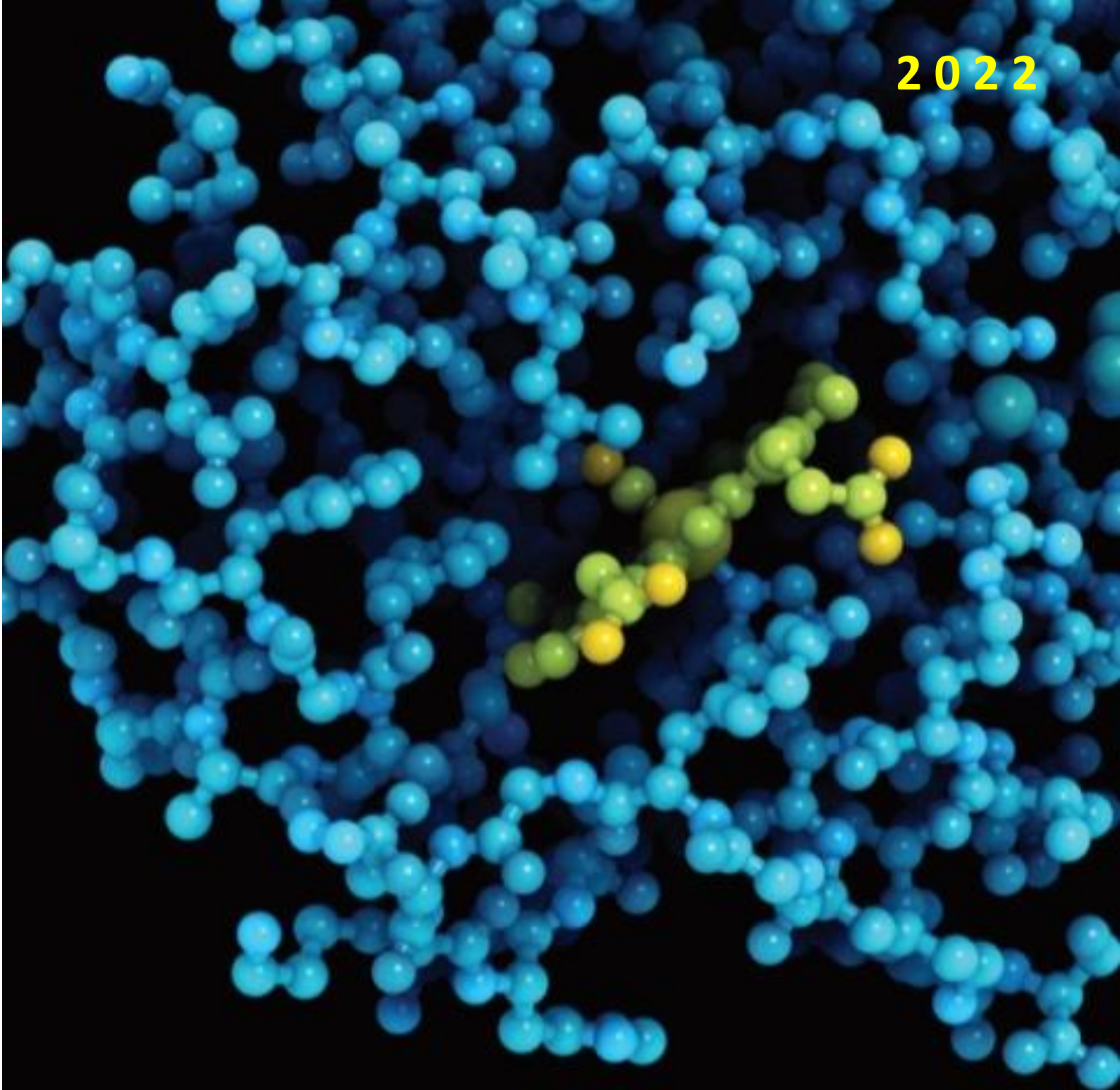

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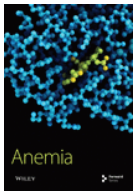

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Research Article

The Prevalence and Pattern of Anaemia in Type 2 Diabetics in Ogbomosho, An Urban Community in Southwestern Nigeria

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Anaemia is a frequent finding in type 2 diabetes, but it is typically seen with established chronic kidney disease and renal insufficiency. Cases, where anaemia predates renal insufficiency, are associated with a worse prognosis for the type 2 diabetes patient and an increased susceptibility to complications. This study aims to determine the prevalence and type of anaemia in persons living with type 2 diabetes without established chronic kidney disease in our environment. The study was a hospital-based cross-sectional study that involved 141 people with known type 2 diabetes as the study group and 140 healthy persons as controls. The study population and the controls were selected using a multistage sampling technique. Data were collected using an interviewer-administered semistructured questionnaire at the Endocrinology clinic, Bowen University Teaching Hospital, Ogbomosho. The data obtained were analyzed using the IBM SPSS version 23.0 (p value ≤0.05 was considered significant). The biochemical (fasting lipids, HBA1C, FBG, serum albumin, creatinine, urea, uric acid, and insulin) and haematological (FBC and red cell indices; PVC, MCV, MCH, MCHC, and RCDW) parameters of the respondents were analyzed using standard methods. The study showed a statistically significant difference in the prevalence of anaemia among subjects, 69.2% as compared to 30.8% of the control group. Normochromic normocytic anaemia was predominant among the subjects, whereas microcytic hypochromic anaemia was the predominant type in the controls. There was no statistically significant difference between MCV and MCHC of both subjects and controls. There was a positive correlation between the incidence of anaemia and the duration of diabetes among the subjects. More people with type 2 diabetes are now living longer, and the addition of haematological parameters should be part of their baseline investigations to aid in the early detection of complications.

1. Introduction

Anaemia is one of the most common preventable public health conditions. Anaemia is defined using the World Health Organization's gender-specific criteria as (Haemoglobin, HB < 13 g/dL in men and <12 g/dL in women) [1]. It is classified into normocytic anaemia when the mean cell volume (MCV) is between 80 and 100 FL, microcytic anaemia when the MCV is less than or equal to 80 FL, and macrocytic anaemia when the MCV is greater than or equal to 100 FL [2].

Diabetes mellitus (DM) is a metabolic disorder with a high prevalence and is associated with disability worldwide. It is defined as persistent hyperglycemia of Glycated haemoglobin A1C, ≥48 mmol/mol (6.5%), fasting plasma glucose level of ≥7.0 mmol/L or random plasma glucose of ≥11.1 mmol/L in the presence of symptoms or signs of diabetes and the presence of relative or absolute insulin

deficiency [3]. About 7% of the world's population has diabetes mellitus and 285 million people have type 2 DM worldwide. It is estimated that by the year 2030, the diabetes patient population worldwide will be about 440 million, with its prevalence increasing fast among developing countries [3, 4].

The disease is classified into two predominant types: type 1 DM, characterized by the absence of endogenous insulin, and type 2 DM, marked by insulin resistance [5, 6]. Anaemia resulting from CKD in type 2 diabetes is due to impaired production of erythropoietin by peritubular fibroblasts, nutritional deficiencies (iron, folate, and B12), inadequate response to erythropoietin, and background proinflammatory conditions. However, anaemia can on occasion predate the onset of nephropathy in T2DM [5, 7]. Normocytic normochromic anaemia may be seen in T2DM before the advent of nephropathy. However, the incidence of anaemia without nephropathy is low as documented by previous studies. Anaemia is an independent factor, for the progression to end-stage renal failure among patients with T2DM, thus, having a prognostic implication for diabetics. Anaemia occurs earlier in patients with diabetic renal disease than in nondiabetic individuals with chronic kidney disease [5, 7].

Anaemia in patients with diabetes mellitus has been found to contribute to the pathogenesis and progression of cardiovascular disease and aggravates diabetic nephropathy and retinopathy [8]. It is therefore clearly important to identify anaemia in patients with T2DM before the onset of renal disease.

The prevalence of anaemia in T2DM without established CKD is not well explored in Nigeria. Therefore, this study was carried out to determine the prevalence and type of anaemia in T2DM in the absence of CKD at the Bowen University Teaching Hospital, Ogbomosho.

2. Methods and Materials

The study was a hospital-based cross-sectional study done over 12 months at the endocrinology clinic of Bowen University Teaching Hospital in Ogbomosho, Nigeria. The study involved all consenting type 2 diabetes patients aged 15–90 years who met the inclusion criteria. Patient with acutely ill conditions, those with concurrent hemoglobinopathies like sickle cell anaemia and G6PD deficiency, patients with documented or on treatment for nutritional anaemia, and patients who have been diagnosed with chronic kidney disease and renal insufficiency and have commenced management were excluded from the study.

2.1. Sample Size Determination and Sampling Technique. The sample size was calculated based on two population mean formulae using G-Power statistical free software version 3.1, by considering the following assumptions: 95% confidence level (2-tailed, $\alpha = 0.05$), 80% power ($\beta = 0.20$), the ratio of sample size (T2DM/control) was 1 : 1, effect size (d) was 0.36 and 10% anticipated nonresponse rate. The sample size was determined to be 141 for the study group and age and sex-matched 140 healthy controls, thus, a total of 281 study participants were included in this study to enhance representativeness. A multistage sampling technique was used.

Stage 1: The researcher made use of information on patients' cards to sort out those who were being managed for diabetes only at the endocrinology clinic.

Stage 2: Involved listing eligible patients determined by assessing their serum urea, creatinine, and urinalysis.

Stage 3: The first eligible respondent was selected by simple random sampling through the balloting method. Then, a systematic random sampling technique was used to select subsequent respondents (Kth respondent) using the sampling interval obtained from the patient's daily lists throughout the investigation at the endocrinology clinic.

The age and sex-matched healthy controls also had a baseline assessment of their serum electrolytes, urea, creatinine, and glomerular filtration rate (GFR) to rule out underlying renal impairment.

2.2. Research Instrument and Data Collection Methods. Sociodemographic and clinical data of the DM and control participants were collected using a semistructured interviewer-administered questionnaire, which was used to obtain important information like age, gender, ethnicity, past and present symptoms of anaemia, past and present treatment of anaemia, and anthropometric measurements, which included height, weight, waist circumference, hip and neck circumference, blood pressure, and body mass index (BMI).

2.2.1. Sample Collection and Preparation. Ten millilitres of venous blood were collected after overnight fasting from the antecubital vein after cleaning with methylated spirit. Each sample collected was separated into three bottles (Two—potassium ethylene diaminotetra acetic, KEDTA, and fluoride oxalate). Whole blood put in bottle 1 was used for full blood count, peripheral blood film, and red cell indices analysis. The second EDTA bottle was separated into plasma by centrifuge and stored frozen for biochemical analysis. Fluoride oxalate was used for fasting plasma glucose.

The laboratory investigations include the full blood count with emphasis on the packed cell volume (PCV) and red cell indices, which are mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RCDW) using the Mindray haematology autoanalyzer BC-10. Biochemical parameters include fasting lipids (total cholesterol, triglycerides, high-density lipoprotein HDL, low-density lipoprotein, LDL), glycated haemoglobin (HBA1C), fasting blood glucose, serum albumin, creatinine, urea, and uric acid obtained using the JENWAY6305 and UNISPEC semiauto chemistry analyzer. Insulin assay was done using a surgifield SM-MR96A

microplate reader. All the parameters were run along with the controls provided by the kit manufacturers.

2.3. Measurement of Main Outcome Variables. The following variables were considered: age, sex, weight, height, waist circumference, hip and neck circumference, blood pressure, body mass index (BMI), full blood count including the packed cell volume (PCV) red cell indices, which are mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RCDW) and patients' diabetes status.

2.4. Data Analysis. Data were checked for completeness and consistency and entered into Statistical Package for the Social Sciences (SPSS) version 23 software (IBM Corporation, SPSS Chicago Inc., IL, USA) for analysis. The results were reported as frequency and percentages for categorical variables and mean ± standard deviation (SD) for normally distributed continuous variables. Statistical differences between the groups were determined by the Pearson chi-square test for categorical variables. Differences were considered to be statistically significant at a p value less than 0.05.

2.5. Ethical Approval and Consent to Participate. Ethical approval was obtained from Bowen University's ethical review committee. Written informed consent was taken from each participant after an adequate explanation of the objectives of the study and the benefits of the study before recruitment into the study. Respondents were told that the participation of persons was voluntary. All other processes of recruitment adhere strictly to the guidelines stipulated by the Helsinki declaration. All information gathered was kept confidential, and participants were identified using only serial numbers.

3. Results

A total of two hundred and eighty-one (281) respondents were recruited for the study. One hundred and forty-one (141) were people living with type 2 diabetes (study), while the remaining one hundred and forty (140) were recruited to serve as controls. The two groups were matched for age and sex.

Table 1 shows the sociodemographic characteristics of the respondents. Most of the respondents were from the Yoruba tribe. Only 33.1% of the subjects had tertiary education, compared to 66.7% in the controls. The majority of the participants 114 (67.5%) in the control group were skilled workers, but only 55 (32.5%) were skilled workers in the subject group. The control group had a 113 (56.5%) predominance of married responders, whereas the widowed were more frequent in subject 47 (90.4%). Most of the respondents in both groups were Christians. The average monthly income was lower in the subjects compared with the control group.

Table 1: Sociodemographic characteristics of diabetics and non-diabetics respondents.

Variables	Subjects n (%)	Controls n (%)
Age (years)		
<40	9 (17.0)	44 (83.0)
>40	132 (57.9)	96 (42.1)
Gender		
Male	51 (47.7)	56 (52.3)
Female	90 (51.7)	84 (48.3)
Ethnicity		
Yoruba	137 (51.5)	129 (48.5)
Igbo	2 (50.0)	2 (50.0)
Hausa	1 (33.3)	2 (66.7)
Others	1 (14.3)	6 (85.7)
Education level		
No formal education	37 (92.5)	3 (7.5)
Primary	26 (72.2)	10 (27.8)
Secondary	25 (73.5)	9 (26.5)
Higher institution	43 (33.1)	87 (66.9)
Postgraduate	8 (22.2)	28 (77.8)
Others	2 (40.0)	3 (60.0)
Occupation		
Unemployed	2 (14.3)	12 (85.7)
Skilled worker	55 (32.5)	114 (67.5)
Semiskilled	59 (86.8)	9 (13.2)
Unskilled worker	19 (79.2)	5 (20.8)
Retired	6 (100.0)	0 (0)
Marital status		
Single	7 (24.1)	22 (75.9)
Married	87 (43.5)	113 (56.5)
Widowed	47 (90.4)	5 (9.6)
Religion		
Christianity	122 (49.0)	127 (51.0)
Islam	19 (67.9)	9 (32.1)
Traditional	0 (0)	3 (100.0)
Others	0 (0)	1 (100.0)
Average monthly income		
<₦20,000	62 (68.1)	29 (31.9)
₦21,000–₦40,000	24 (53.3)	21 (46.7)
>₦40,000	51 (36.4)	89 (63.6)
Retired	4 (100.0)	0 (0)
Year diagnosed with the disease		
<5	68 (48.2)	0
5–10	38 (27.0)	0
>10	35 (24.8)	0

Table 2 shows the past and present symptoms of anaemia in both the subject and the control groups. The past symptoms of anaemia (fatigue, pallor, pedal swelling, palpitation, yellow sclera, and passage of coke-coloured urine) were statistically significant in the study group when compared to the control. p values of <0.001, 0.008, <0.001, <0.001, 0.014, and 0.004, respectively. The present symptoms of anaemia (fatigue, pallor, and pedal swelling) were also more prevalent in the study group. p values <0.01, <0.001 and <0.001 respectively.

Table 3 shows the risk factors for diabetes. The risk factors include a family history of diabetes, smoking, alcohol, physical inactivity, obesity, and stress. The risk factors were more common in the study group than in the controls. p

Table 2: History of past and present symptoms of anaemia diabetics and nondiabetics.

Variables	Subjects (141)	Control (140)	Df	p value
Past				
Fatigue			1	<0.001
Yes	38 (88.4)	5 (11.6)		
No	103 (43.3)	135 (56.7)		
Pallor			1	0.008
Yes	12 (91.7)	1 (8.3)		
No	129 (48.1)	139 (51.9)		
Pedal swelling			1	<0.001
Yes	17 (100.0)	0 (0)		
No	124 (47.0)	140 (53.0)		
Heart beat			1	<0.001
Yes	34 (89.5)	4 (10.5)		
No	107 (44.0)	136 (56.0)		
Yellow sclera			1	0.014
Yes	6 (100.0)	0 (0)		
No	135 (49.1)	140 (50.9)		
Cola urine			1	0.004
Yes	8 (100.0)	0 (0)		
No	8 (100.0)	140 (51.3)		
Present				
Fatigue			1	<0.001
Yes	18 (90.0)	2 (10.0)		
No	123 (47.1)	138 (52.9)		
Pallor			1	<0.001
Yes	6 (100.0)	0 (0)		
No	135 (49.1)	140 (50.9)		
Pedal swelling			1	<0.001
Yes	14 (100.0)	0 (0)		
No	127 (47.6)	140 (52.4)		
Heart beat			1	0.056
Yes	11 (73.3)	4 (26.7)		
No	130 (48.9)	136 (51.1)		
Yellow sclera				0.062
Yes	4 (100.0)	0 (0)		
No	137 (49.5)	140 (50.5)		
Cola urine			1	0.15
Yes	6 (100.0)	0 (0)		
No	135 (49.1)	140 (50.9)		

values of <0.001, 0.007, 0.003, 0.004, 0.016, and <0.001, respectively.

Table 4 shows the anthropometric measurements and the haematological parameters of the respondents. The mean height of the controls was significantly higher than that of the subjects (1.658 ± 0.084 m vs. 1.604 ± 0.102 m; $p < 0.001$). The mean BMI in the subjects was higher than controls (27.44 ± 5.387 kg/m^2 vs. 25.98 ± 5.221 kg/m^2; $p = 0.021$). The mean waist circumference was also higher in the subjects than in the controls (95.957 ± 19.343 cm vs. 87.157 ± 18.288 cm; $p < 0.001$). The mean PCV found in subjects ($36.565 \pm 5.425\%$) was significantly lower than what was found in the controls ($40.751 \pm 5.056\%$) ($p < 0.001$). Similarly, the mean MCH and RCDW of the subjects (26.59 ± 4.784 pg; 15.14 ± 6.435) were significantly lower than what was found in the control group (28.44 ± 6.225 pg; 39.34 ± 6.113) ($p = 0.006$, $p < 0.001$), respectively. There was no statistically significant difference in the mean values for MCV and MCHC between the two groups, with the diabetes group having (81.812 ± 23.820 fl; 329.31 ± 53.926 g/l) compared to (82.252 ± 7.579 fl; 334.90 ± 10.669 g/l), respectively, in the control group.

Table 3: Risk factors for DM.

Variables	Subjects (141)	Controls (140)	df	p value
Family history DM			1	<0.001
Yes	42 (76.4)	13 (23.6)		
No	99 (43.8)	127 (56.2)		
Smoking			1	0.007
Yes	7 (100.0)	0 (0)		
No	134 (48.9)	140 (51.1)		
Alcohol			1	0.003
Yes	13 (86.7)	2 (13.7)		
No	128 (48.1)	138 (51.9)		
Physical inactivity			1	0.004
Yes	8 (100.0)	0 (0)		
No	133 (48.7)	140 (51.3)		
Obesity			1	0.016
Yes	12 (80.0)	3 (20.0)		
No	129 (48.5)	137 (51.5)		
Stress			1	<0.001
Yes	32 (80.0)	8 (20.0)		
No	109 (45.2)	132 (54.8)		
Others			1	0.502
Yes	1 (100.0)	0 (0)		
No	140 (50.0)	140 (50.0)		

Table 5shows the laboratory parameters of the subjects and the controls. The glycated haemoglobin was statistically significant between the two groups (4.9116 ± 1.4443; 5.4451 ± 0.8170. $p < 0.001$). Moreover, the fasting sugar was significantly elevated in the diabetes group (7.2745 ± 4.24105) compared to the control group (4.8438 ± 0.91974) $p < 0.001$. The insulin assay was higher in the subjects than in the controls (10.4489 ± 11.40753 v 8.0871 ± 10.68929), respectively, but not statistically significant $p = 0.074$. The total cholesterol was more in the subjects than in the controls (5.7097 ± 1.63745 v 4.5510 ± 1.43974 mmol/l; $p < 0.001$). The HDL-c is lower in the subjects (1.6356 ± 1.0055 v 1.9162 ± 0.79067 mmol/l; $p < 0.001$) while the LDL is higher in subjects (4.7724 ± 11.1792 v 2.4175 ± 1.39066 mmol/l; $p < 0.001$).

