameroon area, anaemia is still a severe public health problem. Most recent estimates from the World Bank show a prevalence of 62.5% in children under five and 49.3% in pregnant women [9].

Anaemia is not a standalone condition but results from lack of one or more micronutrients, infections such as malaria and hookworm, congenital haemolytic diseases, poor maternal health, socioeconomic status, and even demographic factors [10–12]. Malaria is often associated

with anaemia and is a major contributor to the occurrence and severity of anaemia in malaria endemic zones. Its route of action is seen in the destruction of both parasitized and nonparasitized red cells [13]. The parasites feed on host haemoglobin, and to stop them from acquiring host iron, the recycling of iron by reticuloendothelial (RE) macrophages is impeded [14, 15]. This leads to lack of iron in the cell that may ultimately lead to iron deficiency (ID) and consequently microcytic red cells and anaemia.

Microcytic anaemia which is characterised by smaller than normal circulating red blood cell for age [16] is a common type of anaemia. While iron deficiency is the main cause of microcytic anaemia, thalassaemia trait (which is a defect in haemoglobin synthesis), chronic inflammation (resulting from lack of iron), and other haemoglobinopathies (such as sideroblastic anaemia) are implicated [17]. Microcytic anaemia often goes undetected except incidentally encountered when full blood count is run for other reasons [18], hence, the need for regular monitoring of its occurrence. Another anaemia type of common occurrence is iron deficiency anaemia (IDA) which results from depleted iron stores due to poor absorption or poor or reduced iron intake and poor erythropoiesis [19]. However, a person can be functionally iron deficient without out-rightly manifesting anaemia as often seen in mild to severe anaemia. Nonetheless, as IDA worsens, the red cells of its victims are exposed to oxidation and reduced antioxidant defence [20].

While several studies in the Mount Cameroon area have determined the occurrence of anaemia in conjunction with malaria and other infections in various setting, the type of anaemia which is invaluable in making informed decision when planning control measures has been infrequently examined. Therefore, the objective of this study was to determine the prevalence and identify factors associated with microcytic and malarial anaemia in children ≤15 years living in urban areas of Buea and Limbe in the Mount Cameroon area.

2. Methods

2.1. Study Sites and Participants. The study was conducted in the Buea and Limbe I and III municipalities, both located in the Mount Cameroon area of Fako Division. Buea is situated and characterised as described by previous studies [21]. It is divided into 7 health areas, namely, Bokwango, Bova, Buea Road, Buea Town, Molyko, Muea, and Tole. Limbe has been described by previous studies [22]. In terms of health areas, the Limbe Health District is subdivided further into 8 health areas, namely, Batoke, Bimbia, Bojongo, Bota, Mabeta, Moliwe, Limbe Regional Hospital, and Sanje. These 8 health areas fall under 3 councils: Limbe I (Poh), Limbe II, and Limbe III councils. Malaria parasite transmission in Cameroon is very heterogenous varying with altitude and climate [23]. In the Mount Cameroon area, transmission is holoendemic and perennial with lower altitudes having the highest transmission rates [24]. Entomological inoculation rates (EIR) reported in the area before vector control measures were implemented ranged from 149.0 to 287.0 infectious bites per person per year (ib/p/y) in coastal towns like Limbe, Tiko, and Idenau [25]. This rate witnessed a drop to 0.7–1.4 ib/p/m [26] after the use of long-lasting insecticide-treated bed nets (LLINs) was implemented.

The study population included children 1–15 years living in the study area whose parents or caregivers gave consent to their participation in the study. Those with known HIV status, with active haemorrhage, who had blood transfusion or were operated upon two months prior to the study, and who are or had been on antimalarials two weeks prior to the study were excluded.

2.2. Study Design, Sampling Technique, and Unit. This cross-sectional study was carried out from December 2018 to August 2019. Following administrative and ethical clearances, participants were enrolled into the study at their various communities and at presentation to the hospital following education. Informed consent/assent forms were given to parents/caregivers explaining the purpose, benefits, and risks of the study. Structured questionnaires to obtain information on sociodemographic data, type of accommodation, clinical history, and dietary habits were administered, and clinical evaluation and blood samples were collected thereafter for malaria parasite determination and full blood count (FBC) analyses.

A convenient multistage sampling method was employed in the study. Firstly, for representativeness of each health district, a health area was randomly selected from each of the 3 council areas that make up the Limbe Health District and 2 were selected from the 4 distinct urban settings recognised in the Buea Health District. This was followed by a random selection of representative health facilities and neighbourhoods in the selected health areas. Following education by the community relay agents, potential participants in the selected communities were invited to a specified data collection hall in the neighbourhood on programmed dates coordinated by the neighbourhood head. Concurrently, at presentation to the outpatient department of both Buea Regional and Bota District Hospitals, participants were enrolled prospectively as they fulfilled the inclusion criteria for the study. The sample size for the study was determined using the formula $n = z^2pq/d^2$ [27], where n was the required sample size; z was 1.96, the standard deviation for a 95% confidence interval (CI); P value was 62% which was the anaemia prevalence in the region [21]; q was $(1 - p)$; and d was the acceptable error margin set at 0.05. The optimum sample size for both health districts was 362. To allow for data loss due to incomplete data entry, the sample size was increased by 10% to 400 requiring an average of 200 samples per health district.

2.3. Data Collection. Each child was examined clinically by a trained physician. With the assistance of an interviewer, the parent/caregiver was given a self-administered structured questionnaire to fill, and this included information on sociodemography, clinical symptoms, type of habitation, and dietary habits. Axillary temperature was measured using a clinical thermometer, and fever was defined as temperature ≥37.5°C. Symptoms such as cough, headache, diarrhoea, and body weakness/pain were recorded. Weight and height were

measured using a Terraillon weighing scale and a measuring tape to the nearest 0.1 kg and 1 cm, respectively. Parents of children who could not walk were requested to climb on the balance with the child and then without the child; the difference between both weights was recorded as the child's weight. The recumbent length was measured for children who could not walk. Height-for-age (HA), weight-for-age (WA), and weight-for-height (WH) z-scores were computed based on WHO growth curves [28] using WHO Anthro and AnthroPlus packages [29]. Stunting, underweight, and wasting were, respectively, defined as HA, WA, and WH *z*-scores of <−2. Malnutrition was defined as any *z*-score <−2 [16]. Dietary assessment was done by a recall by parents on how often in a week they consumed the following foods: fruits, vegetables, meat, fish, and plantains. This was scored as (1) 1-2 times, (2) 3-4 times, and (3) >4 times a week.

2.4. Laboratory Methods. Approximately 4 mL of venous blood was collected using sterile techniques into EDTA and dry tubes. Labelled blood samples were transported on ice to the Malaria Research Laboratory of the University of Buea for further analysis. Thin and thick blood films were prepared immediately after dispensing blood into EDTA tubes. Thin films were fixed with absolute methanol and together with the thick films stained for 15 minutes with 10% Giemsa stain and then examined for the detection and identification of the malaria parasite following standard procedures [30]. Slides were considered positive when asexual/gametocyte forms were observed, and parasitaemia was calculated per 200 white cells multiplied by patient's white blood cell (WBC) counts and stretched to 500 leukocytes if gametocytes were seen [30]. Parasite burden was classified per microliter (μL) of blood as low (<1000 parasites), moderate (1000–4999 parasites), high (5000–99,999 parasites), and hyper (≥100,000 parasites) [31].

Blood in the dry tube was centrifuged at 3000 rpm for 5 minutes, and the aliquots were stored at −20°C until use. C-reactive protein (CRP) concentration was determined using the Thermo Fisher ELISA machine as per the manufacturer's instructions. Inflammation was confirmed when acute phase protein (APP) CRP was >5 mg/L [20]. A complete blood count was run using the Nihon Kohden Celltac α haemoanalyser according to manufacturer's instructions to obtain values for WBC, red blood cell (RBC) and platelet (Plt) counts, haemoglobin (Hb), haematocrit (Hct), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width coefficient of variation (RDW-CV), red cell distribution width standard deviation (RDW-SD), mean platelet volume (MPV), and platelet distribution width (PDW). Anaemia was classified based on WHO age-based classification [32] as Hb < 11.0 g/L for children 1–5 years, Hb < 11.5 g/dL for children 6–11 years, and Hb < 12.0 g/dL for children 12–15 years old. Severe anaemia was defined as Hb < 7.0 g/dL for all children. Moderate anaemia was defined as Hb: 7.0–9.9 g/dL for 1–5 years and Hb: 7.0–10.9 g/dL for children 6–15 years, and mild anaemia was defined as Hb: 10.0–10.9 g/dL for children 1–5 years, Hb: 11.0–11.4 g/dL for children 6–11 years, and Hb: 11.0–11.9 g/dL for children 12–15 years. Malaria anaemia (MA) was defined as anaemia + positive blood smear for malaria parasite while nonmalarial anaemia (NMA) was defined as anaemia with negative blood smear for malaria parasites. Microcytosis was defined as MCV <67 fL for children less than 2 years and MCV <73 fL for children 2 to 15 years, and microcytic anaemia was defined as Hb < 11.0 g/dL + MCV< 67 fL and Hb < 11.0 g/dL + MCV <73 fL, respectively [16].

2.5. Data Analysis. Data was keyed into Microsoft Excel 2010 and exported into IBM-Statistical Package for Social Sciences (SPSS) version 20 (SPSS, Inc., Chicago, IL, USA). Continuous variables were summarized as means and standard deviations while categorical variables were reported as percentages and frequencies. Proportion difference was evaluated using Pearson's Chi square test (χ^2), Pearson's ranked correlation (r) for haematological bivariate association, and analysis of variance (ANOVA) to compare group means. Parasite density was log-transformed before analysis. Factors with a P value <0.2 in the bivariate analysis were entered into a multinomial logistic regression to determine risk factors for microcytic and malarial anaemia. Odd ratios (OR) and 95% confidence intervals (CIs) were computed, and $P < 0.05$ value was suggestive of statistical significance.

2.6. Ethical Consideration. Ethical clearance was obtained from the Institutional Review Board hosted by the Faculty of Health Sciences, University of Buea (2018/811–05/UB/SG/IRB/FHS), following an administrative approval from the Regional Delegation of Public Health (R11/MINSANTE/SWR/RDPH/PS/430/940). Authorisations from community heads were also obtained. Only children whose parents consented to their participation in the study and who responded to the questionnaires were enrolled after the purpose, risks, and benefits were clearly explained to them. It was stressed that the study was completely voluntary and that a parent had every right to stop/withdraw their child from the study. Even after the parent's consent to participate in the study, any child who was too scared of a needle was not forced to continue. Data was treated with utmost confidentiality by assigning codes to the patients' samples.

3. Results

3.1. Baseline Characteristics of Study Participants. A total of 614 participants were approached to participate in the study, and 589 of them were enrolled. After data curation, 566 participants were retained of which 47.2% were males and 52.8% were females, living in low (61.5%) and highland (38.5%) areas. The mean (SD) age was 6.4 (4.5) years of whom 51.4% were ≤5 years. As shown in Table 1, most of the participants were enrolled at presentation to hospital (63.3%), had household head between 35 and 50 years old (44.2%), and had secondary level of education (40.6%). Overall, anaemia, malaria, microcytosis, inflammation, fever, and malnutrition prevalence in the study population

Table 1: Characteristics of the 566 participants enrolled in the study.

Parameter	Total
% (*n*)	100 (566)
Gender	
Female	52.8 (299)
Male	47.2 (267)
Age group in years	
1–5	51.4 (291)
6–10	24.9 (141)
11–15	23.7 (134)
Mean age (SD) in years	6.4 (4.5)
Mean height (SD) in cm	112.6 (28.4)
Mean weight (SD) in kg	23.7 (14.6)
Mean Hb conc. in g/dL	10.4 (2.0)
Sociodemographic factors	
Altitude of residence	
Lowland	61.5 (348)
Highland	38.5 (218)
Point of presentation	
Community	36.7(208)
Hospital	63.3 (358)
Health district	
Buea	37.5 (212)
Limbe	62.5 (354)
Age of household head (years)[a]	$N = 407$
≤25	3.9 (16)
26–35	28.5 (116)
36–50	44.2 (180)
>50	23.3 (95)
Education level of household head	
Primary	23.3 (132)
Secondary	40.6 (230)
Tertiary	31.6 (179)
No formal	4.4 (25)
Family size	
≤5	50.5 (286)
6–10	43.8 (248)
>10	5.7 (32)
Clinical factors	
Mean temperature (SD) in °C	37.1 (1.1)
Malaria parasite prevalence	27.7 (157)
Fever prevalence	27.7 (157)
Prevalence of inflammation	58.4 (211)
Anaemia prevalence	68.7 (389)
Microcytosis	48.9 (277)
Malnutrition	15.2 (86)

[a]Calculated for 407 participants.

were, respectively, 62.4%, 27.7%, 48.9%, 58.4%, 27.7%, and 15.2%.

3.2. Malaria Parasite, Nutritional Status, and Dietary Habits. Significantly higher prevalence of malaria parasite was observed in children aged 6–10 years (35.5%, $P = 0.003$), those from low altitude (32.8%, $P = 0.001$), and those who presented themselves at the hospital (33.5%, $P < 0.001$) when compared with their respective contemporaries. The prevalence of malaria parasite and malnutrition was comparable with sex ($P = 0.715$ and $P = 0.421$, respectively). However, significantly higher occurrence of malnutrition was observed in children aged 1–5 years (21.0%; $P < 0.001$), those living at low altitude (18.1%; $P = 0.015$), those whose parents were fishermen (23.3%, $P = 0.025$), and those of other ethnicity (26.5%; $P = 0.029$) as shown in Table 2.

On dietary habits, more children ≤5 years reported not washing hands before meals (19.9%) and consuming fewer plantain meals a week than their older peers. This difference was statistically significant ($P = 0.001$ and $P = 0.038$, respectively). The weekly plantain consumption was significantly linked to parents' education level ($P = 0.049$) with more children whose parents had no formal education consuming plantain ≥3 times weekly (55.8%) when compared with their counterparts as shown in Figure 1.

3.3. Anaemia, Microcytic Anaemia, and Malarial Anaemia Prevalence. Overall, the prevalence of anaemia (68.7%) was highest in children living in low altitude (77.3%), examined in the community (81.2%), and living in homes with 6–10 occupants (71.4%) and whose parents were 25–35 years old (80.2%), had no formal education (75.8%), or were fishermen (86.4%) as well as those of forest ethnicity (100.0%) when compared with their compeers. The differences were statistically significant. In relation to clinical factors, the only significantly higher prevalence of anaemia ($P = 0.011$) was observed in those with inflammation (79.1%) as shown in Table 3.

The prevalence of microcytic anaemia was 36.9% accounting for 53.7% of all the anaemic cases. Significantly, it was highest in children ≤5 years old (43.3%), those living in low altitude (49.1%), those examined within the community (42.8%), those whose parents were 26–35 years old (39.7%) and were fishermen (56.3%), those of forest ethnicity (60.0%), those malaria parasite positive (49.7%), and those who had moderate parasitaemia (62.8%) when compared with their respective contemporaries. While malarial anaemia occurred in 19.6% of the study population, children aged 6–10 years (24.1%), those living at low altitude (23.9%), those whose parents were 26–35 years old (21.6%), and those who were feverish (28.7%) and were malaria parasite positive (68.8%) had significantly higher prevalence than their respective peers (Table 3).

3.4. Effect of Nutritional Status and Dietary Habits on the Different Types of Anaemia. The prevalence of anaemia was significantly higher ($P = 0.010$, $P = 0.047$, and $P = 0.009$) in children who were stunted (83.1%), were underweight (89.5%), and consumed plantains 3-4 times a week (85.3%), respectively, when compared with their equivalents. On the other hand, microcytic anaemia prevalence was significantly higher ($P = 0.048$) in normal (43.7%) than wasted children, while only those who consumed plantains 3-4 times a week had a significantly higher ($P = 0.025$) prevalence of malarial anaemia (20.6%) as shown in Table 4.

3.5. Risk Factors for Anaemia, Microcytic Anaemia, and Malarial Anaemia. As revealed in Table 5, the logistic regression analysis with anaemia as dependent variable showed that children living in lowland ($P = 0.017$), those

Table 2: Prevalence of malaria parasite and malnutrition by sociodemographic factors.

Parameter	Category	N	Malaria parasite prevalence % (n)	χ^2, P value	Malnutrition prevalence	χ^2, P value
Gender	Male	267	28.5 (76)	0.133, 0.715	16.5 (44)	0.648, 0.421
	Female	299	27.1 (81)		14.0 (42)	
Age	1–5	291	28.9 (84)	**11.855, 0.003**	21.0 (61)	**17.110, <0.001**
	6–10	141	35.5 (50)		6.4 (9)	
	11–15	134	17.2 (23)		11.9 (16)	
Altitude	Lowland	348	32.8 (114)	**11.360, 0.001**	18.1 (63)	**5.934 0.015**
	Highland	218	19.7 (43)		10.6 (23)	
Point of presentation	Community	208	17.8 (37)	**16.243, <0.001**	17.8 (37)	1.717 0.190
	Hospital	358	33.5 (120)		13.7 (49)	
Parents' age[c]	≤25	16	6.2 (1)	3.940, 0.268	25.0 (4)	**5.076, 0.166**
	26–35	116	22.4 (26)		17.2 (20)	
	36–50	180	20.6 (37)		13.9 (25)	
	>50	95	14.4 (14)		8.4 (8)	
Education	Primary	132	19.7 (26)	7.288, 0.063	17.4 (23)	2.516, 0.472
	Secondary	230	28.3 (65)		13.9 (32)	
	Tertiary	179	31.3 (56)		14.0 (25)	
	No formal	25	40.0 (10)		24.0 (6)	
Family size	1–5	286	32.5 (93)	**6.605, 0.037**	13.6 (39)	2.629, 0.269
	6–10	248	23.0 (57)		17.7 (44)	
	>10	32	21.9 (7)		9.4 (3)	
Occupation	Farming	123	29.3 (36)	11.239, 0.081	10.6 (13)	**14.495, 0.025**
	Civil servant	77	24.7 (19)		10.4 (8)	
	Petty trading	63	33.3 (21)		9.5 (6)	
	Private worker	92	20.7 (19)		16.3 (15)	
	Fishing	103	28.2 (29)		23.3 (24)	
	Jobless	69	39.1 (27)		23.2 (16)	
	Retired	39	15.4 (6)		10.3 (4)	
Ethnicity	Coastal	212	19.3 (41)	**2.861, 0.581**	10.8 (23)	**10.816, 0.029**
	Grassland	112	17.0 (19)		12.5 (14)	
	Forest	5	20.0 (1)		20.0 (1)	
	Sahel	7	0.0 (0)		14.3 (1)	
	Other ethnic groups	68	23.5 (16)		26.5 (18)	

[c]Calculated for 407 participants. P values in bold are statistically significant.

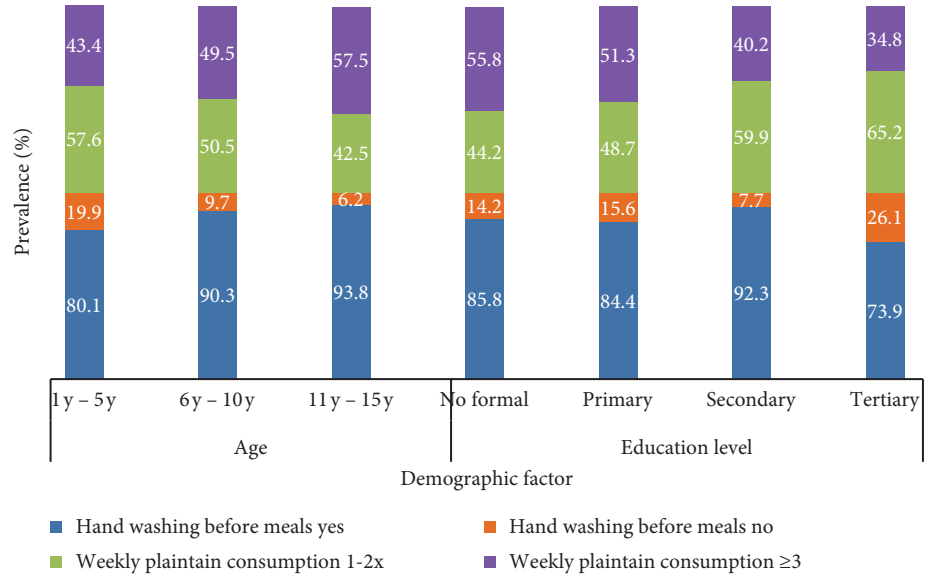

Figure 1: Effect of age and parents' education level on dietary habits.

Table 3: Prevalence of anaemia, microcytic anaemia, and malarial anaemia with respect to sociodemographic and clinical factors.

Parameter	Category	N	Prevalence [% (n)] of					
			Anaemia	P value	Microcytic anaemia	P value	Malarial anaemia	P value
Sociodemographic factors								
Gender	Male	267	70.0 (187)		39.7 (106)	0.196	20.6 (55)	
	Female	299	67.6 (202)	0.525	34.4 (103)		18.7 (56)	0.57.6
Age	1–5	291	69.1 (201)		43.3 (126)		21.6 (63)	
	6–10	141	70.2 (99)	0.781	38.3 (54)	**<0.001**	25.5(35)	
	11–15	134	66.4 (89)		21.6 (29)		9.0 (12)	**0.001**
Altitude	Lowland	348	77.3 (269)		49.1 (179)		24.4 (85)	
	Highland	218	55.0 (120)	**<0.001**	17.4 (38)	**<0.001**	11.9 (26)	**<0.001**
Point of presentation	Community	208	81.2 (169)		42.8 (89)		15.9 (33)	
	Hospital	358	61.5 (220)	**<0.001**	33.5 (120)	**0.028**	21.8 (78)	0.089
Parents' age[c]	≤25	16	68.8 (11)		12.5 (2)		6.2 (1)	
	26–35	116	80.2 (93)	**0.006**	39.7 (46)	**0.004**	21.6 (25)	**0.046**
	36–50	180	70.6 (127)		31.1 (56)		12.8 (23)	
	>50	95	57.9 (55)		18.9 (18)		9.5 (9)	
Family size	1–5	286	69.6 (199)		40.9 (117)		21.7 (62)	
	6–10	248	71.4 (177)	**0.002**	33.9 (84)	0.086	17.3 (43)	0.449
	>10	32	40.6 (13)		25.0 (8)		18.8 (6)	
Education	Primary	132	75.8 (100)		40.2 (53)		13.6 (18)	
	Secondary	230	73.5 (169)	**0.001**	39.1 (90)	0.306	20.9 (48)	0.057
	Tertiary	179	57.0 (102)		31.3 (56)		20.1 (36)	
Occupation	No formal	25	72.0 (18)		40.0 (10)		36.0 (9)	
	Farming	123	71.5 (88)		39.0 (48)		18.7 (23)	
	Civil servant	77	57.1 (44)		18.2 (14)		14.3 (11)	
	Petty trading	63	52.4 (33)		25.4 (16)		19.0 (12)	
	Private worker	92	76.1 (70)		35.9 (33)		16.3 (15)	
	Fishing	103	86.4 (89)	**<0.001**	56.3 (58)	**<0.001**	24.3 (25)	0.094
	Jobless	69	63.8 (44)		46.4 (32)		30.4 (21)	
	Retired	39	53.8 (21)		20.5 (8)		10.3 (4)	
Ethnicity	Coastal	212	68.9 (146)		27.8 (59)		12.7 (27)	
	Grassland	112	61.6 (69)		23.2 (26)		15.2 (17)	
	Forest	5	100.0 (5)	**0.002**	60.0 (3)	**0.002**	20.0 (1)	0.546
	Sahel	7	71.4 (5)		14.3 (1)		0.0 (0)	
	Other ethnic groups	68	88.2 (60)		48.5 (33)		19.1 (13)	
Clinical factors								
Fever	No	409	69.2 (283)		35.2 (144)		15.4 (63)	
	Yes	157	67.5 (106)	0.700	41.4 (65)	0.172	30.6 (48)	**<0.001**
Malaria parasite status	Positive	157	70.7 (111)		49.7 (78)		70.7 (111)	
	Negative	409	60.0 (278)	0.531	32.0 (131)	**<0.001**	0.0 (0)	**<0.001**
Parasite load	Low	92	70.7 (65)		50.0 (46)		70.7 (65)	
	Moderate	43	74.4 (32)	0.665	62.8 (27)	**0.009**	74.4 (32)	0.665
	High	22	63.6 (14)		22.7 (5)		63.3 (14)	
Malnutrition	No	480	11.3 (20)	0.082	14.6 (52)	0.586	14.7 (67)	0.529
	Yes	86	17.0 (66)		16.3 (34)		17.1 (19)	
Inflammation	No	150	67.3 (101)	**0.011**	44.7 (67)	0.436	22.7 (34)	0.902
	Yes	211	79.1 (167)		48.8 (103)		23.2 (49)	

[c]Calculated for 407 participants: P value obtained by χ^2, P values in bold are statistically significant.

whose parents were farmers, worked privately, or were fishermen ($P = 0.042$;$P = 0.013$;$P < 0.001$, respectively), and those who consumed meat 3-4 times weekly ($P = 0.026$) and plantains ($P = 0.034$) were significantly at risk of developing anaemia. They were, respectively, 4, 2.2, 2.7, 5.5, 2.5 and 2.5 times more likely to be anaemic than their counterparts. Significantly, children aged 1–5 years ($P = 0.007$), those of forest ethnicity ($P = 0.007$), those whose parents are farmers ($P = 0.038$) or jobless ($P = 0.009$), and those who had moderate parasitaemia ($P = 0.048$) were 2.4, 15.7, 2.5, 3.4, and 2.6 times, respectively, more likely to have microcytic anaemia. With respect to malarial anaemia, the identified risk factors were the child's age ($P = 0.008$), parent's age ($P = 0.049$) and occupation ($P = 0.023$), and child's weekly consumption of plantain ($P = 0.024$). Children aged 6–10 years, whose parents were 26–35 years old or

Table 4: Prevalence of anaemia, microcytic anaemia, and malarial anaemia with respect to nutritional status and dietary habits.

Parameter	Status	*N*	Prevalence [% (*n*)] of					
			Anaemia	*P* value	Microcytic anaemia	*P* value	Malarial anaemia	*P* value
Stunting	No	494	66.6 (329)		35.0 (173)		18.8 (93)	
	Yes	59	83.1 (49)	**0.010**	47.5 (28)	0.060	23.7 (14)	0.368
Underweight	No	405	67.9(275)		40.7 (165)		22.7 (92)	
	Yes	19	89.5 (17)	**0.047**	47.4 (9)	0.566	21.1 (4)	0.866
Wasting	No	249	67.5(168)		43.7 (107)		21.7 (54)	
	Yes	23	60.9 (14)	0.520	21.7 (5)	**0.048**	13.0 (3)	0.330
Hand washing before meal	Yes	352	68.8 (242)		28.4 (100)		13.6(48)	
	No/seldom	55	80.0 (44)	0.090	40.0 (22)	0.081	18.2 (10)	0.370
Daily meal	1	41	80.5 (33)		39.0 (16)		19.5 (8)	
	2	146	70.5 (103)		30.8 (45)		13.7 (20)	
	3	220	68.2 (150)	0.285	27.7 (61)	0.336	13.6 (30)	0.597
Weekly fruits consumption	1-2	173	74.6 (129)		28.9 (50)		15.6 (27)	
	3-4	57	57.9 (33)		31.6 (18)		14.0 (8)	
	>4	177	70.1 (124)	0.058	30.5 (54)	0.910	13.0 (23)	0.782
Weekly meat consumption	1-2	178	69.7 (124)		29.2 (52)		15.2 (27)	
	3-4	59	83.1 (49)		37.3 (22)		20.3 (12)	
	>4	170	66.5 (113)	0.054	28.2 (48)	0.407	11.2 (19)	0.199
Weekly plantain consumption	1-2	210	65.7 (138)		28.6 (60)		16.2 (34)	
	3-4	68	85.3 (58)		25.0 (17)		20.6 (14)	
	>4	129	69.8 (90)	**0.009**	34.9 (45)	0.289	7.8 (10)	**0.025**

P values in bold are statistically significant.

jobless, and who consumed plantains 3-4 times a week were 3.3, 2.6, 3.8, and 2.9 times, respectively, more likely to have malarial anaemia than their counterparts.

4. Discussion

This study investigates the prevalence and risk factors for microcytic and malarial anaemia in children 1–15 years in urban settings of the Mount Cameroon area. The overall malaria prevalence of 27.7% is like that reported by Teh et al. [33] with the highest burden (35.5%) observed in children in the 6–10 years age group and those living in low altitude. The shift in malaria burden from the under-fives to the 6–10 years observed in this study may be due to effective bed net use in the former corroborating findings elsewhere [34].