Table 6 shows the presence of anaemia in subjects and controls. 69.2% had anaemia in the subjects, whereas only 30.8% had anaemia in the controls. This is statistically significant, with $p < 0.001$. Anaemia is commoner in females in both subjects and controls, as seen in Figure 1.

Table 7 shows the pattern of anaemia in the anaemic subjects, with the majority (66.3%) having normocytic normochromic anaemia.

Table 8 shows the history of DM complications among the anaemic subjects. The complications include retinopathy, diabetic foot, diabetic nephropathy, amputation, diabetic coma, and neuropathy. There was no statistical significance, but complications like retinopathy, diabetic foot, and neuropathy were more common in subjects with anaemia.

Table 4: Anthropometric measurement and red cell indices of diabetics and nondiabetics respondents.

Variables	Categories	Means ± SD	df	p value	95% CI	
					Lower	Upper
Height (m)	Subject Control	1.604 ± 0.102 1.658 ± 0.084	279	<0.001	−0.076	−0.032
BMI (Kg/m²)	Subject Control	27.44 ± 5.387 25.98 ± 5.221	279	0.021	0.222	2.714
BP diastolic (mmHg)	Subject Control	81.170 ± 15.527 80.492 ± 10.865	279	0.672	−2.471	3.826
Waist circumference (cm)	Subject Control	95.957 ± 19.343 87.157 ± 18.288	279	<0.001	4.378	13.221
Weight (Kg)	Subject Control	71.111 ± 13.808 70.431 ± 13.621	279	0.678	−2.541	3.901
Hip circumference (cm)	Subject Control	103.91 ± 15.906 101.12 ± 20.110	279	0.197	−1.458	7.054
Neck circumference (cm)	Subject Control	37.333 ± 5.520 35.650 ± 9.684	279	0.074	−0.166	3.532
PCV (%)	Subject Control	36.565 ± 5.425 40.751 ± 5.056	279	<0.001	−5.417	−2.954
MCV	Subject Control	81.812 ± 23.820 82.252 ± 7.579	279	0.835	−4.597	3.717
MCHC	Subject Control	329.31 ± 53.926 334.90 ± 10.669	279	0.231	−14.725	3.563
M	Subject Control	26.59 ± 4.784 28.44 ± 6.225	279	0.006	−3.149	−0.543
RCDW	Subject Control	15.14 ± 6.435 39.34 ± 6.113	279	<0.001	−25.681	−22.732

Table 5: Biochemical parameters of diabetics and nondiabetics respondents.

Variables	Categories	Mean	SD	df	p value	95% CL	
						Lower	Upper
Total cholesterol (mmol/L)	Subject Control	5.7097 4.5510	1.63747 1.43974	279	<0.001	0.796	1.520
Triglyceride (mmol/L)	Subject Control	0.5645 0.6974	0.76538 0.80927	279	0.158	−0.317	0.052
HDL-C (mmol/L)	Subject Control	1.6356 1.9162	1.00546 0.79067	279	0.01	−0.49313	−0.06810
LDL-C (mmol/L)	Subject Control	4.7724 2.4175	11.17922 1.39066	279	0.014	0.48079	4.22906
Glycated haemoglobin	Subject Control	4.9116 5.4451	1.44434 0.81705	279	<0.001	−0.809	−0.257
Fasting blood glucose	Subject Control	7.2745 4.8436	4.24105 0.91974	279	<0.001	1.70902	3.15277
Serum albumin (mmol/L)	Subject Control	46.9641 44.8421	10.83430 5.19031	279	0.037	0.12463	4.11932
Creatinine (mmol/L)	Subject Control	62.9433 60.1950	31.43787 20.41335	279	0.386	−3.4813	8.9778
Urea (mmol/L)	Subject Control Control	4.1599 4.1293 134.6143	1.97149 1.97059 134.10463	279	0.896	−0.43228	0.49357
Insulin	Subject Control	10.4489 8.0871	11.40753 10.68929	279	0.074	−0.23475	4.95833

Table 6: Presence of anaemia in subjects and controls.

Variables	Subjects	Controls	df	p value
Anaemia	83 (69.2)	37 (30.8)	1	<0.01
Normal	58 (36.0)	103 (64.0)		

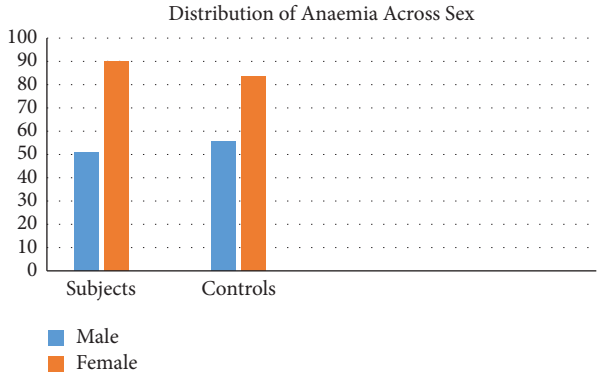

Figure 1: Distribution of anaemia across sex.

Table 7: The pattern of anaemia among the anaemic subjects (83).

Variables	Frequency	Percentage (%)
Anaemia of chronic disorder/normochromic normocytic anaemia	55	66.3
Iron deficiency anaemia	28	33.7
Megaloblastic anaemia	0	0.0

Table 8: History of DM complications among subjects and anaemia.

Variables	Anaemia	Normal	Df	p value
Diabetic retinopathy			1	0.564
Yes	18 (54.5)	15 (45.5)		
No	65 (60.2)	43 (39.8)		
Diabetic foot			1	0.228
Yes	11 (73.3)	4 (26.7)		
No	72 (57.1)	54 (42.9)		
Diabetic nephropathy			1	0.383
Yes	2 (40.0)	3 (60.0)		
No	81 (59.9)	55 (40.4)		
Foot/toe amputation			1	0.230
Yes	0 (0.0)	1 (100)		
No	83 (59.3)	57 (40.7)		
Diabetic coma			1	0.924
Yes	4 (57.1)	3 (42.9)		
No	79 (59.0)	55 (41.0)		
Diabetic neuropathy			1	0.798
Yes	26 (60.5)	17 (39.5)		
No	57 (58.2)	41 (41.8)		
Other complications			1	0.506
Yes	3 (75.0)	1 (25.0)		
No	80 (58.4)	57 (41.6)		

4. Discussion

The study set out to investigate the presence of anaemia in type 2 diabetics who do not have established chronic renal disease. It also classified any anaemia found using red cell indices as a first step towards understanding the pathophysiology of anaemia in type 2 DM.

This study revealed that diabetes mellitus was more prevalent in people older than forty years old and commoner in females than males, a finding supported by Hillier and Pedula [8]. Most of the participants in the subject group are of the Yoruba tribe, with only a few having no formal education. The majority had at least a primary school education; most were skilled and semiskilled; the majority were married and had an average monthly income of <20.000 nairas; and many of the participants had been diagnosed with type 2 diabetes for more than 5 years. These findings were largely linked to the study area (Ogbomosho), where most inhabitants are of Yoruba ethnic descent, Christians, with varying degrees of skilled and semiskilled workers.

Eighty-three out of one hundred and forty-one subjects (69.3%) had anaemia compared to 37 (30.8%) in controls. This is in keeping with previous studies that anaemia is common in patients with diabetes mellitus [9, 10]. This finding is similar to other studies set in similar socioeconomic and geographical locations as Nigeria. This suggests that the similarities in socioeconomic status and geography may have directly or indirectly affected the cause of anaemia in T2DM [11–14].

In some other studies, anaemia was not a prominent finding amongst type 2 diabetes patients that predated chronic kidney disease [10, 15–17]. The reason for the difference in prevalence may be due to environmental factors such as altitude, humidity, the proliferation of

malaria where subclinical infections are common, and the length of time of the disease [5, 18]. It was found that not only was clinical anaemia more common in type 2 diabetes, but the symptoms and the subjective experience of anaemia were more prevalent in type 2 diabetics both past and present. This also corroborates the fact that anaemia and diabetes mellitus might have coexisted before the identification of anaemia [10, 13, 17, 19]. It is also significant to note that a third of the controls also had anaemia. This suggests that anaemia is not only a problem seen in people living with DM but also in the average Nigerian. This may be due to the poor nutritional status found in developing nations like Nigeria [20].

The commonest risk factor for diabetes mellitus in this study is family history. Aravinda in India also found family history and obesity as significant risk factors for the development of diabetes mellitus [14]. However, Barbieri et al. found hypertension and body mass to be significant risk factors. Other significant risk factors were physical inactivity, smoking, alcohol intake, and obesity [13]. All these have been previously documented as significant risk factors by previous studies [14, 19, 21]. The BMI and waist circumference of subjects in this study were statistically significant compared to the control, and this is in agreement with the findings of other authors [19, 21, 22]. These anthropometric parameters are indicators for metabolic syndrome, which is common among people living with diabetes [23, 24].

The packed cell volume, MCH, MCV, and MCHC findings in this study were similar to the findings of Arkew et al. but the RCDW was different [16]. In both studies, anaemia was more prevalent among the type 2 diabetics, but RCDW was much higher in the controls in this study, and significantly increased in their subjects [16]. The reason may be due to different geographical locations and diets. Normochromic normocytic anaemia is the commonest type in this study and it is similar to findings by other authors [13, 25]. This is consistent with anaemia of chronic disease, as found also by Abate et al. [12] pointing out the chronic nature of diabetes mellitus. This is also in consonance with the findings that normochromic normocytic anaemia predates the onset of nephropathy [8, 10]. Microcytic hypochromic anaemia is also seen in about one-third of the subjects with anaemia as found by another author [12] this is also in support of the fact that the aetiology of anaemia is multifactorial [25]. However, megaloblastic anaemia was not detected in either arm of the study. It was discovered that the complications associated with T2DM are more common in those with anaemia. This also supports the fact that quality of life is considered poor in people with T2DM and anaemia [17, 26] and that monitoring and treating anaemia adequately and promptly prevents the development of kidney and cardiovascular diseases [18, 27].

The biochemical parameters in this study showed a statistically significant increase in both glycated haemoglobin and fasting blood sugar in both cohorts in this study. This is in keeping with findings from previous authors [12, 14, 25]. However, this should be within the normal range as found in the controls because the patients are already on one form of treatment or the other. This showed that most patients' blood sugar was not well controlled. Moreover, this is further corroborated by the fact that the insulin assay is higher in subjects than in controls, signifying insulin resistance in T2DM [5]. The total cholesterol and LDL were higher in the subjects than in the controls, while HDL was lower than in the controls. Although the total cholesterol and HDL were statistically significant, LDL and triglycerides were not. This further proves that T2DM had a high prevalence of metabolic syndrome [24].

In conclusion, anaemia in T2DM in this study is high, with anaemia of chronic disease being the most common, and supporting the fact that the anaemia is multifactorial, although the subjects do not have chronic renal disease. This study also supports similar findings that there is insulin resistance in T2DM and that metabolic syndrome is an important component of the disease spectrum. Physicians need to include haematological parameters as part of baseline investigations in T2DM before the onset of complications.

Data Availability

The data can be made available from the corresponding author upon request.

Disclosure

The university had no other role in the study or the decision to submit the article for publication.

Conflicts of Interest

The authors declare that there are no conflicts of interest with this manuscript.

Acknowledgments

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Review Article

Burden and Determinants of Anemia among Under-Five Children in Africa: Systematic Review and Meta-Analysis

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Introduction. Globally, anemia among under-five children is a serious public health problem. Even if there are pocket studies here and there, there is limited evidence on the pooled prevalence of anemia among under-five children in Africa. Therefore, the aim of this study was to determine the pooled prevalence and determinants of anemia. *Methods and Analysis*. This systematic review and meta-analysis was done following the PRISMA guidelines. A comprehensive search was made in PubMed/MEDLINE, Cochrane Library, HINARI, and Ethiopian Journal of Health Development for studies published since 2009. It was supplemented with Google Scholar search. Study selection, data extraction, and quality of studies were assessed by eight reviewers. The Cochrane Q test and I^2 test statistic were used to test the heterogeneity of studies. A random-effects model of DerSimonian-Laird method was used. *Result*. A total of 37 articles were included in this systematic review and meta-analysis. The pooled prevalence of anemia among under-five children in Africa was 59% (95% CI: 55, 63). Being female (AOR = 0.71; 95% CI: 0.57, 0.87), maternal education (AOR = 1.47; 95% CI: 1.31, 1.66), residence (AOR = 0.80; 95% CI: 0.67, 0.95), and family size (AOR = 0.93; 95% CI: 0.89, 0.98) were the determinants of anemia among African under-five children. *Conclusion and Recommendation*. This pooled study revealed that anemia was a severe public health problem. Sex, maternal education, residence, and family size were the determinants of anemia. Therefore, anemia prevention strategy should include sex consideration, educating mothers through youth education, area specific intervention, and encouraging birth spacing.

1. Background

Anemia among under-five children is defined as a hemoglobin level <11 mg/dl or children with hematocrit less than 33% [1]. Worldwide, anemia among under-five children is a major public health problem [2]. Globally, 20 million infants were born with low birth weight (LBW) every year. Nearly, 3.6 million of them died before celebrating their 28 days, of whom almost two-thirds were located in Sub-Saharan Africa and Southern Asia [3]. The effect of anemia can extend up to postpartum period and even newly delivered baby may suffer from a reduced iron store problem up to one year [4]. In developing countries, 46–66% of children under the age of five were affected by anemia [3]. African and Asian regions were the major contributor for a high burden of anemia [5].

The rapid growth and cognitive development of children make them more vulnerable for the development of anemia [6]. The consequences of iron deficiency anemia (IDA) during childhood include growth retardation, reduced school achievement, impaired motor and cognitive development, and increased morbidity and mortality. Mental impairments at early age are thought to be irreversible and the

consequences may continue even after treatment, reinforcing the importance of early detection and prevention [7, 8].

The causes for anemia among under-five children are complex. Among these, low birth weight, undernutrition, poor socioeconomic status, household food insecurity, duration of breast feeding, poor dietary iron intake, poor maternal educational status, diarrhea, fever, poverty, poor sanitation and hygiene, monotonous diet, parent's level of education, and maternal anemia were the commonest contributors for under-five anemia [9–13].

Despite the numerous interventions done so far by the government of African countries and other concerning stakeholders, anemia among under-five children is still a severe public health concern [14–19]. Even though many independent pocket studies have been conducted in the region, the results were inconsistent and the prevalence varies significantly between studies [20–22]. In Africa, the pooled prevalence and determinants of anemia among under-five children have not been yet done. Assessing the pooled result will help to inspire the government's commitment and increase the social and resource mobilization in order to enhance the implementation of evidence based interventions for culminating the effect of anemia among under-five children in particular and the nation in general. Therefore, the aim of this study was to determine the pooled prevalence and determinants of anemia among under-five children in Africa. The findings of this study will help policy makers, program planners, health care providers, and concerned stakeholders to work more on anemia in order to reduce the prevalence of anemia, its consequences, and complication among under-five children.

Prompt identification and treatment of anemia lead to overall improvement of population health outcomes, improved physical exercise performance, and well-being that results in enhanced economic productivity.

2. Methods and Materials

2.1. Patient and Public Involvement. All under-five children in Africa were involved in this study.

2.2. Eligibility Criteria. All studies that reported prevalence and determinants of anemia among under-five children in Africa using English language and gray literatures were included. For estimating the prevalence of anemia, studies with cross-sectional design were included, while, for pooling the determinants of anemia, cross-sectional and case-control studies were included in the study. While studies whose full texts cannot be accessed after trying to contact the primary investigator within 3 months, descriptive studies, systematic reviews of the effects of an intervention, review articles, conference abstract and editorials were excluded from the study.

2.3. Search Strategies. This systematic review and meta-analysis was performed according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines [23]. The study was conducted following the Joanna Briggs Institute (JBI) criteria. Data bases such as MEDLINE (via PubMed), EMBASE, and Cochrane Library, SCOPUS, HINARY, and Google Scholar were used to extensively search the relevant articles conducted since January 1, 2009. Gray literatures were also included by manual search.

2.4. Search Terms Used. The strategy applied to search articles from the electronic data bases was (anemia) OR ("iron deficiency anemia") OR ("low hemoglobin level") AND (determinants) OR ("associated factors") ("Under Five Children") AND (Ethiopia) OR (Eritrea) OR (Kenya) OR (Angola) OR (Benin) OR (Botswana) OR ("Burkina Faso") OR (Burundi) OR (Cameroon) OR ("Cape Verde") OR ("Central African Republic of Chad") OR (Comoros) OR (Congo) OR ("Côte d'Ivoire") OR (Djibouti) OR ("Equatorial Guinea") OR (Gabon) OR (Gambia) OR (Ghana) OR (Guinea) OR ("Guinea-Bissau") OR (Lesotho) OR (Liberia) OR (Madagascar) OR (Malawi) OR (MAli) OR (Mauritania) OR (Mauritius) OR (Mozambique) OR (Namibia) OR (Niger) OR (Nigeria) OR (Réunion) OR (Rwanda) OR ("Sao Tome and Principe") OR (Senegal) OR ("Seychelles") OR (Sierra Leone) OR (Somalia) OR ("South Africa") OR ("Sudan") OR (Swaziland) OR (Tanzania) OR (Togo) OR (Uganda) OR ("Western Sahara") OR (Zambia) OR (Zimbabwe).

2.5. Data Extraction. After obtaining the full text of all articles, duplicates were screened and removed from the citation manger. Based on the eligibility criteria, eight reviewers (SE, AA, TC, AW, MY, FM, FY, and YW) independently reviewed the studies by title, abstract, and full article. Those included and undecided studies were further assessed by reading the full text. Studies that were not eligible based on the full text assessment were excluded and reasons were described for their exclusion in combination with the PRISMA flow diagram to summarize the selection procedure [23]. Studies that passed through this selection process were included in this study. Discrepancies between authors were resolved through discussion and consensus. The study characteristics (author, year of publication, region, target group, sample size, study design, response rate, and children with anemia), subject recruitment procedures, count data with (2×2 tables), crude odds ratio (where count data were not found), and population characteristics were extracted by using extraction sheet developed with Microsoft Excel 2013.

2.6. Quality Assessment and Risk of Bias. The Joanna Briggs Institute (JBI) critical appraisal check list was used to assess the quality of each paper. During data extraction, eight investigators independently performed the quality assessment. The quality scores of six data extractors were averaged. Any disagreement between investigators was solved by discussion and consensus. Finally, studies with higher scores (>50%) were included in the systematic review and meta-analysis.