More than half of the study participants reported washing hands before meal and consuming plantains more than twice weekly. Hand washing increased with age because younger children must be reminded frequently to wash their hands but will form the habit as they grow older. This finding is corroborated by studies elsewhere [35]. In addition, findings from the study revealed that plantain consumption increased with child's age but decreased with parent's level of education. Younger children may prefer the ripe version of the plantain or paste, which is easier for them to chew/swallow but contains more carbohydrates, to the harder unripe plantain [36] consumed easily by older children which has more of iron and vitamin C. On the other hand, increase in the level of schooling increases knowledge on better feeding practices and dietary diversity [37, 38]; also income may account for the decrease in plantain consumption with rise in level of education as participants can probably afford to replace plantains with other iron-rich foods. That notwithstanding, unripe plantains are rich in iron and vitamin C which are two micronutrients that optimize absorption and can aid in avoiding the occurrence of anaemia [39].

The overall anaemia prevalence of 68.7% reveals that anaemia is a serious public health problem in children in this region as elsewhere in the country. This is higher than both the national anaemia prevalence of 60% [9] and that observed by Teh et al. [34] in the same area but lower than the 77.2% reported in Tanzania [111]. The higher prevalence of anaemia among the 6–10 years old compared with their counterparts is not a surprise as a similar higher prevalence of malaria was observed in the same group. Malaria parasite infection has been reported severally as a risk factor of anaemia [21, 22, 34]. In addition, observations from studies in the Mount Cameroon area have revealed that the burden of malaria has shifted from the ≤5 years old to older age groups due to the increased preventive measures in the former group [34, 40, 41]. This age-related change in burden of malaria may have led to the shift in anaemia prevalence from the under-fives to older groups.

That anaemia was more prevalent at lower (Limbe municipality) than higher altitude (Buea municipality) in this study shows the association between malaria, anaemia, and altitude which had long been established in previous studies [11, 33, 42]. Children living at lower altitude were 4 times at odds of being anaemic. Higher temperatures at low altitudes favour breeding of the malaria vector and transmission of malaria parasites [43–45] which in turn leads to increased anaemia prevalence as malaria has been shown to be among the major contributors to the occurrence of anaemia in this setting [34].

Contrary to findings by Sakwe et al. [45] who reported lower anaemia prevalence in a community-based study than

Table 5: Multinomial regression analysis examining sociodemographic, clinical, hygiene, and dietary factors associated with anaemia, microcytic anaemia, and malarial anaemia.

Parameter	Category	Anaemia		Microcytic anaemia		Malarial anaemia	
		AOR (95% CI)	*P* value	AOR (95% CI)	*P* value	AOR (95% CI)	*P* value
		Sociodemographic factors					
Gender	Male	1.0 (0.6-1.6)	0.904	1.2 (0.7–2.0)	0.417	0.7 (0.4–1.4)	0.355
	Female	Reference		Reference		Reference	
Age	1–5	0.9(0.5–1.6)	0.685	2.4 (1.3–4.6)	**0.007**	1.9 (0.8–4.5	0.163
	6–10	1.1 (0.5–2.1)	0.852	2.0 (1.0–3.9)	0.060	3.3 (1.4–8.1)	**0.008**
	11–15	Reference		Reference		Ref	
Altitude	Lowland	4.0 (1.3–12.4)	**0.017**	1.7 (0.5–6.1)	0.401	3.3 (0.8–13.6)	0.096
	Highland	Reference		Reference	—	Reference	—
Presentation	Hospital	1.5 (0.5–4.4)	0.515	0.4 (0.1–1.4)	0.133	2.8 (0.7–11.5)	0.147
	Community	Reference	—	Reference	—	Reference	—
Family size	>10	0.2 (0.1–0.7)	**0.017**	1.2 (0.4–4.7)	0.748	1.0 (0.3–4.1)	0.979
	6–10	1.1 (0.6–1.8)	0.828	1.1(0.6–1.8)	0.833	0.8 (0.4–1.5)	0.446
	1–5	Reference		Reference	—	Reference	—
Parents' age	≤25	1.0 (0.3–3.7)	0.991	0.5 (0.1–2.4)	0.349	0.8 (0.1–7.1)	0.826
	26–35	1.8 (0.9–4.0)	0.117	1.7 (0.8–3.7)	0.163	2.6 (1.0–6.6)	**0.049**
	36–50	1.2 (0.6–2.2)	0.645	1.8 (0.9–3.8)	0.105	1.4 (0.6–3.4)	0.481
	>50	Reference	—	Reference	—	Reference	—
Education level	No formal	1.9 (0.6–5.7)	0.261	0.6 (0.2–1.8)	0.388	0.2 (0.1–0.6)	**0.005**
	Primary	1.5 (0.5–4.4)	0.506	0.5 (0.2–1.5)	0.215	0.2 (0.1–0.6)	**0.003**
	Secondary	1.1 (0.3–3.4)	0.902	0.5 (0.2–1.8)	0.303	0.3 (0.1–0.9)	**0.030**
	Tertiary	Reference	—	Reference	—	Reference	—
Ethnicity	Coastal	1.4 (0.4–5.5)	0.645	1.4 (0.4–4.3)	0.599	0.6 (0.3–1.3)	0.194
	Grassland	2.2 (0.6–8.5)	0.265	0.8 (0.3–2.5)	0.701	0.8 (0.3–1.7)	0.493
	Forest	—	—	15.7 (1.6–155.4)	**0.019**	1.1 (0.1–10.3)	0.961
	Sahel	4.3 (0.4–41.7)	0.210	0.1 (0.0–1.5)	0.098		—
	Others	Reference	—	Reference		Reference	—
Occupation	Farming	2.2 (1.0–4.5)	**0.042**	2.5 (1.1–5.9)	**0.038**	2.0 (0.7—6.2)	0.225
	Civil servant	1.1 (0.5–2.5)	0.735	0.9 (0.3–2.3)	0.762	1.5 (0.4–4.9)	0.543
	Petty trading	0.9 (0.4–2.1)	0.885	1.3 (0.5–3.5)	0.573	2.1 (0.6–56.9)	0.242
	Private worker	2.7 (1.2–6.0)	**0.013**	2.2 (0.9–5.3)	0.087	1.7 (0.5–5.5)	0.373
	Fishing	5.5 (2.3–12.7)	**<0.001**	0.3 (0.0–1.5)	**<0.001**	2.8 (0.9—8.7)	0.073
	Jobless	1.5 (0.7–3.4)	0.313	3.4 (1.4–8.3)	**0.009**	3.8 (1.2–12.1)	**0.023**
	Retired	Reference	—	Reference		Reference	—
		Clinical factors					
Malnutrition	Yes	1.2 (0.1–31.4)	0.903	0.2 (0.0–2.3)	0.196	1.2 (0.0–31.4)	0.903
	No	Reference		Reference		Reference	
Stunting	Yes	0.4 (0.0–12.5)	0.621	7.9 (0.7–84.2)	0.088	0.4 (0.0–12.5)	0.621
	No	Reference		Reference		Reference	—
Underweight	Yes	0.7 (0.0–12.2)	0.831	2.3(0.6–9.5)	0.245	0.7 (0.0–12.2)	0.831
	No	Reference		Reference	—	Reference	—
Wasting	Yes	—	—	1.4 (0.1–12.2)	0.912	—	—
	No	Reference		Reference	—	Reference	—
Parasitaemia	High	0.3 (0.1–1.8)	0.183	0.1 (0.0–1.2)	0.071	0.1 (0.0–1.9)	0.129
	Moderate	0.2 (0.0–1.3)	0.086	2.6 (1.0–6.6)	**0.048**	0.9 (0.2–3.9)	0.932
	Low	Ref		Reference		Ref	
Inflammation	Yes	1.1 (0.3–4.3)	0.937	2.0(0.9–4.4)	0.084	1.1 (0.3–4.3)	0.937
	No	Ref		Ref		Ref	
		Dietary factors					
Weekly fruit consumption	1-2	1.4 (0.8–2.4)	0.240	1.0 (0.6–1.7)	0.995	0.9 (0.5–1.9)	0.861
	3-4	0.4 (0.2–0.8)	**0.015**	1.1 (0.6–2.2)	0.783	0.7 (0.3–1.8)	0.471
	>4	Reference		Reference	—	Reference	—
Weakly meat consumption	1-2	1.2 (0.7–2.1)	0.430	1.1 (0.7–1.9)	0.661	1.3 (0.7–2.6)	0.436
	3-4	2.5 (1.1–5.6)	**0.026**	1.8 (0.9–3.6)	0.077	1.6 (0.7–3.8)	0.346
	>4	Reference		Reference	—	Reference	—
Weekly Plantain consumption	1-2	0.7 (0.4–1.2)	0.174	0.7 (0.2–1.2)	0.158	2.2 (1.0–4.8)	0.052
	3-4	2.5 (1.1–5.6)	**0.034**	0.5 (0.2–1.0)	0.057	2.9 (1.2–7.3)	**0.024**
	>4	Reference	—	Reference	—	Reference	

P values in bold are statistically significant.

in sick children seeking treatment in the hospital, our findings revealed that anaemia was more present in children within the community than at presentation to hospital. This may be due in part to the occurrence of submicroscopic *Plasmodium* infection that was not determined and the prevalence of malaria parasite observed in these apparently healthy children in the community. It is established that malaria causes haemolysis of both parasitized and healthy red cells leading to anaemia [13]. Also, in malaria parasite intense transmission regions, apparently healthy children may have subclinical infection even in the absence of overt disease. Effect of these subclinical infections are usually determined by measuring the concentration of acute phase proteins such as CRP involved in the inflammatory response. Inflammation being primarily protective [46] also causes the retention of iron by the reticuloendothelial macrophages leading to lower iron concentration in circulation and thus anaemia [47].

Parents' age, education level, occupation, and ethnicity as well as family size are sociodemographic factors that are intrinsically linked and significantly associated with childhood anaemia as reported in previous studies [38, 48, 49]. Parents who were ≤35 years old and those with no formal education had more anaemic children than the other parents. Being ≤25 years old may suggest that as first-time parents they are less prepared for parenthood and meeting the nutritional needs of the child. This may also be compounded by lack of knowledge on childcare and anaemia [50, 51]. Also, because a parent has little or no formal education, their chances of getting skilled jobs are also very reduced, leading to low wages and limited access to healthy food sources [52]. The odds of having anaemia was lesser in children in homes with >10 occupants. Contrary to studies where people living in or in proximity to forested areas have normal haemoglobin levels [53], findings from this study reveal that children with forest origin had the highest prevalence of anaemia. It may be that being away from their area of origin keeps them away from wild game and diverse forest foods which have been shown to improve iron intake and limit anaemia [54, 55].

Although not significant, observations from this study reveal that children who were feverish, were malaria parasite positive, and had moderate parasitaemia were more anaemic than their respective counterparts supporting findings from elsewhere [30, 56]. Fever is known as a characteristic symptom of malaria even though nonspecific where other infections are possible [57]. The fever may have been caused by immune responses to malaria or some other underlying disease.

Malnutrition affects all strata of the society but mostly children are more vulnerable because of high nutritional demands required for proper growth [58]. Anaemia was more common in malnourished children and was significantly higher in stunted (83.1%) and underweight (89.5%) children than their counterparts. This finding is in line with studies carried out in Bangladesh by Rahman et al. [59]. However, nutritional deficiencies may not always be directly linked to anaemia but could be the cause that weakens the general health of the children exposing them to other anaemia-causing diseases. This is supported by Khanam et al. [60].

Regarding microcytosis and microcytic anaemia, an overall 48.9% of the study population had microcytosis while microcytic anaemia prevalence was 36.7%. This proportion ranks it as a moderate public health concern according to WHO classification on anaemia burden [32]. This proportion of 36.7% is comparable to that observed in Tanzania [11] but lower than the 49.0% observed by Mah et al. [40] in children seeking treatment in the Yaounde Gynaeco-Obstetric and Paedriatric Hospital. Microcytosis is defined as a lower than normal MCV for age. It is indicative of iron deficiency, and iron deficiency is said to be responsible for half of all anaemia cases [61]. However, since we did not measure ferritin levels and other markers for iron status, we may have underestimated the true depiction of iron deficiency in the population.

Males had higher microcytic anaemia prevalence (70%) than females although this observation may not be a surprise since the males equally had higher prevalence of malaria and anaemia than females. Age-wise, we would have expected children 6–10 years old to have the highest prevalence of microcytic anaemia as with both malaria and anaemia but that was not the case. Instead, the ≤5 years old had significantly higher prevalence of microcytic anaemia than their counterparts. Microcytosis and anaemia each have varied aetiologies, the result of which in combination may lead to microcytic anaemia. The observed microcytic anaemia in this case may have been due to anaemia resulting from nutritional deficiency and not malaria, as children in this age have high iron needs for growth. Furthermore, microcytosis may have been due to the body's protective mechanism to wade off infection with malaria parasite [62], or resulting from some other anaemia-causing factor such as the thalassaemia trait, a common haemoglobinopathy in malaria endemic African populations. It may also result from bacterial, viral, or helminth infections which were not evaluated, as it was out of the scope of this investigation and thus a probable limitation of the study.

A significant association between occupation, ethnicity, and microcytic anaemia is like that observed with anaemia. Parents who were jobless, fishermen, and farmers had children with significantly higher prevalence of microcytic anaemia than their counterparts of other occupation. It is understandable that being jobless predisposes one to lower income and therefore inadequate financial resources to provide nutritious meals to meet the daily nutrient requirement of the household. However, children whose parents/guardians were fishermen and farmers were expected not to have microcytic anaemia because they have access to iron-rich food sources. Nevertheless, observations from this study, congruent to previous studies in this area [21, 34], show that many farmers do not consume the best of the foods they produce but rather sell them for money and consume the less presentable ones. Fish and vegetables are common sources of iron in the area which if consumed in the best state regularly alongside fruits will reduce the occurrence of anaemia. This reasserts reports from other studies [63, 64] that the low or nonconsumption of fruits, vegetables, and iron-rich animal sources increases risks for anaemia.

The overall low prevalence of malarial anaemia in this population (19.6%) reveals that malaria may not be a major contributor to the overall anaemia prevalence. This proportion is lower than the 27.7% previously observed in the same area [34]. Even though submicroscopic infection with *Plasmodium falciparum* infection has been associated with anaemia in other studies [65, 66] which was not investigated in the current study, the lack of association between malaria parasite density and anaemia likely highlights its limited contributions to the burden of anaemia in the study population. Of concern is the significant association of child's age, parent's age, education level, and occupation, with malarial anaemia in the regression analysis which may be directly linked to the influence of both malaria and anaemia. Febrility and malaria positivity influence on malarial anaemia prevalence cannot be overemphasized [21]. Nonetheless, fever, being a nonspecific symptom, may have also resulted from other infections which were not considered in this study.

While the findings of this study may be applicable in several areas in the region and elsewhere with similar environmental conditions and microclimates as well as cultural practices, there is a limitation in its applicability in areas nonendemic for malaria and having variable eating habits. However, the findings provide valuable information that could be used in the development of appropriate, context specific control measures against anaemia, microcytic anaemia, and malarial anaemia by public health authorities.

5. Conclusions

With anaemia prevalence still well above the cut-off value of 40%, it is important to reevaluate childhood determinants that foster its presence and also revisit the implementation strategies that have so far been put in place. An anaemia-monitoring system, especially those aimed at reducing nutritional anaemia, will effectively help in curbing anaemia. While microcytic anaemia is closely moving to the red zone as a public health problem in urban areas of Mount Cameroon, malarial anaemia is of mild public health concern. The high microcytic anaemia prevalence in children aged ≤5 years, those living at low altitude and within the community, shows that some insidious factors other than malaria are at work. While age, parent's/guardian's occupation and ethnicity, and moderate parasitaemia were risk factors for microcytic anaemia, malarial anaemia had as risk factors child's age, parent's age and occupation, and a ≤4 times weekly consumption of plantains. Therefore, when planning interventions to reduce anaemia prevalence in the community, strategies that will elevate the socioeconomic status of the parents should be integrated to aid better and healthy food choices to reduce anaemia due to nutritional deficiencies.

Data Availability

All the datasets generated and analysed during the current study are presented in the paper.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Anemia and Contributing Factors in Severely Malnourished Infants and Children Aged between 0 and 59 Months Admitted to the Treatment Centers of the Amhara Region, Ethiopia: A Multicenter Chart Review Study

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Background. Anemia among severely malnourished children is a double burden that could make the treatment outcome of severe acute malnutrition (SAM) more unfavorable. The burden and the factors are, however, uncovered among children in the Amhara region. Therefore, the study was aimed at determining the prevalence of anemia and identifying contributing factors in severely malnourished children aged between 0 and 59 months admitted to the treatment centers of the Amhara region referral hospitals. *Methods*. A facility-based cross-sectional study was conducted that included 1,301 infants and children, who developed SAM and were admitted to the three referral hospitals of the Amhara region. Data were extracted using a data extraction checklist. The binary logistic regression analysis was employed to show an association between the dependent and independent variables. Multicollinearity was assessed using the variance inflation factor (VIF) and no problem was detected (overall VIF = 1.67). The presence of association was declared based on the p-value (≤0.05), and the adjusted odds ratio with its respective 95% confidence interval was used to report the direction, as well as the strength of association. *Results*. About 41.43% (95% CI: 38.78%–44.13%) of severely malnourished infants and children have developed anemia, of which around half (47%) of them were under six months old. Rural residence (AOR = 1.56; 95% CI: 1.14–2.12) and HIV infection (AOR = 2.00; 95% CI: 1.04–3.86) were significantly associated with higher odds of anemia. Furthermore, being exclusively breastfed (AOR = 0.57; 95% CI 0.39–0.83) remarkably reduced the likelihood of anemia. *Conclusions*. This data confirms that anemia among severely malnourished infants and children is a public health problem in the Amhara region. Infants younger than six months were at a higher risk of anemia. Being a rural resident and contracting HIV infection have elevated the occurrence of anemia, whereas being exclusively breastfed decreased the risk. Therefore, the study gives an insight to policymakers and planners to strengthen the existing exclusive breastfeeding practice. Strategies being practiced to prevent HIV transmission and early detection, as well as treatment, should also be strengthened. Furthermore, mothers/caretakers of infants and children residing in the rural areas deserve special attention through delivering nutrition education.

1. Introduction

Anemia contributes to a wide range of health problems like heart failure, poor cognitive performance, and macro- and micronutrient deficiencies [1, 2]. The burden is the third top-ranked childhood cause of death, which accounted for 8.1% of the total mortalities [3]. Anemia is a hematological disorder that occurs in individuals of all ages though it is most prevalent and severe among reproductive-age women, children younger than five years, sick children, and children suffering from other nutritional deficiency disorders [4]. A sufficient hemoglobin concentration in the human blood is an important indicator of the availability of enough trace minerals, especially iron, as it is a precursor for the synthesis of hemoglobin, which is an important messenger that transports crucial nutrients and oxygen to different parts of the body [5]. A hemoglobin concentration below 11 g/dl is defined as anemia [6]. Anemia is associated with micronutrient deficiencies such as vitamin B-12 and B-9, as well as different infectious diseases that primarily attack the red blood cells [7, 8]. Likewise, iron deficiency shares 50% of all causes of anemia, and it is basically linked with poor dietary intake [9].

SAM and anemia have aninterply association [10, 11]. In other words, childhood anemia might occur as a result of macronutrient deficiency (particularly protein), or it precipitates the occurrence of undernutrition owing to the poor synthesis of macronutrients notably protein [9, 10, 12]. Worldwide, about 18.7 million children are severely malnourished and have prominent micronutrient deficiency, of which 18.5 million are from lower- and middle-income countries [13]. SAM is characterized by the presence of bilateral pitting edema and severe wasting (weight-for-height/length 70%/<−3 standard deviation) and mid upper arm circumference (MUAC) of below 11.5 cm (children older than 6 months) [14]. The concurrent occurrence of anemia and SAM makes the management process more complicated and escalates the likelihood of death. Anemia is indeed the commonest comorbid medical problem seen in children suffering from SAM and commonly referred to as "complicated SAM," which prolongs the recovery time and increases the likelihood of mortality compared with anemic children without SAM and nonanemic children with SAM [15–18]. Surprisingly, the prevalence of anemia among this population ranges from 81.1% to 95% [1, 15, 19]; and in Ethiopia, it is estimated to be between 16.4% and 61.3% [18, 20–22].

In cognizant of the observed nutritional problem among infants and children, Ethiopia is working on improving childhood nutrition through designing various strategies like 1000 days (conception time through to two years of age), Essential Nutrition Action (ENA), Seqota Declaration, and National Nutrition Program (NNP). However, the available studies are not sufficient and thus the role of repeated scientific investigations is significant to show the burden of the problem and contributing factors.

Therefore, a summarized figure that could reflect the burden of anemia and contributing factors among severely malnourished infants and children is imperative to pass a sound decision. In doing so, it would be possible to urge the responsible bodies to recognize the issue and exert appropriate actions to strengthen the strategies that are in place. Similarly, programmers may use this finding as an implementation evaluation. Moreover, the results of this study would stimulate clinicians to stringently follow infants and children suffering from SAM and apply appropriate interventions as early as possible.

2. Methods

2.1. Study Design, Period, and Setting. An institution-based cross-sectional study was conducted among infants and children who were hospitalized between October 2012 and September 2016 at inpatient SAM treatment centers of the Amhara region referral hospitals. Data were gathered from the three hospitals of the region: the University of Gondar, Debre Berhan, and Felege Hiwot referral hospitals. Clinicians working in these hospitals are recommended to follow the national treatment protocol while admitting and treating infants and children. Furthermore, these hospitals have separate inpatient treatment centers whereby severely malnourished children are admitted and receive appropriate nutritional and medical care.

2.2. Study Population and Sample. The study considered all severely malnourished infants and children who were admitted to the SAM inpatient treatment centers of the three aforementioned hospitals. Only children whose anemic status was diagnosed using a hemoglobin test at admission, or before starting the transition phase management, were recruited. This is because children who are at the transition phase obviously start to receive therapies like ready-to-use therapeutic feeding (RUTF), which has trace minerals and could affect the estimation. About 401, 373, and 527 participants were from Felege Hiwot, Debre Berhan, and University of Gondar referral hospitals, respectively, making a total sample size of 1,301.

2.3. Variables of the Study. The outcome variable was anemia and it was ascertained biochemically using hemoglobin status. Sociodemographic factors, such as the health facility's name, children's sex, children's age, and residence were included. Breastfeeding status, vaccination status, presence of diarrhea, presence of pneumonia, type of SAM, HIV status, antibiotic intake, folic acid supplementation, and vitamin A supplementation were the independent variables.

2.4. Anemia and Anthropometry Measurements. Infants and children whose hemoglobin concentration was below 11 g/dl were labeled as anemic [6]. Although the abovementioned criterion is for infants and children younger than six months, the same cut-off point was used for children older than six months, as there is no other standard for this group

of population and it is commonly applicable in the clinical set-up [23].

Infant's and children's nutritional status was measured using the national guideline for management of SAM. Accordingly, SAM was diagnosed as either the presence of severe wasting (weight-for-height/length 70%/<−3 SD) or bilateral pitting edema of both feet or MUAC of below 11.5 cm (for only children older than six months) [14, 24].

2.5. Breastfeeding and Immunization Status. Breastfeeding status was taken from the chart and children who were breastfed for the first six months without adding other foods other than the prescribed medicines were considered as exclusively breastfed [25]. Immunization status was explained as "unimmunized," "fully immunized," "unknown," and "incomplete," according to the World Health Organization (WHO) [26]. Children who did not receive any vaccine during their immunization period were considered "unimmunized," according to the evidence recorded in the medical recordings. Infants and children who had missed receiving at least one of the recommended vaccines were categorized as "incompletely immunized," whereas "fully vaccinated" was defined as infants and children who have completed and received the vaccine according to their age. Unknown vaccination status was declared when there was no vaccination history recorded in the medical record. However, for infants younger than 12 months, their immunization status was considered as "fully immunized" provided that they received age-appropriate vaccines, and it was considered "incomplete" if they missed one of the immunization schedules.

2.6. Clinical Forms of Malnutrition. The types of SAM were described using the clinical presentations[27]. Accordingly, infants and children who had no edema but had fat wastage and other supplementary clinical presentations diagnosed with marasmus. Infants and children who had edema, muscle wastage, and other clinical manifestations were diagnosed with kwashiorkor. Lastly, infants and children who had mixed forms of clinical presentations of the above-mentioned malnutrition forms were diagnosed as having both marasmus and kwashiorkor.

2.7. HIV and Tuberculosis Infection. The HIV infection status was considered using the confirmatory tests as per the national test algorism of the country. Infants and children aged under eighteen months had their HIV status examined using polymerization chain reaction (PCR) and those whose results were positive were assigned the status positive for HIV infection, whereas children older than eighteen months had their HIV status examined using any confirmatory antibody test and those whose results were positive were assigned the status positive for HIV infection [28]. Similarly, only confirmed tuberculosis (TB) infection was considered.

2.8. Data Collection Procedure, Quality Assurance, and Extraction Procedure. The data were collected using a data extraction sheet comprised of all independent and dependent variables. Prior to the commencement of the data collection, training of two days was given for two data collectors and one supervisor in each hospital aiming at briefing about the objectives of the study and what kind of data should be extracted. A pretest was performed in order to understand the variables that are available in the medical registering chart. Data reported the hemoglobin status analyzed through HemaCue-HB 201, and hematological analyzer machine was considered. The completeness of the data was checked on a daily basis. Finally, the data sets from the three hospitals were merged.

2.9. Data Processing and Analysis. The collected data were entered into Epi-data version 4.4.3.1 and exported to STATA version 14, and coding, cleaning, and analyses were done accordingly. All continuous independent variables were categorized. The outcome variable was dichotomized and coded as "0" and "1," representing not anemic and anemic, respectively. For continuous variables, such as age, the histogram was used to determine which measure of central tendency is appropriate. Descriptive statistics such as frequency, percentages, and measures of central tendency with their appropriate corresponding measure of dispersion were used. Tables and texts were used to present the findings.

Furthermore, the binary logistic regression analysis was applied to identify factors associated with anemia. Variables with a p value of less than 0.2 in the bivariable analysis were transferred to multivariable analysis to control the possible effects of confounder/s and identify the significant variables. Hosmer and Lemeshow goodness-of-fit test was used to examine the model adequacy, and it was insignificant (p value= 0.79).

The interaction of independent variables was checked by a multicollinearity test using the variance inflation factor (VIF), and no problem was detected (overall VIF = 1.67). Finally, the presence of an association between the independent and dependent variables and its direction and strength were established by the AOR with its corresponding 95% CI for variables with p-value <0.05. The difference in outcome among hospitals was checked by intraclass correlation (ICC), and no significant difference was observed. As a result, the model without considering variability was used.

2.10. Ethical Consideration. Ethical clearance was obtained from the ethical review committee of the three referral hospitals, and a permission letter was also obtained from the respective hospitals. As the study was conducted through a review of records, no consent was asked from the mothers, or caregivers of the study subjects. The confidentiality and privacy of the patient record was ensured by avoiding names and identification number from extraction form and using codes instead.

3. Results

3.1. Sociodemographic Characteristics of Children with SAM. The medical records of 1,301 children with SAM were reviewed. The median age of children was 16 months

(interquartile range (±IQR) of 9 to 24 months). Of all respondents, 527 (40.51%) were from the University of Gondar referral hospital. More than half (54.57%) of children were females and nearly two-thirds (64.43%) of them were from rural residence. A small proportion (6%), two-fifths (40%), and just over one-third (33.59%) of them were fully vaccinated, had developed diarrhea, and contracted pneumonia, respectively. One hundred seven (8.22%) and 54 (4.15%) children with SAM had TB and HIV, respectively. Moreover, just more than four of every ten (42.86%) children took antibiotics, while 65.82% were supplemented with folic acid (Table 1).

3.2. Prevalence of Anemia. The prevalence of anemia among under-five-year-old infants and children suffering from SAM was 41.43%, 95% CI: (38.78%, 44.13%). The magnitude varied throughout the age categories and a third of severely malnourished infants and children younger than six months were anemic: 36.15%, 95% CI: (27.92%, 45.04%). Furthermore, nearly equal proportion of them aged 6–23 months and ≥23 months had experienced anemia: 41.73% (95% CI: 38.42, 45.10) and 42.81% (95% CI: 37.19, 48.56), respectively.

3.3. Factors Associated with Anemia. In the bivariable logistic regression analysis, age, residence, exclusive breastfeeding, type of SAM, pneumonia, tuberculosis infection, HIV status, taking antibiotics, and supplemented vitamin A were entered into a multivariable logistic regression model. After the adjustment, residence, exclusive breastfeeding, and HIV status remained significantly associated with anemia.