2.7. Data Synthesis and Analysis. Data were analyzed using STATA version 14.0. The pooled proportion was calculated to estimate the prevalence of anemia. The pooled odds ratio (OR) with 95% CI was determined to estimate the

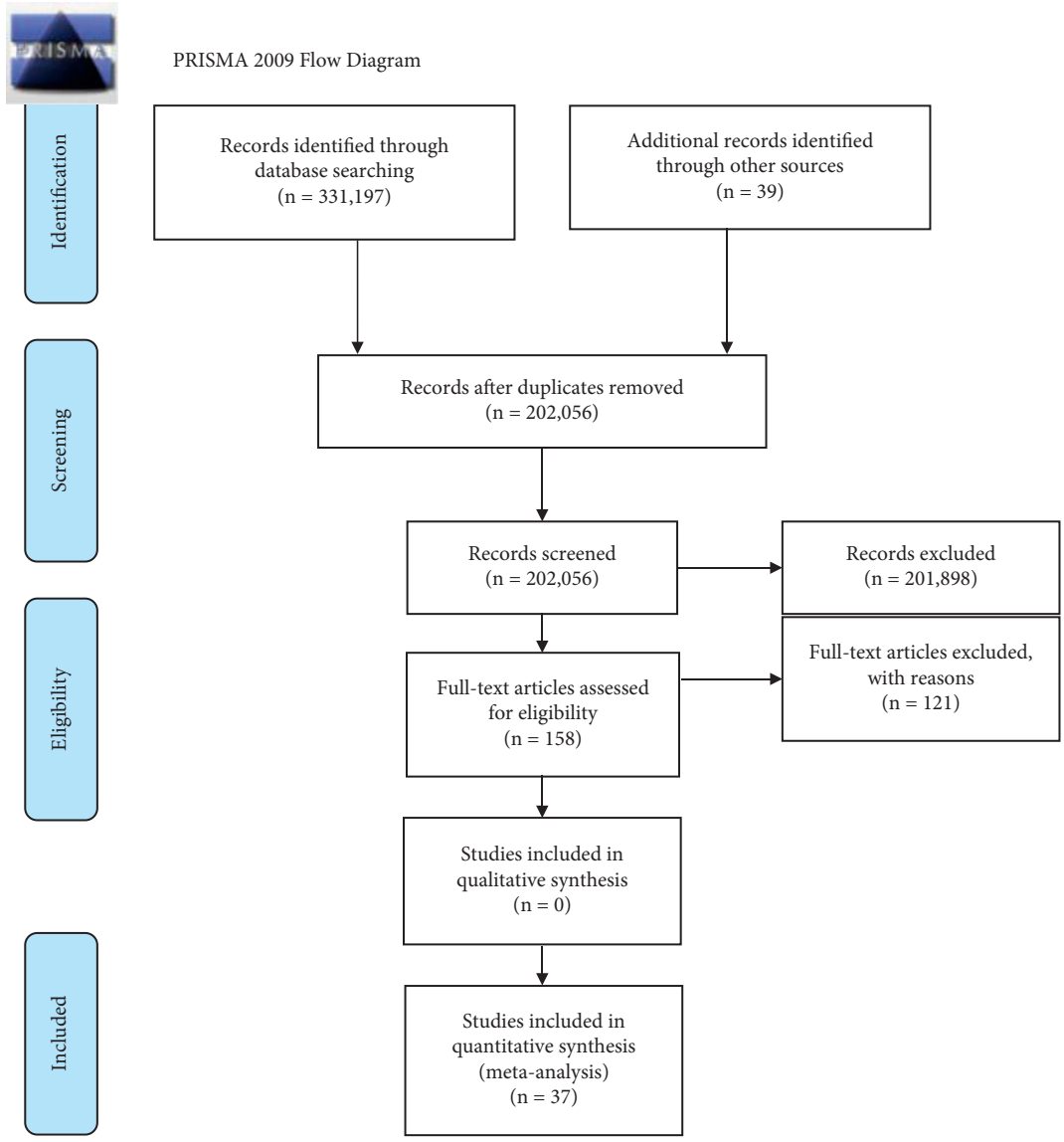

Figure 1: Preferred reporting items for systematic review and meta-analysis, June 2020.

determinants of anemia among under-five children. The degree of heterogeneity was checked by Cochran Q and I^2 statistics. The Cochrane Q statistic was considered significant, if the Pvalue is <0.10, while the I^2 statistics at least 50% was considered to be significant [24, 25]. Since the variation between the study findings is significant, a random-effects model with 95% confidence interval was used. Heterogeneity was checked by running metaregression, subgroup analysis, and sensitivity analysis. Subgroup analysis was performed based on sex and study setting (region). Funnel plots analysis, Egger weighted regression, and Begg rank correlation tests were done to detect publication bias ($P < 0.05$ was considered as a suggestive of statistically significant publication bias) [25, 26].

2.8. Registration and Reporting. This systematic review and meta-analysis was registered in the PROSPERO with a CRD number of 42020150881.

2.9. Ethical Clearance. This study was reviewed and approved by institutional review board of College of Medicine and Health Sciences, Wollo University.

3. Result

A total of 331,236 articles were retrieved by literature search (Figure 1). Of these, 129,180 were excluded because of duplication, 201,898 did not have any relation with the aim of this study, and 121 did not meet the eligibility criteria. Finally, only 37 articles were included in this systematic review and meta-analysis. All included articles were full text and done using cross-sectional study design with one cohort [27] and three case controls [28–30]. The sample population varied from 210 [28] to 8,260 [31] children aging between 0 and 59 months. In this study, a total of 67,647 under-five children were included. The overall information regarding the prevalence of anemia was obtained from twenty African

Table 1: Summary of extracted studies on anemia among under-five children in Africa, 2009–2020.

Author	Publication year	Study setting	Target group	Study design	Sample size	Prevalence	Quality
Gaston et al.	2009	Lesotho	Under five	Cross-sectional	1295	49	76.5
Gaston et al.	2014	Lesotho	Under five	Cross-sectional	1139	54	76.5
Guled and Mamat	2017	Ethiopia	6–59 months	Cross-sectional	397	72.0	67
Amugsi	2019	Ghana	6–59 months	Cross-sectional	2451	68.0	88
Woodruff et al.	2018	Guinea	Under five	Cross-sectional	5681	34	79
G/Egziabiher et al.	2014	Ethiopia	6–59 months	Cross-sectional	568	37	70.57
Simbauranga et al.	2015	Tanzania	Under five	Cross-sectional	448	77	58.9
Muchie	2016	Ethiopia	6–59 months	Cross-sectional	7636	50	61.8
Melako et al.	2019	Ethiopia	6–23 months	Cross-sectional	477	52.0	66.5
Kateera et al.	2015	Rwanda	6–59 months	Cross-sectional	1882	7.0	75.55
Melku et al.	2018	Ethiopia	6–59 months	Cross-sectional	707	29	80.3
Thorne	2013	Guinea	6–59 months	Cross-sectional	872	82.0	64.3
Diouf et al.	2013	Senegal	9–15 months	Cross-sectional	245	87	73.5
Woldie et al.	2015	Ethiopia	6–23 months	Cross-sectional	346	66	56.4
Van Buskirk et al.	2014	Ghana	0–36 months	Cross-sectional	861	83	61.5
Ntenda et al.	2017	Malawi	6–59 months	Cross-sectional	2597	63	67.8
Gebreweld et al.	2019	Ethiopia	6–59 months	Cross-sectional	404	41	65.9
Wasihun et al.	2020	Ethiopia	6–9 months	Cross-sectional	610	58	68.8
Mghanga et al.	2017	Tanzania	0–59 months	Cross-sectional	303	83	74
Habte et al.	2013	Ethiopia	6–59 months	Cross-sectional	8260	50	57.5
Ali	2018	Uganda	Under five	Cross-sectional	1808	50	66.5
Petry et al.	2019	Gambia	Under five	Cross-sectional	1354	50	78.4
Ojoniyi et al.	2019	Tanzania	Under five	Cross-sectional	7916	58	82.1
Kejo et al.	2018	Tanzania	6–59 months	Cross-sectional	436	85	67.5
Wirth et al.	2016	Sierra Leone	Under five	Cross-sectional	710	76	64.3
Kuziga et al.	2017	Uganda	6–59 months	Cross-sectional	376	59	52.9
Nambiema et al.	2019	Togo	6–59 months	Cross-sectional	2890	63	55.6
Semedo et al.	2014	Cape Verde	6–59 months	Cross-sectional	993	52	60.8
Alaofè et al.	2017	Benin	6–59 months	Cross-sectional	681	82	53.5
Roba et al.	2013	Ethiopia	6–23 months	Cross-sectional	216	54	72.1
Jemal et al.	2016	Ethiopia	6–59 months	Cross-sectional	399	52	66
Bahizire et al.	2017	Congo	6–59 months	Cross-sectional	838	47	71
Foote et al.	2013	Kenya	6–35 months	Cross-sectional	858	72	79
Wangusi et al.	2016	Kenya	6–23 months	Cross-sectional	227	76	68.1
Menon and Yoon	2015	Uganda	Under five	Cross-sectional	3878	61	80.1
Ojoniyi O	2017	Tanzania	Under five	Cross-sectional	6592	57	84
Engle-Stone et al.	2017	Cameroon	12–59 months	Cross-sectional	291	45	63.3
Total sample size					69,253	59.0	

countries. These countries were Benin [32], Cameroon [33], Cape Verde [34], Congo [22], Ethiopia [14, 15, 31, 35–42], Gambia [43], Ghana [18, 28, 44], Guinea [20, 45], Kenya [19, 46], Lesotho [47], Malawi [48], Mozambique [29], Nigeria [49, 50], Rwanda [21], Senegal [17], Sierra Leone [51], South Africa [27], Tanzania [16, 30, 52–55], Togo [56], and Uganda [57–59] (Table 1).

3.1. Prevalence of Anemia among Under-Five Children in Africa. The overall pooled prevalence of anemia among under-five children in Africa was 59% (95% CI: 55, 63). The true variability among studies other than chance was 100% ($I^2 = 100\%$, Pvalue = 0.000). The lowest prevalence was observed in Rwanda 7% (95% CI: 7%, 7%), while the highest prevalence was observed in Senegal 87% (95% CI: 86%, 87%) (Figure 1). A study done in Rwanda did not include the milder form of anemia. This may be the reason for the lowest report of anemia prevalence in Rwanda (Figure 2).

To deal with the possible sources of heterogeneity, subgroup analysis was done by sex and region (study setting). The analysis result showed that heterogeneity still exists in both parameters mentioned above. In terms of region, the sources of heterogeneity were Ethiopia, Tanzania, Lesotho, Ghana, and Uganda.

The following funnel plot appears asymmetric; even if it indicates the presence of publication bias, it was not statistically significant (Figure 3).

3.2. Sensitivity Analysis

3.2.1. Determinants of Anemia among Under-Five Children in Africa. The result of this systematic review and meta-analysis indicated that sex of a child, maternal educational status, residence, and family size were the pooled determinants of anemia among under-five children in Africa. Being female is a protective against anemia among under-

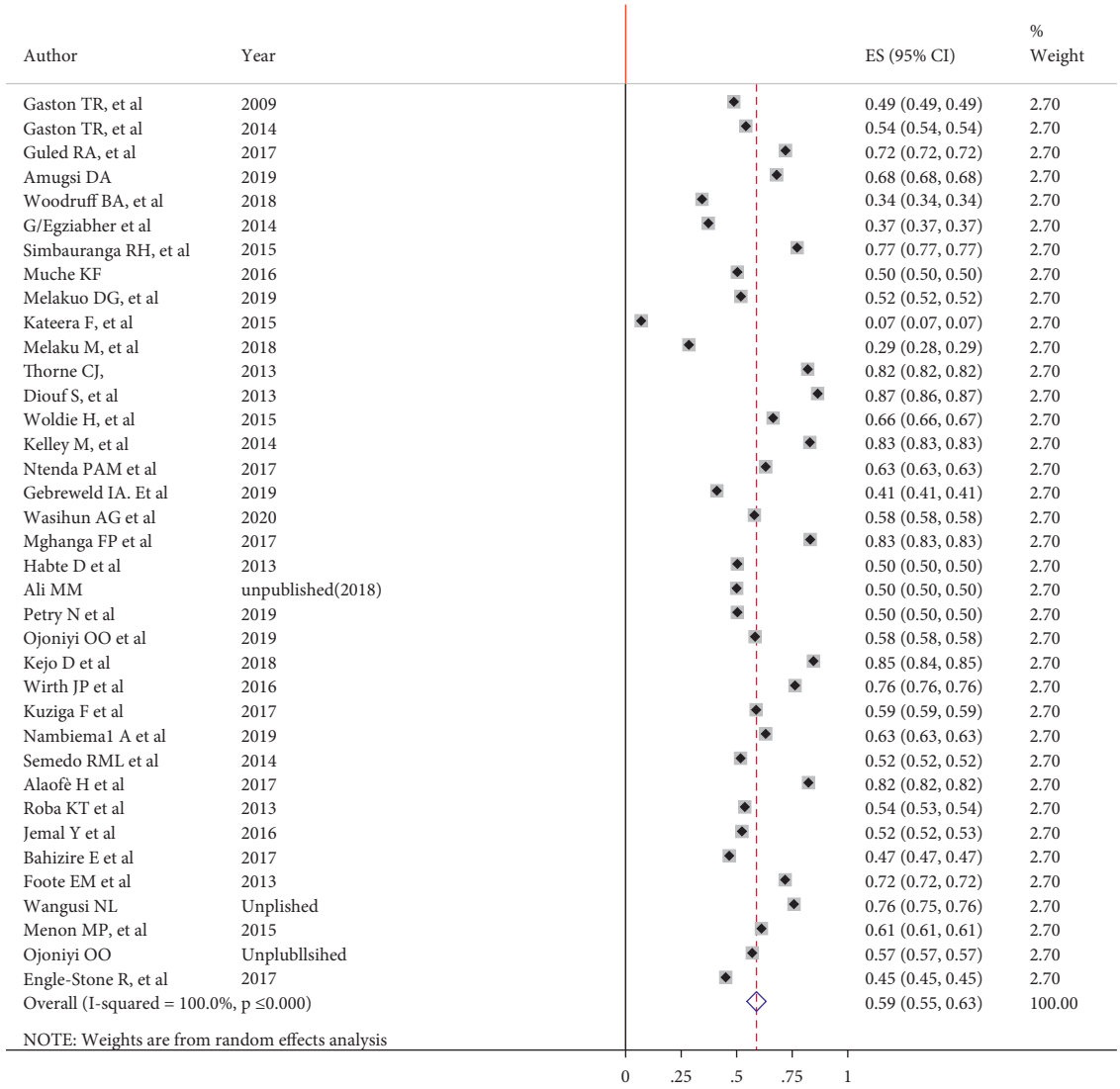

Figure 2: Forest plot for pooled prevalence of anemia among under-five children in Africa, 2009–2020.

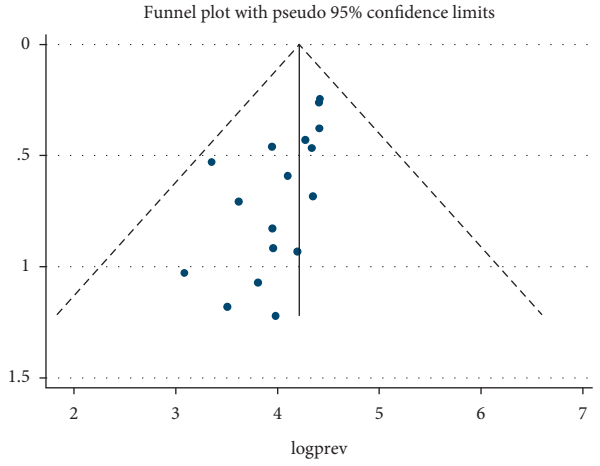

Figure 3: Funnel plot to detect the publication bias regarding prevalence of anemia, 2009–2020.

five children (AOR = 0.71; 95% CI: 0.57, 0.87), Mothers who were unable to read and write were 53% times more likely to have anemic child (AOR = 1.47; 95% CI: 1.31, 1.65). Those children from rural setting were 20% less likely to be affected by anemia as compared to children from urban setting (AOR = 0.80; 95% CI: 0.67, 0.95). Under-five children from a family size of less than five were 7% less likely to be affected by anemia (AOR = 0.93; 95% CI: 0.89, 0.98) (Table 2).

4. Discussion

This study was aimed at estimating the pooled prevalence and determinants of anemia among under-five children in Africa by reviewing the existing pocket studies. Based on the finding of this study, the pooled prevalence of anemia among under-five children in Africa was 59%. This finding is in line with a global prevalence of anemia [60]. According to the classification of World Health Organization (WHO), it was

Table 2: Pooled determinants of anemia among under-five children in Africa, 2009–2020.

Variables		Pooled AOR (95% CI)	Heterogeneity			*P* value
			I^2	*Q* statistic	*P* value	
Malaria falciparum	Yes	1.31 (0.85, 1.78)	95.5%	15.74	0.000	0.22
	No	1				
Sex	Female	0.71 (0.57, 0.87)	94.1	240.3	0.000	0.001
	Male	1				
Stunting	Yes	1.16 (0.94, 1.43)	0.00	2.79	0.99	0.17
	No	1				
Maternal education	Informal	1.47 (1.31, 1.65)	0.00	5.69	0.89	0.00
	Elementary	1.05 (0.89, 1.25)	0.0	2.00	0.99	0.62
	High school	1				
Diarrhea	Yes	1.44 (0.86, 2.42)	93.9	245.06	0.00	0.17
	No	1				
ANC follow-up	Yes	1.68 (0.46, 6.23)	0.0	0.08	0.43	0.43
	No	1				
Residence	Rural	0.80 (0.67, 0.95)	0.0	5.14	0.64	0.000
	Urban	1				
IFA intake	Yes	0.99 (0.63, 1.57)	0.0	0.00	0.73	0.44
	No	1				
Family size	<5	0.93 (0.89, 0.98)	0.0	4.19	0.52	0.004
	≥5	1				
Occupation	Unemployed	1.14 (0.86, 1.51)	0.0	1.77	0.98	0.38
	Employed	1				

categorized under severe public health problem [61]. This finding suggests that, based on the current pace, it is difficult to achieve the global 50% reduction of anemia by 2025 in Africa [62].

This study showed that sex was a significant predictor of anemia among under-five children. Being female is protective against anemia among under-five children. The possible explanation for anemia discrepancy by sex could be due to the state of rapid growth of male children compared to females in the first months of life which increases their micronutrient requirement including iron, which cannot be met by diet alone [63]. If this physiological state is not compensated with iron rich complementary foods, risk of iron deficiency anemia will be higher in male children as compared to females.

This finding revealed that maternal education was a significant predictor of anemia among under-five children. Mothers with informal education were 53% more likely to have child with anemia. This finding is in line with a systematic review and meta-analysis study conducted in Ethiopia [64]. This might be because mothers with no formal education may not understand the introduction of scientifically sound feeding practices and are less likely to follow the recommended child feeding practices [65]. In addition, mothers with no education were negatively affecting the socioeconomic status of households which in turn limits food purchasing power and is a strong predictor for nutritional outcomes of children. Hence, their access to hem iron source food is limited [66]. In order to tackle the effect of anemia in children, nutrition education is suggested for mothers [67].

This study indicated that residence was a significant predictor of anemia among under-five children. Those children from rural setting were 20% less likely to be affected by anemia as compared to children from urban setting. This may be because mothers in the rural setting will breastfed their children exclusively till six months of age and continue breastfeeding till 24 months and more. Since iron in breast milk is more likely to be absorbed and utilized by the child's body, it will contribute for the normal stores of iron, which will help in reducing anemia among under-five children. In order to turn on the health loss due to anemia in children, UNICEF and WHO jointly recommended adequate breastfeeding practices [67]. But the finding of this study is inconsistent with a systematic review and meta-analysis study conducted in Ethiopia [64].

This study showed that family size was a significant predictor of anemia among under-five children in Africa. Those children from a household size of <5 were 7% less likely to be anemic as compared to their counterparts. This could be because large family size is associated with food insecurity. The lesser the families are, the more likely adequate and diversified diet can be afforded, which is rich in iron [68].

Some of the limitations of this study were articles published only in English language that were included. This may affect the prevalence estimation of anemia. Another limitation of this study was articles which were conducted among pediatrics that were not included. The data were obtained from twenty African countries. However, the analyzed pooled prevalence may not fully represent the prevalence of anemia in Africa because there is lack of evidences in some parts of the region.

To conclude, based on this systematic review and meta-analysis, anemia was a severe public health problem among under-five children in Africa. Sex, maternal education, residence, and family size were the determinants of anemia among under-five children. Therefore, adequate

intervention should be designed by considering sex and residence difference, addressing maternal illiteracy through youth education and nutrition education, and promoting birth spacing.

Data Availability

All the required data is included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

SE and AA participated in the conceptualization, searching, and selection, data extraction and analysis, writing, and approving the manuscript. TC participated in data extraction and analysis, report writing-up, writing, and approving the manuscript. AW and FY participated in the conceptualization, searching, and selection, writing, and approving the manuscript. FM participated in the conceptualization, editing, and approving the manuscript. MY took part in searching and selection, data analysis, revising, and approving the manuscript. YW contributed to searching and selection, data extraction and analysis, writing the manuscript, and approving the manuscript.

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Research Article

Molecular Characterization and Genotype-Phenotype Correlation of G6PD Mutations in Five Ethnicities of Northern Vietnam

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzyme disorder and is caused by G6PD gene mutations. To date, more than 400 variants in the G6PD gene have been discovered, and about 160 identified variants are associated with a significant decrease in the G6PD enzyme activity. However, the molecular characterization and epidemiological study of G6PD deficiency are still limited in Vietnam. Therefore, we conducted this study to determine the G6PD variants among the Vietnamese populations and evaluate their correlation to G6PD enzyme activity. A total of 339 patients (302 males and 37 females) were enrolled in this study. The G6PD variants were identified by Sanger sequencing. Our results indicate that males are more severely deficient in G6PD than females. This enzyme activity in males (1.27 ± 1.06 IU/g·Hb) is significantly lower than in females (2.98 ± 1.57 IU/g·Hb) ($p < 0.0001$). The enzyme activity of the heterozygous-homozygous females and heterozygous females-hemizygous males was found to be significantly different ($p < 0.05$), which is interpreted due to random X-inactivation. For G6PD molecular characteristics, *Viangchan* (c.871G>A), *Canton* (c.1376G>T) and *Kaiping* (c.1388G>A) variants were the most dominant, accounting for 24.48%, 17.70%, and 22.42%, respectively, whereas the highest frequency of complex variants was observed in *Viangchan/Silent* with 20.35%. In terms of G6PD activity, the *Union* variant presented the lowest mean value (1.03 IU/g·Hb) compared to the other variants ($p < 0.05$). Computational analysis using Polyphen-2 tool investigated that all variants were relative to G6PD deficiency and separated the levels as benign and damaged. The result will establish effective methods to screen G6PD variants in Vietnam.

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is a key cytosolic enzyme in the pentose phosphate pathway (PPP) to produce NADPH which plays an important role in protecting the red blood cells from oxidative stress by reducing glutathione dimers oxidized and sulfhydryl groups [1]. The lack of the G6PD enzyme can cause hemolytic and related disorders such as clinical acute hemolysis, neonatal jaundice, and congenital hemolytic anemia [2,3]. G6PD deficiency, also known as favism, is the most prevalent enzyme disorder and is found worldwide [4–6]. An estimated 400 million people were influenced globally by G6PD, and an average of 4100 people died every year from 1990 to 2013 [7]. Based on the residual enzyme activity and clinical manifestations, G6PD deficiency is categorized into five groups by the WHO. Class I (less than 1% of normal activity) has been considered the most serious among classes and is specifically

associated with chronic nonspherocytic hemolytic anemia (CNSHA). Class II (1 to 10% of normal) is highly associated with acute hemolytic anemia, while Class III (10 to 60% of normal) is normally associated with occasional acute hemolytic anemia, and Class IV (60 to 150% of normal) and Class V (>150% of normal) are mostly asymptomatic [8].

G6PD is encoded by the G6PD gene which is located in the telomeric region of the X chromosome (Xq28). Thus, G6PD deficiency has been inherited as the X-linked incomplete dominant. While males are always hemizygous because of having only one X chromosome, females with this disorder may be heterozygous or homozygous and have less severe clinical manifestations [9]. The G6PD gene is 18.5 kb in size with 13 exons and codes for 515 amino acids of the G6PD enzyme. To date, more than 400 variants in the G6PD gene have been discovered, and about 160 identified variants show a significant decrease of the enzyme in erythrocytes [10,11]. The vast majority of G6PD variants are single-base substitutions and are distributed as follows: 85.4% are missense, 8% are multiple mutations, 5.3% are deletions, and 1% are mutations within introns [12]. Moreover, many variants present genetic characteristics within specific populations, geographic regions, and ethnic groups [13]. For example, the *G6PD Mediterranean* (c.563C>T) variant is widely distributed in Southern Europe, the G6PD A-variant is predominant in African origins, and the G6PD *Mahidol* (c.487G>A) and *Viangchan* (c.871G>A) variants are mostly associated with Asians, especially in Myanmar and Cambodian populations [14–16]. Furthermore, the correlation between these variants and the deficiency of the G6PD enzyme is being investigated. The G6PD variants such as *Canton* (c.1376G>T), *Kaiping* (c.1388G>A), and *Gaohe* (c.95A>G) have been identified to reduce enzyme activity by up to 90% in the Chinese population. Different variants can cause varying enzyme activities [17,18].

In Vietnam, G6PD deficiency is also a prevalent genetic disorder with an incidence rate of about 8.9% and a diverse distribution according to ethnic groups and regions [19,20]. Several G6PD variants are characterized by being detected in the Vietnamese population, such as *Vietnam1* (c.7G>A), *Vietnam2* (c.197T>G), and *BaoLoc* (c.352T>C) [21,22]. However, the correlation between specific G6PD variants and these activity genotypes has not been reported. Also, the molecular epidemiology of G6PD deficiency is still limited in Vietnam. To provide more information to diagnose this disorder, we performed this study to carry out the prevalence of G6PD deficiency and G6PD variants in the Vietnamese population by direct sequencing. These data will contribute to prenatal genetic counseling to reduce morbidity, reduce consequences for the patient's family and society, and improve the quality of health care in the community.

2. Materials and Methods

2.1. Sample Collection. To screen G6PD variants, 339 pediatric patients were selected from 25 provinces of Northern Vietnam and confirmed G6PD deficiency with an enzyme activity less than 6 IU/g·Hb by Vietnam National Children's Hospital from 2017 to 2020. The patients belonged to five different ethnic groups: Kinh, Mong, Muong, Thai, and Tay. The ages were arranged between 1-month-old and 24-month-olds. The participants consented to enroll before the study´s commencement. Whole blood samples were obtained in K2-EDTA tubes with a concentration of 1.5 mg/mL, then the G6PD enzyme activity was measured by an automated biochemistry analyzer AU5800/AU680 (Beckman Coulter, USA) at the Department of Biochemistry, Vietnam National Children's Hospital.

2.2. Molecular Characteristic Analysis of G6PD Variants. Genomic DNA was extracted from peripheral blood samples by following the Wizard Genomic DNA purification kit instruction (Promega, USA). The primers for amplifying the G6PD gene were designed according to Nguyen Thi Hue et al. (2009) with minor modifications [21]. For PCR, the mixture contains GoTaq Hot Start Master Mix (2X), primer set (1 μM), DNA template (50 ng/μL), and sterile water. The PCR conditions were performed by initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min then holding at 4°C. After purification, PCR amplicons were sequenced by an ABI 3500xl Genetic Analyzer (Applied Biosystems, France). To identify G6PD variants, the sequencing results were analyzed by CLC Main Workbench software and assembled with the G6PD sequence on GenBank (NG_009015).

2.3. Damaging In Silico Analysis. The estimated damage score was evaluated by using the PolyPhen-2 web server (genetics.bwh.harvard.edu/pph2/index.shtml) [23]. For PolyPhen-2, the predicted function of a variant is classified as benign, possibly damaging, or probably damaging, with the scale score arranged from 0 to 0.5, 0.5–0.9, and 0.9–1, respectively. The G6PD query protein sequence from UniProtKB (P11413) was mapped as a reference.

2.4. Statistical Analysis. Statistical analysis was evaluated by GraphPad Prism ver.9 software. Comparison among groups was conducted using one-way ANOVA. Variables such as age, detection rate, and genotype were described by descriptive statistics. A chi-square test was applied for the comparison of frequencies of G6PD deficiency between both genders. A $p$value < 0.05 was considered statistically significant.

3. Results

3.1. Patient Clinical Characteristics. A total of 339 patients were enrolled in this study, which contained 302 males (89.09%) and 37 females (10.91%) from five different ethnicities: Kinh, Muong, Nung, Thai, and Tay. The participant characteristics are listed in Table 1. The infants' age was distributed from less than 1-month old (79.06%) to over 24-months old (0.88%). There was a significant difference in age between males and females ($p = 0.0089$) (Table 1). The G6PD enzyme activity was measured as 1.46 ± 1.24 IU/g·Hb

Table 1: Participant characteristics in this study.

	Male	Female	*Total*	*p*
		Age		
<1 month	244 (71.98)	24 (7.08)	268 (79.06)	0.0089*
1–6months	50 (14.75)	9 (2.61)	59 (17.41)	
>6–12 months	5 (1.48)	1 (0.29)	6 (1.77)	
>12–24 months	1 (0.29)	2 (0.59)	3 (0.88)	
>24 months	2 (0.59)	1 (0.29)	3 (0.88)	
N	**302**	**37**	**339**	
		Enzyme activity (IU/g·Hb)		
Average	1.27 ± 1.06	2.98 ± 1.57		<0.0001*
		The G6PD deficiency level of participants		
High (<0.6 U/g·Hb)	78 (23.01)	1 (0.29)	79 (23.30)	<0.0001*
Medium (0.6–3.6 IU/g·Hb)	212 (62.54)	21 (6.20)	233 (68.74)	
Low (3.6–9 IU/g·Hb)	12 (3.54)	15 (4.42)	27 (7.96)	
N	**302**	**37**	**339**	
		Genotype		
No mutation	11 (3.25)	0	11 (3.25)	<0.0001*
Homozygous	0	8 (2.36)	8 (2.36)	
Hemizygous	291 (85.84)	0	291 (85.84)	
Heterozygous	0	29 (8.55)	29 (8.55)	
N	302	37	339	

on average. A significant difference was valued between both genders with 1.27 ± 1.06 IU/g·Hb in males and 2.98 ± 1.57 IU/g·Hb in females ($p < 0.0001$) (Figure 1(a)). In normal conditions, the G6PD reference range at 37°C is 6–20.5 IU/g·Hb. The level of G6PD deficiency was categorized as high level (<0.6 IU/g·Hb) with 79 patients (23.30%), medium level (0.6–3.6 IU/g·Hb) with 233 patients (68.74%), and low level (3.6–9 IU/g·Hb) presented in 27 patients (7.96%). There was a significant difference in these levels between genders ($p < 0.0001$). Among 339 cases, 332 cases were found to carry G6PD genetic variants in both males and females including homozygous (2.36%), hemizygous (85.84%), and heterozygous (8.55%) ($p < 0.0001$). No G6PD variants were recorded in 11 cases (3.25% in males) (Table 1).

3.2. Prevalence of the G6PD Enzyme Activity in Five Ethnic Groups. In our study, the five selected ethnicities were distributed in Northern Vietnam. The majority of samples were arranged in Kinh, followed by Muong, Tay, Nung, and Thai with different prevalences of 61.6%, 16.5%, 10.7%, 6.5%, and 4.7%, respectively (Figure 2(a)). Also, the Tay population presented the highest enzyme activity (1.61 ± 1.37 IU/g·Hb), and the lowest enzyme activity was observed in the Muong population (1.26 ± 1.16 IU/g·Hb) (Figure 2(b)). However, no significant difference was recorded between the enzyme activity and ethnic groups ($p = 0.6487$).

3.3. Identification and Function Prediction of G6PD Variants. With 339 participants, 14 G6PD variants were detected by using the Sanger sequencing method and categorized into two types: missense and silent (Table 2) (Figure 3). Of these, the *Viangchan* (c.871G>A), *Canton* (c.1376G>T), and *Kaiping* (c.1388 G > A) variants were the most dominant across the five ethnic groups, accounting for 24.48%, 17.70%, and 22.42%, respectively (Figures 3(h), 3(m), 3(n)). A silent variant (c.1311C>T) in exon 11 was also found with a high frequency (25.66%) (Figure 3(o)). Moreover, the *NanKang* (c.517 T > G), *Mediterranean* (c.563C>T), *Coimbra Shunde* (c.592C>T), and *Taiwan-2* (c.1330G>A) variants were relatively rare and were only detected in one sample each (0.29%) (Figures 3(e), 3(f), 3(g), 3(j)). In addition, the coexistent variants were found in our samples with variable frequencies, mostly together with *Silent* variants (c.1311C>T), presented in *Valladolid/Silent* variant (0.59%), *Viangchan/Silent* variant (20.29%), *Union/Silent* variant (2.06%), *Canton/Silent* variant (0.59%), and *Kaiping/Silent* variant (0.88%) (Table 2). A unique variant between *Canton* and *Kaiping* was found in one tested individual (0.29%) (Figure 3(o)).

PolyPhen-2 is a useful automatic tool for the prediction of the possible impact of an amino acid substitution on the structure and function of a human protein [23]. In this study, computational analysis was performed to estimate the risk of disease among G6PD variants. A total of 14 variants, four of them were identified to have the maximum damaging score (DS = 1) including *Valladolid*, *NanKang*, *Coimbra Shunde*, and *Union*, and the benign score was observed in *Mediterranean* (0.371), *Chinese-5* (0.205), and *Taiwan-2* (0.127) (Table 2). A high-risk score was also accessed in the remaining variants, arranging from 0.860 to 0.998. The *Silent* variant (c.1311C>T) did not give any score by Polyphen-2 because it was a silent variant.

3.4. Correlation between the Genotype and the G6PD Activity Phenotype. According to the WHO instruction, the 13 identified G6PD variants in our study were predominantly identified in Class II and III, except for the silent variant. Among these variants, *Gaohe*, *Orissa*, *Quing Yan*, *Chinese-5,* and *Taiwan-2* were categorized as Class III, while *Valladolid*,

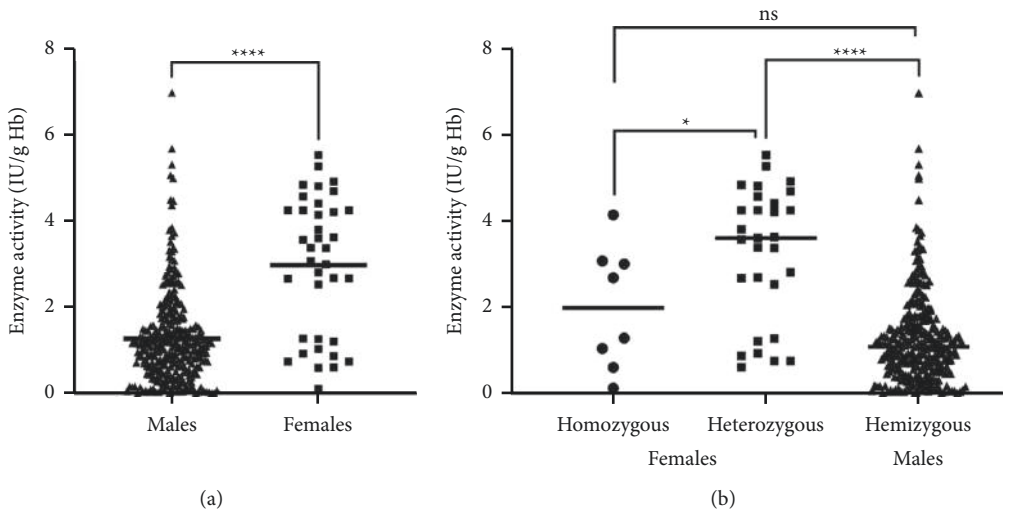

Figure 1: Comparative distribution of G6PD activity by genders and genotypes. Each dot represents the G6PD enzyme activity of each subject. (a) G6PD activities between males and females. (b) G6PD activities among genotypes in both males and females.

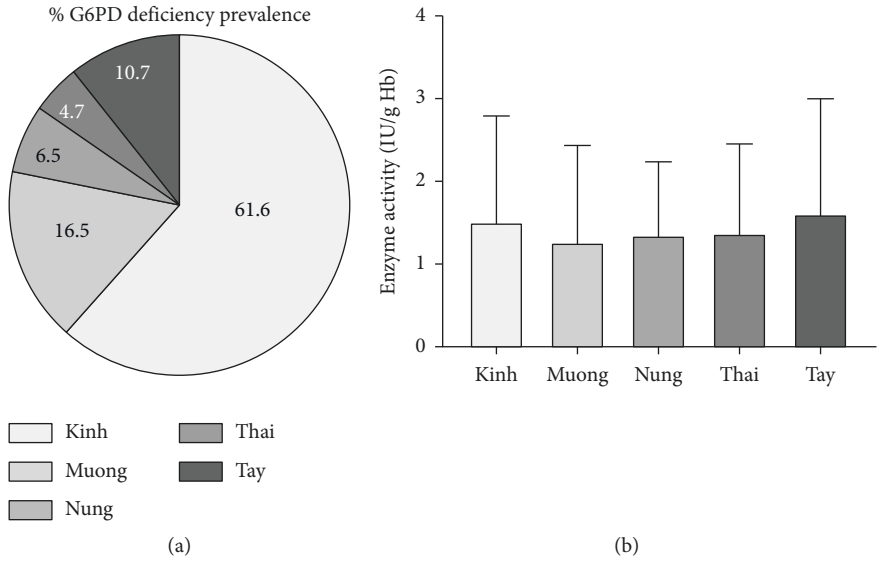

Figure 2: The prevalence and G6PD enzyme activity among Vietnamese ethnics. (a) The prevalence of G6PD deficiency among five different ethnicities. (b) Distribution of G6PD activities according to ethnic groups.

NanKang, *Mediterranean*, *Coimbra Shunde*, *Viangchan*, *Union*, *Canton*, and *Kaiping* were categorized as Class II (Table 3). The G6PD variant genotype was mainly found in hemizygous (294/332) males (1.26 ± 1.04 IU/g·Hb), while homozygous (8/332) and heterozygous genotypes (29/332) were commonly observed in females with the enzyme activity arranged 1.99 ± 1.33 IU/g·Hb and 3.26 ± 1.52 IU/g·Hb, respectively. The lower activity was significantly observed in hemizygous males than in heterozygous females ($p < 0.0001$), whereas a statistical difference was evaluated when a comparison of activity between homozygous females and heterozygous females was made ($p = 0.04$) (Figure 1(b)). In terms of G6PD activity, the *Union* variant presented the lowest mean value (1.03 IU/g·Hb), followed by *Canton* (1.4 IU/g·Hb) and *Kaiping* variant (1.35 IU/g·Hb) (Figure 4(a)). Variant groups in which there were ≥2 representatives were shown in Figure 4. There was a significant difference between these variants and the enzyme activity ($p = 0.0088$). In addition, the activity of the cooccurred variants was also presented, but no significant difference was recorded ($p = 0.8139$) (Figure 4(b)). Among the coexistent variants, we found that only the *Viangchan/Silent* variants presented a correlation to the G6PD enzyme activity (r=0.3186, $p = 0.0033$), while the other variants did not show the relationship.

4. Discussion

G6PD deficiency is a common enzyme abnormality that affects approximately 5% of the world´s population and causes some diseases related to erythrocytes [24]. Some

Table 2: Distribution of G6PD variants in this study.