The odds of anemia among rural dwellers was 56% higher than urban residents (AOR = 1.56; 95% CI: (1.14, 2.12)). Similarly, infants and children who had contracted HIV infection were two times higher to develop anemia compared with their counterparts (AOR = 2.00; 95% CI: (1.04–3.86)). The odds of anemia among exclusively breastfed children was decreased by 43%, compared with their nonexclusively breastfed counterparts (AOR = 0.57; 95%CI: (0.39, 0.83)) (Table 2).

Table 1: Sociodemographic characteristics of children with SAM in the Amhara region, northwest Ethiopia, 2016.

Variables	Frequency	Percentage
Location of health facility		
Felege Hiwot Referral Hospital	401	30.82
Debre Berhan Referral Hospital	373	28.67
Gondar Referral Hospital	527	40.51
Child's sex		
Male	591	45.43
Female	710	54.57
Child's age		
Under 6 months	130	9.99
6–23 months	865	66.49
2 years and above	306	23.52
Residence		
Rural	837	64.43
Urban	462	35.57
Exclusive breastfeeding		
No	241	18.52
Yes	1,060	81.48
Immunization status		
Unimmunized	90	6.92
Incompletely immunized	495	38.05
Fully immunized	81	6.23
Unknown	635	48.80
Diarrhea		
No	794	61.03
Yes	507	38.97
Pneumonia		
No	864	66.41
Yes	437	33.59
Type of SAM		
Marasmus	888	68.26
Kwashiorkor	273	20.98
Marasmus and kwashiorkor	140	10.76
Tuberculosis		
Yes	107	8.22
No	1,194	91.78
HIV status		
Positive	54	4.15
Negative	885	68.02
Unknown	362	27.82
Antibiotics		
Given	393	42.86
Not given	524	57.14
Folic acid		
Given	859	65.82
Not given	446	34.18
Vitamin A		
Given	763	83.30
Not given	153	16.70

4. Discussion

Malnutrition is a lingering and important public health problem, affecting the lives of multiple children and women in developing countries like Ethiopia. Anemia is an auxiliary nutritional problem that could interfere with recovery from illness. Carrying out studies among this disadvantaged population is imperative to understand the problem and take corrective measures accordingly. Hence, the aim of this study was to assess the prevalence of anemia and factors among under-five-year-old children with SAM in the Amhara region.

The prevalence of anemia among children with SAM aged between 0 and 59 months was 41.43% (38.78%–44.13%), which is a public health pressing problem requiring urgent attention of clinicians and policymakers. The finding is lower than that of a study from Vavuniya, Sri Lanka (55.5%) [29]. Sir Lanka's study was done in a single rural district (Vavuniya), in which more than 70% of children with SAM were from a socially deprived society. However ththe current study is mainly conducted in cities presumably wealthier although there was some possibility of incorporating referral cases from rural areas. Similarly, the finding of the study is lower than a study from Turbo, Columbia (51.1%) [30]. This could be due to the inclusion of children under six months old in the current study, who are at a lower risk of being anemic compared with children older than six months [31, 32].

Table 2: Factors associated with anemia among children with SAM aged 0–59 months in the Amhara regional state, northwest Ethiopia, 2016.

Variables	Anemia		COR (95% CI)	AOR (95% CI)	p value
	Yes	No			
Age					
<24 months	333	504	1	1	
≥24 months	205	257	1.21 (0.96, 1.52)	1.08 (0.81,1.45)	0.34
Residence					
Rural	300	372	1.51 (1.12, 2.04)	1.56 (1.14, 2.12)	0.031
Urban	89	167	1.	1	
Exclusively breastfed					
No	121	120	1	1	
Yes	418	642	0.65 (0.49, 0.86)	0.57 (0.39, 0.83)	0.02
Type of SAM					
Marasmus	334	554	1	1	
Kwashiorkor	140	133	1.75 (1.33, 2.29)	1.12 (0.76, 1.63)	0.43
Marasmus and kwashiorkor	65	75	1.44 (1.01, 2.06)	1.24 (0.81, 1.89)	0.31
Pneumonia					
Absent	371	493	1	1	
Present	168	269	0.83 (0.66, 1.05)	0.87 (0.64, 1.22)	0.11
Tuberculosis					
No	488	706	1	1	
Yes	51	56	1.32 (0.89, 1.96)	1.38 (0.87, 2.19)	0.21
HIV					
Negative	355	530	1	1	
Positive	31	23	2.01 (1.15, 3.51)	2.00 (1.04, 3.86)	0.01
Unknown	153	209	1.09 (0.85, 1.40)	1.03 (0.72, 1.46)	0.22
Antibiotics					
Given	181	212	1.31 (1.01, 1.72)	1.25 (0.93, 1.66)	0.35
Not given	206	318	1	1	
Vitamin A					
Not given	133	151	1	1	
Given	254	379	0.76 (0.57, 1.01)	0.86 (0.63, 1.19)	0.37

This finding is also lower than that of a study from Guinea-Bissau (80.2%) [1]. Guinea-Bissau's study was conducted in a rural area in which most children would have a greater possibility of working in agricultural areas which create a potential risk of acquiring soil-transmitted hookworm infections that could increase the risk of anemia [33].

As far as factors contributing to anemia is concerned, rural residence, being exclusively breastfed, and having HIV infection contributed to the development of anemia. The study highlighted that rural residence was associated with higher odds of anemia, depicting that those rural children are disadvantageous over their peers residing in an urban area in experiencing extra nutritional complications, including (but are not limited to) macro and micronutrient deficiencies that could lead to death [34]. Studies have shown that the sequelae of anemia are diversified like deficiencies of other important micronutrients and extra nutrition problems [35]. Consequently, these children could encounter poor neurodevelopment and are incapable of carrying out tasks demanding cognitive performance [2, 36]. This finding is supported by a multicenter study from Burkina Faso, Ghana, and Mali [37]. Furthermore, a study from Uganda showed that rural residence introduced a higher risk of anemia due to poor access to health services, including health education [38], which would help residents maintain their self and environmental hygiene. Additionally, the poor availability and accessibility of nutrient-rich food items in the area related to food insecurity and other attributes [39].

The current data suggest that exclusive breastfeeding decreases the odds of anemia. Alternatively, previous studies revealed that the duration of exclusive breastfeeding is one of the risk factors for the ongoing development of anemia [40]; the longer the duration of breastfeeding, the higher the risk of experiencing anemia. Likewise, existing literature speculated that exclusive breastfeeding contributes to developing anemia; it is known that as the child grows, the nutrient requirements become higher, and the infant iron storage acquired from the mother through the placenta become depleted, which might not be compensated by the breast milk only as it contains a small amount of iron [41, 42]. To that end, studies suggest that supplementing iron at this stage could limit the development of childhood anemia [43, 44]. Taking all the arguments in the current and the previous studies into consideration, further investigations that involve high-level studies are highly recommended.

Furthermore, HIV infection has doubled the odds of developing anemia. There are a wide range of mechanisms to how HIV infection could lead to anemia: it results in excessive RBC destruction and ineffective RBC production as a result of invading the hematopoiesis sites such as bone marrow [45–47]. The other mechanism is that the infection reduces the erythropoietin performance [48, 49]. Likewise,

some antiretroviral therapy (ART) drugs like zidovudine (AZT) usually cause anemia by interfering with the production of RBCs [50, 51]. The cell proliferation of organs in this segment of the population is less competitive compared with healthy counterparts. Furthermore, vitamin B12, an indispensable vitamin that supports the role of iron in the synthesis of hemoglobin, is deficient among HIV-infected individuals [52]. This vitamin deficiency is commonly seen in malnourished individuals secondary to gastric malfunction and the problem is a vicious cycle. Therefore, it is possible to draw an inference that HIV infection worsens childhood anemia among children with SAM because of the dual effects of poor RBC production and the deleterious effect of AZT. The study implies that apart from weakening the immunity and opening a great opportunity for other opportunistic infections, HIV infection jeopardizes the body's capability of generating RBCs and the production of hemoglobin. In addition, HIV infection shortens the lives of individuals, and it is notable that this infection would significantly reduce the survival of children with SAM. Thus, to limit the occurrence of opportunistic infection and facilitate the RBC production, which are both significant to prevent anemia, early treatment and prevention of HIV infection is recommended as usual.

Considering that multicentered sites of the region could reflect the strength of the study and thus could fortify the generalizability of the findings to the region. Similarly, as the data were gathered from different sites, the study considered a clustering effect. However, this study was based on secondary data; therefore, the study suffered from incomplete data, and as a result, some charts of the children were not considered.

5. Conclusions

The study suggests that just greater than a third of severely malnourished children aged between 0 and 59 months admitted to the treatment centers of the Amhara region referral hospitals have developed anemia, echoing a public health problem. Being a rural resident and having an HIV infection have elevated the occurrence of anemia, whereas exclusive breastfeeding reduced the likelihood. Therefore, it is valuable for policymakers and planners to strengthen the preventive strategies of HIV infection and give a special focus to rural residents. In addition, clinicians working in maternal and child health departments are recommended to strengthen the treatment of HIV infection before causing further damages. Although the current study has come up with evidence revealing the protective effect of exclusive breastfeeding on anemia, it contradicts the existing literature and it is quite impossible to draw a conclusion basing this study. Therefore, future scholars are recommended to conduct a study that helps solve the observed contradiction.

Abbreviation

AIDS: Acquired Immunodeficiency Syndrome
AOR: Adjusted odds ratio
COR: Crude Odds Ratio
CI: Confidence Interval
HIV: Human Immunodeficiency Virus
IQR: Interquartile Range
MUAC: Midupper Arm Circumference
SAM: Severe Acute Malnutrition
SD: Standard Deviation
TB: Tuberculosis
WHO: World Health Organization
WHZ: Weight-for-Height *Z* Score.

Data Availability

All the relevant data used to present the study are available; however, the corresponding author will supply the data upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Anaemia in the Hospitalized Elderly in Tanzania: Prevalence, Severity, and Micronutrient Deficiency Status

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Introduction. Anaemia is a common problem in sub-Saharan Africa. While most literature has focused on children, women of childbearing age, and pregnant women, data for the elderly population are relatively scarce. Anaemia exhorts negative consequences to functional ability of elderly patients, both physically and cognitively. The purpose of this study was to determine the prevalence of anaemia, severity, and micronutrient deficiency status in the elderly hospitalized patients in Tanzania. *Methods*. A total of 156 hospitalized adults aged 60 years and above were enrolled in this study. A structured questionnaire was used to capture sociodemographic and clinical characteristics. Blood samples were collected, and a complete blood count, serum cobalamin, serum ferritin, and serum folate levels were measured to assess anaemia and micronutrient deficiency status in all participants who had anaemia. *Results*. The prevalence of anaemia was 79.5% (124/156) with severe anaemia in 33.9% (42/124) of participants, moderate anaemia in 42.7% (53/124) of participants, and 23.4% (29/124) of all participants had mild anaemia. Micronutrient deficiency was found in 14.5% (18/124) of all participants with anaemia. Combined deficiency (either iron and vitamin B12 deficiency or iron and folate deficiency) was the most common micronutrient deficiency anaemia with a frequency of 33.3% (6/18), followed by isolated iron and folate deficiencies at equal frequency of 27.8% (5/18) and vitamin B12 deficiency at 11.1% (2/18). *Conclusion*. The prevalence of anaemia in the hospitalized elderly population is high warranting public health attention and mostly present in moderate and severe forms. Micro-nutrient deficiency anaemia is common in this age group and is mostly due to combined micronutrient deficiency.

1. Introduction

Anaemia is a condition that presents with a decrease in the population of red blood cells in the body [1]. The widely used method for establishing anaemia is through measurement of hemoglobin concentration in the blood [1, 2]. The World Health Organization (WHO) defines anaemia as hemoglobin of less than 13 g/dl in men and less than 12 g/dl in women [2, 3]. It further classifies the anaemia severity into mild, moderate, and severe based on the hemoglobin levels [2]. Whilst this definition is being applied for all populations, different studies argue that the data used excluded individuals above 65 years of age [4–6]. Nevertheless, in adults aged 60 years and above, anaemia has notable adverse consequences of impaired functionality, cognition, increased hospital admissions, and increased morbidity and mortality [7, 8]. The WHO estimates that roughly 24% of older adults (over 60 years of age) globally have anaemia [3]. In developed countries, the prevalence of anaemia in the elderly aged 60 years and above ranges between 3% and 63% with a higher prevalence found in those who are hospitalized or in nursing homes [5, 9–11]. Prevalence of anaemia in the elderly population is higher in developing countries, ranging between 20.6% and 49.5% in community based studies

[12–16] and those in nursing homes having a prevalence as high as 68.7 [17, 18]. A community survey done in Uganda in 2013 revealed 20% of adults aged over 50 years were anaemic [19]. There is generally a paucity of data for the prevalence of anaemia in hospitalized elderly from developing countries, particularly in Africa. Anaemia has been extensively studied in children, women of reproductive age, and pregnant women in Tanzania and found to be high and mostly attributable to nutritional causes [20–22]. However, to the best of our knowledge, there are no studies that have been done on anaemia in the elderly population in Tanzania.

2. Materials and Methods

2.1. Study Design. This was a hospital-based descriptive cross-sectional study which recruited patients aged 60 years and above admitted at Muhimbili National Hospital (MNH), between September 2015 and February 2016. MNH is the main national referral hospital receiving patients from all over Tanzania.

2.2. Participant Enrollment and Data Collection. Participants were enrolled to the study if they were 60 years or older, admitted at MNH, and consented to participate in the study. During the study period, 321 patients over the age of 60 were admitted. Of these, only 156 were eligible for inclusion in the study. Participants were excluded from the study if (1) they had received a blood transfusion seven days prior to their admission (27 participants); (2) they were on treatment for nutritional causes of anaemia such as oral iron, folate, or vitamin B_{12} (103 participants); or (3) they were not able to communicate (29 participants). Six participants did not give consent to participate in the study (Figure 1). The study was ethically approved by the Muhimbili University of Health and Allied Sciences (MUHAS) Research Ethics Committee, and written informed consent was obtained from all participants prior to enrollment. A structured questionnaire was filled for each participant, recording sociodemographics characteristics and clinical parameters.

2.3. Sample Collection and Laboratory Methods. Ten milliliters of venous blood was drawn for laboratory tests (haematology and biochemistry) from each participant within 24 hours of admission. Blood was collected into sterile vacutainers for haematological tests (EDTA anticoagulant) and biochemical tests (vacutainers without additives). Haematological tests were run at the Central Pathology Laboratory at MNH. Full blood counts were run on a 3700 CELL DYN machine. Serum vitamin B12 and serum ferritin tests were done on STAT FAX 303 ELISA SYSTEM, and serum folate levels were measured on ARCHITECT PLUSCI 4100 machine. The quantitative determination of serum ferritin was done using the DRG Ferritin ELISA assay (EIA 4292).

2.4. Definition of Key Terms. Anaemia was defined as hemoglobin levels of less than 13.0 g/dl in men and less than 12.0 g/dl in women, based on the WHO definition. Severity of anaemia was categorized based on the WHO classification, whereby mild anaemia was denoted by hemoglobin levels of 11 g/dl–11.9 g/dl in women and hemoglobin 11–12.9 g/dl in men. Moderate anaemia is denoted if hemoglobin levels were between 8 and 10.9 g/dl in both men and women and severe anaemia if hemoglobin levels were less than 8 g/dl in both men and women [3].

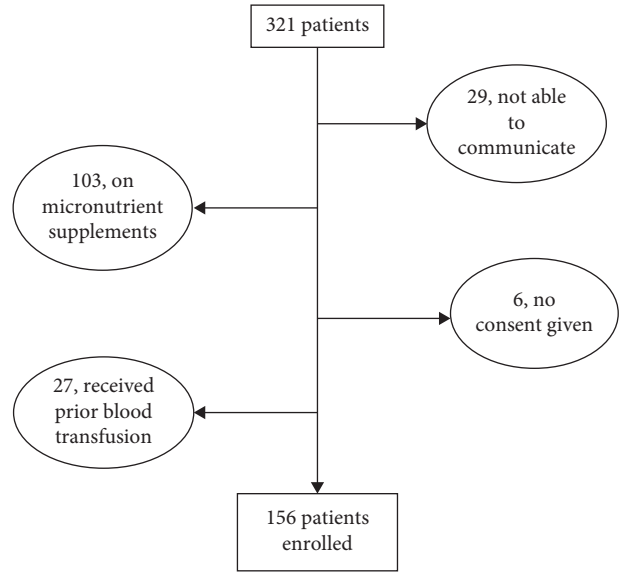

Figure 1: Enrollment Flow chart.

Iron deficiency anaemia was denoted by hemoglobin levels <12 g/dl in females and <13 g/dl in males accompanied with a serum ferritin of less than 30 μg/ml [23].

Vitamin B12 deficiency anaemia was considered in patients with hemoglobin levels <12 g/dl for women and <13 g/dl for men accompanied with a serum vitamin B12 of less than 148 pmol/L [24, 25].

Folate deficiency anaemia was defined as hemoglobin levels <12 g/dl in women and <13 g/dl in men accompanied with a serum folate <3 ng/ml [25].

Nutritional deficiency anaemia was considered in patients with hemoglobin levels <12 g/dl in women and <13 g/dl in males accompanied with either iron deficiency and folate deficiency or vitamin B12 deficiency [3].

2.5. Data Management and Statistical Analysis. Collected data were screened for quality, and coding was done prior to entering into R-studio statistical programs, which was used for analysis. Data analysis included calculation of means and standard deviations for numerical data which was normally distributed. Medians and interquartile ranges were computed for data which was not normally distributed. Categorical data were summarized by frequencies and proportions. Hypothesis testing was further undertaken using the Student's t test and chi-squared test for numerical and categorical variables, respectively. A p value <0.05 was considered statistically significant.

3. Results

3.1. Study Participants. The study enrolled 156 participants, of whom 95 (60.9%) were males. The median age was 66

years, with the oldest participant being 90 years old. More than two thirds were married, and half of them had no formal education. Overweight (BMI between 25.0 and <30) and obesity (BMI ≥30) were recorded in 44.8% of all participants, majority being males (71.4%) (Table 1).

3.2. Prevalence of Anaemia. The overall prevalence of anaemia was 79.5% (95% CI 72.5–85.1%) (Figure 2). There was no significant difference in proportion of males with anaemia and females with anaemia, 80% (95% CI = 70.9–86.8%) versus 78.7% (95% CI = 66.9–87.1%), respectively, (P value = 1). There was no evidence of a trend in proportion of anaemia between males and females within different age groups (<70 years, 70–79 years, and 80+ years).

3.3. Severity of Anaemia. Moderate anaemia was found in 53 (42.7%) anaemic participants. It was followed by severe anaemia in 42 (33.9%) and mild anaemia in 29 (23.4%) anaemic participants. Moderate anaemia was the most common type of anaemia among participants aged 60–69 years. For participants who were older than 80 years, severe anaemia was more common (Figure 3).

3.4. Nutritional Deficiency Anaemia. Among the participants with anaemia, a total of 18 (14.5%) had nutritional deficiency. Of those with nutritional deficiency, majority (6/18 (33.3%)) had combined deficiency (either iron and vitamin B12 deficiency (5/18) or iron and folate deficiencies (1/18)) (Figure 4).

4. Discussion

Despite the high prevalence of anaemia in different groups studied in Tanzania, data on the elderly population are scarce. Our study provides baseline data in a population of elderly hospitalized patients. The high prevalence (79.5%) of anaemia in the hospitalized elderly revealed in this study is similar to a study done in India in patients attending a geriatric clinic which reported the prevalence of anaemia in elderly to be 71% [26]. These findings are consistent with studies in both developed and developing countries which reveal a higher prevalence of anaemia in hospitalized elderly [27], in contrast to studies done in community elderly where the prevalence ranges between 10.6% and 23% [9, 12, 15, 16, 19, 28]. The higher prevalence in hospital-based studies is not surprising as it is well known that anaemia is a common finding in most disease states particularly if the condition is serious enough to require hospital admission. Developing countries that are still battling the high burden of communicable disease and the concomitant lower socioeconomic status present in these countries are expected to see more hospital admissions of the elderly and are likely to have a higher prevalence of anaemia in the elderly.

More than two thirds of participants in the present study presented with either moderate or severe anaemia. This is in contrary to findings observed from studies done in both community elderly and those in institutions (old age homes), where mild anaemia was the most common type of anaemia [18, 29, 30]. This may be attributed to the hospital-based nature of our study; our population already had relatively progressed illnesses that required admission. However, it may also be possible that there is already a significant proportion of the elderly population in our community that has anaemia in its milder forms. When they acquire conditions that force them to seek medical care, the anaemia would have worsened and thus present with a moderate anaemia or severe anaemia. It is important to also note that the diagnostic criteria used to define anaemia in the elderly in the present study is based on the WHO criterion which was extrapolated from epidemiologic data collected from those under the age of 65 years. It has been argued that these criteria may not be appropriate for the elderly population [5]. Studies conducted on healthy elderly individuals showed a decline in hemoglobin and red cell counts with increasing age in males [6]; it is therefore possible that what we are considering as anaemia in the elderly may in actual sense be the norm in this population. Studies performed on a larger data-base' with participants over 60 years of age are needed to develop a clearly defined diagnostic criteria of anaemia in the elderly. Community-based studies to determine the prevalence and severity of anaemia in healthy Tanzanian elderly population would also add value to what has been observed from this hospital-based study.

In our study, almost a quarter of all participants with anaemia were found to have a nutritional deficiency. Studies in developed countries have reported nutritional deficiency to be the most common cause of anaemia in the elderly (one third of all cases of anaemia) [23]. Although our study did not ascertain other causes of anaemia, we would expect similar findings in a country where nutritional deficiency plays a prime causal role in the development of anaemia in other population groups such as under-fives, adolescent girls, and pregnant women [20, 21, 31]. However, in the present study, the most frequent type of nutritional deficiency was combined deficiency anaemia (iron deficiency and vitamin B12 deficiency or iron deficiency and folate deficiency). Frequency was similar in isolated iron deficiency and isolated folate deficiency. This is contrary to findings from other studies where iron deficiency has dominated the picture in nutritional deficiency anaemia [32, 33]. In the developing world, however, the frequencies are highly variable. For instance, a study in India reported vitamin B12 deficiency as the most frequent cause of nutritional deficiency anaemia [15], whilst in Zimbabwe, folate deficiency was reported as the most frequent cause [34] and a study in Uganda reported iron deficiency as the most frequent cause of nutritional deficiency anaemia [19]. It is however important to interpret these findings in light of the fact that the cutoff levels for diagnosis of IDA in a geriatric hospitalized population in regions with high prevalence of infectious diseases have not been clearly established, an observation that was also previously made in a study by Mugisha et al. [19]. Furthermore, in the present study, only serum ferritin was used as an indicator of iron deficiency anaemia. Serum ferritin is an acute phase reactant whose levels have been

Table 1: Description of study participants.

	All (*N* (%))	Males (*N* = 95) (*N* (%))	Females (*N* = 61) (*N* (%))
Age (years)			
60–69	98 (62.8)	55 (56.1)	43 (43.9)
70–79	42 (26.9)	29 (69.0)	13 (31.0)
80+	16 (10.3)	11(69.8)	5 (31.2)
Marital status			
Married	110 (70.5)	76 (69.1)	34 (30.9)
Unmarried	46 (29.5)	19 (41.3)	27 (58.7)
Education level			
None/informal	24 (15.4)	9 (37.5)	15 (62.5)
Primary	54 (34.6)	35 (64.8)	19 (35.2)
Secondary	62 (39.7)	40 (64.5)	22 (35.5)
Higher learning	16 (10.3)	11 (68.75)	5 (31.25)
BMI (kg/m^2)			
Underweight	4 (2.6)	2 (50.0)	2 (50.0)
Normal	82 (52.6)	43 (52.4)	39 (47.6)
Overweight	64 (41.0)	45 (70.3)	19 (29.7)
Obese	6 (3.8)	5 (83.3)	1 (16.7)
Blood Pressure			
Normal	82 (52.6)	52 (63.4)	30 (36.6)
Prehypertensive	42 (26.9)	21 (50.0)	21 (50.0)
Stage I hypertension	27 (17.3)	19 (70.4)	8 (29.6)
Stage II hypertension	5 (3.2)	3 (60.0)	2 (40.0)

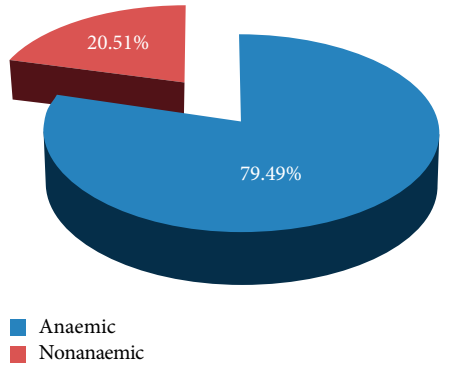

Figure 2: Prevalence of anaemia.

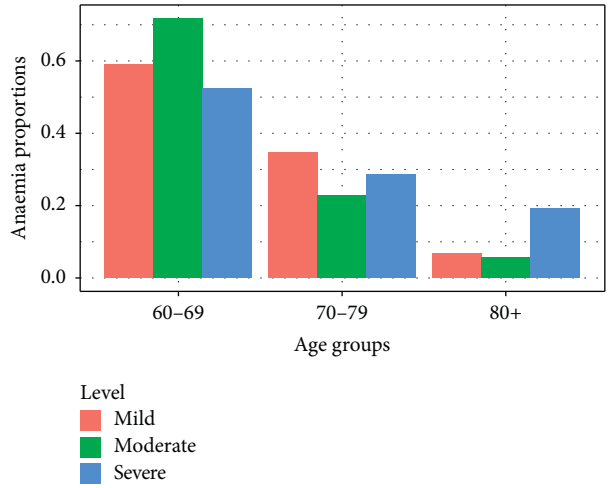

Figure 3: Severity of anaemia with age groups.

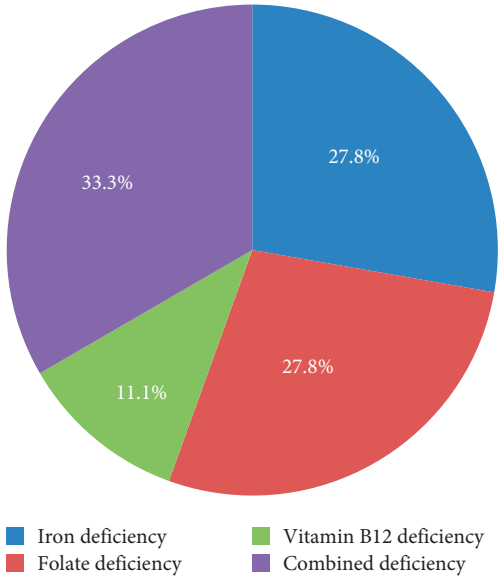

Figure 4: Proportion of nutritional deficiency anaemia, $N = 18$.

shown to increase with age and may be elevated in inflammatory conditions [35, 36]. Nevertheless, different socio-ultural practices (veganism, alcoholism, etc) may play roles in the variations observed, and further research is necessary to establish causal factors. A follow-up epidemiological study on causes of anaemia in the elderly in Tanzania would be of great value.

5. Conclusion

The prevalence of anaemia in the hospitalized elderly population in Tanzania is very high and mostly present in moderate and severe forms. Nutritional deficiency anaemia is common, accounting for a quarter of the diagnosed anaemia in the hospitalized elderly. Combined deficiency anaemia (either iron and vitamin B12 deficiency or iron and folate deficiency) is the leading subtype of micronutrient deficiency anaemia. Larger community-based studies are required to define criterion for the diagnosis of anaemia in older individuals and to establish the magnitude of anaemia in the elderly population. Furthermore, the high prevalence calls for a follow up study on the aetiological profile of anaemia in this population.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Establishment of Hematological Reference Values among Healthy Adults in Bamenda, North West Region of Cameroon

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The use of the reference range of values of a laboratory test is highly significant in diagnostic accuracy. However, race and ethnic variations may affect the safe use of reference ranges from a different setting/population. Because the establishment of reference ranges for the Cameroonian population will possibly improve the quality of health care, this study was designed to establish hematological reference ranges among healthy adults in Bamenda, North West region of Cameroon. This was a cross-sectional study carried out within the period of five months from February 2020 to June 2020, at the Bamenda Regional Hospital. A total of 350 (139 females and 211 males) study participants who met the inclusion criteria were included in the study. The Urit 3300 autoanalyzer (Urit Medical Electronic (Group) Co., Ltd, Guilin, China) was used to analyze the hematological parameters. The general health questionnaire for donors, for verification of reference range study and laboratory tests, was used for data collection. Descriptive statistics were used to calculate reference ranges, means, and medians at 95% confidence intervals. Maximum and minimum reference ranges were computed at 97.5th and 2.5th percentiles. The nonparametric test (Mann–Whitney test) was used to determine the significance of the difference in hematological values between the male and female groups. Three (MID%, LYM#, and MID#) out of the 19 hematological parameters were verified, while sixteen (WBC, LYM%, GRAN%, GRAN#, RBC, HGB, HCT%, MCV, MCH, MCHC, RDW_CV, RDW_SD, PLT, MPV, PDW, and PCT%) were established. The currently used reference intervals do not represent the population of the North West region. Therefore, other regional hospitals in Cameroon should establish reference intervals applicable to their respective regions.