Variant name	Position	Amino acid substitution	Type of variant	Exon	Case	Frequently (%)	In silico analysis
Gaohe	c.95A>G	H32A	Missense	2	24	7.08	Damaging score: 0.998
Orissa	c.131C>G	A44G	Missense	3	3	0.88	Damaging score: 991
Quing Yan	c.392G>T	G131V	Missense	5	12	3.54	Damaging score: 0.826
Valladolid	c.406C>T	L142C	Missense	5	2	0.59	Damaging score: 1
NanKang	c.517T>G	F173L	Missense	5	1	0.29	Damaging score: 1
Mediterranean	c.563C>T	S188P	Missense	5	1	0.29	Benign score: 0.371
Coimbra Shunde	c.592C>T	R198C	Missense	6	1	0.29	Damaging score: 1
Viangchan	c.871G>A	V291M	Missense	9	83	24.48	Damaging score: 0.996
Chinese-5	c.1024C>T	L342F	Missense	9	14	4.13	Benign score: 0.205
Taiwan-2	c.1330 G > A	V444I	Missense	11	1	0.29	Benign score: 0.127
Union	c.1360C>T	R454C	Missense	11	51	15.04	Damaging score: 1
Canton	c.1376G>T	R459L	Missense	12	60	17.70	Damaging score: 0.910
Kaiping	c.1388G>A	R463H	Missense	12	76	22.42	Damaging score: 0.860
Silent	c.1311C>T	T437T	Silent	11	87	25.66	N/A
Valladolid/Silent	c.406C>T c.1311C>T	A142C T437T	Missense /Silent	5, 11	2	0.59	N/A
Viangchan/ Silent	c.871G>A c.1311C>T	V291M T437T	Missense /Silent	9, 12	69	20.35	N/A
Union/Silent	c.1360C>T c.1311C>T	R454C T437T	Missense /Silent	11	7	2.06	N/A
Canton/Silent	c.1376G>T c.1311C>T	R459L T437T	Missense /Silent	11, 12	2	0.59	N/A
Kaiping/Silent	c.1388G>T c.1311C>T	R463H T437T	Missense /Silent	11, 12	3	0.88	N/A
Canton/Kaiping	c.1376G>T c.1388G>A	R459L, R463H	Missense	12	1	0.29	N/A

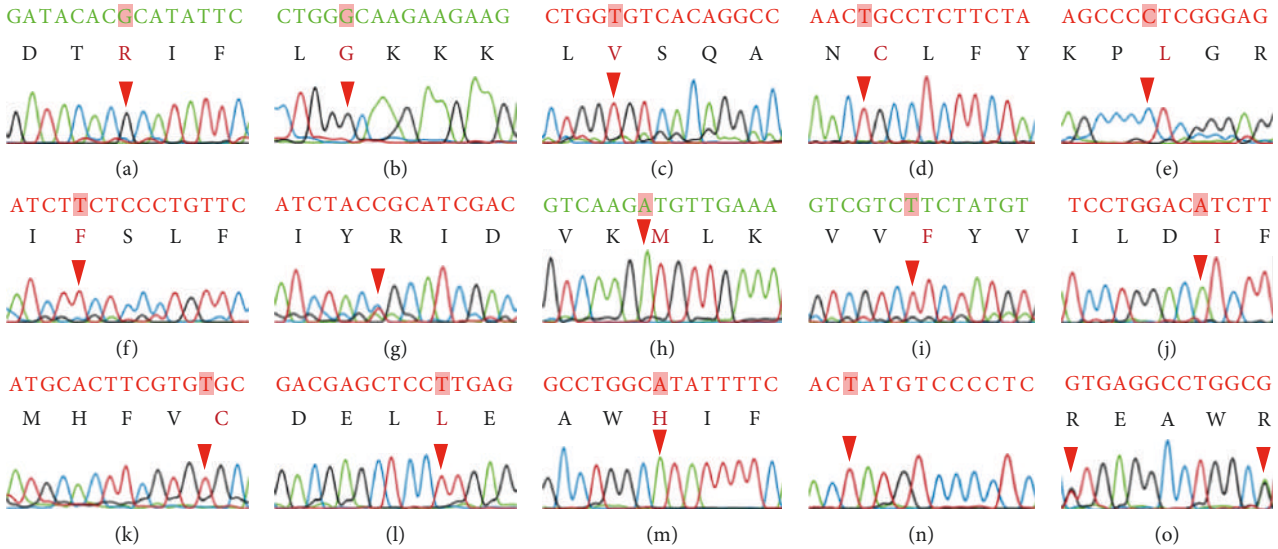

Figure 3: The electropherogram of G6PD variants. (a) Gaohe (c) 95A > G), (b) Orissa (c.131 C > G), (c) Quing Yan (c.392 G > T), (d) Valladolid (c.406 C > T), (e) NanKang (c.517 T > G), (f) Mediterranean (c.563 C > T), (g) Coimbra Shunde (c.592 C > T), (h) Viangchan (c.871 G > A), (i) Chinese-5 (c.1024 C > T), (j) Taiwan-2 (c.1330 G > A), (k) Union (c.1360 C > T), (l) Canton (c.1376 G > T), (m) Kaiping (c.1388 G > A), (n) Silent (c.1311 C > T), and (o) Canton/Kaiping (c.1376 G > T/c.1388 G > A).

Table 3: Variant patterns of G6PD and corresponding enzyme activities.

Variant patterns	Class	Male		Female			
		Hemi	Enzyme activity (IU/g·Hb)	Homo	Enzyme activity (IU/g·Hb)	Hete	Enzyme activity (IU/g·Hb)
Valladolid	II	2	1.98	0	—	0	—
NanKang	II	1	1.7	0	—	0	—
Mediterranean	II	1	0.65	0	—	0	—
Coimbra Shunde	II	0	—	0	—	1	2.67
Viangchan	II	73	1.31	4	1.83	6	3.24
Union	II	41	0.66	1	0.12	9	2.8
Canton	II	55	1.26	0	—	5	2.98
Kaiping	II	72	1.32	1	1.28	3	2.13
Gaohe	III	22	1.34	0	—	2	4.15
Orissa	III	2	1.04	1	3.07	0	—
Quing Yan	III	10	2.05	0	—	2	5.19
Chinese-5	III	11	1.38	1	4.14	2	4.19
Taiwan 2	III	1	5.06	0	—	0	—

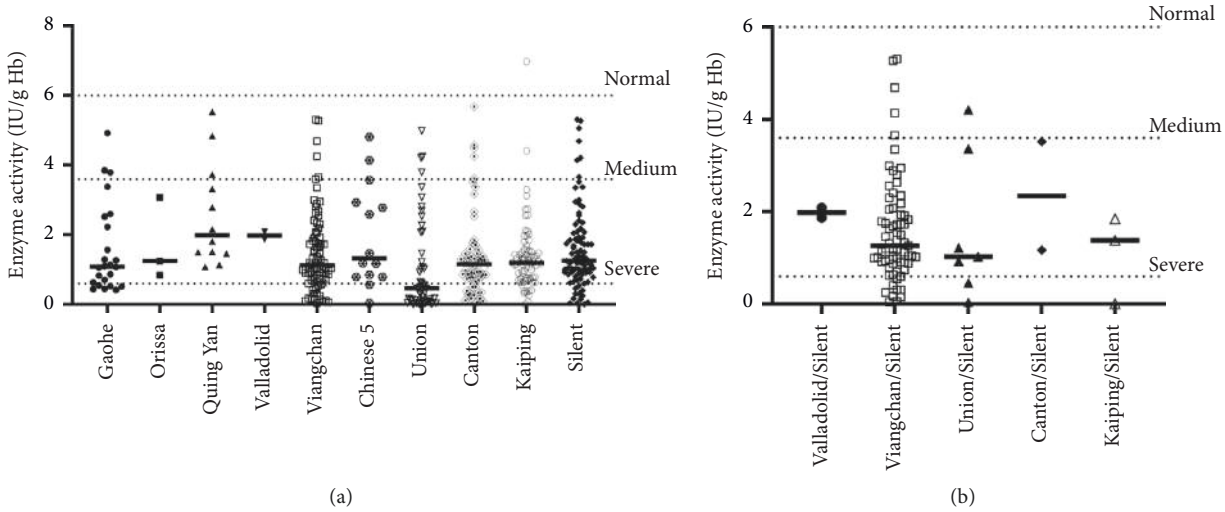

Figure 4: Distribution of G6PD activities according to mutation types. Only variant groups in which there were ≥ 2 representatives are shown. (a) G6PD activities of single identified variants. (b) G6PD activities of compound variants.

mutations on the G6PD gene are being investigated to be associated with this deficiency of the G6PD enzyme activity. Therefore, identifying G6PD variants plays an important role in screening and estimating the risk variants in communities.

The distribution of G6PD deficiency is variable across ethnic groups and geographical regions [13]. In our study, the 339 blood samples were selected from five ethnicities in Northern Vietnam including Kinh, Muong, Nung, Thai, and Tay. The results showed that while the Kinh ethnic group had the highest prevalence of G6PD deficiency (~60%), the Thai ethnic group carried the lowest G6PD distribution compared to the others (Figure 2(a)). The main distribution of G6PD deficiency in the Kinh ethnic group can be explained by the predominance of this ethnic group in Vietnam, accounting for approximately 86% of the population [25]. However, no statistically significant difference was observed between the G6PD incidence and ethnic groups ($p > 0.05$). Similar results were reported in the Kinh and S'Tieng ethnic groups, according to Nguyen Thi Hue et al. (2009). Although the frequency of G6PD deficiency in Southern Vietnam was rather high, accounting for 11.3%, this proportion in the Kinh and S'Tieng populations was only 8.7% and 14%, respectively ($p = 0.07$) [21]. Likewise, compared to Myanmar ethnicities, the Kachin people have a higher level of G6PD prevalence (29.6%) compared to other local groups such as Mon (12%), Burmese (10%), Karen (12.9%), and Burman [26–28]. Also, the variable of G6PD distribution was observed among the Lue ethnicities in Thailand. Although the rate of G6PD in the Lue ethnic group was 13.51%, the different local languages show the variation in Ta-Kadai (9.69%), Sino-Tibetan (4.51%), Austroasiatic (7.58%), and Hmong-Mien groups (1.77%) [29]. An extreme distribution of G6PD deficiency in the Great Mekong Subregion (GMS) countries can be understood because these countries were seriously affected by the malaria pandemic [30]. It could be a possible evolutionary factor to increase the prevalence of G6PD deficiency in the population.

Furthermore, our results indicated that males were more severely deficient in G6PD than females. This enzyme activity in males (1.27 ± 1.06 IU/g·Hb) was significantly lower than in females (2.98 ± 1.57 IU/g·Hb) ($p < 0.0001$) (Table 1; Figure 1(a)). Because the G6PD gene is located on the X chromosome, its expression can be different between both genders. Males have only one X chromosome and will be hemizygous with G6PD mutations [31]. Therefore, G6PD deficiency can express fully in this phenotype compared to that in females, which can be caused by X-inactivation [32, 33]. Females with G6PD heterozygous genotypes present a wide range decrease of G6PD activity, a range from 20–80% with the normal [34].

In this study, we identified 13 G6PD variants by Sanger sequencing. The majority of variants are chiefly discovered in China, India, and they have been established as Asia variants; the other is found in European origin countries and Mediterranean areas such as *Valladolid* and *Mediterranean* [12,13]. The finding supports the notion that the genetic drift event occurred in the Asian population in the prehistoric period. For example, the migration of Chinese to Vietnam has been recorded for a long time and gradually Vietnamized to be Hoa ethnics [35]. Ethnic migration is investigated to play a crucial role in regular genetic trait distribution and the characteristics of populations according to the gene flow process [36–38]. In Yuzhong Zhen's study, the heatmap for distribution of the G6PD-deficient allele indicates that Canton, Kaiping, and Gaohe are highly related to the Chinese population, the G6PD Viangchan and Mahidol were mostly related to the Southern Asian population. The ethnic migration suggested that the Chinese variants occurred before the formation of these Chinese ethnic populations [39].

Of 13 G6PD variants, we found that *Viangchan* variants had the highest frequency among our ethnic groups with 24.48% (Table 1). It has been considered the most common mutation in Asia with diverse distribution between regions and ethnicities. In Southern Vietnam, this variant is highly detected in the Kinh and K'Ho ethnic groups with 44%, and 75% is observed in the Raglai and Pako ethnic groups [22, 40]. In several countries of GMS, the *Viangchan* variant is found in Laotians (100%), Cambodians (97.9%), and Thais (67.7%) [16, 41, 42]. The sharing of G6PD *Viangchan* among Southeast Asian populations reveals insight into old ancestral sources in these countries. In addition, the *Canton* and *Kaiping* variants are the most prevalent in South West China with 20% and 79.16%, respectively, found in 17.7% and 24.2% of our samples [43]. Likewise, in Thailand, *Canton* and *Kaiping* are observed in 15.4% and 14.4% of G6PD deficiency cases, respectively [5]. The G6PD *Union,* which was presented at 15.04% in this study, is determined at 100% in the Khomu population and 9.5% in Thailand [20, 44]. On the other hand, G6PD *Gaohe* is also an important Chinese variant with an incidence rate ranging from 8.8% to 14.2% in different studies and was identified at about 7.08% in this study [45–47]. The Chinese variants including *Orissa* (0.88%), *Quing Yan* (3.54%), *NanKang* (0.29%), *Taiwan-2* (0.29%), *Chinese-5* (0.29%), and *Coimbra Shunde* (0.29%), and European variants such as *Valladolid* (0.59%) and *Mediterranean* (0.29%) were rarely detected in Northern Vietnam but were observed in several studies with various frequencies [5, 18, 29, 48–51]. Moreover, *Silent* variants are the most common polymorphism of G6PD gene and have a high rate of distribution among populations [18, 21, 29].

To date, *in silico* analysis has been applied to estimate the pathogenic mutations for disease. In the current study, the Polyphen-2 tool reported *Mediterranean*, *Chinese-5*, and *Taiwan-2* variants as benign, whereas the other variants were damaged, almost similar to the G6PD classification of WHO (Tables 2, 3). These results suggest that all G6PD variants can be caused by G6PD deficiency. The application of bioinformatics tools in G6PD mutations has been investigated in different populations [52–54]. In Chinese ethnicity, the lowest enzyme activity is G6PD *Canton* variant, which was recorded in the *Union* variant of our data [18]. In addition, the *Mediterranean* variant, which is considered more severe by the WHO classification, was found to be benign through bioinformatic analysis in this study. The different results can be understood by the variable of gene expression within and between populations [55]. Although the *Silent* variant is a silent mutation in the intronic region, it has been investigated relatively to G6PD deficiency [56]. Some hypotheses are postulated to interpret the expression of enzyme activity of this mutation. By predicting the secondary structure of G6PD mRNA, the mutant *Silent* presents the stable structure at the start codon boundary, therefore causing a negative effect on mRNA translation [57]. Or this single mutation may be located in the enhance region, where nucleotide alteration can change the function and lead to reduced gene expression [58]. Thus, further studies should be performed to clarify the mechanism of the *Silent* variant. Among coexistent variants, the linkage disequilibrium between G6PD *Viangchan* and *Silent* was the most common, occurring in Thai, Vietnamese, and Chinese populations [42, 58, 59]. However, the correlation of these covariants to the G6PD enzyme activity is not fully understood.

5. Conclusions

In this study, we successfully identified 13 G6PD variants related to G6PD deficiency in Northern Vietnam by Sanger sequencing. G6PD *Viangchan, Canton, Kaiping,* and *Union* variants were the most prevalent across Vietnamese ethnic groups, accounting for 79.64% of samples. In addition, the six cooccurred variants were also observed at different frequencies. The correlation between these single variants and G6PD deficiency was investigated by a bioinformatic tool, further studies should be performed on coexistent mutations. The result will contribute to the diagnosis and screening of G6PD deficiency in Vietnam, reduce consequences for the patient's family and society, as well as improve the quality of health care in the community.

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 there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

Thi Thao Ngo and Thinh Huy Tran contributed equally to this work.

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Research Article

Hematological Parameters in Individuals with Beta Thalassemia Trait in South Sumatra, Indonesia

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Background. β-Thalassemia has a very wide clinical variation, depending on the severity of the patient's condition. Individuals with β-thalassemia traits are usually asymptomatic; however, laboratory examination will show mild anemia with microcytic hypochromic erythrocytes morphology with wide variation depending on the genotype. This study was conducted to determine the reference value of hematological parameters and hemoglobin (Hb) analysis based on the phenotype of β-thalassemia (β^0 and β^+) and determine the differences of hematological characteristics between the two phenotypes. *Methods*. This cross-sectional study was conducted by evaluating the hematological parameters and Hb analysis of the β-thalassemia trait in the family of thalassemia patient population. The subjects were divided into β^0 and β^+. The subject with normal Hb analysis with or without iron deficiency was excluded. *Results*. A total of 203 subjects with thalassemia traits were included from the families of thalassemia patients, consisting of 101 subjects with β^0-thalassemia, 82 subjects with β^+-thalassemia, and the mutation had not been found in 20 subjects. There was a relationship in the mean/median of hematological parameters, HbA_2 and HbF, between β^0-thalassemia and β^+-thalassemia ($P < 0.05$). ROC for each hematological parameter, HbA_2 and HbF, showed that the highest diagnostic value based on the area under the curve was mean corpuscular hemoglobin (MCH) (0.900) and mean corpuscular volume (MCV) (0.898). The cutoff point of MCH for β^0-thalassemia trait was ≤20.5 pg (sensitivity 85%, specificity 90%) and MCV was ≤66.8 fL (sensitivity 87%, specificity 87%). *Conclusion*. MCH values can be used as a screening tool for predicting β^0-thalassemia in the relatives of thalassemia patients in the South Sumatra population.

1. Introduction

Thalassemia is an autosomal recessive genetic disorder characterized by hemolytic anemia. β-Thalassemia is caused by abnormalities of the single β-globin gene that interfere with the synthesis of the globin chains of hemoglobin; this interference can cause total (β^0) or partial (β^+) disruption of globin chain synthesis [1, 2]. Currently, thalassemia is the most widespread monogenic disease in the world. Based on the WHO, in 2008, at least 5.2% of the world's population were thalassemia carriers, 1.1% of which were couples that had a risk of having children with hemoglobin disorders [3]. The prevalence of trait carriers was found to be very high in the populations of Africa, Southeast Asia, the Eastern Mediterranean, and the Western Pacific [4]. Southeast Asia is the region with the most complex thalassemia genotype compared to other countries. South Sumatra is one of the areas with the highest carrier rates for hemoglobinopathies in Indonesia, with the prevalence being 15%, consisting of 9% carriers of β-thalassemia trait and 6% HbE [5].

β-Thalassemia has a very wide clinical variation, depending on the severity of the patient's condition and age at the time of diagnosis. Thalassemia traits is asymptomatic; however, laboratory examination will show variation of Hb

levels; it could be normal to up to 2 g/dL, with microcytic hypochromic erythrocytes [6]. Hemoglobin analysis will demonstrate an increase in HbF and HbA2 levels. The Indonesian thalassemia management guidelines recommend the routine use of erythrocyte index as a screening tool in patients with thalassemia. Based on this guideline, patients with MCV <80 fL and MCH <27 pg should be evaluated further. In Indonesian Guideline for Management of Thalassemia (2018), possibility of thalassemia traits is identified by HbA_2 examination: an HbA_2 level between 3.6 and 4.2% suggests mild β^+-thalassemia and levels between 4 and 9% suggest heterozygote β^0-thalassemia and severe β^+-thalassemia [7]. This study was conducted to determine the appropriate reference value of hematological parameters and Hb analysis based on the phenotype of β-thalassemia (β^0 and β^+) and determine the differences of hematological characteristics between the two phenotypes.

2. Methods

The cross-sectional study was conducted at the Thalassemia Center in Mohammad Hosein General Hospital (RSMH), Palembang, from October 2020 to December 2020. RSMH is the tertiary hospital which cover the South Sumatera region. The ethical clearance of this study was approved by RSMH (Letter No.: 111/kepkrsmh/2020) and RSCM-FKUI (Letter No.: KET-1369/UN2.F1/ETIK/PPM.00.02/2020) Ethical Committee. Research subjects are parents and siblings of thalassemia patients who routinely receive blood transfusions. The method uses consecutive sampling; subjects were recruited until the minimum sample size was reached, until it meets the number of samples, or until the end of the study.

Written consent was obtained from all subjects who were willing to participate. Subjects were enrolled consecutively from relatives of patients who received regular blood transfusion in RSMH. For children under 18 years of age, consent was obtained from their parents. The screening was carried out in the form of interviews, physical examination, and blood collection for hematological examination, Hb analysis, and DNA analysis. Interviews were conducted to determine the identity, ethnic origin, and history of transfusion. The ethnic groups were grouped based on the origin of the South Sumatran and non-South Sumatran tribes. Physical examination included an examination for clinical symptoms such as pallor and splenomegaly. 15 mL of blood was collected intravenously for hematological examination, Hb analysis, iron status, and DNA analysis. We excluded the subjects with normal Hb analysis with or without iron deficiency. Hb analysis was normal if HbA_2 is <3.5%, HbF <1%, and HbA >97% [7]. Iron deficiency was defined if ferritin serum was <12 ng/mL for under 5 years old subjects and <15 ng/mL in subjects ≥5 years old [8].

DNA analysis was not performed on subjects who had normal Hb analysis or evidence of iron deficiency. β-Thalassemia mutation type was determined by DNA analysis using the PCR-RFLP technique followed by DNA sequencing. Examination of mutations in exons 1 and 2 and intron 1 that often appears in Indonesia was carried out on all subjects. In subjects whose mutations were unidentified, the examination was continued on exon 3. The types of globin gene mutation were grouped according to the β-globin synthesis phenotype. β^0-Thalassemia is the phenotype of a mutation that causes the β-globin chains do not produce, such as mutations in IVS1-nt5 (G ⟶ C), IVS1-nt1 (G ⟶ T), CD15 (TGG ⟶ TAG), CD17 (AAG ⟶ TAG), CD30 (+C), CD 35 (−C), CD41-42 (−TCTT), CD8/9 (+G), IVS1-nt2 (T ⟶ C), and CD 26 (GAG ⟶ TAG), whereas in β^+-thalassemia, β-globin chains were formed, but has declined in function, which included the mutations in CD 19 (AAC ⟶ AGC) and CD-26 (GA<u>G</u> ⟶ <u>A</u>AG)/HbE. [9].

2.1. Statistical Analysis. Descriptive data in the form of mean, median, and range were used to describe the distribution of numerical data (Hb, erythrocyte index, Hb analysis). Data analysis was conducted to determine the relationship between thalassemia mutation phenotype with Hb levels, MCV, MCH, MCHC, RDW, and Hb analysis using an independent *t*-test. The assessment of the cutoff point of each variable that had a relationship was using ROC curve. The diagnostic value (sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio positive, and likelihood ratio negative) was calculated. The analysis was carried out using the SPSS version 20 for Windows.

3. Results

There were 224 subjects in the current study, consisting of 203 subjects who were carriers based on Hb analysis and DNA analysis and 21 subjects who had normal Hb analysis, in which 3 had iron deficiency anemia.

The carriers consisted of 154 subjects who are parents, 24 subjects are siblings, 4 subjects are uncles, and 1 subject is a cousin of thalassemia patients who has routinely received transfusions at RSMH. The characteristics of the subjects are given in Table 1. Most of the subjects carrying the trait were females (64.5%); the median age was 37.3 (2.3–64.5) years and came from the South Sumatran ethnic group (70.4%). Most of the subjects lived outside of Palembang city (56.7%). Based on DNA analysis, 20 subjects had no mutation identified yet, but the hematologic features, Hb analysis, and iron status suggested to thalassemia condition, consisting of 18 subjects are parents and 2 subjects are siblings.

The type of mutation grouped by the β-globin chain synthesis is given in Table 2. We identified two types of heterozygotes, β^+-thalassemia mutations and 10 types of β^0-thalassemia mutations. The most common mutations found were mutations in IVS1-nt5 heterozygotes in 59 (32.2%) subjects, followed by HbMalay heterozygotes in 47 (25.7%) subjects, HbE heterozygotes in 35 (19.1%) subjects. IVS1-nt1 heterozygotes in 16 (8.7%) subjects, CD 41–42 (−TCTT) heterozygotes in 14 (7.7%) subjects, CD 8/9(+G) heterozygotes in 4 (2.2%) subjects, CD 35(−C) heterozygotes in 3 (1,6%) subjects, and CD15, CD26 (G ⟶ T0), and CD 30 heterozygotes mutation each in one subject (2.5%).

Table 3 provides hematological parameters and Hb analysis by type of mutation. There were statistically

Table 1: Characteristics of the subject of the trait carrier ($n = 203$).

Characteristics	N	%
Age group (years)		
2–10	15	7.4
>10	188	92.6
Gender		
Male	72	35.5
Female	131	64.5
South Sumatra ethnic group		
Yes	143	70.4
Not	60	29.6
Mutation type		
β^0-Thalassemia heterozygotes	101	49.7
β^+-Thalassemia heterozygotes	82	40.3
The mutation was not found[†]	20	10

[†]Mutation was not found in exon 1, exon 2, exon 3, and several introns that are often populated by Indonesia.

Table 2: Types of β-thalassemia mutations found in the present study ($n = 183$).

Mutation type	$N = 183$	%
β^0-Thalassemia heterozygote		
IVS1-nt5 (G ⟶ C)	59	32.2
IVS1-nt1	16	8.7
CD 41–42 (−TCTT)	14	7.7
CD 8/9 (+G)	4	2.2
CD 35 (−C)	3	1.6
CD 15	1	0.5
CD 26 (G > T)	1	0.5
CD 30	1	0.5
CD 71/72 (+A)	1	0.5
IVS1-nt2	1	0.5
β^+-Thalassemia heterozygote		
HbMalay	47	25.7
HbE	35	19.1

Table 3: Hematological variables among β^0 and β^+-thalassemia carrier.

Variable	β^0-Thalassemia ($N = 101$)	β^+-Thalassemia ($N = 82$)	P value
Hematological parameter			
Hb (g/dL)*	11.4 ± 1.2	12.4 ± 1.4	<0.001‡
MCV (fL)**	63.6 ± 4.8	72.05 ± 5.3	<0.001‡
MCH (pg)**	19.5 ± 1.8	23.1 ± 2.3	<0.001‡
MCHC (g/dL)	30.7 ± 0.9	31.9 ± 1.4	<0.001‡
RDW (%)**	17.4 ± 1.7	15.6 ± 1.7	<0.001‡
Hemoglobin analysis			
HbA_2 (%)**	5.02 ± 0.8	4.3 ± 0.6	<0.001‡‡
HbF (%)**	0,91 ± 1,8	0.8 ± 2.8	0,040‡‡
HbA (%)**	93.7 ± 3.05	85.9 ± 11.6	0.078‡‡

*Normal distribution,**abnormal distribution, ‡Independent t-test,‡‡Mann–Whitney test.

significant differences in the mean/median Hb, MCV, MCH, MCHC, RDW, HbA_2, and HbF, where Hb, MCV, MCH, and MCHC levels were lower in β^0-thalassemia compared to β^+-thalassemia. Meanwhile, the RDW level was higher. Besides, based on Hb analysis, HbA_2 and HbF levels were higher in β^0-thalassemia compared to β^+-thalassemia with a P value <0.05.

Table 4 provides the hematological parameters and Hb analysis of each genotype (CD 35 (−C), CD 41–42 (−TCTT), CD8/9 (+G), IVS1-nt1, IVS1-nt5, HbE, and HbMalay). HbE and HbMalay was frequent mutation in South East Asia region; this study showed that Hb, MCV, and MCH level of HbE trait was greater than HbMalay trait, while the RDW was lower.

The diagnostic values (sensitivity, specificity, positive and negative predictive value, likelihood ratio positive and negative, and characteristic ROC) in connection with the cutoff in this population for differential diagnosis of β^0-thalassemia compared to β^+-thalassemia are given in Table 5.

Table 4: Distribution of hematological parameters and Hb analysis for each genotype.

Genotype	β^0 heterozygotes			β^+ heterozygotes			
	CD 35 (−C)	CD 41–42 (−TCTT)	CD 8/9 (+G)	IVS1-nt1	IVS1-nt5	HbE	HbMalay
	$n = 3$	$n = 14$	$n = 4$	$n = 16$	$n = 59$	$n = 35$	$n = 47$
Hematological parameter							
Hb (g/dL)**	12.1 (10.8–12.3)	10.9 (8.8–13.5)	12.1 (11.1–13.0)	11.0 (9.4–13.3)	11.4 (9.3–14.6)	12.7 (9.5–15.1)	12.3 (8.3–15.5)
MCV (fL)**	65.9 (63.3–68.3)	60.2 (55.6–63.4)	61.3 (61.1–61.5)	62.5 (54.4–85.3)	63.9 (51.9–79.5)	75.2 (61.9–83.3)	71.2 (53,4–81,3)
MCH (pg)**	20.0 (20.0–21.0)	18.5 (17.0–20.0)	18.0[a]	19.0 (17.0–28.0)	20.0 (15.0–26.0)	24.0 (18.0–30.0)	22.0 (16.0–25.0)
RDW (%)**	15.6 (14.5–17.7)	18.0 (15.4–20.8)	18.2 (17.6–19.1)	17.5 (12.1–20.1)	17.2 (12.6–20.2)	14.6 (12.3–18.3)	15.6 (13.5–20.6)
Hemoglobin analysis							
HbA2 (%)**	5.4 (5.1–5.4)	5.5 (4.9–6.6)	4.8 (4.7–5.3)	5.2 (2.7–6.1)	5.0 (1.9–6.4)	3.7 (2.9–5.7)	4.5 (3.7–6.0)
HbF (%)**	0 (0–1.4)	0.4 (0–2.9)	0.7 (0–0.4)	0.7 (0–4.1)	0 (0–12.7)	0 (0–24.5)	0.2 (0–3.9)
HbA (%)**	94.6 (93.2–94.9)	93.9 (90.5–95.1)	95.2 (94.3–95.3)	93.6 (90.7–96.8)	94.6 (70.9–98.1)	71.1 (68.0–94.8)	95.1 (91.4–96.3)
HbE (%) ($n = 35$)**					1 subject: 25.4	25.2 (23.0–27.0)	

**Abnormal distribution (median). [a]MCH value in CD 8/9(+G) mutation has the constant value (4 subjects) = 18 pg.

Table 5: Evaluation of different hematological parameters and Hb analysis in the differentiation of β^0-thalassemia compared to β+-thalassemia.

	Sensitivity	Specificity	PPV (%)	NPV (%)	LR +	LR −	Cutoff for β^0 in our population	AUC (95% CI)
Hematology parameter								
Hb	78	56	69	68	1.78	0.39	≤12.3	0.702 (0.625–0.778)
MCV	87	87	89	85	2.71	0.29	≤66.8	0.898 (0.847–0.948)
MCH	85	90	91	83	8.73	0.16	≤20.5	0.900 (0.849–0.952)
MCHC	86	60	73	78	2.14	0.23	≤31.5	0.784 (0.716–0.851)
RDW	79	71	77	73	0.29	2.71	≥16.15	0.211 (0.142–0.280)
Hemoglobin analysis								
HbA_2	88	74.4	81	84	3.44	0.16	≥4.65	0.844 (0.781–0.906)
HbF	58	61	65	54	1.50	0.68	≥0.35	0.583 (0.500–0.667)

PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; AUC, area under the curve.

Figure 1 shows ROC for hematological parameter calculation, and Figure 2 shows ROC for Hb analysis calculation. From Table 5, Figures 1 and 2, the highest diagnostic value was related to MCH to differentiate β^0-thalassemia compared to β^+-thalassemia. There was no significant difference in the AUC of HbF levels between these types of mutation.

4. Discussion

Southeast Asia region has the most complex genotypes of thalassemia in the world as the result of various combinations of globin chain gene mutations from interethnic marriage [10]. South Sumatra has a high carrier prevalence of β-thalassemia trait as much as 15% [11], so that screening is necessary to prevent the birth of children with thalassemia major. In the current study, analysis of 183 subjects was conducted to compare the hematological parameters between β^0-thalassemia compared to β^+-thalassemia carriers in the families of thalassemia patients to determine the predictive value that can be used as a screening tool. The current study found 12 types of mutations in 183 subjects, with the most common mutations being IVS1-nt5 (32.2%), followed by HbMalay (25.7%) and HbE (19.1%). A previous study in 2003 that examined trait-carrying mutations in the Malay ethnicity of South Sumatra in the general population found that the most common mutations were HbE (36.3%), followed by HbMalay (34.09%) and IVS1-nt5 (9.09%) [5].

Erythrocyte index (MCV and MCH) is a parameter used in screening for thalassemia, based on the International Thalassemia Federation (TIF) guidelines. Levels of MCV <78 fL and MCH <27 pg, with peripheral blood features of microcytic, hypochromic, and anisopoikilocytosis can be suspected as carriers [1]. Based on Indonesia Guidelines for Management of Thalassemia, the suspected carriers in general population, if the MCV value was <80 fL and the MCH value was <27 pg [7].

In the current study, the mean of Hb, MCV, MCH, and MCHC in β^0-thalassemia was lower than β^+, besides the RDW, HbA_2, and HbF levels were higher. These data could explain the differences between the two phenotypes to define cutoff values for each variable. Indonesia, especially South

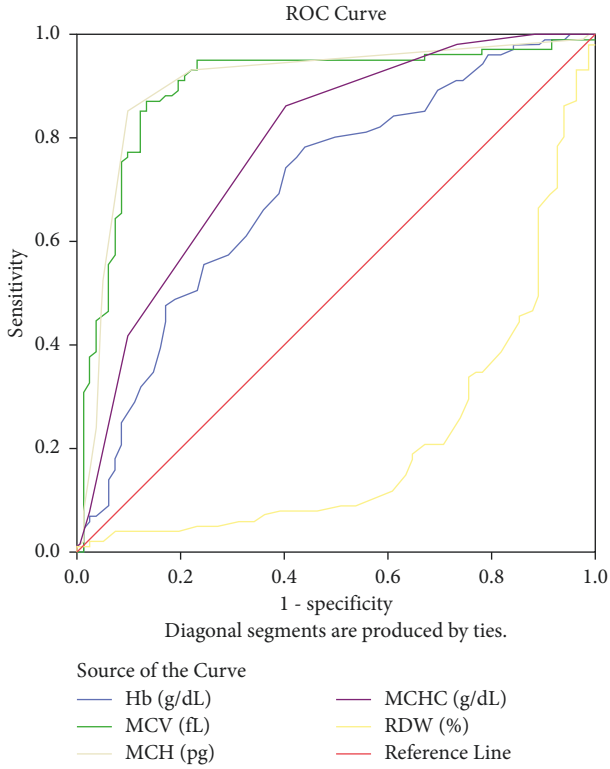

Figure 1: Receiver operative characteristic curves (ROC) of hematological parameters (Hb, MCV, MCH, MCHC, and RDW) (P value of each formula <0.001).

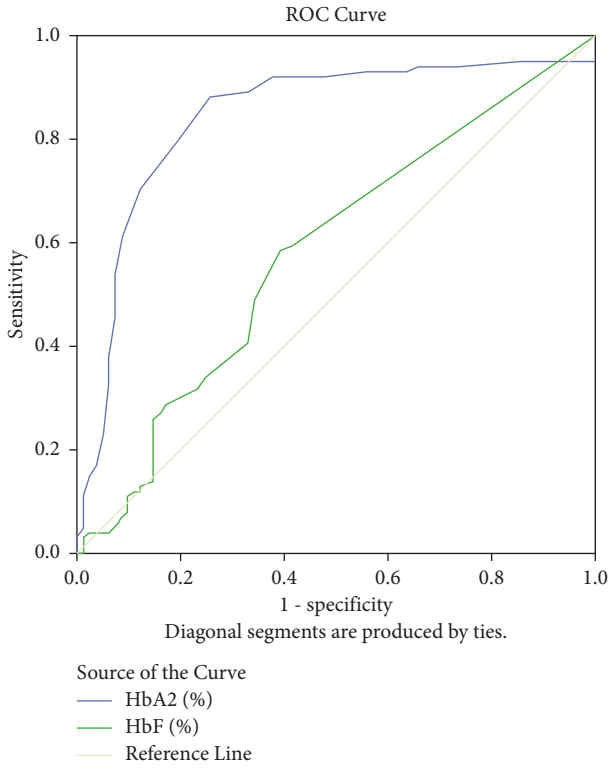

Figure 2: Receiver operative characteristic curves (ROC) of HbA_2 ($P < 0.001$) and HbF ($P = 0.053$).

Sumatra, have various ethnic groups. The cutoff points of hematological parameters and Hb analysis can be useful to differentiate the type of thalassemia mutation in the carrier population in South Sumatra for screening purposes in the families of thalassemia patients. Evaluation of hematological parameters and Hb analysis in this study was performed and compared according to the ROC curve. The result shows that MCH has the largest AUC (0.900).

Hematological characteristics of β-thalassemia heterozygotes have wide variation based on the type of mutation [12, 13]. Baliyan et al.' [14] study in India showed that MCV <74 fL combined with MCH <28 pg can be used as a cutoff for the screening test of thalassemia in antenatal anemic woman in South Asian region, if the HPLC in the facility does not exist (sensitivity 95%, specificity 16%). Another study in Iran showed that MCH <27 pg was more sensitive compared with MCV <80 fL for screening of β-thalassemia traits in the general population [15]. The study in Israel [16] showed that there was a relationship between MCH and type of mutation with cutoff point 20.94; however, MCH was less sensitive to differentiate between β^0 and β^+ populations. This present study found that MCH value ≤ 20.5 pg was predicted as β^0-thalassemia (sensitivity 85%, specificity 90%, PPV 91%, NPV 83%, and likelihood ratio positive 8.73).

A study in Israel showed that MCV lower than 66.96 fL could predict the possibility of β^0 mutation (sensitivity 77% and specificity 91%) [16]. Almost similar to this present study, we found the cutoff point of MCV was ≤66.8 fL (sensitivity 87%, specificity 87%) for β^0 and >66.8 for β^+.

The RDW examination is usually done to differentiate between iron deficiency anemia and thalassemia independent of transfusion. The cutoff > 14% suggests thalassemia trait carriers [17]. In the present study, we found significant differences in the levels of RDW between β^0-thalassemia and β^+-thalassemia with $P < 0.05$. The median RDW is known to be lower in β^0-thalassemia compared to β^+-thalassemia. In the study done in Medan, North Sumatra (2019) [18], the RDW level in carriers of β-thalassemia trait was between 15.7% and 16.5%, while this present study has a wider range of RDW which was 12–20.8%.

Based on Hb analysis, we found that there are significant differences between the levels of HbA_2 and HbF in β^0-thalassemia and β^+-thalassemia, where the levels of HbA_2 in β^0-thalassemia were between 1.7 and 6.6% with a median of 5.1%. The lower levels found in the current study compared to the threshold used by the guidelines used for the screening of thalassemia in which levels of HbA_2 in thalassemia suggestive of β^0-heterozygotes were 4–9% and in β^+-thalassemia between 2.9 and 6% with a median of 4.3%. HbA_2 levels can also be affected by iron status, whereas in deficiency anemia, HbA_2 levels will decrease [7]. The HbA_2 level used in screening to detect thalassemia is >3.5%. However, in this study, 6.6% of subjects had $HbA_2 < 3.5\%$. We calculated the cutoff point of HbA_2 of β^0-thalassemia in this study using the ROC curve, finding that HbA_2 level ≥ to 4.65% was predicted to be β^0-thalassemia, with sensitivity 88% and 74% of specificity. The HbA_2 value in this subject could be affected by iron status; there were 5 subjects who

had iron deficiency, and those are 2/101 subjects with β^0-thalassemia and 3/82 subjects with β^+-thalassemia.

From the current study, we concluded that MCH can be used as a screening tool to differentiate the type of β-thalassemia mutation (β^0 or β^+) in populations of carriers consisting of relatives of patients with thalassemia in South Sumatra.