1. Introduction

A reference range of values of a laboratory test is the basis for results interpretation and patient management; therefore, it is highly significant in diagnostic accuracy [1]. Due to variation in topographical, social, and health status, it is unsafe to use reference ranges from a different setting/population (race and ethnicity) [2]. Other causes of variation in reference ranges include body mass index, sex, age, genetics, altitude, and environmental factors like pathogens [2–4]. Studies have shown differences in hematological parameters among different populations [5–8]. For example, differences in normal hematological values have been reported between subjects with African and non-African lineage [6, 7]. Hence to ensure accurate diagnosis and clinical research the ethnicity of study participants should be considered [8]. In Africa, clinical trials of preventive interventions for particularly malaria, HIV, and tuberculosis are common. The use of inappropriate reference intervals for trial screening may cause excessive elimination of prospective participants. Cameroon has a population with diverse ethnic backgrounds and reference values among medical laboratories across the nation [9]. The establishment of reference ranges for the Cameroonian population will possibly improve the quality of health care and clinical trials. Moreover, the Clinical and Laboratory Standard Institute (CLSI) endorses the establishment of reference ranges by each laboratory [4]. Until now, no reference ranges have been established for the laboratory of the Bamenda Regional Hospital. Therefore, this study was designed to establish

hematological reference ranges among healthy adults in Bamenda, North West region of Cameroon.

2. Method

2.1. Study Site and Population. The study was carried out at the Bamenda Regional Hospital. Bamenda is situated at a height of about 1258 meters above the sea level, and it is located along 10.15 longitude and 5.96 latitudes. Bamenda being the regional headquarter for the North West region is one of the ten regional headquarters in Cameroon. The North West region has seven divisions: Boyo, Bui, Donga-Mantung, Mezam, Menchum, Momo, and Ngoketunjia divisions. There are many ethnic groups in the North West region, including immigrants from other regions and countries like Nigeria. The main ethnic groups of the North West regions are of Tikar origin, which includes the Tikari, Moghamo, Fulani, and Widikum [10]. Bamenda has an estimated 337,036 inhabitants [11].

2.2. Ethical Considerations. Ethical approval and written informed consent for this study were obtained from the Ethical Review Committee of the University of Bamenda (2020/0115H/UBa/IRB: 2020/0149H/UBa/IRB) and all the study participants, respectively.

2.3. Study Participants/Study Period. This was a cross-sectional study carried out within the period of five months from February 2020 to June 2020. According to the Clinical and Laboratory Standards Institute Guidance Document C28A2A [12], a minimum sample size of 300 (120 males and 120 females) was targeted for this study. The study participants were consecutively recruited from apparently healthy voluntary nonremunerated blood donors between the ages of 18 and 60 years, who gave their consent to be part of the study. A total of 350 (139 females and 211 males) study participants who met the inclusion criteria were included in the study.

2.4. Exclusion Criteria. All pregnant/lactating/menstruating women, those who were underweight (body mass index of ≤18.5), had lost ˃10% body weight in the past 6 months, had jaundice in the past 12 months, had a blood transfusion, sickle cell anemia, on any medication, and/or had an unexplained fever in the past 3 months were excluded from the study. Also, all those who tested positive for hepatitis B virus (HBV), hepatitis C virus (HCV), malaria (*Plasmodium falciparum* antigen histidine-rich protein 2 rapid diagnostic test), *Treponema pallidum* hemagglutination (TPHA), and human immunodeficiency virus 1/2 antibodies (HIV 1/2 antibodies) were excluded from the study.

2.5. Laboratory Analysis. Screening for confounding factors was performed for each study participant [12]. 5 mL of venous blood was collected into the K3EDTA test tubes. Using whole blood/serum, laboratory tests were carried out for the diagnosis of malaria (PfHRP2), HBV (HBsAg RDTs), HCV (anti-HCV antibody detection), TPHA, HIV 1/2 antibodies, and the determination of ABO blood group and genotype [13, 14].

2.6. Blood Cell Count Analysis. The validated hematology analyzer the Urit 3300 autoanalyser (Urit Medical Electronic (Group) Co., Ltd, Guilin, China) was used. Validation of the analyzer was done to confirm that the level and procedure of measurement is satisfactory and correct and that the calibration was appropriately done. The detection principle of the analyzer is electrical impedance (for WBC/RBC/PLT) and photoelectric colorimetry (for HGB). The hematology analyzer was validated by checking its performance characteristics, which was compared against the manufacture's claim as per the package insert. These characteristics included precision, accuracy, linearity, reportable range—analytical measurement range (AMR) and clinical reportable range (CRR), carryover, sensitivity, and specificity [4]. Following the manufacturer's instructions, the analyzer was calibrated and maintained, and appropriate internal quality controls were done daily.

2.7. Data Analysis. The general health questionnaire for donors for verification of reference range study was used to collect demographic data. Laboratory tests were done to collect data based on the analysis of hematological parameters and confounding factors. Hematological parameters for 40 participants were initially analyzed. For each parameter, if ˃10% of the values were out of the manufacturer's reference range, it was considered unverified [12]. If otherwise, hematological parameters were considered verified. Categorical data were presented as frequencies and percentages. Descriptive statistics were used to calculate reference ranges, means, and medians at 95% confidence intervals. Maximum and minimum reference ranges were computed at 97.5th and 2.5th percentiles. The nonparametric test (Mann–Whitney test) was used to determine the significance of the difference in hematological values between the male and female groups. GraphPad Prism version 9.0.1 and Microsoft Excel were used for the data analysis.

2.8. Definition of the Analyzed Hematological Parameters. WBC: white blood cell, LYM%: lymphocyte%, MID%: midsized cells (monocytes)%, GRAN%: granulocyte%, LYM#: lymphocyte#, MID#: midsized cells (monocytes)#, GRAN#: granulocyte#, RBC: red blood cell, HGB: hemoglobin, HCT%: hematocrit%, MCV: mean corpuscular volume, MCH: mean cell hemoglobin, MCHC: mean cell hemoglobin concentration, RDW_CV: red blood cell volume distribution width-CV, RDW_SD: red blood cell volume distribution width-SD, PLT: platelet, MPV: mean platelet volume, PDW: platelet distribution width, PCT%: plateletcrit%, CV: coefficient of variation, SD: standard deviation, #: number, and %: percentage.

3. Results and Discussion

The ≥18 to 30 years age group was the most (75.14%) represented, while the ˃50 to 60 years age group was the least represented. Most (43.71%) of the study participants were from Mezam Division. 55.14% of the study participants had the O blood group, and 96% were Rhesus positive. The male : female sex ratio of the study participants was 1.52 : 1 (Table 1).

For both the female and male groups, three (MID%, LYM#, and MID#) out of the 19 hematological parameters were verified. For the female group, the out-of-range percentage varied from 0% (MID#) to 95% (RDW_SD). However, the out-of-range percentage for the male group ranged from 0% (MID#) to 82.5% (MPV) (Table 2). The combined out-of-range percentage for the hematological parameters extended from 0% (MID#) to 95% (RDW_SD) for the current study but extended from 3.5% (RBC) to 46.7% (MCV) in Asmara, Eritrea [15]. For both sexes, group reference values were lower for WBC, GRAN%, GRAN#, MCV, and RDW_SD and higher for Lym%, RBC, HGB, MCHC, PLT, MPV, and PCT% parameters, compared to the manufacturer's reference values. The reference values for HCT% were higher for females. However, for males, the HCT% reference values exceeded both limits of the manufacturer's reference range. Similarly, in both sex groups, the reference values of MCH, RDW_CV, and PDW exceeded both limits of the manufacturer's reference range.

The hematological profile of the current study population differs from what was reported for the central province of Iran (Southwestern Asia) [16]. The median values of HGB, WBC, and GRAN% values were lower, but the median values of PLT and LYM% were higher, compared to that reported for Iran. It was established that compared to European-Americans, African-Americans have lower values of HGB, HCT%, RBC, PLT, MCV, WBC, GRAN#, and LYM % [6, 7]. Similar to the African-Americans, reference values for the current study were lower for WBC, GRAN#, and MCV, but contrarily, the values were higher for HGB, RBC, PLT, MCV, and LYM%, compared to the standard. However, compared to subjects from the USA, subjects from Southern and Eastern Africa had higher HCT%, HGB levels, WBC, and neutrophil counts [7]. Like in the current study, significantly lower MCV and MCH were reported among the Kenyan population [17]. Comparing the current results with that reported for Southern and Eastern Africa, although HGB and HCT% levels were also higher, WBC and GRAN# were rather lower. This indicates discrepancies among the reference values of African-Americans, Southern, and Eastern Africans and Cameroonians of the North West region. In Nigeria, based on locally established reference values, only 10% of study participants were classified abnormal compared to more than 40% who were classified by US DAIDS [18] and hence the need for locally established reference values.

Twelve out of the 19 (63.16%) hematological parameters were significantly different between the males and females (see Tables 3 and 4). The difference in three (RBC, HGB, and HCT%) out of the 12 (25%) parameters agrees with the differences indicated in the manufacturer's reference values. The median values of RBC, HGB, and HCT% were significantly higher in the male group. Similarly, the median values of RBC, HGB, and HCT% were significantly higher in the male group in a study carried out five years ago, in the same study area as the current study [19]. However, the difference in 9 (MID%, GRAN%, MID#, GRAN#, MCV, MCH, PLT, MPV, and PCT%) out of the 12 (75%) parameters disagree with the uniformity (among male and female values) indicated in the manufacturer's reference values. The median values of MID%, MID#, MCV, MCH, and MPV were significantly higher in the male group, whereas the median values of GRAN%, GRAN#, PLT, and PCT% were significantly higher in the female group. The median values of GRAN# and PLT were also significantly higher in the female group in the previous study of the same study area [19]. The median hemoglobin concentration was 15.2 g/L in men and 12.9 g/L in women ($p < 0.001$).

Studies have equally reported significantly higher values of reticulocyte counts, RBC counts, HGB, and HCT% in adult males compared to the females [1, 2, 9, 17, 20–22]. B12 deficiency and comorbidities were more common in elderly men [23], while in a population-based study among healthy Ugandan population, RBC counts, HGB, and HCT% were higher in adult males than females [2]. Similarly, RBC counts, HGB, and HCT% were higher in adult males than females in three regions in Ghana [20]. Median HGB concentration was equally higher among the men than the women in Yaounde, Cameroon [9]. HGB levels were lower, while platelet counts were higher in females among the healthy adult population in Kenya [17]. From many other studies, PLT counts were found to be higher across different female age groups, as compared to the males [1, 15, 17, 21]. Among normal Nigerian adults, platelets were found to be significantly higher among females than males [1]. Likewise in North West Ethiopia, platelets were found to be significantly higher among females than males [21]. In fact, in Asmara Eritrea, except platelet counts which was higher among the females, all the elevated hematological analytes were found to be higher in males [15]. This is also in line with findings from the Central Region of Cameroon [9]. Generally, the trend on the differences in hematological parameters between males and females is similar across most African countries.

WBC and neutrophil counts were also found to be significantly lower among black women when four indigenous groups based in the United Kingdom were studied [24]. In North West Ethiopia [21], Yaounde Cameroon [9], and in the current study (Bamenda Cameroon), WBC counts were higher among the females. Also, LYM#, NEUT#, and MID# counts were all higher among females in North West Ethiopia [21]. This is however contrary to what was reported in Asmara Eritrea, where values of all hematological analytes (except PLT count) were found to be higher in males than females [15] (see Table 5). Topographical and ethnic variances could have accounted for these differences.

There are many confounders when analyzing hematological parameters in the general population. Some

Table 1: Sociodemographic factors, blood group, and body mass index of the study participants.

Variables		Male (%)	Female (%)	Total (%)
Age (years)	≥18–30	142 (67.3)	121 (87.1)	263 (75.1)
	>30–40	50 (23.7)	17 (12.2)	67 (19.1)
	>40–50	14 (6.6)	0	14 (4)
	>50–60	5 (2.4)	1 (0.7)	6 (1.7)
Division of origin	Mezam	98 (46.5)	55 (39.6)	153 (43.7)
	Bui	24 (11.4)	31 (22.3)	55 (15.7)
	Momo	27 (12.8)	14 (10.1)	41 (11.7)
	Donga-Mantung	14 (6.6)	5 (3.4)	19 (5.4)
	Ngonketunjia	14 (6.6)	6 (4.3)	20 (5.7)
	Menchum	10 (4.7)	5 (3.6)	15 (4.3)
	Boyo	7 (3.3)	2 (1.4)	9 (2.6)
	Non-North west	17 (8.1)	21 (15.1)	38 (10.9)
Blood group	O	111 (52.6)	82 (59)	193 (55.1)
	A	46 (21.8)	28 (20.1)	74 (21.1)
	B	46 (21.8)	25 (18)	71 (20.3)
	AB	8 (3.8)	4 (2.9)	12 (3.4)
Rhesus factor	Positive	205 (97.2)	131 (94.2)	336 (96)
	Negative	6 (2.8)	8 (5.8)	14 (4)
Body mass index	Underweight (≤8.5)	0	0	0
	Normal weight (>8.5–24.99)	102 (48.3)	59 (42.5)	161 (46)
	Overweight (>24.99–29.99)	81 (38.4)	44 (31.7)	125 (35.7)
	Obesity (>29.99)	28 (13.3)	36 (25.9)	64 (18.3)
Total (%)		211 (60.3)	139 (39.7)	350

Sources: compiled by authors.

Table 2: Obtained reference intervals and manufacturer's reference intervals of hematological parameters.

Parameter	Unit	RI for males, $N = 211$	Manufacturer's RI for males	Out of range (%)	RI for females, $N = 139$	Manufacturer's RI for females	Out of range (%)
WBC	$10^3/\mu L$	2.86–8.54	4–10	18	3.0–10	4–10	30
LYM%	%	27.89–62.42	20–40	73	27.2–62.38	20–40	75
MID%	%	6–17.58	1–15	5*	5.05–13.42	1–15	10*
GRAN%	%	29.09–59.31	50–70	77.5	31.02–68.43	50–70	65
LYM#	$10^3/\mu L$	1.2–4.5	1–4.1	2.5*	1.04–4.86	1–4.1	7.5*
MID#	$10^3/\mu L$	0.2–1.1	0.1–1.8	0*	0.2–1.3	0.1–1.8	0*
GRAN#	$10^3/\mu L$	1.2–4.4	2–7.8	37.5	1.2–5.2	2–7.8	30
RBC	$10^6/\mu L$	4.4–7.0	4–5	80	3.6–7.3	3.5–5	25
HGB	g/dL	12.1–18.2	12–16	17.5	8.8–19.24	11–15	22.5
HCT%	%	35.1–51.69	42–49	45	25.1–55.6	37–43	67.5
MCV	fL	63.21–89.81	82–92	57.5	57.41–90.1	82–92	50
MCH	pg	22.4–31.9	27–31	30	20.09–33.46	27–31	50
MCHC	g/dL	32.1–37.9	32–36	17.5	31.79–38.66	32–36	22.5
RDW_CV	%	10.4–15.4	11.5–14.5	37.5	10.6–16.51	11.5–14.5	25
RDW_SD	fL	24.8–43.7	37–54	80	24.17–44.3	37–54	95
PLT	$10^3/\mu L$	104.4–338.8	100–300	12.5	102–656.9	100–300	37.5
MPV	fL	8.33–14.1	7.4–10.4	82.5	8.1–14.78	7.4–10.4	82.5
PDW	fL	9.07–17.04	10.0–14	37.5	8.6–16.96	10–14	42.5
PCT%	%	0.11–0.39	0.1–0.28	30	0.1–0.76	0.1–0.28	47.5

Sources: compiled by authors. *Out of range percentage for verified hematological parameter.

inconsistencies in the values of hematological parameters may be caused by confounding factors such as cold agglutinin disease [25, 26]. For example, initial hemogram test results were significantly affected by cold agglutinin disease, causing incorrect results [26]. However, those with cold agglutinin disease-associated symptoms like jaundice were excluded from the current study. Seasonal variation was also demonstrated in the levels of white blood cell counts and neutrophils in a large population-based study [27]. And even after having adjusted for possible confounders, higher depression and anxiety were shown to cause increase RDW and WBC levels [28]. Plasma markers of inflammation were also shown to have a strong independent association with increased levels of RDW [29]. WBC levels in the current study were rather generally lower than the manufacturer's reference range. Although, the neutrophil-lymphocyte ratio

Table 3: Calculated hematological (RBC and RBC indices) reference intervals for the screened population.

Parameter (unit)	Sex	*N*	Mean	95% CI for mean	Median	95% CI for median	Range	2.5th–97.5th %	*P* value (gender)
RBC ($10^6/\mu$L)	Female	139	4.74	4.61–4.87	4.68	4.56–4.77	3.23–8.77	3.57–7.23	<0.0001*
	Male	211	5.4	5.33–5.48	5.35	5.3–5.43	3.91–7.31	4.41–6.99	
HGB (g/dL)	Female	139	12.94	12.62–13.26	12.9	12.6–13.1	7.6–20	8.8–19.2	<0.0001*
	Male	211	15.34	14.99–15.69	15.2	15–15.4	11.5–47.4	12.13–18.15	
HCT% (%)	Female	139	39.92	35.85–37.99	36.6	35.6–37.3	33.3–69.6	25.1–55.6	<0.0001*
	Male	211	43.39	42.85–43.93	43.3	42.4–44	32.7–56.4	35.1–51.69	
MCV (fL)	Female	139	78.2	76.96–79.45	78.8	78–80.3	54.5–92	57.45–90.1	0.0073*
	Male	211	80.23	79.29–81.17	80.2	79.7–82.4	35.4–93.4	63.21–89.81	
MCH (pg)	Female	139	27.41	26.96–27.87	27.6	27.3–28	19.5–35.2	20.1–33.45	0.0030*
	Male	211	28.14	27.84–28.43	28.2	28–28.7	20.3–34.6	22.38–31.92	
MCHC (g/dL)	Combined	350	35.24	34.92–35.39	35.2	35–35.2	28.5–85.1	32–38.65	0.4309
	Female	139	35.2	34.95–35.54	35.2	35–35.4	28.5–42	31.79–39.53	
	Male	211	35.23	34.73–35.74	35.1	34.8–35.2	31.5–85.1	32.09–37.92	
RDW_CV (%)	Combined	350	12.7	12.54–12.85	12.45	12.3–12.6	10–19.3	10.5–15.82	0.4467
	Female	139	12.85	12.57–13.13	12.4	12.3–12.8	10.2–19.3	10.6–16.5	
	Male	211	12.6	12.42–12.77	12.5	12.2–12.7	10–17.3	10.43–15.37	
RDW_SD (fL)	Combined	350	33.31	32.71–33.9	33.9	33–34.7	22.2–50.6	24.8–44.3	0.6062
	Female	139	33.15	32.16–34.15	33.9	29.7–34.8	23.1–45.4	24.2–44.3	
	Male	211	33.41	32.66–34.16	33.9	33–34.7	22.2–50.6	24.8–43.68	

Sources: compiled by authors. *Significant *P* values.

Table 4: Calculated hematological (WBC and platelet) reference interval for the screened population.

Parameter (unit)	Sex	*N*	Mean	95% CI for mean	Median	95% CI for median	Range	2.5th–97.5th %	*P* value (gender)
WBC ($10^3/\mu$L)	Combined	350	5.29	5.13–5.45	5	4.9–5.2	1.7–12.6	3–9.42	0.0877
	Female	139	5.47	5.18–5.77	5.1	4.9–5.5	1.7–12.6	3.3–10.25	
	Male	211	5.17	4.98–5.36	4.9	4.8–5.2	2.6–10.8	2.86–8.54	
LYM% (%)	Combined	350	44.15	43.24–45.06	43.95	42.7–44.8	4.5–70.4	27.59–61.66	0.3727
	Female	139	43.63	42.19–45.07	43.55	42.1–44.8	14.9–70.4	27.2–62.38	
	Male	211	44.53	43.35–45.71	44.4	42.7–45.5	4.5–64.8	27.89–62.42	
MID% (%)	Female	139	8.33	7.98–8.69	8.1	7.8–8.4	0.2–16.9	5.05–13.42	<0.0001*
	Male	211	10.17	9.75–10.58	9.6	9-10	4.3–24.4	6–17.58	
GRAN% (%)	Female	139	48.03	46.51–49.54	47.6	46.8–49	15.7–78.2	31.02–68.43	0.0034*
	Male	211	45.02	43.94–46.1	45.2	43.5–47.3	13.1–70.2	29.09–59.31	
LYM# ($10^3/\mu$L)	Combined	350	2.35	2.26–2.43	2.2	2.1–2.3	0.5–5.6	1.2–4.7	0.4327
	Female	139	2.39	2.24–2.54	2.2	2.1–2.3	0.5–5.5	1.04–4.86	
	Male	211	2.32	2.21–2.42	2.1	2.1-2.2	0.9–5.6	1.2–4.48	
MID# ($10^3/\mu$L)	Female	139	0.46	0.43–0.5	0.4	0.4-0.4	0.2–1.4	0.2–1.3	0.0007*
	Male	211	0.52	0.49–0.55	0.5	0.4-0.5	0.2–1.3	0.2–1.07	
GRAN# ($10^3/\mu$L)	Female	139	2.6	2.45–2.76	2.4	2.3–2.5	0.7–5.8	1.2–5.2	0.0034*
	Male	211	2.33	2.23–2.43	2.2	2.1–2.3	1.8–5.1	1.2–4.37	
PLT ($10^3/\mu$L)	Combined	139	294	274.8–313.2	281	263–307	64–870	102–656	<0.0001*
	Female	211	222.8	214.8–230.9	224	217–231	29–453	104.4–338.8	
	Male								
MPV (fL)	Combined	139	10.99	10.67–11.32	11	10.6–11.8	7.7–15.6	8.1–14.75	<0.0001*
	Female	211	11.98	11.65–12.31	12.5	12.2–12.6	7.8–36.6	8.33–14.1	
	Male								
PDW (fL)	Combined	350	12.78	12.56–13.01	12.6	12.2–13.3	6.8–23	8.91–16.81	0.0623
	Female	139	12.59	12.2–12.99	12.2	11.8–14	8.2–23	8.6–16.95	
	Male	211	12.91	12.64–13.18	12.9	12.2–13.3	6.8–18.3	9.09–16.99	
PCT% (%)	Female	139	0.31	0.29–0.33	0.29	0.27–0.31	0.09–0.93	0.11–0.75	<0.0001*
	Male	211	0.27	0.24–0.31	0.26	0.25–0.28	0.02–3.6	0.11–0.39	

Sources: compiled by authors. *Significant *P* values.

Table 5: Comparison of obtained reference intervals and reference intervals in Yaounde and other African countries.

Parameter (unit)	Sex	Obtained RI	Man.'s RI	Out of range (%)	Bamenda Cameroon (2015)	Yaounde Cameroon	Eritrea	Tanzania	Ghana	Ethiopia
RBC ($10^6/\mu$L)	Female	3.57–7.23	3.5–5	25	4.12–5.48	3.4–5.3	4–5.7	3.84–5.59	3.09–5.3	3.45–6.25
	Male	4.41–6.99	4-5	80	4.42–6.13	4–5.9	4.2–6.07	4.41–6.27	3.79–5.96	3.53–6.93
HGB (g/dL)	Female	8.8–19.2	11–15	22.5	10.9–14.5	9.89–13.7	12.5–17.6	11.1–15.7	8.8–14.4	11–16.7
	Male	12.13–18.15	12–16	17.5	12.4–16.4	11–16	12.6–17.8	13.7–17.7	11.3–16.4	15.3–18
HCT% (%)	Female	25.1–55.6	37–43	67.5	32.8–44.2	29.7–42	37.9–52	36.2–46.8	26.4–45	32.1–56.6
	Male	35.1–51.69	42–49	45	37–49.8	34.6–47.61	40.5–55	40.2–53.7	33.2–50.2	36.2–58.6
MCV (fL)	Combined	—	82–92	—	—	—	—	—	—	85–100
	Female	57.45–90.1	—	50	71.6–92.7	72–96	85.5–100	77.7–97.9	73–96	—
	Male	63.21–89.81	—	57.5	68.2–93.3	70–97	85.7–100	76.4–98.8	70–98	—
MCH (pg)	Combined	—	27–31	—	—	—	—	—	—	—
	Female	20.1–33.45	—	50	23.1–30.5	23.22–31.2	26.5–32.6	24.2–33.1	22.3–33.6	25.8–32.8
	Male	22.38–31.92	—	30	22.4–31.6	22.8–33.4	28–33	23.1–33.2	22.7–33.5	26.6–33.3
MCHC (g/dL)	Combined	—	32–36	—	—	—	—	—	—	—
	Female	31.79–39.53	—	22.5	31.2–34.4	30–34.2	30–33.7	30.4–34.8	30.4–36.5	28.5–34.4
	Male	32.09–37.92	—	17.5	31.8–34.6	29.9–35.3	30.4–33.7	30.6–35.1	22.3–33.6	29.5–34.4
WBC ($10^3/\mu$L)	Combined	3–9.4	4–10	—	3.2–8.3	—	—	—	—	3.2–8.8
	Female	3.3–10.25	—	30	3.6–8.3	2.8–6.7	3.3–8.9	3.2–8	3.4–9.3	—
	Male	2.86–8.54	—	18	3.0–8.2	2.6–6.81	3.7–9.3	2.8–7.9	3.5–9.2	—
PLT ($10^3/\mu$L)	Combined	102.6–459.5	100–300	—	142–354	—	—	—	—	
	Female	102–656	—	37.5	148–367	143–369	145.4–351.6	151–425	89–403	128–432
	Male	104.4–338.8	—	12.5	140–346	133–339	128.4–318.4	147–356	88–352	

Sources: compiled by authors and [9, 15, 19]. Man.'s RI = manufacturer's reference interval.

is an inflammatory marker for major adverse cardiac events [30], due to stringent exclusion criteria set for the current study, participants with major adverse cardiac events could not have been enrolled into the study.

3.1. Limitations. The study was conducted using blood donors. Although blood donors are generally considered as healthy, they are prone to developing iron deficiency, especially in repeat donors with insufficient iron intake. Although iron deficiency does not necessarily translate into deviations of hematology parameters, the possibility cannot be excluded either.

4. Conclusion

Only three (MID%, LYM#, and MID#) out of the 19 hematological parameters considered in this study were verified. Sixteen (WBC, LYM%, GRAN%, GRAN#, RBC, HGB, HCT%, MCV, MCH, MCHC, RDW_CV, RDW_SD, PLT, MPV, PDW, and PCT%) hematological parameters have been established, demonstrating that the currently used reference intervals do not represent the population. Based on the commendation by the Clinical and Laboratory Standard Institute (CLSI) that reference ranges be established by each laboratory, published reference intervals (literature and manufacturer) should not be used without local verification. Therefore, to ensure adequate clinical care and perhaps successful subsequent clinical trials in Cameroon, further studies to establish hematological parameters for other regions in Cameroon are recommended.

Data Availability

The data used to support the findings of this study are available from the corresponding author on request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Factors Associated with Anemia among Pregnant Women of Underprivileged Ethnic Groups Attending Antenatal Care at Provincial Level Hospital of Province 2, Nepal

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Background. This study aims at determining the factors associated with anemia among pregnant women of underprivileged ethnic groups attending antenatal care at the provincial level hospital of Province 2. *Methods*. A hospital-based cross-sectional study was carried out in Janakpur Provincial Hospital of Province 2, Southern Nepal. 287 pregnant women from underprivileged ethnic groups attending antenatal care were selected and interviewed. Face-to-face interviews using a structured questionnaire were undertaken. Anemia status was assessed based on hemoglobin levels determined at the hospital's laboratory. Bivariate and multiple logistic regression analyses were used to identify the factors associated with anemia. Analyses were performed using IBM SPSS version 23 software. *Results*. The overall anemia prevalence in the study population was 66.9% (95% CI, 61.1–72.3). The women from most underprivileged ethnic groups (Terai Dalit, Terai Janajati, and Muslims) were twice more likely to be anemic than Madhesi women. Similarly, women having education lower than secondary level were about 3 times more likely to be anemic compared to those with secondary level or higher education. Women who had not completed four antenatal visits were twice more likely to be anemic than those completing all four visits. The odds of anemia were three times higher among pregnant women who had not taken deworming medication compared to their counterparts. Furthermore, women with inadequate dietary diversity were four times more likely to be anemic compared to women having adequate dietary diversity. *Conclusions*. The prevalence of anemia is a severe public health problem among pregnant women of underprivileged ethnic groups in Province 2. Being Dalit, Janajati, and Muslim, having lower education, less frequent antenatal visits, not receiving deworming medication, and having inadequate dietary diversity are found to be the significant factors. The present study highlights the need of improving the frequency of antenatal visits and coverage of deworming program in ethnic populations. Furthermore, promoting a dietary diversity at the household level would help lower the prevalence of anemia. The study findings also imply that the nutrition interventions to control anemia must target and reach pregnant women from the most-marginalized ethnic groups and those with lower education.