Data Availability

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

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

Anemia Burden among Hospital Attendees in Makkah, Saudi Arabia

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Background. Anemia is a major health problem in Saudi Arabia and has multiple etiologies. Many studies have been conducted in Saudi Arabia in specific population groups like school children, adolescents, university students, and females in the reproductive age group, and most have reported high prevalence of anemia. This study was conducted in a specialist hospital in Makkah city and includes all outpatients aged 15 years and above. *Objective*. To study the burden of anemia among hospital attendees, its stratification based on gender and age, and its severity along with the morphological types of anemia. *Methods*. This is a study conducted at a specialist hospital in Makkah city and one-month data were collected retrospectively from the laboratory database and include demographic and routine hematological results of complete blood count (CBC). *Results*. A total of 21,524 patients were included, out of which 9444 (43.9%) were males and 12020 (56.1%) were females. The overall prevalence of anemia was 38.7% (8339). Prevalence was very high in females, accounting for 68.2% (5689), whereas it was 31.8% (2650) in males. There were 39.6% (3301), 43.9% (3657), and 16.6% (1381) cases of mild, moderate, and severe anemia, respectively. In females, anemia was more prevalent in the age group of 15 to 49, which is considered as the reproductive age group. Microcytic anemia was the most prevalent type observed in this age group, accounting for 40.7% of all anemia cases. Normocytic anemia was more prevalent in the males, accounting for 52%. *Conclusion*. Our study showed high prevalence of anemia among the patients attending outpatient departments in a specialist hospital. Females have high prevalence of anemia when compared to male population. Microcytic anemia was the most common anemia type among females and was seen in the 15–49 age group. There is an increase in prevalence of anemia with age for males, whereas, in females, increased prevalence is observed in the reproductive age groups and the anemia prevalence maintained a steady decrease towards the 5th to the 9th decades. Normocytic anemia was more prevalent in the 5th to the 9th decades, indicating that there are more etiologies other than iron deficiency in the causation of anemia. Macrocytic anemia was the least reported anemia type. Anemia of mild and moderate severity was predominant in both genders, although severe anemia showed higher prevalence in females as compared to males. *Conclusion*. Anemia is highly prevalent in adolescents, adults, and the elderly in Makkah region. The most common cause is thought to be iron deficiency, although other causes are not uncommon. The authorities need to address the problem of prevention and reduction in anemia prevalence by taking effective measures and interventions.

1. Introduction

Anemia is a global health issue affecting a quarter of the global population [1]. It affects almost every country and occurs in all age groups but is more prevalent during pregnancy and childhood [2]. In many developing countries, anemia has reached the level of an epidemic [3]. It is reported that half of the cases of anemia are due to iron deficiency, which is the most common micronutrient deficiency [4, 5]. Along with nutritional deficiencies, malaria, parasitic infections, blood loss, hemoglobinopathies, and bone marrow suppression or replacement are common etiologies [6]. Anemia is defined by WHO as a condition in which hemoglobin concentration is below 120 g/L in nonpregnant females and below 130 g/L in males [2]. Iron deficiency causes chronic fatigue and tiredness and can affect

cognitive functions, as well as motor and mental development along with visual and auditory functions of people [7]. Anemia is found in varying degrees across the world, depending on the age group and geographic location. Anemia affects one out of every four people, with pregnant women and children under the age of five being the most vulnerable. With two-thirds of preschool-aged children and half of all women impacted, the WHO areas of Africa and Southeast Asia are the most at risk [2]. In terms of numbers, the majority of the burden is concentrated in South-East Asia, where roughly 40% of anemic preschool-age children and nonpregnant women, as well as approximately 30% of pregnant women, reside [8].

WHO has reported global prevalence of 30.2% among nonpregnant women (15–49.99 years) and about 33% in Asia and 44.4% in Africa. Among men (aged 15–59.99 years) and the elderly (≥60 years), the global anemia prevalence is 12.7% and 23.9%, respectively [2].

Anemia is a common problem in low- and middle-income countries, particularly among adolescent females, women of reproductive age, pregnant women, and children. Anemia is expected to be reduced in women of reproductive age (15–49 years) by 50 percent by 2025, according to the second of the world's six global nutrition objectives. Given the fact that anemia affects half a billion women of reproductive age around the world, eliminating anemia is essential for the health as well as their economic production. According to the Global Health Observatory, anemia prevalence among women of reproductive age ranged from 9.1 percent in Australia to 69.6 percent in Yemen in 2016 [8].

According to reports, anemia is a significant health burden in the Gulf countries, with a high frequency of anemia in females between the ages of 17 and 24 years, as well as males, being reported [4]. Preschool children, pregnant women, and nonpregnant women all have high prevalence of anemia in Saudi Arabia, which, according to the World Health Organization's report on the worldwide prevalence of anemia, is considered a moderate health problem in the country, with prevalence ranging between 20.0 and 39.9 percent [2].

According to the findings of a study conducted on Saudi women aged 15–49 years, anemia was found in 40% of the participants [9]. Another investigation by Al Quaiz found a significant frequency of anemia among females from Riyadh, with an estimated 37 percent of females suffering from the condition [7]. Many studies have been undertaken in various population groups such as school children, teenagers, university students, and females in the reproductive age group in Saudi Arabia, and the findings have revealed a high incidence of anemia in these categories in the country [5, 10–13]. In this study, we aimed to determine the prevalence of anemia, its stratification based on gender, its severity, and the morphological type of anemia in all of the study patients.

2. Materials and Methods

This study was done using the patient's data collected retrospectively for one month from a specialist hospital in Makkah city. The data were collected from the laboratory database and include demographic and routine hematological results of complete blood count (CBC) performed on a fully automated hematology analyzer. Hematological data from all patients aged 15 years and above and attending the routine outpatient departments over a period of one month from January 1, 2019, to January 31, 2019, were included for analysis.

The hemoglobin cut-off (g/L) for the diagnosis of anemia and its categorization based on severity into mild, moderate, and severe anemia was done as per WHO recommendation as shown in Table 1 [14].

Statistical Analysis was done using IBM SPSS Statistic (Statistical Package for the Social Sciences, version 20, Armonk, New York, USA). P value <0.05 was considered statistically significant. Continuous variables were expressed as mean ± standard deviation and categorical data were presented as median and interquartile range (IQR). Odds ratios (ORs) and 95% confidence intervals were obtained by logistic regression to determine the impact of age on anemia.

3. Results

The specialist hospital lab receives between 400 and 500 blood samples for routine hematological tests every day from patients attending the outpatient clinics. A total of 21,524 patients were included, out of which 9444 (43.87%) were males and 12020 (56.2%) were females (Figure 1). The mean age for male patients was 48.83 ± 18.7, with a range of 15 to 89 years, and for females it was 46.88 ± 17.8, with a range of 15 to 88 years. We included 21,524 hemoglobin estimations and none of the patients had repeated hemoglobin value. The overall prevalence of anemia was 38.7% (8339). Among all the anemia cases, prevalence was very high in females, accounting for 68.2% (5689), whereas it was 31.8% (2650) in males. Table 2 shows the hematological variables for male and female patients. Overall, there were 39.6% (3301), 43.9% (3657), and 16.6% (1381) cases of mild, moderate, and severe anemia, respectively. The mean hemoglobin concentration was 125.29 ± 25.7. The mean hemoglobin in males was 100.12 ± 19.2 g/l and in females it was 98.8 ± 18.2 g/l. Tables 3 and 4 along with Figures 2 and 3 present the age-wise distribution of hemoglobin and stratification into mild, moderate, and severe anemia based on WHO recommendation for males and females, respectively [14].

All the anemia cases were categorized into microcytic, normocytic, and macrocytic anemia based on the reference cut-off for mean corpuscular volume (MCV). Microcytic anemia was the most prevalent type among females, whereas normocytic anemia was more prevalent in the males, accounting for 52% of the anemia cases in males (Tables 5 and 6; Figures 4 and 5). The prevalence of macrocytic anemia was very low in both genders. Among the male patients, high prevalence of mild and moderate anemia is observed in all age groups, although the 6th, 7th, and 8th decades showed increased prevalence, whereas severe anemia was distributed uniformly over all the age groups. In females, anemia was more prevalent in the age group of 15 to 49 which is considered as the reproductive age group. Microcytic anemia was the most prevalent type observed in this age group,

Table 1: Hemoglobin levels for stratification into mild, moderate, and severe anemia [14].

	Nonanemia	Anemia		
		Mild	Moderate	Severe
Nonpregnant women (15 years of age and above)	120 or higher	110–119	80–109	<80
Men (15 years of age and above)	130 or higher	110–129	80–109	<80

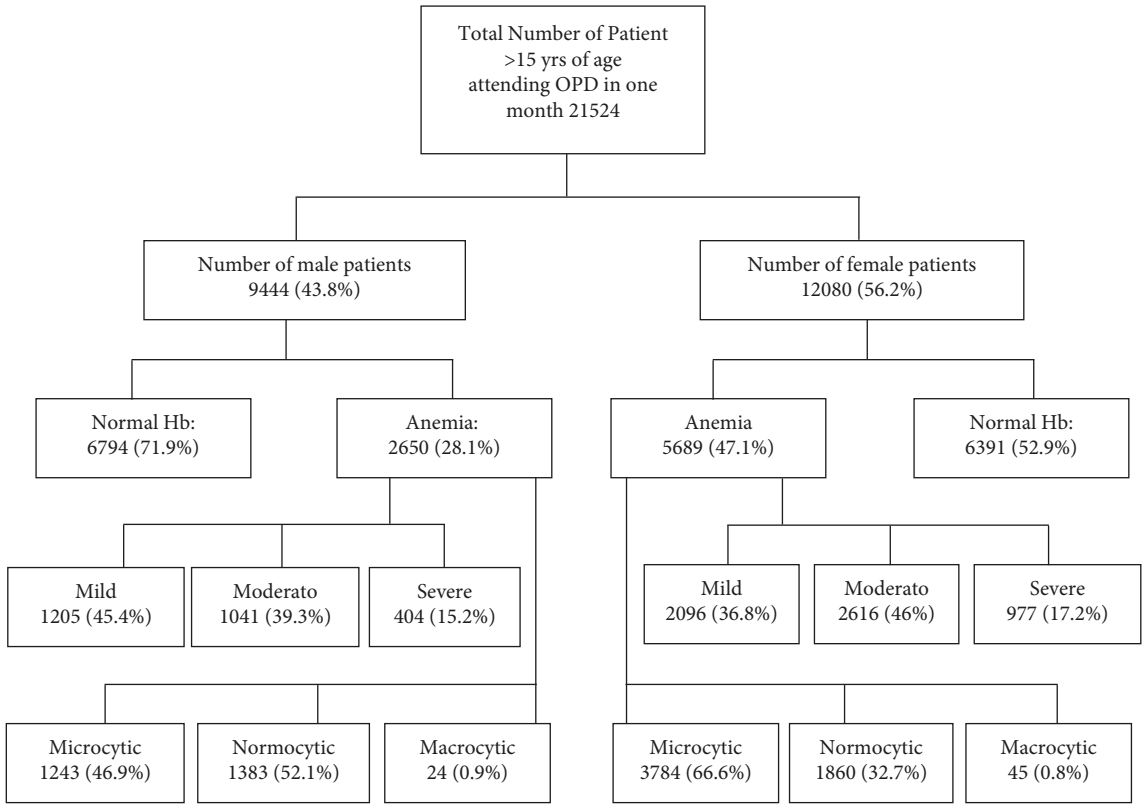

Figure 1: Flow chart showing the patient selection and stratification.

accounting for 40.7% of all anemia cases (Table 6). After performing regression analysis for determining the impact of age on anemia, the ORs (95% CI) for severe, moderate, and mild anemia were 1.007 (1.004–1.01, $P < 0.001$), 1.011 (1.009–1.013, $P < 0.001$), and 1.013 (1.011–1.016, $P < 0.001$), respectively. The OR for microcytic anemia was 0.984 (0.982–0.985, $P < 0.001$) and for macrocytic anemia it was 1.014 (1.002–1.025, $P = 0.022$).

4. Discussion

This study was done using the patient's data collected retrospectively for one month from a specialist hospital in Makkah city. In this study, we attempted to analyze the anemia prevalence among the outpatient attendees in various speciality departments. Anemia is a complex disease with nutritional and nonnutritional variables and mechanisms at play. Saudi Arabia, along with the Gulf Arab countries and others, is located in the eastern Mediterranean. In the eastern Mediterranean region, anemia prevalence ranges from 22.6 percent to 63 percent among pregnant women and is 69.6 percent among women of reproductive age [15]. Anemia was found to be prevalent in a high proportion of the patients in this study, according to the findings. The significant prevalence of microcytic anemia in females in the reproductive age range was identified, indicating that iron deficiency is the most likely cause of the condition. It is possible that malnutrition, increased blood loss due to pregnancy or menstruation, and a lack of iron absorption are the primary causes of this condition [6]. Because the serum ferritin levels were not available, we did not corroborate the findings. There have been numerous studies in Saudi Arabia which have revealed a significant frequency of iron deficiency anemia (IDA) in women of reproductive age in the country. IDA is prevalent in 41.6 percent of women between the ages of 18 and 40, according to Alswalem AM [16], and 38.3 percent among Saudi university female medical students, according to another report by Alsheikh [3]. According to the World Health Organization, 2 billion people are anemic worldwide, with iron deficiency accounting for half of all anemia cases. Iron deficiency is the most common micronutrient deficiency in the world [4, 17]

Owaidah et al. [18] described regional variation in the prevalence of iron deficiency and IDA and reported

Table 2: Hematological variables and demographic data.

Variable	Mean	SD	Minimum value	Maximum value	Median
Age (years)					
Male	48.84	18.17	15	89	46
Female	46.88	17.8	15	88	47
Hb level (g/dl)					
Male	100.12	19.2	3.12	21.5	14.3
Female	98.8	18.2	2.56	23.3	12.1
Mean cell volume (μm^3)					
Male	83.86	6.56	47.8	138.9	84.5
Female	81.77	7.80	46.6	120.6	82.88
MCH (pg)					
Male	28.08	2.71	12.3	54.6	28.58
Female	26.65	3.27	13.15	59.60	27.3
MCHC (g/dl)					
Male	32.4	38.12	8.26	64.3	33.35
Female	31.3	39.57	7.57	70.5	32.45
RDW (%)					
Male	14.13	2.03	10.9	35.5	13.6
Female	14.91	2.31	11	32.7	14.28
RBC (m/μl)					
Male	5.05	0.75	1.35	10	5.12
Female	4.54	0.56	1.75	7.43	4.56

Table 3: Severity of anemia across age groups in males.

			Age groups								Total
			≤19	20–29	30–39	40–49	50–59	60–69	70–79	>80	
HGB categories	Severe anemia	Count	29	64	65	45	55	73	48	25	404
		% of total	1.1%	2.4%	2.5%	1.7%	2.1%	2.8%	1.8%	0.9%	15.2%
	Moderate anemia	Count	59	126	130	119	159	205	141	102	1041
		% of total	2.2%	4.8%	4.9%	4.5%	6.0%	7.7%	5.3%	3.8%	39.3%
	Mild anemia	Count	68	100	129	118	202	273	210	105	1205
		% of total	2.6%	3.8%	4.9%	4.5%	7.6%	10.3%	7.9%	4.0%	45.5%
Total		Count	156	290	324	282	416	551	399	232	2650
		% of total	5.9%	10.9%	12.2%	10.6%	15.7%	20.8%	15.1%	8.8%	100.0%

Table 4: Severity of anemia across age groups in females.

			Age groups								Total
			≤19	20–29	30–39	40–49	50–59	60–69	70–79	>80	
HGB categories	Severe anemia	Count	48	146	176	201	149	116	92	49	977
		% of total	0.8%	2.6%	3.1%	3.5%	2.6%	2.0%	1.6%	0.9%	17.2%
	Moderate anemia	Count	117	346	527	536	370	375	225	120	2616
		% of total	2.1%	6.1%	9.3%	9.4%	6.5%	6.6%	4.0%	2.1%	46.0%
	Mild anemia	Count	91	353	371	388	356	303	157	77	2096
		% of total	1.6%	6.2%	6.5%	6.8%	6.3%	5.3%	2.8%	1.4%	36.8%
Total		Count	256	845	1074	1125	875	794	474	246	5689
		% of total	4.5%	14.9%	18.9%	19.8%	15.4%	14.0%	8.3%	4.3%	100.0%

highest prevalence of iron deficiency in the Makkah region. We could not assess the iron deficiency status in our patients wherein IDA appears to be highly prevalent. The overall anemia prevalence reported by AlAssaf [19] was 21% in females and 2.3% in males, whereas AlQuaiz et al. [17] reported anemia prevalence of 40% in women of reproductive age group in Riyadh. The only study reporting low prevalence of 12.5% was done in Tabuk in female university students in the age group of 19–25 years [1]. Majority of the studies conducted in Saudi Arabia have included selected population groups like children below the age of 5, school-going children, young adults/adolescents, pregnant women, and women in reproductive age group. [1, 4, 17, 20–23]. There are regional variations of anemia prevalence in Saudi Arabia within a specific group. Very few studies are conducted in the general population particularly in adults and the elderly [24, 25].

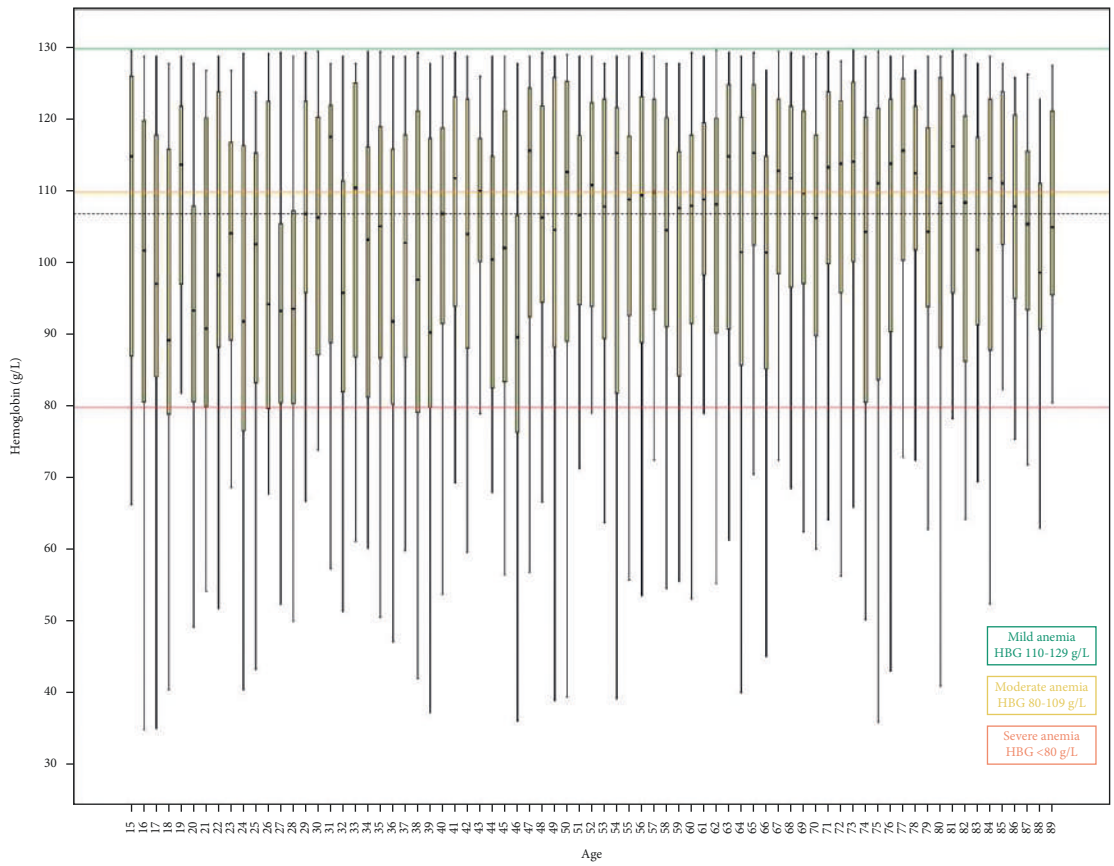

Figure 2: Anemia groups stratified based on hemoglobin concentration for male gender and age.