1. Background

Anemia, a condition with low blood hemoglobin concentration and/or red blood cells (RBCs), is a global public health problem that mostly affects low- and middle-income countries (LMICs) [1]. Anemia in pregnancy poses a greater risk for low birth weight, preterm birth, and perinatal and neonatal mortality [2]. Besides, the severity of anemia is associated with higher rates of maternal mortality [3]. Anemia affects over half a billion women of reproductive age worldwide. It is estimated to affect 38% (32.4 million) of pregnant women globally with highest prevalence in the World Health Organization (WHO) regions of South-East Asia (48.7%) and Africa (46.3%) [4]. Evidence from various

low- and middle-income countries suggests that anemia is disproportionately concentrated in the low socioeconomic group [5] with poorest, ethnically disadvantaged, and least educated at the greatest risk.

In Nepal, 41 percent of reproductive age women are anemic with highest prevalence in Province 2 (58%) [6]. The anemia prevalence in women is disproportionately higher among Terai Dalit (so-called untouchable Terai inhabitants), Terai Janajati (indigenous Terai people), Terai/Madhesi Brahmin/Chhetri (upper caste Terai inhabitants), and Muslims [7]. These ethnic groups constitute over three-fourths of the population in Province 2 [8]. Yet, they are historically marginalized and have performed poorly as measured by indicators of poverty, health, nutrition, education, and women's empowerment. The utilization of health services by Terai Dalit, Terai Janajati, Muslim, and Madhesi is consistently poor for the past many years [7]. Furthermore, studies have also shown that Terai women are more likely to be anemic than women in other regions [9–11].

Reducing anemia prevalence among women and children has been a longstanding priority for Nepal. The Government of Nepal is determined to meet various global and national targets for anemia reduction based on its commitments towards National Strategy for Control of Anemia among Women and Children in Nepal 2002, Multi-Sectoral Nutrition Plan II (2018–2022), Sustainable Development Goals (SDG) 2030, and World Health Assembly Global Nutrition Targets of achieving 50% reduction in anemia among women of reproductive age by 2025. In this context, the Ministry of Health and Population has made many remarkable efforts to reduce anemia in women through activities such as universal, daily iron-folic acid (IFA) supplementation to pregnant and lactating women, deworming program to reduce the burden of parasitic infections, and mandatory fortification of wheat flour with iron, folic acid, and vitamin A [12, 13]. Yet, anemia is still a public health problem in Nepal. This is more challenging as women from the poorest and marginalized groups are the most affected [7].

The 2030 agenda for sustainable development has urged countries to place special emphasis on those left furthest behind and the most excluded with a strong focus on leaving no one behind. This agenda also requires the country to reach its goals and targets for all people and all segments of the society [14]. The national strategy for reaching the unreached (2016–2030) has also clearly highlighted that the reduction of health and nutrition inequalities and achievement of a universal health coverage in the country can only be realized if unreached populations are systematically targeted [15]. In this context, nutrition programs, actions, and strategies are necessary that target the most vulnerable communities bearing a disproportionate burden of anemia [11]. This warrants an appropriate investigation among marginalized ethnic groups and those furthest behind. Although a wide range of research studies in Nepal have attempted to examine the factors associated with anemia, majority of these analyses are specifically limited to the general population such as women, children, and adolescents. Disaggregated analysis involving key subpopulations particularly the ethnic groups which are underprivileged and have greater vulnerability to anemia has not yet been available for Nepal. It is against this background that this study was conducted. The study aimed at assessing the factors associated with anemia among pregnant women of underprivileged ethnic groups who attended antenatal care at the provincial level hospital of Province 2. A better understanding of the local determinants of anemia is considered crucial to identify and implement evidence-based and contextually appropriate strategies [5].

2. Methods

2.1. Study Design and Settings. This was a hospital-based cross-sectional study conducted at Janakpur Provincial Hospital, located at the Janakpurdham, the capital of Province 2, Nepal. Province 2, one of the seven provinces, consists of eight districts that extend in the southeastern flat plains (Terai region) of Nepal. Despite its ecological richness, Province 2 fares poorly in various socioeconomic and health indicators including but not limited to literacy, teenage pregnancy, nutrition, contraceptive use, immunization coverage, and exposure to domestic violence. As per the Nepal Demographic Health Survey, the prevalence of anemia among women of reproductive age was reported the highest in Province 2 [6]. Janakpur Provincial Hospital is the largest referral level public hospital in Province 2 offering a wide range of healthcare services including antenatal, maternal, and newborn care. This hospital receives patients and clients largely from Dhanusha and surrounding four districts (Siraha, Mahaottari, Sarlahi, and some parts of Sindhuli).

2.2. Study Population. This study was carried out among pregnant women who attended antenatal care (ANC) in the Janakpur Provincial Hospital. Women in the second and third trimester of pregnancy and belonging to the underprivileged ethnic groups were included in the study. However, women with severe obstetric complications and those with the history of intake of steroidal drugs were excluded from the study. The underprivileged groups in this study constituted of Terai Dalit, Terai Janajati, Muslim, and Madhesi. These groups have historically suffered oppression, discrimination, and social-segregation and are politically, economically, and socially backward [16, 17]. They are often unable to enjoy social services and facilities and face significant inequalities in the utilization of health care [7, 18]. Terai Dalits are ascribed the lowest position in the caste-ethnicity hierarchical structure and represent the most depressed category among all ethnic groups. They have suffered from acute landlessness and caste-based discrimination, including untouchability [16, 19]. Madhesi caste group grips a relatively better advantage as compared to the other three groups [20].

2.3. Study Design and Sampling Procedure. The sample size was calculated using Epi Info StatCalc software assuming 95% level of confidence, 0.06 margin of error, and 57.8%

anemia prevalence among reproductive age women of Province 2 [6]. A minimum sample of 261 was estimated and it was increased to 287 considering the nonresponse rate of 10%. The study participants attending antenatal care between 10 am and 4 pm were consecutively enrolled until the planned sample size was achieved.

2.4. Data Collection. A structured questionnaire was developed based on the study objectives. The standard food and dietary recall questionnaire developed by Food and Nutrition Technical Assistance (FANTA) Project was used to assess the dietary diversity status [21]. The questionnaire was divided into four broad sections: sociodemographic information, preventive health practices, dietary practices, and hemoglobin level. Data collection was carried out between November and December 2017 using a face-to-face interview with the pregnant women at the antenatal care (ANC). Interviews were conducted in a separate room after the participants received their antenatal services. The interview was administered by the first author who could speak both Nepali and Maithili (local) languages. In order to determine the status of anemia, blood was drawn from each participant with the help of a certified lab technician. The blood samples were collected and tested in the laboratory of Janakpur Provincial Hospital. The collected blood samples were checked for hemoglobin level using the cyanmethemoglobin method.

3. Measurement of Variables

3.1. Anemia. The pregnant women were considered anemic if they had a hemoglobin concentration less than 11.0 g/dl [22]. Anemia was further categorized as mild (hemoglobin = 10.0–10.9 g/dl), moderate (hemoglobin = 7.0–9.9 g/dl), and severe (hemoglobin <7.0 g/dl) [22].

3.2. Dietary Diversity Status. This was a dichotomous indicator of whether or not women have consumed at least five out of ten defined food groups within 24 hours. In order to determine this, we used a 24-hour dietary recall questionnaire gathering information on all foods and beverages consumed by the participants in the previous day and night. The foods consumed were aggregated into 10 recommended food groups: starchy staples, pulses (beans, peas, and lentils), and nuts and seeds; dairy; meat, poultry, and fish; eggs and dark green leafy vegetables; vitamin A-rich fruits and vegetables; other vegetables; and other fruits. For each food group the pregnant women consumed from, a score of 1 was provided and 0 otherwise. The scores from all ten food groups were added to obtain the total dietary diversity score ranging from 0 to 10. The dietary diversity score thus obtained was categorized into 2 groups to derive a dietary diversity status for pregnant women. The dietary diversity score of five or more was considered adequate (coded as 1), and the score below five was inadequate (coded as 0) [21, 23].

3.3. Ethnicity. A caste/ethnicity classification used by the Health Management Information System (HMIS) of the Ministry of Health and Population, Nepal, was adapted for this study. This system uses six caste groups: Dalits, Janajati, Muslim, Madhesi, Brahmin/Chhetri, and others, of which the first four groups were taken as they are considered belonging to the underprivileged groups. Among Dalit and Janajati, only Terai Dalits and Terai Janajati were present during the period of data collection.

3.4. Data Management and Analysis. The data were entered into EpiData Entry version 3.1 and then transferred into IBM SPSS version 23 software for analyses. In the first stage, descriptive analyses were performed. Frequency tables with percentage were generated for categorical variables, while mean and standard deviation (SD) were calculated for continuous variables. In the next stage, bivariate and multivariate analyses were performed to determine the factors associated with anemia. Variables that were significant at 15% significance level in bivariate analyses using Pearson's chi-square test were entered into a multiple logistic regression model [24]. Before performing the regression analysis, a test of multicollinearity was done. One of the variables "gravida" showed multicollinearity (variance inflation factor (VIF) >5) and was excluded from the analysis. Model fit was measured with the Hosmer and Lemeshow goodness-of-fit test; the model was found to be a good fit with $p > 0.05$. Odds ratios (OR) were presented with their corresponding 95% confidence intervals (CIs), and a probability value (p value) of less than 0.05 was considered statistically significant.

3.5. Ethical Considerations. The study protocol was approved by the Institutional Review Committee of the Institute of Medicine, Tribhuvan University (Reference no. 46(6–11E)2/074/075). The study was fully abided by the ethical guidelines of Nepal Health Research Council. Approval to conduct this study was also obtained from Janakpur Provincial Hospital (previously known as Janakpur Zonal Hospital). All participants were above the age of 18. A written informed consent (in Nepali language) was obtained from the participants before the interview and blood sample collection. In case of illiterate participants, the consent form was read out and thumb impressions were obtained in the presence of a literate witness. The participation in the study was voluntary, and no incentives were provided. Also, the participants did not have to pay any charges for laboratory tests. Furthermore, the study participants had the right to withdraw from the study at any time. Personal identifier was omitted in the questionnaire to maintain anonymity and to ensure confidentiality of information.

4. Results

4.1. Characteristics of the Study Population. The age of the women ranged from 18 to 37 years. The mean age of the respondents was 22.6 years (SD = 3.9 years). Majority of the women were in the age group 20–24 years (45.6%), were

Madhesi (65.2%), and followed Hinduism (90.2%). Only one-fourth of pregnant women (27.2%) had completed their secondary level education. Greater majority of the pregnant women lived in joint and extended families (81.5%) and were homemaker (89.9%). At the time of the interview, 41.5 and 58.5 percent were in their second and third trimesters of pregnancy, respectively. More than half (53.7%) of the pregnant women were multigravida. Among the multigravida women, more than one-fourth (26.6%) had a birth interval of more than two years. About one in ten (9.4%) pregnant women had a history of miscarriage/abortion (Table 1).

4.2. Preventive Health Practices. In our study, only about a fourth of pregnant women (28.8%) had their four ANC visits completed. More than four in five women had consumed iron-folic acid (88.1%) and deworming medicine (81.8%). All pregnant women (100%) used mosquito net while sleeping (Table 2).

4.3. Dietary Practices. The starchy staple foods were consumed by all pregnant women (100%). A greater majority of the women consumed pulses (97.6%), dairy products (90.9%), and other vegetables (84.0%). About half of the respondents consumed other fruits (52.6%) while less than half consumed dark green leafy vegetables (44.3%), meat, poultry, and fish (30.3%), and eggs (15.7%). One-fifth (21.9%) consumed other vitamin A-rich fruits and vegetables while only one-tenth (11.5%) consumed nuts and seeds. More than two-thirds of the respondents (69.3%) had adequate dietary diversity as they consumed at least five of the ten food groups. More than 1 in 10 women (15.7%) avoided certain food groups in pregnancy for cultural reasons (Table 3).

4.4. Prevalence of Anemia. The overall prevalence of anemia (hemoglobin <11.0 g/dl) was 66.9% (95% CI, 60.3%–71.2%). In terms of severity, mild anemia was 64.8% (95% CI, 59.0%–70.3%), moderate anemia was 1.7% (95% CI, 0.57%–4.02%), and one woman was severely anemic (Table 4).

4.5. Factors Associated with Anemia. The anemia status of pregnant women was compared with sociodemographic characteristics, preventive health practices, and dietary diversity status. In the bivariate analyses, a statistically significant association was found with ethnicity ($p < 0.01$), religion ($p = 0.008$), education ($p < 0.001$), place of residence ($p = 0.019$), gravida ($p = 0.006$), frequency of antenatal visits ($p < 0.001$), consumption of iron-folic acid ($p = 0.032$) and deworming medicines ($p = 0.001$), and dietary diversity status ($p < 0.001$). However, the association was not statistically significant for age, occupation, family type, pregnancy trimester, birth interval, history of miscarriage/abortion, and practice of food avoidance (Table 5).

The regression analysis showed that the anemia status in pregnant women was significantly associated with ethnicity, education, history of consumption of deworming medicines, frequency of antenatal visits, and dietary diversity status. The odds of anemia were higher among pregnant women of underprivileged ethnic group and those with lesser

Table 1: Sociodemographic characteristics of the study population ($n = 287$).

Variables	Number	Percent
Age group (years)[a]		
≤19	64	22.3
20–24	131	45.6
25–29	66	23.0
≥30	26	9.1
Ethnicity		
Madhesi	187	65.2
Terai Dalit	55	19.2
Muslim	29	10.1
Terai Janajati	16	5.5
Religion		
Hindu	259	90.2
Muslim	28	9.8
Educational status of the respondent		
Below secondary level	209	72.8
Secondary level and above	78	27.2
Family type		
Joint/extended	234	81.5
Nuclear	53	18.5
Occupation		
Homemaker	258	89.9
Employed (job, business, and labor)	29	10.1
Place of residence		
Urban	214	74.6
Rural	73	25.4
Pregnancy trimester at interview		
Second	119	41.5
Third	168	58.5
Gravida		
Primigravida	133	46.3
Multigravida	154	53.7
Birth interval ($n = 154$)		
≤2 years	113	73.4
>2 years	41	26.6
History of miscarriage/abortion		
No	260	90.6
Yes	27	9.4

[a]Mean ± SD = 22.6 ± 3.9.

Table 2: Preventive health practices ($n = 287$).

Variables	Number	Percent
Number of antenatal visits		
<4 times	206	71.8
≥4 times	81	28.2
Consumed iron-folic acid		
Yes	252	87.8
No	35	12.2
Consumed deworming medicine		
Yes	234	81.5
No	53	18.5
Used mosquito net while sleeping		
Yes	287	100

Table 3: Dietary practices of the pregnant women ($n = 287$).

Variables	Number	Percent[a]
Food groups		
Starchy staples (grains, white roots, and tubers)	287	100.0
Pulses (beans, peas, and lentils)	280	97.6
Nuts and seeds	33	11.5
Dairy	261	90.9
Meat, poultry, and fish	87	30.3
Eggs	45	15.7
Dark green leafy vegetables	127	44.3
Other vitamin A-rich fruits and vegetables	63	21.9
Other vegetables	241	84.0
Other fruits	151	52.6
Dietary diversity		
Adequate	199	69.3
Inadequate	88	30.7
Food avoidance during pregnancy		
Yes	45	15.7
No	242	84.3

[a]The total adds of percentage are more than 100% as the pregnant women consumed food items from multiple food groups.

Table 4: Prevalence of anemia among pregnant women ($n = 287$).

Variables	Number	Percentage	95% CI
Anemia	192	66.9	61.1–72.3
Severity of anemia			
Mild anemia	186	64.8	59.0–70.3
Moderate anemia	5	1.7	0.57–4.02
Severe anemia	1	0.3	—

education. The underprivileged women such as Terai Dalit, Janajati, and Muslims were 2 times more likely to be anemic (AOR, 2.34; 95% CI, 1.06–5.17) compared to Madhesis. Similarly, those with education below secondary level were about 3 times more likely to be anemic (AOR, 2.87; 95% CI, 1.52–5.45) than those having at least secondary level education. It was also observed that the pregnant women who did not complete their four antenatal visits were two times more likely to be anemic (AOR, 2.28; 95% CI, 1.23–4.22) than their counterparts who had completed all four visits. Pregnant women who had not consumed deworming medicines were three times more likely to be anemic (AOR, 3.03; 95% CI, 1.20–7.65) that those who had consumed the tablets. Furthermore, women who did not have adequate dietary diversity were four times more likely to be anemic (AOR, 4.25; 95% CI, 1.81–9.98) compared to women having adequate diversity in their diet (Table 6).

5. Discussion

In the present study, two in three pregnant women (66.9%) were anemic, signifying a severe public health problem [25]. This figure is more than two times higher than the prevalence reported among pregnant women from midwestern Nepal (28.3%) [26]. The anemia prevalence in our study is also higher than both the national (41%) and provincial estimates (58%) for the women of reproductive age [6]. The discrepancies in the prevalence might be due to hospital-based study setting, inclusion of only the women from underprivileged ethnic groups, different study periods, and regional variations in the socioeconomic status and dietary practices. Being a referral level hospital, it is also possible that some of the pregnant women attending ANC at the study hospital were referred from peripheral health facilities after being suspected for pregnancy-related complications including anemia. However, such information was not explored in this study. The anemia prevalence in our study was comparable with the study conducted among pregnant women in a similar setting [27]. The PoSHAN community studies' baseline report had also reported similar findings among the pregnant women in the Terai region [28].

In our study, the factors associated with anemia among underprivileged pregnant women were ethnicity, education, intake of deworming medication, antenatal visits, and dietary diversity status. Based on the available evidence from Nepal, it is clear that pregnant women from underprivileged or disadvantaged ethnic groups are more likely to be anemic compared to the upper caste groups. Our study further revealed that the burden of anemia is unevenly distributed even within the underprivileged ethnic groups. The odds of anemia in pregnancy were two times higher among the most-marginalized groups (Terai Dalit, Terai Janajati, and Muslims) compared to Madhesi women. This finding is quite obvious as Terai Dalits and Muslims occupy the lowest position in the human development and poverty indices [29, 30] and suffer caste-based discriminations much higher than other groups. The underlying structural factors such as poor literacy, caste-based discriminations, lesser autonomy in decision making, and other cultural restrictions might have predisposed these women towards greater anemia risks. Furthermore, both the per capita consumption expenditure and land ownership are reported to be lowest among the Terai Dalit caste groups [31], suggesting their limited capacity to consume adequate and diverse foods. This finding warrants the need for special targeting to women from the most disadvantaged ethnic groups with nutritional programs and interventions in order to "leave no one behind" as pledged in the 2030 agenda [14].

Our study found significantly higher odds of anemia among pregnant women with lower education levels. Women who had education below the secondary level were about three times more likely to be anemic than others. This is consistent with the findings of similar studies where anemia was inversely associated with maternal education [32–35]. This is perhaps due to the benefits associated with education. For example, higher education can contribute to higher productivity and earnings which in turn might have positive influences on women's dietary practices. Studies have also documented the positive impact of educational attainment on the quality of women's diet [36]. Women's education might also be associated with women's autonomy and empowerment. Autonomous women are likely to obtain more information and make better decisions regarding their nutrition, improve healthcare seeking, and influence intrahousehold food distribution [37, 38]. Benefits of completing secondary education on the nutritional status of women must therefore be recognized.

Table 5: Association of anemia status with various characteristics.

Sociodemographic characteristics, preventive health practices, and dietary diversity status	Anemic no. (%) ($n = 192$)	Nonanemic no. (%) ($n = 95$)	p value
Age group			
≤19	45 (23.4)	19 (20.0)	0.297
20–24	82 (42.7)	49 (51.6)	
25–29	44 (22.9)	22 (23.2)	
≥30	21 (10.9)	5 (5.3)	
Ethnicity			
Terai Dalit/Janajati/Muslim	86 (44.8)	15 (14.7)	<0.001[a]
Madhesi	106 (55.2)	81 (85.3)	
Religion			
Hindu	167 (87.0)	92 (96.8)	0.008
Muslim	25 (13.0)	3 (3.2)	
Educational status			
Below secondary level	160 (83.3)	49 (51.6)	<0.001[a]
Secondary level and above	32 (16.7)	46 (48.4)	
Family type			
Nuclear	34 (17.7)	19 (20.0)	0.638
Joint/extended	158 (82.3)	76 (80.0)	
Occupational status			
Homemaker	177 (92.2)	81 (85.3)	0.067
Employed (job, business, and labor)	15 (7.8)	14 (14.7)	
Place of residence			
Urban	135 (70.3)	16 (16.8)	0.019[a]
Rural	57 (29.7)	79 (83.2)	
Pregnancy trimester at interview			
Second	85 (44.3)	34 (35.8)	0.127
Third	107 (55.7)	61 (64.2)	
Gravida			
Multigravida	114 (59.4)	40 (45.0)	0.006[a]
Primigravida	78 (40.6)	55 (57.9)	
Birth interval ($n = 154$)			
≤2 years	88 (77.2)	25 (62.5)	0.70
>2 years	26 (22.8)	15 (37.5)	
History of miscarriage/abortion			
No	175 (91.1)	89 (89.5)	0.648
Yes	17 (8.9)	11 (10.5)	
Number of antenatal visits			
<4 times	153 (79.7)	53 (55.8)	<0.001[a]
≥4 times	39 (20.3)	42 (44.2)	
Consumption of iron-folic acid			
Yes	163 (84.9)	89 (93.7)	0.032[a]
No	29 (15.1)	6 (6.3)	
Consumption of deworming medicine			
Yes	146 (76.0)	88 (92.6)	0.001[a]
No	46 (24.0)	7 (7.4)	
Food avoidance during pregnancy			
Yes	31 (16.1)	14 (14.7)	0.757
No	161 (83.9)	81 (85.3)	
Dietary diversity			
Inadequate	80 (41.7)	8 (8.4)	<0.001[a]
Adequate	112 (58.3)	87 (91.6)	

[a]Statistically significant at $p < 0.05$.

Low dietary diversity significantly predicted anemia in our study population. Pregnant women who did not have adequate diversity in their diet were four times more likely to be anemic compared to women whose dietary diversity was adequate. A number of previous studies from Nepal and other LMICs have also confirmed the association of low dietary diversity with anemia [39–42]. The role of dietary diversity in ensuring the adequate hematological status of

Table 6: Multivariate analysis of factors associated with anemia in pregnancy.

Variable	Adjusted OR	95% CI
Ethnicity		
Terai Dalit/Janajati/Muslim	2.34	1.06–5.17[a]
Madhesi	1	1
Religion		
Muslim	1.59	0.38–6.68
Hindu	1	1
Educational status		
Below secondary level	2.87	1.52–5.45[a]
Secondary level and higher	1	1
Occupational status		
Homemaker	1.75	0.65–4.73
Employed (job, business, and labor)	1	1
Place of residence		
Rural	1.12	0.54–2.32
Urban	1	1
Frequency of antenatal visits		
<4 times	2.28	1.23–4.22[a]
≥4 times	1	1
Consumption of iron-folic acid tablets		
No	1.71	0.62–4.73
Yes	1	1
Consumption of deworming medicines		
No	3.03	1.20–7.65[a]
Yes	1	1
Dietary diversity		
Inadequate	4.25	1.81–9.98[a]
Adequate	1	1

[a]Statistically significant at $p < 0.05$.

pregnant women and thus its contribution in reducing the likelihood of anemia has been well documented [43]. Nonetheless, the anemia prevalence in our study was higher although more than two-thirds of pregnant women were found to have an adequate dietary diversity. This might be because although the pregnant women reported consuming items from diverse food groups, they might have been consumed in low frequency and small portion sizes [28, 44–46]. Women often suffer the most from inequitable intrahousehold food distribution and are often the last in the household serving order [44–46]. Furthermore, one study conducted among Tharu and Musahar populations (indigenous groups of Terai) revealed that the diet in Terai is dominated by rice (cereals) with only tiny portions of side dishes such as lentils and vegetables which are insufficient to meet the recommended dietary allowance for micronutrients [47]. Although green vegetables and other diverse foods are readily grown and available in the Terai region, people do not regard them as food items that should be consumed in large amounts [47]. This highlights the need for strong targeted behavior change communication (BCC) intervention that promotes not only dietary diversity but also the nutritional value of locally available foods and need for optimal intake of diets from each group (dietary adequacy). Additionally, the possibility that lower dietary diversity in our study population might have been influenced by household food insecurity cannot be undermined. Results of national survey data found significantly higher odds of experiencing household food insecurity by Dalit women [48]. The interventions to reduce micronutrient deficiency in the region should therefore aim to address dietary diversity, dietary adequacy, and food security targeting the ethnically disadvantaged populations.

Evidence shows that low coverage of deworming medications during pregnancy results in increased parasitic infections [49, 50] and is associated with higher rates of anemia in pregnant women. Not having a deworming medication was one of the associated factors among our study population. Respondents who had not taken deworming medicines in their current pregnancy had three times higher odds of anemia than their counterparts. This is comparable with the evidence from previous studies [41, 51, 52]. Further in our study, fewer ANC visits were associated with increased chances of anemia among the study population. This can be explained by the fact that late and infrequent ANC visits might deny women or delay the provision of iron-folic acid supplementation, deworming medication, and/or malaria prophylaxis whereas women who seek ANC frequently are more likely to benefit from counseling and advices concerning nutrition, preventive health behaviors, and healthful dietary practices. Our finding is consistent with previous studies which have reported an association between infrequent ANC visits and anemia [32, 41, 53]. Promoting the coverage and frequency of antenatal visits is therefore considered vital to reducing anemia in pregnant women.

There are some notable limitations which must be taken into account while interpreting the results of the study. First, the cross-sectional nature of our study makes it difficult to establish the temporal relationships. Second, the assessment of dietary diversity status was based on 24-hour dietary recall, which may not always represent the actual intake. Moreover, the possibility of recall bias also cannot be ruled out completely. Third, our prevalence estimate was based on participants enrolled in the hospital-based setting, which may differ from that of community-based studies. Lastly, it is important to recognize that underprivileged ethnic groups from hill/mountain such as hill Dalit and hill Janajati could not be represented in this study as they were not present at ANC during the data collection period.

6. Conclusions

The prevalence of anemia is a severe public health problem among pregnant women of underprivileged ethnic groups in Province 2. Being Dalit, Janajati, and Muslim, having lower education, less frequent antenatal visits, not receiving deworming medication, and having inadequate dietary diversity are found to be the significant factors. The present study highlights the dire need of improving the frequency of antenatal visits and coverage of deworming program in ethnic populations. Furthermore, promoting a dietary diversity at the household level would help lower the prevalence of anemia. The study findings also imply that the nutrition interventions to control anemia must target and

reach pregnant women from the most-marginalized ethnic groups and those with lower education.

Abbreviations

ANC: Antenatal care
OR: Odds ratio
SD: Standard deviation
SPSS: Statistical Package for Social Sciences
WHO: World Health Organization.

Data Availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Yadav UK. contributed to the design of the study and collection of data. Ghimire P. performed the statistical analyses, drafted the manuscript, and led the writing of the paper. Both Amatya A and Lamichhane A. contributed to the conception and design of the study and provided important critical revisions in the manuscript. Ghimire P. has the primary responsibility for the final content. All authors read and approved the final manuscript.

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Research Article

Prevalence of Anaemia and Its Associated Factors among Type 2 Diabetes Mellitus Patients in University of Gondar Comprehensive Specialized Hospital

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Background. Anaemia is one of the commonest blood disorders seen in patients with diabetes. In Ethiopia, chronic illnesses are tremendously raising with their complications. But very little research has been conducted, particularly on anaemia among diabetes mellitus (DM) patients. Therefore, this study aimed at assessing the prevalence of anaemia and associated factors among type 2 diabetes mellitus patients in Northwest Ethiopia. *Methods*. A cross-sectional study design was employed at University of Gondar Comprehensive Specialized Hospital from March 1 to April 15, 2019, among 372 type 2 diabetes mellitus patients (T2DM). Multivariable logistic regression analysis was fitted, and the corresponding adjusted odds ratio (AOR) and 95% CI were used to identify factors associated with anaemia. Level of significance was declared at the p value less than 0.05. *Results*. The study revealed 8.06% (95% CI: 5.68–11.31%) of the participants were anaemic. Being male (AOR = 2.74, CI: 1.02, 7.38), combined type of treatment (AOR = 8.38, CI: 1.66, 42.25), having diabetes-related microvascular complications (AOR = 3.24, CI: 1.14, 9.26), and hypertension (AOR = 0.01, CI: 0.002, 0.06) were the significant factors associated with anaemia. *Conclusions*. The finding of the current study revealed low prevalence of anaemia among T2DM patients. Sex, type of treatment, diabetes-related microvascular complications, and hypertension were factors associated with anaemia. Assessment of haemoglobin levels among T2DM patients may help to prevent ensuing microvascular complications. Incorporate anaemia screening into the routine assessment of diabetic complication particularly for those who are hypertensive and took combined treatment to allow early appreciation and treatment of anaemia and later improve the overall care of patients with diabetes.

1. Introduction

Anaemia is a condition in which the number of healthy red blood cells is lower than normal in the body and/or lower than the normal amount of haemoglobin in the red blood cells [1–4]. Anaemia is an indicator of both poor nutrition and poor health [5].

Anaemia is a serious global public health problem that affects populations in both rich and poor countries. Globally, anaemia affects 1.62 billion people which correspond to 24.8% of the population [6]. It occurs at all stages of the life cycle but is more prevalent in pregnant women (40%) and young children (42%) [2, 6].

In Ethiopia, 57% of children aged 6–59 months suffered from some degree of anaemia. Twenty-five percent of children are classified with mild anaemia, 29% with moderate anaemia, and 3% with severe anaemia. Twenty-four percent and fifteen percent of women and men in Ethiopia are anaemic, respectively. Eighteen percent of women are classified as mildly anaemic, 5% moderately anaemic, and 1% severely anaemic [7].

There is considerable evidence that anaemia exacerbates severity and impairs the outcome of peripheral small vessel disease in diabetic patients [8]. Anaemia can make certain complications (diabetic neuropathy, diabetic nephropathy, and diabetic retinopathy) more likely to occur, and it can

worsen the kidney, heart, and artery diseases, which are more common in people with diabetes [8, 9].

There is a significant association between haemoglobin concentration and fasting blood glucose. High incidence of anaemia is likely to occur in patients with poorly controlled diabetes and in patients with diabetes and renal insufficiency [10]. Some studies have shown that reduced erythropoietin production and anaemia happen earlier in people with diabetes and kidney disease than in those with kidney disease and no diabetes [1].

Anaemia in DM patients is a common condition, but it often goes unrecognized and not treated. Its symptoms are vague and easily mistaken for symptoms of other serious or chronic diseases. But even mild anaemia can significantly lower one's quality of life, and untreated anaemia can have serious long-term health effects [1, 11].

Even though several studies have been conducted in different parts of the world on anaemia among T2DM patients, its magnitude varies among their findings up to 63% in Pakistan [12], 41.4% in Cameroon, 39.4% in Malaysia, 18% in India, 55.5% in Saudi, and 34.8% in Ethiopia [13]. Such a difference will make the understanding of the exact magnitude of anaemia among T2DM patients.

In Ethiopia, chronic illnesses are tremendously raising with their complications. But very little research has been conducted, particularly on anaemia among DM patients, although it has been reported as the common complication of DM. Thus, knowing its current magnitude is very important for policy makers to design an intervention and strengthen regular screening and management of anaemia among DM patients and can be used as evidence for early sign of kidney problem and other diabetes-related complications and improving DM patient's health-related quality of life through prevention of diabetes-related complications. Hence, this research aimed to determine the prevalence of anaemia and its associated factors among T2DM patients at University of Gondar Comprehensive Specialized Hospital (UGCSH) in Northwest Ethiopia.

2. Methods and Materials

2.1. Study Design, Setting, and Population. A cross-sectional study design was employed at UGCSH from March 1 to April 15, 2019. UGCSH is found in Gondar town of Amhara regional state, which is located 743 km northwest of the capital Addis Ababa, Ethiopia, and it serves Gondar and surrounding zones. Type 2 DM patients attending the outpatient's clinic of the hospital for routine follow-up during the study period were included. All patients with a known hematologic disease, those who received a blood transfusion in the preceding 3 months, and those who were pregnant women were excluded.

2.2. Sample Size Determination. The required sample size of the study was determined using single population proportion formula by considering 34.8% prevalence rate of anaemia based on a previous study in people with diabetes in Ethiopia [13], Z = the level of statistical significance with a 95% confidence interval (CI) of 1.96, and precision level of 0.05. Then, the minimum sample size obtained was 348. After adding 10% to account for nonrespondents, a total of 382 diabetic patients were included in the study. A systematic random sampling technique (i.e., every two patients) was employed to select the study participants.

2.3. Data Collection. Data were collected by using a structured data extraction checklist. Patient intake form, follow-up card, and DM registration book were used as data sources. Sociodemographic characteristics, baseline, and follow-up clinical and laboratory data were collected from patient cards. Four data collectors and one supervisor who are health professionals were recruited. Two-day training was given for the data collectors and supervisor on how to retrieve records as per data extraction sheet.

2.4. Operational Definitions. Patients were classified as anaemic according to the World Health Organization (WHO) criteria (Hg < 12 g/dl for females and <13 g/dl for males) [3]. Microvascular complications of diabetes are those long-term complications that affect small blood vessels. These typically include retinopathy, nephropathy, and neuropathy. Macrovascular complications of diabetes are primarily diseases of the coronary arteries, peripheral arteries, and cerebrovasculature [14]. For good glycaemic control, an average of four consecutive fasting blood glucose measurement was ≤130 mg/dl, and for poor glycaemic control, an average of four consecutive fasting blood glucose measurement was >130 mg/dl [15].

2.5. Statistical Analysis. The data were checked for inconsistencies, coding error, completeness, clarity, and missing values before they were entered. The data were entered using Epi-info 7 and exported to STATA 14.1 statistical software for further data cleaning and statistical analysis. Descriptive statistical analysis such as frequency, percentage, cross tabulation, mean, and standard deviation were performed. Multivariable logistic regression analysis was fitted, and the corresponding adjusted odds ratio (AOR) and 95% CI were used to identify factors associated with anaemia. A p value < 0.05 was used to characterize statistically significant results.

3. Results

3.1. Characteristics of Study Participants. A total of 372 T2DM patients, of which 230 (61.83%) were females, were included in the study. More than half of the participants (206 (55.38)) were aged above 60 years. From the total number of the participants, 138 (37.10%) had at least one of diabetes-related microvascular complications, whereas fifty-six of them (15.05%) had at least one diabetes-related macrovascular complications. The duration of DM ranged from 2 up to 19 years, with a mean (±SD) of 8.87 ± 3.69 years. One hundred (26.88%) participants were hypertensive, with 76 (20.43%) and 45 (12.10%) participants having SBP of >140 mmHg and DBP

of >90 mmHg, respectively. The average of four consecutive fasting blood sugar levels (FBS) during study periods is with a mean (±SD) of 204.50 ± 57.10 mg/dl (Table 1).

3.2. Prevalence of Anaemia among T2DM Patients. The overall prevalence of anaemia in the study participants was found to be 8.06% (95% CI: 5.68–11.31%); 10.56% of diabetic males and 6.52% of diabetic females were found to be anaemic. Of these 372 patients, 14.49% patients with diabetes-related microvascular complications and 17.86% patients with diabetes-related macrovascular complications had anaemia.

3.3. Factors Associated with Anaemia among T2DM Patients. Table 2 shows the factors associated with anaemia among T2DM patients. Sex, type of treatment, diabetes-related microvascular complications, and hypertension were the significant factors associated with anaemia. Diabetic male patients were 2.74 times more likely to be anaemic than diabetic female patients. The odds of developing anaemia in patients with at least one diabetes-related microvascular complication were 3.24 times more likely as compared with those without any microvascular complications. The study also showed that the odds of developing anaemia for patients who take combined treatment were 8.38 times higher than those patients who took oral glycaemic agent only. In addition, there was a statistically significant relationship between hypertension and anaemia.

Table 1: Sociodemographic and clinical characteristics of T2DM patients at UGCSH, Northwest Ethiopia, 2019 ($n = 372$).

Variables	Frequency ($n = 372$)	Percentage (%)
Sex		
Male	142	38.17
Female	230	61.83
Age (years)		
≤60	166	44.62
>60	206	55.38
Duration of DM		
≤5	65	17.47
6–10	193	51.88
>10	114	30.65
Hypertension		
Yes	100	26.88
No	272	73.12
SBP		
≤140	296	79.57
>140	76	20.43
DBP		
≤90	327	87.90
>90	45	12.10
Microvascular complication		
No	234	62.90
Yes	138	37.10
Macrovascular complication		
No	316	84.95
Yes	56	15.05
Glycaemic control		
Poor	346	93.01
Good	26	6.99
Type of treatment		
Oral hypoglycaemic agent	329	88.44
Combined	43	11.56

DBP, diastolic blood pressure; SBP, systolic blood pressure. Combined both insulin and oral hypoglycaemic agents.

4. Discussion

The current study demonstrated the prevalence of anaemia among T2DM patients in UOGCSH to be 8.06%. This number is lower than the finding of a hospital-based cross-sectional study performed in Harari region, Eastern Ethiopia (34.8%), and northeast of the country (20.1%) [13, 16]; this prevalence is also lower than previous studies in Iran (30.4%), India (18%), and Malaysia (39.4%) [17–19]. Such variations in the magnitude of anaemia among diabetic patients might be due to differences in the cutoff value used to measure it, sample size, and variations in the overall characteristics of the study area that could be related to the prevalence of anaemia among T2DM patients.

The finding of the current study showed that gender was significantly associated with anaemia. Anaemia is more likely to occur in male patients compared to female patients. Similar finding have been reported in Ethiopia [13]. However, this finding was contraindicating with another study performed in Pakistan [12]. The possible explanation could relate to differences involving genetic factors and number of female patients who are menopause because of the effect of menstruation on iron stores.

In this study, diabetes-related microvascular complication is significantly associated with the occurrence of anaemia. Consistent with the present finding, the increased ratio for developing anaemia has also been found in the previous study conducted in China [20], Egypt [9], Cameroon [21], and Iran [17]. This could be explained as anaemia is associated with a reduction in both the number of red blood cells and antioxidant potential of erythrocytes, which may lead to characteristics diabetic complications [22]. In addition, some studies reported that anaemia may modulate the activity of molecular signaling pathways that lead to progressive organ damage [23]. The odds of anaemia were higher among patients who were taking combined treatment compared to those who were taking oral hypoglycaemic agents. The possible explanation could be due to malabsorption of vitamin B12 and the destruction of red blood cell due to the adverse effect of the drugs and also the synergetic effect of the two drugs.

In this study, we found that hypertension was the strongest risk factor for anaemia among T2DM patients. The main cause could be, in diabetic patients, the risk of renal impairment, thus increasing the subsequent development of anaemia. In addition, nutritional deficiencies especially iron deficiency and chronic inflammation can be the cause [24]. This finding is consistent with a cross-sectional study performed in Ethiopia [25]. The study has its limitation as this

Table 2: Multivariable logistic regression of variables associated with anaemia among T2DM patients in UGCSH, Northwest Ethiopia, 2019.

Variables	Anaemia		COR (95% CI)	AOR (95% CI)
	Yes	No		
Sex				
Female	15	215	1	1
Male	15	127	1.69 (1.08, 3.58)	2.74 (1.02, 7.38)*
Age				
≤60	12	154	1	1
>60	18	188	1.23 (0.57, 2.63)	0.60 (0.22, 1.64)
SBP				
≤140	25	271	1	1
>140	5	71	0.57 (0.19, 1.71)	0.66 (0.15, 2.95)
DBP				
≤90	24	303	1	1
>90	6	39	0.49 (0.11, 2.16)	0.22 (0.03, 1.51)
Duration of DM				
≤5 years	5	60	1	1
6–10 years	18	175	1.44 (1.27, 7.88)	1.45 (0.26, 8.24)
>10 years	7	107	1.55 (0.39, 6.09)	0.54 (0.09, 3.33)
Type of treatment				
Oral hypoglycaemic agent	25	304		1
Combined	5	38	1.68 (1.25, 4.43)	8.38 (1.66, 42.25)**
Microvascular complications				
No	10	224	1	1
Yes	20	118	3.79 (1.72, 8.37)	3.24 (1.14, 9.26) *
Macrovascular complications				
No	20	296	1	1
Yes	10	46	3.21 (1.42, 7.31)	1.15 (0.36, 3.63)
Glycaemic control				
Good	5	21	1	1
Poor	25	321	0.45 (0.14, 1.39)	0.24 (0.05, 1.21)
Hypertension				
Yes	6	266	1	1
No	24	76	0.03 (0.008, 0.10)	0.01 (0.002, 0.06)***

*** p value < 0.001; ** p value < 0.01; * p value < 0.05. CI, confidence interval; DBP, diastolic blood pressure; SBP, systolic blood pressure; AOR, adjusted odds ratio; COR, crude odds ratio.

study was conducted based on secondary data, data on some potentially important predictors which help to know that the dietary pattern of the patient was not assessed. Moreover, since this study used a cross-sectional study design, we cannot report the cause and effect relationship of microvascular complications and anaemia.

5. Conclusion

The finding of the current study revealed low prevalence of anaemia among T2DM patients. Sex, type of treatment, diabetes-related microvascular complications, and hypertension were factors associated with anaemia. Assessment of haemoglobin levels among T2DM patients may help to prevent ensuing diabetes-related microvascular complications. Incorporate anaemia screening into the routine assessment of diabetic complication particularly for those who are hypertensive and took combined treatment to allow early appreciation and treatment of anaemia and later improve the overall care of patients with diabetes.

Abbreviations

AOR: Adjusted odds ratio
COR: Crude odds ratio
DBP: Diastolic blood pressure
DM: Diabetes mellitus
FBS: Fasting blood sugar
HR: Hazard ratio
HTN: Hypertension
SBP: Systolic blood pressure
T2DM: Type 2 diabetes mellitus.

Data Availability

The data used to support the findings of this study are included within this article.

Ethical Approval

Before the commencement of the study, ethical clearance was obtained from the Institutional Review Board of the University of Gondar. Then, permission letters from officials of University of Gondar Comprehensive Specialized Hospital, Department of Internal Medicine, were processed before data collection. To ensure confidentiality, patient

names were not included; instead, code numbers were assigned to depict the results.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Conception of the work, design of the work, acquisition of data, and analysis and interpretation of data were done by SA. Data curation, drafting the article, revising it critically for intellectual content, validation, and final approval of the version to be published were done by SA, BS, and AB. All authors read and approved the final manuscript.

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Research Article

Genotype-Phenotype Correlation of G6PD Mutations among Central Thai Children with G6PD Deficiency

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Background. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common X-linked inherited erythroenzymopathy in Thailand. The clinical and hematological manifestations of G6PD deficiency are variable. *Objective*. This study aimed to characterize the genotype-phenotype correlation of G6PD mutations in Thai pediatric patients who were followed-up in Phramongkutklao Hospital, a tertiary center in central Thailand. *Material and Method*. A total of 102 children including 73 males (71.6%) and 29 females (28.4%) were included in our study. Mutation analysis was performed by direct DNA sequencing of all coding exons of the G6PD gene. Ninety-one patients (89.2%) were presented with neonatal hyperbilirubinemia and 11 patients (10.8%) were presented with acute hemolytic anemia beyond the neonatal period. *Results*. Molecular analysis of the G6PD gene in 102 G6PD-deficient Thai children identified 12 different mutations. G6PD Viangchan (871G > A) and G6PD Canton (1376G > T) were the first (46.2%) and the second (15.4%) most common identified mutations among both male and female G6PD-deficient individuals, respectively. All affected males were hemizygous for G6PD mutations and had an average G6PD level of 16.7 ± 11.5 (3–76) IU/ml.RBC. Majority of female patients (27 in 29, 93.1%) were heterozygous for G6PD mutations and had an average G6PD level of 133.6 ± 43.4 (9–195) IU/ml.RBC. Two female patients (6.9%) were either homozygous or compound heterozygous for the mutations and had G6PD level in the affected male range (35 and 10 IU/ml.RBC). Only 1 in 27 heterozygous females (3.7%) had G6PD level in the affected male range (9 IU/ml.RBC) which is possibly explained by nonrandom X-chromosome inactivation. The correlation of genotypes, G6PD levels, and clinical phenotypes was not demonstrated in our study in which all of the included G6PD-deficient patients were presented with neonatal hyperbilirubinemia and acute hemolytic anemia, since the genotype-phenotype correlation is normally demonstrated in chronic nonspherocytic hemolytic anemia (CNSHA) G6PD-deficient individuals. *Conclusion*. This study characterizes the molecular heterogeneity of G6PD variants causing G6PD deficiency in Thai children. Our study demonstrated the efficiency of direct DNA sequencing which can identify 12 missense mutations in Thai children.

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency (OMIM 300908) is the most common X-linked inherited erythroenzymopathy in Thailand. The prevalence of G6PD deficiency in Thai populations was 3 to 18% [1]. G6PD deficiency is caused by mutation in the G6PD gene (OMIM 305900), located on the long arm of the X chromosome (Xq28). The G6PD gene consists of 13 exons and encodes a 515-amino acid G6PD enzyme (OMIM 305900). Mutation of the G6PD gene results in diminished functionality or stability of the enzyme, giving rise to a wide range of

biochemical heterogeneity and clinical manifestations. To date, more than 180 different mutations in the G6PD gene have been reported in the literature [2, 3]. The majority of the mutations are single-base substitutions leading to missense variants. Many of these mutations occur at relatively high frequencies within specific populations, geographic regions, and ethnic groups [4].

The clinical and hematologic manifestations of G6PD deficiency vary; in most cases, neonatal hyperbilirubinemia and acute hemolytic anemia precipitated by exogenous oxidative stress such as infections, drugs, and fava beans (favism) are principle clinical presentations. In Thailand, most children affected with G6PD deficiency presented with neonatal hyperbilirubinemia [5]. As for X-linked inheritance, symptomatic patients are mostly hemizygous affected males but less common homozygous/compound heterozygous females. Heterozygous females can have a wide range of G6PD activity ranging from normal to severe deficiency due to skewed X-chromosome inactivation. Thus, heterozygous females are also at increased risk of neonatal hyperbilirubinemia and acute hemolysis [3, 5]. Comprehensive analysis of the G6PD mutation and genotype-phenotype correlation of G6PD deficiency in Thai populations have never been studied.

In this study, the genotyping of the G6PD gene and the genotype-phenotype correlation among 102 pediatric patients affected with G6PD deficiency was characterized in Phramongkutklao Hospital, a tertiary care center in central Thailand.

2. Material and Methods

2.1. Patient Selection. To determine the molecular characterization of the G6PD gene, 102 pediatric patients with G6PD deficiency including 73 males (71.6%) and 29 females (28.4%) who were admitted to Pediatrics Department, Phramongkutklao Hospital, between June 2017 and May 2018 were enrolled in our study. The ages ranged from 1 day to 13 years. Clinical manifestations of our patients included neonatal hyperbilirubinemia and acute hemolysis related to oxidative stress. Chronic nonspherocytic hemolytic anemia associated with G6PD deficiency was not identified in this study. The study protocol was approved by the Institutional Review Board of Royal Thai Army, Thailand. The consent was obtained by the study participants prior to study commencement.

2.2. Mutation Analysis of the G6PD Gene. After informed consent was obtained from the patients or parents, a total of 102 EDTA blood samples from all individuals were collected. Genomic DNA was extracted from peripheral blood lymphocytes according to the manufacturer's instructions. All 13 coding exons and exon-intron boundaries of the G6PD gene were amplified by PCR using primers as previously described [6]. The PCR condition was initial denaturing at 95°C for 5 min, followed by denaturing at 95°C for 30 sec, annealing at 62–72°C for 60 sec, extension at 72°C for 60 sec for 30 cycles and final extension at 72°C for 5 min. Final 50 μL PCR reaction mixture contained 100–200 ng genomic DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.25 μM of each primer, and 0.2 unit Taq DNA polymerase (Thermo Scientific, CA, USA). The PCR products were visualized on 1% agarose gel electrophoresis. All PCR products were purified and directly sequenced in both directions. The reference sequences were NM_001360016.1 and NP_001346945.1 for G6PD cDNA and G6PD amino acid positions, respectively.

2.3. G6PD Quantitative Enzymatic Activity. The activity of G6PD enzyme was performed by the enzyme kinetic method (Modified Calbiochem) subsequent to hospital admission and repeated at the steady state approximately 3 months after neonatal jaundice and acute hemolysis. The G6PD enzymatic activity was expressed as international units (IU)/ml.RBC. The normal G6PD enzymatic activity in our laboratory for both males and females was 159–297 and 197–331 IU/ml.RBC, respectively. The G6PD enzymatic activity below the normal range was considered as G6PD deficiency.

2.4. Statistical Analysis. Baseline values of selected variables were calculated as mean, standard deviation, and range. Continuous variables were compared between the two groups using the unpaired *t*-test for data with a parametric distribution. Statistical analysis was performed using IBM SPSS Software, Version 23 (IBM, NY, USA), and p value <0.05 was considered to be statistically significant.

3. Results

A total of 102 children including 73 males (71.6%) and 29 females (28.4%) were included in our study. Clinical manifestations of our G6PD-deficient children were classified as 91 individuals (89.2%) with neonatal hyperbilirubinemia (66 males and 25 females) and 11 individuals (10.8%) with acute hemolytic anemia (7 males and 4 females) (Table 1). The majority (80 patients, 78.4%) of our patients were residing in central Thailand. The remaining patients lived in northeastern (17 patients, 16.7%), northern (4 patients, 3.9%), and southern (1 patient, 1%) Thailand.

Molecular analysis of the G6PD gene in G6PD-deficient Thai children identified a total of 12 different mutations (Table 2 & Figure 1). Among 104 G6PD alleles including 73 alleles from hemizygous males, 27 alleles from heterozygous females, and 4 alleles from homozygous/compound heterozygous females with G6PD deficiency, G6PD Viangchan (871G > A) was the most common mutation and was detected in 48 chromosomes (46.2%). G6PD Canton (1376G > T) was the second most common and was identified in 16 chromosomes (15.4%), followed by G6PD Kaiping (1388G > A) 15 chromosomes (14.4%) and G6PD Mahidol (487G > A) 9 chromosomes (8.6%). Eight uncommon G6PD mutations including 4 alleles (3.8%) of G6PD Quing Yan (392G > T), 2 alleles (1.9%) of G6PD Coimbra (592C > T), G6PD Union (1360C > T), G6PD Songklanagarind (196T > A), G6PD Valladolid (406C > T)

Table 1: Clinical phenotypes of 102 G6PD-deficient Thai children.

Clinical phenotype (*n*)	Male *n* (%)	Female *n* (%)	Total (102)
Neonatal jaundice (91)	66 (90.4)	25 (86.2)	91 (89.2)
Acute hemolytic anemia (11)	7 (9.6)	4 (13.8)	11 (10.8)
Total (102)	73 (71.6)	29 (28.4)	102

Data shown as number (percent).

Table 2: The genotyping of 104 G6PD alleles in 102 G6PD-deficient Thai children.

Genotype of G6PD mutation	Number *n* (%)	Male	Female	
		Hemizygous *n* (%)	Heterozygous *n* (%)	Homozygous/compound heterozygous *n* (%)
Viangchan (871G > A)	48 (46.2)	34 (46.6)	11 (40.7)	3 (75%)
Canton (1376G > T)	16 (15.4)	9 (12.3)	6 (22.2)	1 (25%)
Kaiping (1388G > A)	15 (14.4)	12 (16.4)	3 (11.1)	—
Mahidol (487G > A)	9 (8.6)	6 (8.2)	3 (11.1)	—
Quing Yan (392G > T)	4 (3.8)	1 (1.3)	3 (11.1)	—
Aures (143C > T)	2 (1.9)	2 (2.7)	—	—
Coimbra (592C > T)	2 (1.9)	2 (2.7)	—	—
Songklanagarind (196T > A)	2 (1.9)	2 (2.7)	—	—
Union (1360C > T)	2 (1.9)	2 (2.7)	—	—
Valladolid (406C > T)	2 (1.9)	1 (1.3)	1 (3.7)	—
Chinese-5 (1024C > T)	1 (1)	1 (1.3)	—	—
Mediterranean (563C > T)	1 (1)	1 (1.3)	—	—
Total	104	73	27	4

Data shown as number (percent).

and G6PD Aures (143C > T), and 1 allele (1%) of G6PD Chinese-5 (1024C > T) and G6PD Mediterranean (563C > T) were identified. G6PD Valladolid and G6PD Aures were firstly identified in Thai populations in our study. All 104 G6PD alleles were characterized by direct DNA sequencing. According to sex classification, all 12 mutations were detected among male patients, whereas only 6 mutations were identified among female patients potentially due to the low prevalence and smaller sample size of female patients (Table 2). Nevertheless, G6PD Viangchan was still the most common G6PD mutation identified among both male and female patients. Of the 29 female patients, the majority (27 patients, 93.1%) were identified to be heterozygous for G6PD mutations. Only 2 patients (6.9%) were identified to be either homozygous or compound heterozygous for G6PD mutations including 1 homozygous of G6PD Viangchan and 1 compound heterozygous of G6PD Viangchan and G6PD Canton.

Our study demonstrated the variability of G6PD levels ranging from very low detectable activities to some residual activity (Table 3). The average G6PD levels were 16.7°±°11.5 (3–76) IU/ml.RBC among affected males and 133.6°±°43.4 (9–195) IU/ml.RBC among heterozygous females. Two female patients who were homozygous and compound heterozygous for G6PD mutations had 35 and 10 IU/ml.RBC of G6PD level, respectively. G6PD levels, categorized by each type of G6PD mutations, are shown in Table 3. Interestingly, almost all affected males had G6PD levels less than 45 IU/ml.RBC except for one patient who was hemizygous for G6PD Valladolid presenting G6PD level 76 IU/ml.RBC. On the other hand, nearly all heterozygous females had G6PD levels more than 45 IU/ml.RBC, except for one individual (1 in 27 patients, 3.7%) who was heterozygous for G6PD Kaiping presenting G6PD level 9 IU/ml.RBC, which was in the affected male range.

Concerning genotype-phenotype correlation study, G6PD level was categorized by each clinical phenotype (Table 4). In G6PD-deficienct males, the average G6PD activities were 17.26°±°11.7 (3–76) IU/ml.RBC among neonatal jaundice patients and 11.8°±°8.9 (3–25) IU/ml.RBC among acute hemolytic patients. In G6PD-deficient heterozygous females, the average G6PD activities were 136.1°±°44.6 and 113.7°±°32.2 IU/ml.RBC among individuals presenting with neonatal jaundice and acute hemolytic anemia, respectively. Although G6PD levels among acute hemolytic individuals were slightly lower than those among neonatal jaundice individuals, no significant difference was found in the mean G6PD levels between these two major clinical phenotypes for both affected males (p value 0.135) and heterozygous females (p value 0.279).

4. Discussion

G6PD deficiency is seen primarily in populations across the Mediterranean, Africa, Middle East, and Southeast Asia where malaria was endemic, as the deficiency confers selective survival advantage from infection with *Plasmodium falciparum*. Consequently, the prevalence of G6PD deficiency is high, ranging from 5 to 20% in these regions [7]. In Thailand, the prevalence was 3 to 18% [1]. In this study, we aimed to characterize the genotype-phenotype correlation of the G6PD mutation in 102 unrelated Thai pediatric patients affected with G6PD deficiency including 73 males (71.6%) and 29 females (28.4%). As previously reported, the majority (89.2%) of our patients presented with neonatal hyperbilirubinemia followed by acute hemolytic anemia (10.8%)

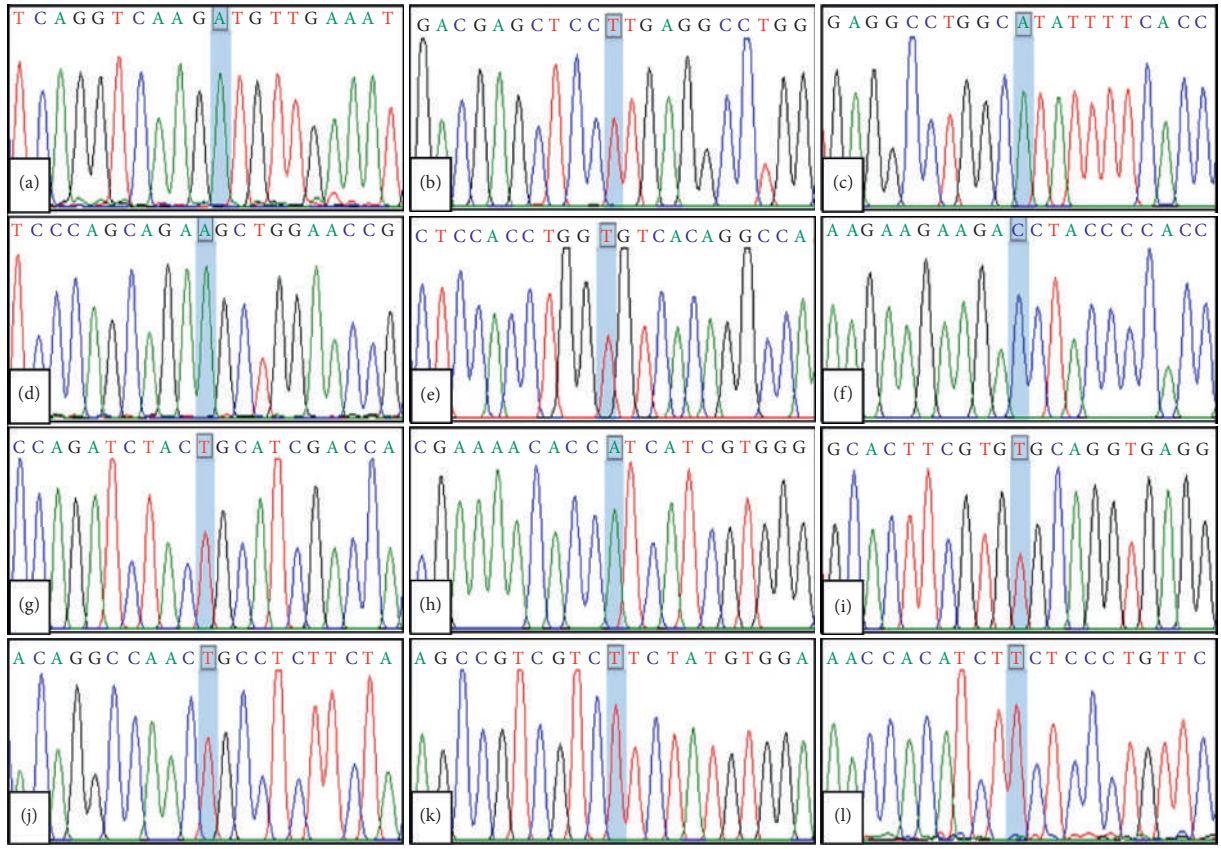

FIGURE 1: Electropherogram of 12 G6PD mutations identified in this study; (a) G6PD Viangchan (c.871G>A, p.Val291Met), (b) G6PD Canton (c.1376G>T, p.Arg459Leu), (c) G6PD Kaiping (c.1388G>A, p.Arg463His), (d) G6PD Mahidol (c.487G>A, p.Gly163Ser), (e) G6PD Quing Yan (c.392G>T, p.Gly131Val), (f) G6PD Aures (c.143T>C, p.Ile48Thr), (g) G6PD Coimbra (c.592C>T, p.Arg198Cys), (h) G6PD Songklanagarind (c.196T>A, p.Phe66Ile), (i) G6PD Union (c.1360C>T, p.Arg454Cys), (j) G6PD Valladolid (c.406C>T, p.Arg136Cys), (k) G6PD Chinese-5 (c.1024C>T, p.Leu342Phe), and (l) G6PD Mediterranean (c.563C>T, p.Ser188Phe).

[5, 8]. Our study recruited patients with G6PD deficiency who were hospitalized and developed acute hemolysis or neonatal jaundice according to inclusion criteria described in the manuscript. This limitation might underestimate the actual prevalence of G6PD deficiency in Thailand due to overlooking those with mild symptoms or asymptomatic and could be the reason for the difference of prevalence between our study and the previous study [9, 10] that enrolled all patients who presented with intravascular hemolysis found 81% had G6PD deficiency at Gaza Strip, Palestine.

To date, more than 180 mutations are associated with G6PD deficiency [2, 3, 11]. Most mutations (85%) are missense variants which reduce G6PD enzyme stability and activity. In our study, 12 missense mutations affecting coding regions of the G6PD gene were identified. The three most common mutations were G6PD Viangchan (46.2%), G6PD Canton (15.4%), and G6PD Kaiping (14.4%). G6PD Viangchan has been previously reported to be the most common mutation in central (53.8%) and southern (31.3%) Thailand [5, 8]. In Southeast Asia, G6PD Viangchan is also the most common mutation identified in Cambodian (97.9%), Laotians (100%), Vietnamese (44%), and Malaysian Malay (37.2%) populations [12–15]. G6PD Mahidol which is the predominant variant in Myanmar (91.3%) and northern Thailand (20%) and also the common mutation among Malaysian Malays (15.1%) [15–17] was identified in 8.6% of G6PD-deficient patients in our study. The high prevalence of G6PD Viangchan and the existence of G6PD Mahidol among Thais probably suggested a common ancestral origin of the Thais and other Southeast Asian populations. G6PD Canton and G6PD Kaiping are the two commonest mutations among the Chinese [18] and were found to be the second (15.4%) and the third (14.4%) most common variants in our study, respectively. Other less common Chinese mutations including G6PD Quing Yan (3.8%), G6PD Union (1.9%), and G6PD Chinese-5 (1%) were also identified in smaller numbers. The discovery of these Chinese variants possibly indicated the descendants of Chinese immigrants in the Thai population. In addition, low frequency of three previously reported mutations among Thai G6PD-deficienct patients including G6PD Coimbra (1.9%), G6PD Songklanagarind (1.9%), and G6PD Mediterranean (1%) was also identified in this study.

Although novel G6PD mutation was not identified in our study, G6PD Aures and G6PD Valladolid were firstly identified among our Thai children affected with G6PD deficiency. G6PD Aures causing mild G6PD deficiency

Table 3: Genotype of G6PD mutations and G6PD levels in 73 affected males and 27 heterozygous females.

Genotype of G6PD mutation (*n*)	G6PD level (IU/ml.RBC) (mean±SD (min–max))	
	Affected males	Heterozygous females
Viangchan (45)	17.18 ± 9.35 (5–45)	138.63 ± 34.2 (61–189)
Canton (15)	12.6 ± 7.72 (3–26)	137.17 ± 53.04 (84–195)
Kaiping (15)	17.92 ± 10.91 (7–36)	9, 129, 180
Mahidol (9)	15.5 ± 7.77 (8–29)	108, 109, 136
Quing Yan (4)	13	100, 152, 177
Aures (2)	3, 10	—
Coimbra (2)	23, 34	—
Songklanagarind (2)	3, 28	—
Union (2)	9, 10	—
Valladolid (2)	76	159
Chinese-5 (1)	25	—
Mediterranean (1)	15	
Total (100)	16.7 ± 11.5 (3–46)	133.6 ± 43.4 (9–195)
	(73)	(27)

Data shown as mean±SD or number (percent).

Table 4: G6PD levels categorized by the genotype of G6PD mutations and clinical phenotypes of 73 affected males and 27 heterozygous females.

Genotype of G6PD mutation	G6PD level categorized by clinical phenotype (IU/ml.RBC) (mean±SD (min–max))			
	Neonatal Jaundice		Acute hemolytic anemia	
	Affected male (66)	Heterozygous female (24)	Affected male (7)	Heterozygous female (3)
Viangchan	16.8 ± 9.6 (5–45)	125.2 ± 53.7 (61–189)	8	148
Canton	12.3 ± 8.2 (3–26)	123.7 ± 76 (85–195)	3	84
Kaiping	11.8 ± 11.3 (7–34)	9, 129, 180	12, 25	-
Mahidol	11.75 ± 4.5 (8–17)	108, 136	—	109
Quing Yan	13	100, 152, 177	—	—
Aures	10	—	3	—
Coimbra	34	—	23	—
Songklanagarind	3, 28	—	—	—
Union	10	—	9	—
Valladolid	76	159	—	—
Chinese-5	25	—	—	—
Mediterranean	15	—	—	—
G6PD level (IU/ml.RBC) (mean±SD (min–max))	17.26 ± 11.7 (3–76)	136.1 ± 44.6 (9–195)	11.8 ± 8.9 (3–25)	113.7 ± 32.2 (84–148)

Data shown as mean+_SD or number (percent).

associated with favism was firstly discovered in Algeria in 1993 [19]. G6PD Valladolid was firstly identified in two unrelated Spanish males in 1997 [20]. This mutation causes mild hemolytic anemia. How these two mutations occurred among G6PD-deficient Thai children remains unclear. These mutations may arise independently, because no historical linkage was found between Spain, Algeria, and Thailand. Interestingly, both patients who carried the G6PD Valladolid variant live in northeastern Thailand. Because no study of G6PD variants has been conducted in northeastern Thailand, G6PD Valladolid may have a high prevalence in this geographic region.

Concerning X-linked inheritance, G6PD deficiency occurs more frequently among males than females as in our study for which the majority (71.6%) of patients comprised males (Table 1). Among hemizygous males and less common homozygous/compound heterozygous females, G6PD deficiency is fully expressed. Almost all affected males and two females who were either homozygous or compound heterozygous for G6PD mutations in our study exhibited G6PD levels less than 45 IU/ml.RBC. Only one affected male who carrying G6PD Valladolid had G6PD level 76 IU/ml.RBC suggesting a mildly affected G6PD variant [20]. Among heterozygous females, a combination of normal and G6PD-deficient cells could be identified due to X-inactivation in either one of the two X chromosomes during the early embryonic period making a wide range of G6PD enzymatic activity among carrier females [3]. In our study, the majority (93.1%) of female patients who had low G6PD levels was heterozygous for G6PD mutations. Nearly all heterozygous females had G6PD levels ranging from

61 to 195 IU/ml.RBC, which are higher than that in the affected male range. Only one heterozygous female (3.7%) carrying G6PD Kaiping had G6PD level 9 IU/ml.RBC, which is in the affected male range and possibly explained by nonrandom X-chromosome inactivation, which has been previously described in this type of mutation [21].

Concerning genotype-phenotype correlation, our data suggest no association between the genotypes, G6PD enzymatic activities, and clinical phenotypes among G6PD-deficient Thai children. The variability of G6PD activity is far from being explained by the type of mutation and is uncorrelated with clinical phenotypes including neonatal jaundice and acute hemolytic anemia which were the only two major clinical features identified in this study. Although G6PD levels remained poorly correlated with the genotype affected with neonatal jaundice and acute hemolytic anemia, it appeared to be better correlated with the genotype presented with chronic nonspherocytic hemolytic anemia (CNSHA) as described in related studies [22–24]. The lack of genotype-phenotype correlation in our study could be a consequence of a complex multifactorial mechanism probably related to both environmental factors and genetic modifiers such as infection, medications, and dietary pattern of G6PD-deficient individuals and the X-chromosome inactivation pattern among heterozygous [21].

Although various studies of G6PD mutation have been conducted in Thai populations, DNA sequencing of the G6PD gene was firstly used to comprehensively identify the mutation spectrum among Thai G6PD-deficient children. Polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP) have been used to detect common G6PD mutations in Thai populations in central, southern, and northern Thailand [5, 8, 17]. The mutations were unidentified in approximately 9 to 23% of subjects suggesting a greater genetic heterogeneity than previously anticipated. Our study demonstrated the efficiency of Sanger sequencing which could identify mutations among all 102 patients. In contrast to PCR-RFLP assays which are limited to detecting only known variants, direct sequencing is more comprehensive and reliable for detecting sequence variations within the genes.

5. Conclusion

In conclusion, this study characterizes the molecular heterogeneity of G6PD variants causing G6PD deficiency among Thai children. All of the G6PD mutations have been characterized by direct DNA sequencing of G6PD genes. Twelve different G6PD mutations were identified in our study. G6PD Viangchan, G6PD Kaiping, and G6PD Canton were the three most common mutations identified in our study and accounted for more than 75% of our patients. Although genotype-phenotype correlation was not demonstrated in our study, molecular analysis of the G6PD gene remains useful for diagnostic confirmation and carrier detection leading to appropriate genetic counseling for patients and their family members in the future.

Data Availability

The data used to support the findings of this study are included within the article and available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Prevalence of Anemia and Its Associate Factors among Women of Reproductive Age in Lao PDR: Evidence from a Nationally Representative Survey

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Introduction. Anemia continues to be a major public health problem significant among women of reproductive age (WRA) in developing countries, including Lao People's Democratic Republic (Lao PDR), where the prevalence of anemia among women remains high. This study aimed to assess the prevalence of anemia and its associated factors among WRA 15–49 years in Lao PDR. *Methods*. We conducted a cross-sectional study, using the Lao Social Indicator Survey II, 2017 dataset. A total of 12,519 WRA tested for anemia were included in this study, through multistage sampling approaches. Binary logistic regression was used to determine the associated factors of anemia. *Results*. Of 12,519 women, 4,907 (39.2%) were anemic. Multivariate logistic regression revealed that living in central provinces (aOR: 2.16, 95% CI: 1.96–2.38), rural area (aOR: 1.1, 95% CI: 1.00–1.20), large family size with more than 6 persons (aOR: 1.14, 95% CI: 1.01–1.29), pregnancy (aOR: 1.46, 95% CI: 1.22–1.74), having any adverse pregnancy outcomes (aOR: 1.14, 95% CI: 1.03–1.25), poor drinking water (aOR: 1.24, 95% CI: 1.10–1.39), and poor sanitation facility (aOR: 1.15, 95% CI: 1.03–1.28) were significantly associated with an increased risk of anemia. Conversely, four factors were associated with anemia preventively, including being aged 25–34 years (aOR: 0.81, 95% CI: 0.74–0.90), postsecondary education (aOR: 0.76, 95% CI: 0.60–0.97), Hmong-Mien ethnicity (aOR: 0.48, 95% CI: 0.39–0.59), and watching television almost daily (aOR: 0.84, 95% CI: 0.75–0.95). *Conclusion*. Anemia continues to be a major public health challenge in Lao PDR. Interventions should be considered on geographic variations, improving safe water and sanitation facility, promoting of iron supplements during pregnancy, and health education through mass media for women in rural areas.

1. Introduction

Anemia is a condition of red blood cells insufficient to meet the body's physiological demands. According to the World Health Organization (WHO), the threshold hemoglobin (Hb) level for anemia is less than 120 g/l for nonpregnant women and 110 g/l for pregnant women aged 15 years and above [1]. Anemia is a global public health problem, with major consequences for human health as well as adverse impacts on social and economic development [2]. The WHO estimates two billion anemic persons and anemia is responsible for one million deaths a year, and about three-quarters of cases occur in Africa and Southeast Asia [3]. The global prevalence of anemia in pregnant and nonpregnant women was 38% and 29%, respectively, in 2011, translated to about half a million anemic nonpregnant women and 32 million anemic pregnant women. Worldwide, 50% of anemia cases are caused by iron deficiency, and other important causes, including infections, nutritional deficiencies, and genetic conditions [4].

Several studies had found various factors associated to increase the risk of developing anemia in women of reproductive age (WRA) [5–7] such as consumption of wells water as the sources of drinking water, having permanent sterilization, living in small and medium-sized cities, aged between 30–39 years, no or occasional smoking, spring and winter seasons, ethnicity, and having low education level. Moreover, older age, limited knowledge of anemia, pregnancy during the second and third trimesters, multiparity, and experience of abortions were more likely reasons of anemia in pregnant women and lactating women [5, 8–10].

Anemia is a crucial public health problem in Lao People's Democratic Republic (Lao PDR). Although the prevalence of anemia among pregnant and nonpregnant women was reduced from 56.5% to 36.0% and from 46.1% to 31.0% in 2006 and 2011, respectively [2, 11], it remained higher than the global average (29%) for nonpregnant women [12]. The improvement of anemia in the country might have been attributed to the program for intermittent iron and folic acid supplementation that targeted women of childbearing age [13]. However, there are few studies on anemia that have been conducted in Lao PDR, mostly focusing on preschool children [14] and adolescent women [15], and all of them were carried out on a small scale and limited to a specific region of the country. Moreover, the associated factors for the prevalence of anemia in WRA have not been investigated. Therefore, this study aimed to assess the prevalence of anemia and to identify its associated risk factors among WRA 15–49 years in Lao PDR.

2. Materials and Methods

2.1. Study Design and Data Sources. We used the data from the Lao Social Indicator Survey (LSIS) II, 2017, a nationally representative cross-sectional survey conducted in July to November 2017 by the Lao Statistic Bureau in collaboration with the Ministry of Health and the Ministry of Education and Sport, as part of the Global Multiple Indicator Cluster Survey (MICS) Program developed by the United Nations Children's Fund (UNICEF). It combines the MICS and the Demographic and Health Survey (DSH).

2.2. Sampling and Study Population. LSIS II used the multistage, stratified cluster sampling approach for the selection of the survey sample. The sampling frame for this survey consisted of a list of all villages, considered as enumeration areas (EAs) in the country. The version used as the sampling frame was the village register of December 2015. They were stratified by the type of residence (urban, rural with the road, and rural without road areas) in every 18 provinces, defined as sampling strata. A two-stage sampling unit was used to ensure that estimates were representative at the national level. At the first stage, 1,170 EAs (373 from urban areas, 687 from rural with road areas, and 110 from rural without road areas) were selected from each sampling strata by using systematic probability proportional to size sampling procedures. At the second stage, 20 households were randomly selected from each EA, resulting in a total of 23,400 households; more details were described elsewhere [16]. Anemia was tested for in WRA 15–49 years in half of the selected households from the general survey. On-site hemoglobin analysis was performed by using a battery-operated portable HemoCue analyzer to estimate the Hb level in grams per deciliter blood. WRA 15–49 years who were tested for Hb and had the result of anemia testing were included in this study The LSIS II data were collected based on the MICS6 model questionnaire by qualified and trained interviewers. Details of the questionnaires can be found in the report of LSIS II, 2017 [16].

2.3. Study Variables. The outcome variable was the presence of anemia during the survey, which is defined as Hb level below 11.0 g/dl and 12.0 g/dl for pregnant and nonpregnant women, respectively, in accordance with WH [1]. The independent variables included in the analysis were identified based on previous literature, including individual characteristics such as age, level of education, ethnolinguistic group, religion, health insurance coverage, marital status, pregnancy status (pregnant versus nonpregnant), and experience of adverse pregnancy outcomes for the last pregnancy; environmental and health-related factors, including exposure to mass media: reading newspapers/magazine, listening to radio, and watching television (TV); main sources of drinking water, type of toilet facility, tobacco/cigarette smoking, and alcohol drinking; and household factors such as the area of residence (urban, rural with road, and rural without road), the region of residence (northern, central, and southern provinces), household wealth status, and size of the family.

2.4. Data Analyses. We summarized anemia status in accordance with each independent variable by conducting descriptive statistics analyses. Pearson's chi-squared test was used to assess the association between anemia status and independent variables. A variable found statistically

significant in bivariate analyses with p-values <0.05 was further analyzed in multivariate analysis, after checking for multicollinearity by examining the variance inflation factors (VIF), and only variables with VIF less than 2 were included. Binary logistic regression was performed to determine the associated factors of anemia among WRA. The results of univariate and multivariate logistic regression analyses were reported in crude odds ratio (OR) and adjusted odds ratio (aOR) with 95% confidence intervals (CI), respectively. Women's sampling weights were available in the dataset and were included in all analyses for adjusting the effects of the stratified cluster sampling approach. All data were analyzed on SPSS, version 25 (IBM Corp., Armonk, NY).

2.5. Ethics Statement. The study protocol was reviewed and approved by the National Ethics Committee for Health Research, the Ministry of Health, Lao PDR (ID: 2020.33.NW). Verbal consent was obtained for each respondent participating prior to the data collection and each participant was informed of the voluntary nature of participation and the confidentiality and anonymity of information.

3. Results

3.1. Characteristics of Study Population and Prevalence of Anemia. A total of 12,519 WRA 15–49 years were included in this study. The mean age of the participant was 30.1 (±9.76) years. Around 35.1% of women had primary education and were living in the richest households (23.2%), with more than 6 people (24.5%). More than half of the women were Lao-Tai (65.1%), were married (73%), resided in rural with road area (57%), and had improved source of drinking water (85.9%) and improved sanitation facility (77.9%) (Table 1).

An overall prevalence of anemia among WRA15–49 years was 39.2% (95% CI, 38.3%–40%). The prevalence of anemia was particularly high among women who resided in the southern provinces (45.4%), were uneducated (42.3%), were married (43.6%), were pregnant (47.3%), experienced any adverse pregnancy outcomes (43%), ever smoked (42.1%), and had not watched TV at all (42%) (Table 1).

3.2. Associate Factors of Anemia. Multivariate analyses highlighted that women living in central provinces (aOR 2.16; 95% CI 1.96–2.38), southern provinces (aOR 2.05; 95% CI 1.82–2.31), and a rural area with the road (aOR 1.10; 95% CI 1.0–1.20) and with large family size with more than 6 people (aOR 1.14; 95% CI 1.01–1.29) were significantly associated with an increased risk of developing anemia as compared to those living in northern provinces and urban areas and with less than 4 household members, respectively. Similarly, pregnant women (aOR 1.46; 95% CI 1.22–1.74) and women who experienced any adverse pregnancy outcomes (aOR 1.14; 95% CI 1.03–1.25) had higher odds of developing anemia compared to their counterparts. In addition, increased odds of developing anemia were found among women who had an unimproved source of drinking water (aOR 1.24; 95% CI 1.10–1.39) and unimproved toilet facility (aOR 1.15; 95% CI 1.03–1.28). Conversely, compared to women aged 15–24 years, women aged 25–34 years (aOR 0.81; 95% CI 0.74–0.90) were negatively associated with developing anemia. Similarly, a low risk of anemia was found among women who had postsecondary education (aOR 0.76; 95% CI 0.60–0.97), belonged to Hmong-Mien ethnicity (aOR 0.48; 95% CI 0.39–0.59), and watched television almost daily (aOR 0.84; 95% CI 0.75–0.95) (Table 2).

4. Discussion

This study aims to assess the prevalence of anemia and to comprehend the influence of various factors, including individual characteristics and environmental and household factors on the prevalence of anemia among WRA 15–59 years in Lao PDR and to therefore identify its associated risk factors. This study revealed the prevalence of anemia among WRA remained high (39.2%). However, the prevalence of anemia among Lao women was lower than many other countries in the same region [17, 18], as well as developing countries on average [19]. However, it is still considered as a public health problem significant particularly among pregnant women as severe public health concerns, according to WHO classification [1]. Various associate factors for the prevalence of anemia were found after controlling for available independent variables.

At the individual level, we found that women who had any adverse pregnancy outcomes have higher odds of developing anemia. It is well documented that maternal anemia leads to poor neonatal outcomes such as low birth weight, stillbirth, preterm delivery, and early neonate death [20], women who had abortion reported a higher risk of developing anemia [8, 21]. Also, this might be due to iron deficiency [20, 22]. In Lao PDR, pregnant women usually receive iron supplementation during their antenatal care (ANC) visits. However, ANC visit rate in Lao PDR was increased from 35% to 54% in 2006 and 2011, respectively, but only half of those women took iron pills during their pregnancy [23]. Moreover, a high prevalence of insufficient iron intake (91%) was reported in a recent study [24]. In addition, current pregnancy shows an increased odd of anemia, this finding was found in other different settings [25–27]. Physiologically, the plasma volume increases progressively during pregnancy, causing a two-to three-fold and 10- to 20-fold increase in the requirement for iron and folate, respectively. For these reasons, the intake of iron and folic acid supplementation is necessary to maintain Hb at normal levels [28]. Therefore, interventions should highlight the promotion of iron and other micronutrient supplements during pregnancy.

On the other hand, our study found that women aged 25–34 had a lower risk of being anemic than those of younger ages. A similar finding was found in India [9]. As 60% of Lao people are younger than 25 years and early-age married, that resulted in early childbearing being very common among young women, with high birth rates at 15–19 years [29]. Women younger than 18 years had received less ANC visits [30]; consequently, this could be mean

Table 1: Sociodemographic characteristics and prevalence of anemia among WRA in Lao PDR.

Characteristics	Total (weighted)	Nonanemia *n* (%)	Anemia *n* (%)	*p*-value
Age, years		**12519**		
15–24	4285 (34.2)	2539 (59.3)	1746 (40.7)	**<0.001**
25–34	3911 (31.2)	2490 (63.7)	1421 (36.3)	
35–49	4323 (34.5)	2587 (59.8)	1736 (40.2)	
Education		**12519**		
None	1996 (15.9)	1151 (57.7)	845 (42.3)	
Primary	4391 (35.1)	2696 (61.4)	1695 (38.6)	
Lower secondary	2726 (21.8)	1661 (60.9)	1065 (39.1)	**0.02**
Upper secondary	1750 (14)	1067 (61)	683 (39)	
Postsecondary/nontertiary	418 (3.3)	272 (65.1)	146 (34.9)	
Tertiary	1238 (9.9)	770 (62.2)	468 (37.8)	
Ethnolinguistic group		**12518**		
Lao-Tai	8154 (65.1)	4843 (59.4)	3311 (40.6)	
Mon-Khmer	2878 (23)	1729 (60.1)	1149 (39.9)	**<0.001**
Hmong-Mien	1061 (8.5)	780 (73.5)	281 (26.5)	
Chinese-Tibetan	307 (2.5)	198 (64.5)	109 (35.5)	
Other	118 (0.9)	65 (55.1)	53 (44.9)	
Religion		**12518**		
Buddhist	8404 (67.1)	4993 (59.4)	3411 (40.6)	
Animist	3904 (31.2)	2501 (64.1)	1403 (35.9)	**<0.001**
Christian	186 (1.5)	108 (58.1)	78 (41.9)	
Other	24 (0.2)	13 (54.2)	11 (45.8)	
Area of residence		**12519**		
Urban	4202 (33.6)	2646 (63)	1556 (37)	**<0.001**
Rural with road	7140 (57)	4207 (58.9)	2933 (41.1)	
Rural without road	1177 (9.4)	763 (64.8)	414 (35.2)	
Region of residence		**12519**		
Northern provinces	3887 (31)	2842 (73.1)	1045 (26.9)	**<0.001**
Central provinces	6253 (49.9)	3474 (55.6)	2779 (44.4)	
Southern provinces	2379 (19)	1299 (54.6)	1080 (45.4)	
Wealth quintiles		**12519**		
Poorest	2145 (17.1)	1268 (59.1)	877 (40.9)	
Poorer	2272 (18.1)	1381 (60.8)	891 (39.2)	0.36
Middle	2414 (19.3)	1474 (61.1)	940 (38.9)	
Richer	2785 (22.2)	1727 (62)	1058 (38)	
Richest	2903 (23.2)	1766 (60.8)	1137 (39.2)	
Size of family		**12517**		
≤3 persons	2040 (16.3)	1279 (62.7)	761 (37.3)	**0.01**
4–6 persons	7409 (59.2)	4529 (61.1)	2880 (38.9)	
>6 persons	3068 (24.5)	1807 (58.9)	1261 (41.1)	
Source of drinking water		**12519**		
Improved	10760 (85.9)	6660 (61.9)	4100 (38.1)	**<0.001**
Unimproved	1759 (14.1)	956 (54.3)	803 (45.7)	
Type of toilet facility		**12518**		
Improved	9752 (77.9)	6094 (62.5)	3658 (37.5)	**<0.001**
Unimproved	2766 (22.1)	1521 (55)	1254 (45)	
Health insurance status		**12518**		
Uninsured	10640 (85)	6436 (60.5)	4204 (39.5)	0.06
Insured	1878 (15)	1179 (62.8)	699 (37.2)	
Marital status		**12519**		
Never married	2826 (22.6)	1738 (61.5)	1088 (38.5)	0.07
Currently married	9145 (73)	5569 (60.9)	3576 (39.1)	
Formerly married	548 (4.4)	309 (56.4)	239 (43.6)	
Pregnancy status		**12519**		
None	11961 (95.5)	7322 (61.2)	4639 (38.8)	**<0.001**
Currently pregnant	558 (4.5)	294 (52.7)	264 (47.3)	

Table 1: Continued.

Characteristics	Total (weighted)	Nonanemia *n* (%)	Anemia *n* (%)	*p*-value
Adverse pregnancy outcomes		**12519**		
No (live births)	10158 (81.1)	6261 (61.6)	3897 (38.4)	**<0.001**
Yes (stillbirths, miscarriage, abortion)	2361 (18.9)	1355 (57.4)	1006 (42.6)	
Reading newspaper/magazine		**12518**		
None	10613 (84.8)	6420 (60.5)	4193 (39.5)	
Less than once a week	976 (7.8)	607 (62.2)	369 (37.8)	0.13
At least once a week	718 (5.7)	463 (64.5)	255 (35.5)	
Almost daily	211 (1.7)	125 (59.2)	86 (40.8)	
Listening to radio		**12520**		
None	9210 (73.6)	5571 (60.5)	3639 (39.5)	
Less than once a week	1118 (8.9)	710 (63.5)	408 (36.5)	0.27
At least once a week	1141 (9.1)	692 (60.6)	449 (39.4)	
Almost daily	1051 (8.4)	643 (61.2)	408 (38.8)	
Watching TV		**12518**		
None	2398 (19.2)	1395 (58.2)	1003 (41.8)	
Less than once a week	550 (4.4)	340 (61.8)	210 (38.2)	**0.02**
At least once a week	1461 (11.7)	907 (62.1)	554 (37.9)	
Almost daily	8109 (64.8)	4973 (61.3)	3136 (38.7)	
Ever smoked		**12519**		
None	11422 (91.2)	6981(61.1)	4441 (38.9)	**0.03**
Yes	1097 (8.8)	635 (57.9)	462 (42.1)	
Ever drank alcohol		**12519**		
None	2071 (16.5)	1217 (58.8)	854 (41.2)	**0.03**
Yes	10448 (83.5)	6398 (61.2)	4050 (38.8)	

that these young women did not receive iron supplements during their pregnancy. Also, our study revealed that education was significantly associated with less risk of anemia among women who had postsecondary education compared to those who had no education. Several studies in different settings supported this finding [9, 31, 32].

Several environmental factors were found to be a positive risk of anemia. Unimproved sources of drinking water and unimproved toilet facility were about one time more likely to cause anemia as compared to the improved source of drinking water and sanitation facility. Naturally, arsenic enriched in groundwater had been found in tube well/hand pump drinking water supplies in Southeast Asia, including Lao PDR [33, 34], particularly in central and southern provinces of the country [35]. A previous study found an increased risk of anemia among women who had been exposed to arsenic from their drinking water [36, 37]. Although Lao PDR has made good progress in the provision of clean drinking water across the country, gaps still exist in rural villages [38], because 60% of the communities used dug wells as the main sources of water [39]. Moreover, Lao PDR had low rates of access to sanitation facilities in rural areas, where 73% of the population live [40]. Thus, we cannot neglect the probability of environmental contamination with parasites that causes anemia [41]. Therefore, interventions should strongly continue the provision of access to safe drinking water and improved sanitation facility in rural communities. Our findings were consistent with previous studies in other settings, including Myanmar [42], Nepal [6], Uganda [25], and Tanzania [26]. However, mass media exposure is a good source of receiving wider information, including health promotion programs, and increase chances of iron and folic acid supplements intake [43]. Our study showed that increasing the frequency of watching television is a significant protective factor against anemia in women; a similar finding was also found in India [44].

At the household level, geographic area of residence was found to be associated with anemia among women who lives in rural with road area and central and southern provinces who were one to two times more likely to be anemic, respectively. Similar associations between anemia and geographic location have been found in previous studies conducted in different settings of the same region [17, 27], and different region [6]. The southern provinces and some central provinces in Lao PDR are considered to be high malaria-endemic regions particularly in remote festered areas [45, 46]. The majority of malaria infections were asymptomatic and half of them were associated with anemia in women [47]. Nevertheless, the reduction of malaria confirmed cases more than 50% [48] and the high proportion of insecticide-treated bed nets coverage in endemic areas contributed to the reduction of malaria prevalence in Lao PDR [46, 49]; however, the effectiveness of treatment outcome for malaria by chloroquine, used as the first-line therapy for uncomplicated malaria and its resistance [50], and improper usage of insecticide-treated bed nets in rural areas during farming seasons [49] are a major challenge for malaria control in Lao PDR. Also, *Schistosoma mekongi* is an endemic parasite in a limited area of the southern province [51]. It is well documented that schistosomiasis infection is

Table 2: Associate factors with anemia among WRA in Lao PDR.

Characteristics	Crude		Adjusted	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age, years				
15–24	Reference		Reference	
25–34	**0.83 (0.75–0.90)**	**<0.001**	**0.81 (0.74–0.90)**	**<0.001**
35–49	0.97 (0.89–1.06)	0.57	0.94 (0.85–1.04)	0.29
Education				
None	Reference		Reference	
Primary	**0.85 (0.76–0.95)**	**0.005**	0.89 (0.78–1.00)	0.06
Lower secondary	**0.87 (0.77–0.98)**	**0.02**	0.91 (0.79–1.05)	0.21
Upper secondary	**0.87 (0.76–0.99)**	**0.04**	0.91 (0.77–1.07)	0.26
Postsecondary/nontertiary	**0.73 (0.58–0.91)**	**0.006**	**0.76 (0.60–0.97)**	**0.03**
Higher	**0.82 (0.71–0.95)**	**0.001**	0.90 (0.75–1.08)	0.27
Ethnolinguistic				
Lao-Tai	Reference		Reference	
Mon-Khmer	0.97 (0.89–1.06)	0.51	0.94 (0.82–1.08)	0.45
Hmong-Mien	**0.52 (0.45–0.60)**	**<0.001**	**0.48 (0.39–0.59)**	**<0.001**
Chinese-Tibetan	0.80 (0.63–1.02)	0.07	1.23 (0.94–1.61)	0.11
Other	1.18 (0.82–1.71)	0.35	0.97 (0.66–1.41)	0.87
Religion				
Buddhist	Reference		Reference	
Animist	**0.82 (0.75–0.88)**	**<0.001**	1.04 (0.91–1.19)	0.49
Christian	1.05 (0.78–1.41)	0.72	1.14 (0.83–1.56)	0.41
Other	1.27 (0.57–2.82)	0.54	1.43 (0.63–3.25)	0.38
Area of residence				
Urban	Reference		Reference	
Rural with road	**1.18 (1.09–1.28)**	**<0.001**	**1.10 (1.00–1.20)**	**0.03**
Rural without road	0.92 (0.80–1.05)	0.24	0.90 (0.77–1.05)	0.21
Region of residence				
Northern provinces	Reference		Reference	
Central provinces	**2.17 (1.99–2.37)**	**<0.001**	**2.16 (1.96–2.38)**	**<0.001**
Southern provinces	**2.26 (2.03–2.51)**	**<0.001**	**2.05 (1.82–2.31)**	**<0.001**
Size of family				
≤3 persons	Reference		Reference	
4–6 persons	1.06 (0.96–1.18)	0.19	1.08 (0.97–1.20)	0.12
>6 persons	**1.19 (1.06–1.35)**	**0.004**	**1.14 (1.01–1.29)**	**0.03**
Source of drinking water				
Improved	Reference		Reference	
Unimproved	**1.36 (1.23–1.51)**	**<0.001**	**1.24 (1.10–1.39)**	**<0.001**
Type of toilet facility				
Improved	Reference		Reference	
Unimproved	**1.36 (1.25–1.48)**	**<0.001**	**1.15(1.03–1.28)**	**0.01**
Pregnancy status				
None	Reference		Reference	
Current pregnant	**1.41 (1.19–1.68)**	**<0.001**	**1.46 (1.22–1.74)**	**<0.001**
Adverse pregnancy outcomes				
No (live births)	Reference		Reference	
Yes (stillbirths, miscarriage, abortion)	**1.19 (1.08–1.30)**	**<0.001**	**1.14 (1.03–1.25)**	**0.008**
Watching TV				
None	Reference		Reference	
Less than once a week	0.86 (0.71–1.04)	0.12	0.84 (0.69–1.03)	0.10
At least once a week	**0.85 (0.74–0.97)**	**0.01**	**0.80 (0.69–0.92)**	**0.003**
Almost everyday	**0.87 (0.80–0.96)**	**0.006**	**0.84 (0.75–0.95)**	**0.005**
Ever smoking				
None	Reference		Reference	
Yes	**1.14 (1.00–1.29)**	**0.03**	0.99 (0.87–1.13)	0.94
Ever drunk alcohol				
None	Reference		Reference	
Yes	**0.90 (0.82–0.99)**	**0.03**	0.92 (0.83–1.03)	0.18

due to one of the parasites causing chronic blood loss, resulting in anemia [52, 53]. Even though schistosomiasis was selected for elimination by WHO, reservoir animals and insufficient safe sources of water in the endemic area make a control strategy challenging [51]. Thus, the interventions should be considering the malaria control strategies to ensure the availability and propriety of insecticide-treated mosquito bed nets, and deworming programs in affected geographic areas should be prioritized.

Also, our study found that a large family size with more than 6 people was significantly associated with an increase in the risk of anemia. A similar outcome was found in a previous study conducted in Ethiopia [54]. This might be due to food insecurity and distribution for large family size. In addition, access to foods is another issue, especially in a rural area, because less than 2% have permanent markets available in rural villages and poor quality road infrastructure, particularly during the rainy season which makes access to foods more challenging [38]. Moreover, a recent study in Lao PDR reported a high prevalence of insufficient diversity of food consumption (90.1%) and iron intake (61.8%) among WRA [24]. However, a low risk of developing anemia was found among women who belonged to the Hmong-Mien ethnolinguistic group. Geographically, this ethnolinguistic group mostly live in mountainous areas of the northern part of the country [38]. Physiological hemoglobin demands are greater among people living at high altitudes due to the low concentration of oxygen in the atmosphere [12]. Thus, the hemoglobin increases with an increase of altitude which is reported in a study conducted in Peru [55], particularly when the altitude is above 1,000 meters above sea level [56]. A study in Myanmar reported lower odds of anemia among women who lived in the hilly zone compared to those living in another zone of the country [27].

Our study has some limitations. Due to the cross-sectional nature of the survey, it is not possible to analyze the cause-effect relationship between anemia and the predictor variables. Also, this study was not able to assess other risk factors for anemia such as the family history of thalassemia, parasitic infections, contraceptive use, nutritional status, and micronutrient deficiencies such as folate, iron, and B-12, which might have potentially an important impact on the development of anemia.

5. Conclusions

The anemia continues to be a major public health challenge in Lao PDR. The prevalence of anemia among WRA was high (39.2%). Living in a rural area, in southern provinces, and with large family size, unimproved water and unimproved sanitation facility, being pregnant, and experiencing any adverse pregnancy outcomes were significantly associated to increase the risk of being anemia. Conversely, being aged 25–34 years and having postsecondary education and daily exposure to television were protective factors against anemia. Therefore, interventions should be considered on geographic variations, improving the provision of safe water and sanitation facilities, and promoting family planning and ANC by providing iron and acid folic supplements during pregnancy. Providing health education through mass media should be enhanced for women in rural areas.

Data Availability

The secondary data used in this analysis are available on reasonable request to the Lao Statistics Bureau, Ministry of Planning and Investment in Lao PDR. https://www.lsb.gov.la/en/home/

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

S. Keokenchanh conceptualized and designed the study, analyzed the data, and wrote the manuscript. A. Morita advised for data analyses. S. Kounnavong advised for ethical review in Lao PDR. S. Kounnavong, A. Tokinobu, W. Ikeda, and K. Midorikawa contributed to the critical revision of the manuscript. S. Keokenchanh has the primary responsibility for the final content. All authors read and approved the final manuscript.

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Research Article

Anemia among Women Who Visit Bost Hospital for Delivery in Helmand Province, Afghanistan

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Background. Anemia is a global public health problem that affects a large number of pregnant women worldwide. In developed and developing countries, the number of pregnant women who become anemic ranges between 18% and 56%, respectively. The aim of this study was to determine the prevalence of anemia and factors associated with anemia among pregnant women who visit Bost Hospital for delivery in Helmand province, Afghanistan. *Methods*. This was a hospital-based cross-sectional study that included 787 pregnant women who visited Bost Hospital for delivery services from January to June 2019. Data was collected in a self-structured questionnaire, which included sociodemographic, obstetrics, and laboratory information. Data was analyzed using SPSS 21.00 Statistical software. The prevalence of anemia was presented as a percentage. Bivariate analysis and binary logistic regression were used to identify the predictors of anemia among pregnant women. *Results*. The overall prevalence of anemia in this study was 51% (95% CI = 48.7%–54.7%). The mean hemoglobin concentration among the study participants was 10.8 (±1.8) g/dL. On bivariate analysis, age group 30 years and above, rural residency and unemployment/housewives, multiparity, and no previous use of contraceptive were found to be associated with anemia. Binary logistic regression showed that multiparity (AOR = 3.09, 95% CI = 1.81–5.29) and no contraceptive use (AOR = 1.53, 95% CI = 1.08–2.16) were the independent predictors of increased anemia among pregnant women. *Conclusion*. Anemia was found to be a severe public health problem in the study area. Policymakers in Afghanistan must accelerate interventions to promote family planning. The need for prospective studies is also suggested to identify other factors associated with anemia among pregnant women.

1. Background

Anemia among pregnant women has proven to be a global public health problem, mainly affecting low- and middle-income countries. It is defined by the World Health Organization (WHO) as a hemoglobin level less than 11gr/dl at any time during pregnancy [1]. It is classified as severe, moderate, and mild when concentrations of hemoglobin (Hb) are <7 g/dL, 7–9.9 g/dL, and 10-11 g/dL, respectively [1, 2].

Anemia can affect anyone at any stage of life. However, it mainly affects pregnant women and children under the age of five. Globally, it affected more than 1.62 billion (25%) people; of these, 56 million cases were among pregnant women [3, 4]. The prevalence of anemia among pregnant women varies in different parts of the world. For instance, its global prevalence is estimated at 41.8%, with the highest prevalence in sub-Saharan Africa (61.3%) [5, 6]. Due to the conflict and instability in Afghanistan, anemia among pregnant women has been less researched. WHO

publications and Demographic Health Surveys (DHS) have summarized that anemia is prevalent in 38.2 % of cases among pregnant women in Afghanistan [5].

Anemia among women who are pregnant has significant adverse health effects. It can deteriorate women's health and raise neonates' risk of adverse health outcomes [7]. In addition, several studies indicated that low-birth-weight, preterm birth, infection, and hemorrhage are caused by anemia during pregnancy [7–9]. WHO recommends full blood count testing for the diagnosis of anemia during pregnancy. However, on-site hemoglobin testing with a portable hemoglobinometer is faster, simpler, and less expensive, and hence, it is recommended for diagnosing anemia in settings where full blood count testing is not available [10].

Various studies have reported anemia among pregnant women [1, 5, 11–19]. In developed and developing countries, the proportion of pregnant women who become anemic varies from 18% to 56%, respectively [5, 18]. Maternal age, marital age, gestational age, residency, gravidity, parity, maternal level of education, inadequate access to iron/folic supplements, and insufficient birth spacing have been found to be associated with anemia among pregnant women [1–3, 8–19]. Evaluation of anemia status and understanding of the factors associated with anemia among pregnant women is critical in reassuring maternal health as well as neonatal health. Monitoring of the Hb level is very important to treat and prevent anemia. Few studies reported in Afghanistan on anemia among pregnant women. Therefore, this study aimed to assess the proportion of anemia and identify factors that are associated with anemia among pregnant women who visit Bost Hospital for delivery in Helmand province.

2. Methods

2.1. Study Setting and Period. This facility-based study was conducted in Bost Hospital of Helmand province, Afghanistan, from January to June 2019. Bost Hospital, a 300-bed facility run by the Ministry of Public Health (MoPH) in collaboration with the Médecins Sans Frontières (MSF), is located at the center of Lashkargah city. This provincial hospital provides a variety of curative and preventive services with special emphasis on curative services to approximately 500000 urban and rural populations.

2.2. Study Design and Population. This study was based on cross-sectional data analysis. Study participants were pregnant women who visited Bost Hospital for delivery in Helmand province. According to WHO recommendations, pregnant women with hemoglobin values below 11 g/dl were considered anemic.

Inclusion and exclusion criteria were as follows: pregnant women who visited Bost Hospital for delivery during the study period were included. Pregnant women with antepartum hemorrhage, who recently transfused blood, who receive therapy for anemia, and who were unable to respond due to severe illness or unwillingness to participate in the study were excluded.

2.3. Sample Size and Sampling Procedures. A total of 787 pregnant women were included in the study. The sample size was determined using the single population proportion's general formula, with the following assumptions: prevalence of anemia in the study area (P) of 38.5% [5], confidence level (CI) of 95%, and margin of error of 5%. We were given a sample size of 363 by this estimate. For the accuracy and validity of the analysis, the sample size was increased to 787.

With regard to the sampling methods, we consecutively included all pregnant women who visited Bost Hospital for delivery services during the study period.

2.4. Data Collection Methods. A self-structured and pretested questionnaire was used to obtain study participants' sociodemographic information, history of obstetrics, and level of Hb. The questionnaire was first prepared in English; later, it was translated to Pashtu and back to English in order to obtain the validity of contents. A face-to-face interview was used to collect data. As data collectors, three clinical nurses, one laboratory technician, and one supervisor were involved. In the health facility, suspected cases of anemia were subjected to Hb level measurement. However, we measured the Hb level of all pregnant women during the study period using the portable device Acon Mission Plus HB meter. As per WHO recommendations, pregnant women with hemoglobin values below 11 g/dl were considered anemic and they were classified as mild (10–10.9 g/dl), moderate (7–9.9 g/dl), severe (below 7 g/dl), and very severe anemic (below 4 g/dl). [1].

2.5. Data Analysis. Collected data were coded and entered into Microsoft Excel (2016). Data quality was checked for consistency, completeness, and accuracy. The data was analyzed using IBM SPSS version 21 [20]. Prevalence of anemia among pregnant women was presented as percentages. Bivariate analysis was used for the possible factors associated with anemia. To determine the strength of association, the Odds Ratio (Chi-square test, Mantel–Haenszel statistics) was estimated. Stepwise multiple logistic regression (forward LR method) was carried out to identify independent determinants for anemia. The factors, which had an association with anemia on bivariate analysis, were included in the multiple regression analysis.

2.6. Ethical Consideration. Ethical approval of the study was obtained from the research committee of the Medical Faculty, Kandahar University. This research has also been approved by the Helmand Public Health Directorate. Informed consent was sought from all study participants. To maintain the confidentiality of the participants in the study, unique identifiers were removed from the data analysis.

3. Results

A total of 787 pregnant women who visited Bost Hospital for delivery were included in the analysis. The mean age of pregnant women was 30.48 years (±7.02). Of the total, 61.4% were of age above 30 years and 64.4% belonged to a rural residence. More than half of the pregnant women (53.3%) had no formal education and 93.8 % were housewives. With reference to the education of the husband, 51.7% of them were uneducated. Over half of households (53.3%) had monthly income ranging from 5000 to 10000. 42.4% and 3.3% of households had >10000 and <5000 monthly incomes, respectively, Table 1 shows sociodemographic information of study participants at baseline.

All pregnant women who participated in this study were presented in the third trimester. Most of the pregnant women (89.1%) had single parity. Approximately 70.1% of women in the past had not used contraception. With reference to the birth interval, most study participants (93.3%) had no birth interval information. In just nine cases, more than two years of the birth interval was documented, while 44 cases had less than two years of birth interval. Table 2 shows obstetrics and medical-related characteristics of study participants at baseline.

The overall prevalence of anemia in this study was 51% (95% CI = 48.7%–54.7%) (Figure 1). The mean (SD) hemoglobin concentration among the study participants was 10.8 (±1.8) g/dL. Of the anemic pregnant women, 171 (42.6%), 213(53.1%), and 17 (4.2%) had mild anemia (Hb ranges 10.0–10.9 g/dL), moderate anemia (Hb ranges 7.0–9.9 g/dL), and severe anemia (Hb < 7.0 g/dL), respectively (Figure 2).

Bivariate analysis of the factors associated with anemia among pregnant women is shown in Table 3. Age 30 years and above ($p = 0.002$, COR = 1.19), rural residency ($p = 0.002$, COR = 1.71), unemployment/housewives ($p = 0.017$, COR = 1.04), multiparity ($p \leq 0.0001$, OR = 2.80), and no previous use of contraceptives were significantly associated with anemia. These factors were included in the binary logistic regression model. It was found that multiparity (AOR = 3.09, 95% CI = 1.81–5.29) and no previous uses of contraceptives (AOR = 1.53, 95% CI = 1.08–2.16) were significantly associated with anemia.

Table 1: Socioeconomic and demographic characteristics of the pregnant women visiting Bost Hospital for delivery in Helmand province, 2019 ($n = 787$).

Characteristic	No. of cases	Percentage
Age group (Years)		
1. < 20	29	3.7
2. 20–24	131	16.6
3. 25–29	144	18.3
4. 30–34	215	27.3
5. 35–39	178	22.6
6. >40	90	11.4
Type of residence		
1. Urban	280	35.6
2. Rural	507	64.4
Maternal education		
1. Illiterate	391	53.3
2. Can read and write	177	24.1
3. Primary	77	10.5
4. Secondary (9–12)	41	5.5
5. Baccalaureate and above	47	6.4
Occupation status		
1. Housewife	738	93.8
2. Employed	47	6.2
Husband education		
1. Educated	301	38.2
2. Uneducated	407	51.7
Household monthly income (in Afghanis)		
1. <5000	26	3.3
2. 5000–10000	424	53.9
3. >10000	334	42.4
4. Don't disclose	3	0.4
Family size		
1. <5	85	10.8
2. >5	702	89.2

Table 2: Obstetrics and medical-related characteristics of the pregnant women visiting Bost Hospital for delivery in Helmand province, 2019 ($n = 787$).

Characteristic	No. of cases	Percentage (%)
Parity		
1. Single	701	89.1
2. Multiple	86	10.9
Previous use of contraceptive		
1. Yes	235	29.9
2. No	552	70.1
Birth interval		
1. <2 years	44	5.6
2. > 2 years	9	1.1
3. No child	133	16.6
4. Don't disclose	601	76.3

4. Discussion

This study was carried out to determine the prevalence of anemia and identify factors that are associated with anemia among pregnant women who attend Bost Hospital for delivery in the Helmand province of Afghanistan. Monitoring of hemoglobin level provides very important inputs for preventing and treating anemia among pregnant women. It is of particular importance in countries like Afghanistan that have been affected by the high Maternal Mortality Ratio (MMR) and conflict-affected weak health systems.

In this study, the proportions of pregnant women who become anemic were 51%. According to the WHO classification of public health significance of anemia, the severity indicates that it is a severe public health problem among pregnant women in the study area [5]. Studies from different developing countries found that 15–59% of pregnant women suffer from anemia during pregnancy [11–18]. However, the prevalence of anemia in this study was higher than that in other studies conducted in Afghanistan [5]. This may be an overestimation of actual

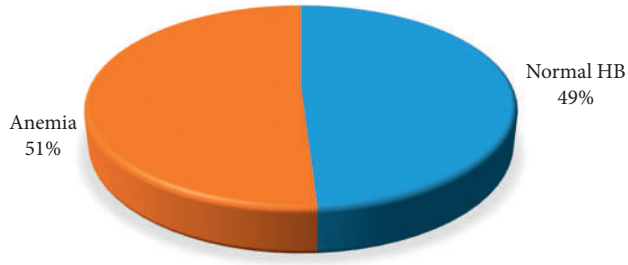

Figure 1: Prevalence of anemia among pregnant women who visit Bost Hospital for delivery ($n = 787$).

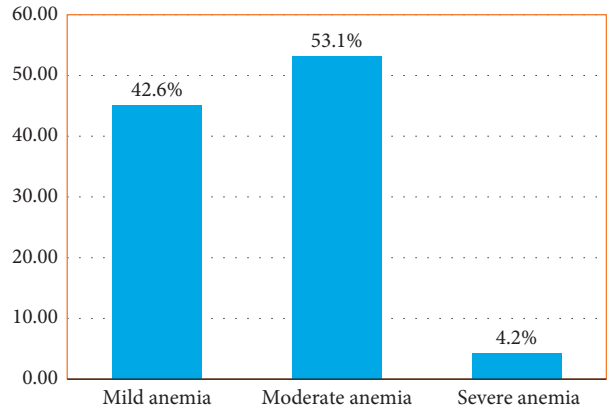

Figure 2: Percentage of anemia by severity among anemic pregnant women ($n = 401$).

Table 3: Factors associated with anemia among pregnant women attending Bost Hospital for delivery in Helmand province, 2019: unadjusted and adjusted odds ratio.

Independent variable		Unadjusted odds ratio (95% CI)	p value	Adjusted odds ratio (95% CI)	p value
Age	<30	1	0.002	1	0.336
	≥30	1.19		1.18 (0.83–1.67)	
Residency	Urban	1	0.002	1	0.317
	Rural	1.71 (1.09–2.45)		1.18 (0.85–1.64)	
Occupation	Employed	1	0.017	1	0.019
	Unemployed	1.04		0.47 (0.25–0.88)	
Parity	Single	1	<0.001	1	<0.001
	Multiple	2.80 (2.34–3.51)		3.09 (1.81–5.29)	
Previous use of contraceptive	Yes	1	0.002	1	0.015
	No	1.15 (1.09–1.45)		1.53 (1.08–2.16)	

figures in this study as this population is only pregnant women who visit the hospital for delivery, which is dominated by those who are in the third trimester. According to published literature, anemia among pregnant women was significantly associated with the third trimester of gestational age [1, 3, 11, 13–16]. Although the prevalence of anemia, in this study, was lower than that in other studies conducted in Pakistan (57.7%) [11] and Sudan (53%) [16], it was higher than that in Iran (16.8 %) [12], India (33.9%) [1], and Bangladesh (34.7%) [13]. Variation in prevalence of anemia reported in different studies can be attributed to variation in socioeconomic status, geographic locations, research methodology, dietary habits of study participants, and other nonexplored factors.

This study revealed that multiple parities (AOR = 3.09, 95% CI = 1.81–5.29) and no previous use of contraceptives (AOR = 1.53, 95% CI = 1.08–2.16) were the independent predictors of anemia among pregnant women. Multiparity and no previous use of contraceptives were documented as important risk factors of anemia among pregnant women in the literature. Several studies have found multiple parities and no previous use of contraceptives as important predictors of anemia among pregnant women (Ethiopia, Ghana, and India—multiparity [14, 15, 19], and Tanzania and Nepal—no previous use of contraceptives [22, 23]). Consistent with previous studies, every pregnancy can increase the risk of hemorrhage before, during, and after pregnancy. Hence, multiparty aggravates the risk of hemorrhage.

Furthermore, iron and other nutrients are depleted during increased and repeated pregnancies. The use of contraceptives not only reduces the number of parities but also has noncontraceptive benefits. The use of contraception as protection against anemia has been documented in several studies. Women with multiparity and no previous use of contraceptives are at higher risk of anemia, develop maternal complications, and are associated with higher adverse health outcomes in neonates [7–9]. Hence, all families should be educated on the importance of family planning at the earliest to avoid anemia during pregnancy.

In addition to multiparity and no previous use of contraceptives, we also identified an association between anemia and old age, rural residency, and unemployment. These factors did not achieve significance in binary logistic regression. Many studies in the past have found that anemia was higher in women of rural dwelling [13, 15, 24]. The higher prevalence of anemia among pregnant women residing in rural areas may be due to inaccessibility to health care centers, lack of anemia-causing factors information, and inappropriate dietary habits.

In this study, anemia was preponderant among unemployed housewives (93.8%). This finding is inconsistent with studies conducted in Ethiopia [25], Uganda [26], and Pakistan [27]. Financial constraints, illiteracy, and not having early access to health care services may play an important role. In conflict-affected zones, social and financial barriers can act as major obstacles for women in seeking care.

Pregnant women of advanced age showed a higher risk of anemia in this study. This result is in agreement with findings from Ethiopia [28], Tanzania [29], and China [30]. However, contradictory findings are reported in studies from Mexico [31], Tabas [32], and Malawi [33]. Anemia in pregnancy is widely believed to increase with parity and maternal age.

This study found no significant association between anemia and monthly income, level of maternal education, and birth interval. In previous studies, however, a significant association was reported [13–18, 21, 33]. Differences in findings of previous reports and this study may be due to differences in monthly income and educational levels of study participants.

4.1. Limitations and Strengths. Firstly, this study was an institutional-based one. Therefore, the results of this study may not reflect what is going on at the level of the community. Secondly, some predictors of anemia were not deeply investigated. Thus, many considerations need to be taken into account in future research, such as antenatal care, history of bleeding, iron/folic acid supplementation, food security, dietary diversity, and marital age. Although this study has been an institutional-based one, which restricts its generalizability, it is the first study of its kind from Afghanistan populations.

5. Conclusion

In this study, the prevalence of anemia among pregnant women was 51 percent, which indicates a severe public health problem in the study area. Multiparity and no previous use of contraceptives were the independent factors that could significantly predict anemia among pregnant women. Hence, Afghanistan's policymakers must accelerate interventions to promote family planning in the country to reduce the prevalence of anemia among pregnant women. The results also suggest the need for prospective studies to identify other factors associated with anemia among pregnant women.

Data Availability

The dataset is available and will be presented on request.

Conflicts of Interest

The authors declare that they do not have any conflicts of interest.

Authors' Contributions

ZA, HS, WW, and AW designed the study. HS, KF, and ZA analyzed the data and prepared the initial manuscript. All authors discussed the results and critically commented on the manuscript at all stages. All authors read and approved the final manuscript.

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