Numerous risk factors for the development of IDA in Saudi women of reproductive age have been identified [4]. Dietary habits, menorrhagia, a history of NSAID use, and a personal or family history of IDA are all risk factors [16, 17, 23, 25]. There is compelling evidence for a negative link between limited meat consumption and an increased risk of developing IDA [3]. As observed in earlier studies, irregular meals and skipping meals, particularly skipping breakfast, are risk factors for IDA [4]. Regular breakfast consumption has an effect on the prevalence of anemia [23]. Vitamin C has been shown to be an effective booster of iron absorption in nonheme meals, and citrus fruits are high in vitamin C [1]. Numerous studies indicate that a family history of genetic disorders is highly associated with IDA, and additional research is needed to confirm this [23]. Another factor for the high incidence is that the Saudi population consumes fewer multivitamin and mineral supplements than many other countries [26]. Microcytic anemia is more prevalent in females due to malnutrition, increased blood loss associated with pregnancy and menstruation, and a deficiency of iron absorption, whereas normocytic anemia is more common in males due to blood loss and chronic disorders [6]. Our results are in agreement with other studies. In our study, the prevalence of anemia in the elderly was 11.68 percent, which is consistent with a report by Alsaeed, who found that the prevalence of anemia in the elderly (>60 years) was 12.9 percent [25]. Males had increased prevalence of anemia with age in our study, whereas females had elevated prevalence in reproductive age groups and a continuous decline in anemia prevalence from the 5th to the 9th decades. Interestingly, normocytic anemia was more widespread in the fifth to ninth decades, demonstrating that anemia is caused by more than iron shortage. Macrocytic anemia was the least reported kind of anemia, accounting for fewer than 2% of cases, which is consistent with data from another Saudi Arabian investigation [13].

The cause of anemia in the senior population must be determined, and fresh studies must be conducted to investigate it. Consumption of tea, which contains a high concentration of polyphenols, has been linked to anemia in the elderly. Polyphenols block nonheme iron absorption. Tea drinking is a widespread ritual in Saudi Arabia and is typically drunk before and after meals [1, 22]. According to a study conducted in China, the high prevalence of anemia among the middle-aged population and elderly was caused by factors such as insufficient consumption of citrus fruits, reduced consumption of red meat, eggs, vegetables, and dairy, and excessive consumption of cereals, cooking oil, and salt, all of which were prevalent among the middle-aged and elderly population in China [16]. Another element to examine is the drastic shift in the Saudi population's food habits from the traditional diet of dates, milk, rice, fresh vegetables, and seafood to junk foods and fewer green vegetables and fruits [20]. Other well-established risk factors for anemia include obesity and malnutrition [20, 27]. Obesity and overweight are considered chronic

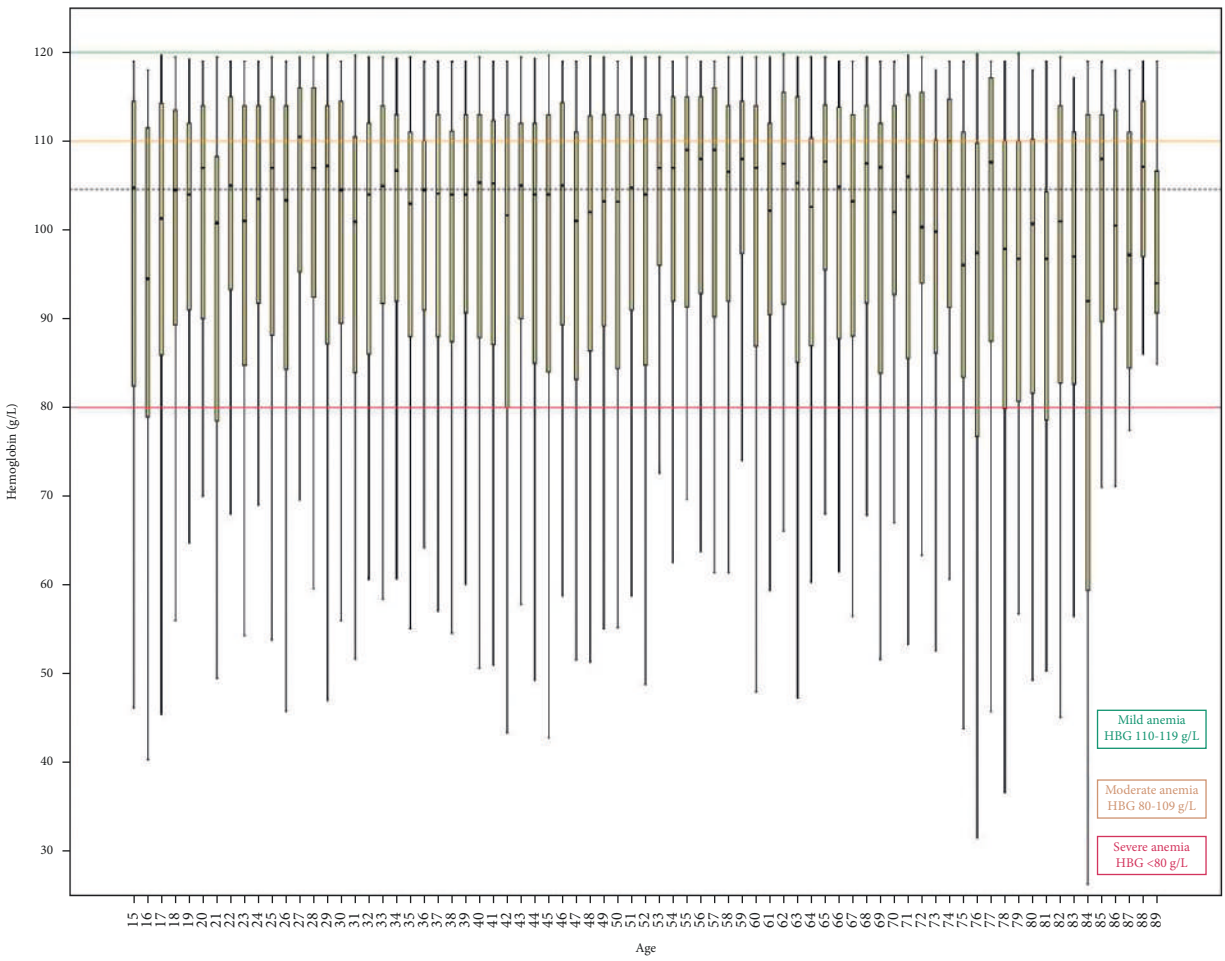

Figure 3: Anemia groups stratified based on hemoglobin concentration for female gender and age.

Table 5: Prevalence of morphological types of anemias across age groups in males.

			Age groups								Total
			≤19	20–29	30–39	40–49	50–59	60–69	70–79	>80	
MCV groups	Microcytic	Count	109	167	159	142	182	260	146	84	1249
		% of total	4.1%	6.3%	6.0%	5.4%	6.9%	9.8%	5.5%	3.2%	47.1%
	Normocytic	Count	47	123	158	135	234	286	249	145	1377
		% of total	1.8%	4.6%	6.0%	5.1%	8.8%	10.8%	9.4%	5.5%	52.0%
	Macrocytic	Count	0	0	7	5	0	5	4	3	24
		% of total	0.0%	0.0%	0.3%	0.2%	0.0%	0.2%	0.2%	0.1%	0.9%
Total		Count	156	290	324	282	416	551	399	232	2650
		% of total	5.9%	10.9%	12.2%	10.6%	15.7%	20.8%	15.1%	8.8%	100.0%

inflammatory processes and hence play a role in the development of anemia [27]. Anemia is a prevalent disorder among the elderly, and it is connected with an increased risk of death, disability, and impaired physical performance [25]. Numerous factors such as ethnic origin, smoking status, dietary inadequacy, and altitude of residence all influence a person's red cell characteristics, and early detection of anemia in the elderly is critical for rapid intervention and management. The symptoms of anemia are not always obvious in the elderly, and efforts should be taken to determine the most likely cause of anemia. Folate, vitamin B12, serum ferritin, and serum erythropoietin levels should be determined. Additionally, Saudi Arabia has high prevalence of risk factors such as obesity and an unhealthy lifestyle [20].

The World Health Assembly (WHA) has designated anemia reduction as a global dietary priority for 2025 [15]. There is a dearth of research documenting regional progress toward reducing anemia burden and the techniques and interventions being adopted. The trend in anemia prevalence over a ten-year period does not indicate a major decline, and anemia prevalence has remained stable in Saudi Arabia over the last decade [15]. Low and below-average public awareness initiatives can be blamed for the nation's failing health [22]. Saudi Arabia should take a multisectoral,

Table 6: Prevalence of morphological types of anemias across age groups in females.

			Age groups								Total
			≤19	20–29	30–39	40–49	50–59	60–69	70–79	>80	
MCV groups	Microcytic	Count	202	629	824	857	540	419	215	98	3784
		% of total	3.6%	11.1%	14.5%	15.1%	9.5%	7.4%	3.8%	1.7%	66.5%
	Normocytic	Count	53	209	245	261	325	369	254	144	1860
		% of total	0.9%	3.7%	4.3%	4.6%	5.7%	6.5%	4.5%	2.5%	32.7%
	Macrocytic	Count	1	7	5	7	10	6	5	4	45
		% of total	0.0%	0.1%	0.1%	0.1%	0.2%	0.1%	0.1%	0.1%	0.8%
Total		Count	256	845	1074	1125	875	794	474	246	5689
		% of total	4.5%	14.9%	18.9%	19.8%	15.4%	14.0%	8.3%	4.3%	100.0%

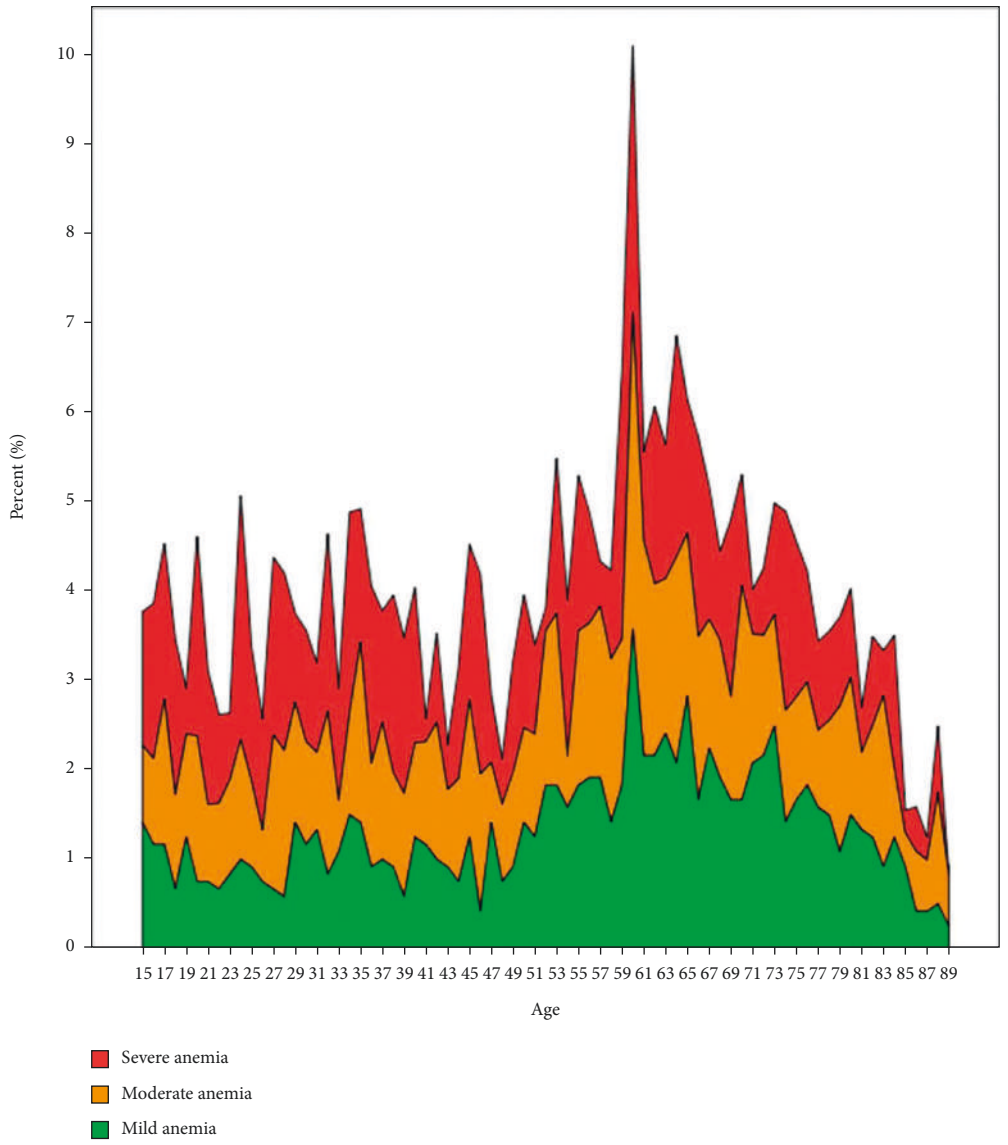

Figure 4: Age-wise prevalence of mild, moderate, and severe anemia in males.

community-based strategy to prevent and control anemia. Along with dietary inadequacies, nonnutritional causes of anemia such as acute and chronic parasite infections and genetic illnesses such as thalassemia, G6PD deficiency, and sickle cell trait must be addressed [15].

4.1. Limitations. Our study has some limitations. We included patients who visited a hospital, which may have inflated the numbers, but we excluded hospitalized patients. However, our findings are consistent with those of other research conducted in Saudi Arabia.

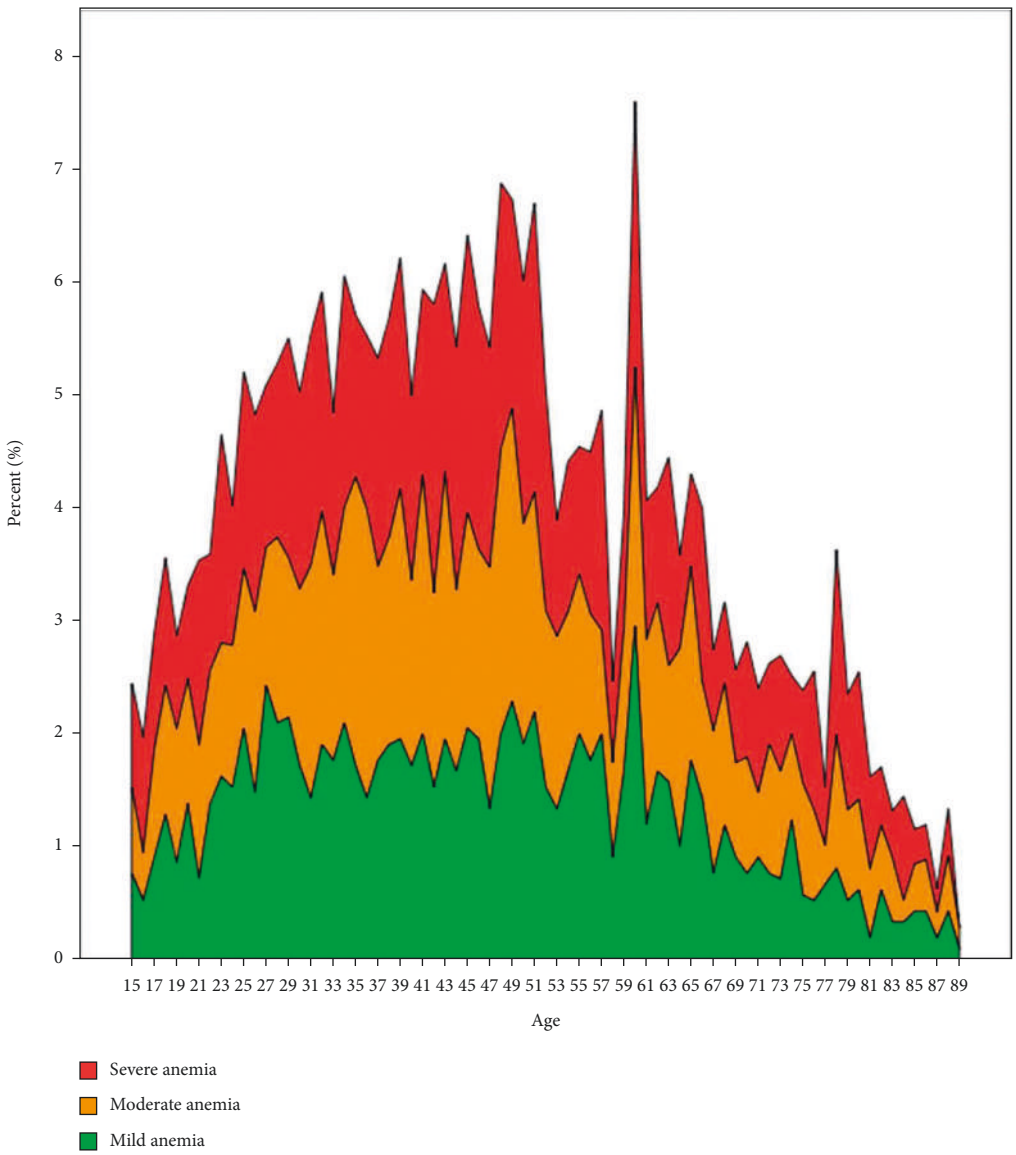

Figure 5: Age-wise prevalence of mild, moderate, and severe anemia in females.

5. Conclusion

Our study showed high prevalence of anemia among the patients attending outpatient departments in a specialist hospital. Females have high prevalence of anemia when compared to male population. Microcytic anemia was the most common anemia type among females and was prevalent in the 15–49 age group considered as the reproductive age group, whereas normocytic anemia was more common among the male gender. Anemia of mild and moderate severity was predominant in both genders, although severe anemia showed higher prevalence in females as compared to males. The authorities need to address the problem of prevention and reduction in anemia prevalence in Saudi Arabia by taking effective measures and interventions.

Data Availability

Our conclusions arise from the evaluation of the demographic and laboratory data accessed from the hospital records and are described in this study. These data cannot be released due to patient confidentiality.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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Research Article

A Retrospective Study Using Mentzer Index for Prevalence of Iron Deficiency Anemia among Infants Visiting Maternal Centers at the Age of One Year

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Anemia, defined as a hemoglobin level two standard deviations below the mean for age, is prevalent in infants and children worldwide. Characterizing anemia as microcytic and normocytic depends on the mean corpuscular volume (MCV), which is an important parameter in differentiating many types of anemia. Microcytic anemia due to iron deficiency is the most common type of anemia in children. In this study, we aimed to assess the Mentzer index used by the Ministry of Health (MOH) in Palestine as a useful tool in differentiating between iron deficiency anemia (IDA) and thalassemia. We assessed for the prevalence of IDA among infants at the age of one year visiting the mother centers from seven West Bank provinces in Palestine. Medical records and hematology laboratory data of 3262 infants were retrospectively analyzed from the years of 2018 to 2020. The Mentzer index applied to all population by dividing mean corpuscular volume (MCV, in fL) by the red blood cell count (RBC, in millions per microliter). A corrected Mentzer index was further calculated among anemic infants to include only microcytic (MCV with less than 72 fl) and hypochromic (mean corpuscular hemoglobin concentration (MCHC) with less than 32 g/L) indices. Mentzer index calculations for the whole population showed that 29.1% were anemic (hemoglobin (HGB) less than 11 g/dl): 21.1% had mild anemia, 7.6% had moderate anemia, while 0.2% had severe anemia. The corrected Mentzer index calculations showed a prevalence of 5.9% and 3.2% among IDA and thalassemia infants, respectively. Severity of anemia was correlated with low body weight and infants born through cesarean mother birth with no interference with gender influence. CBC indices of RBC count, HGB, MCV, and mean corpuscular hemoglobin (MCH) showed a significant difference (p values < 0.05) between IDA and thalassemia infants' populations following the corrected Mentzer index. With the corrected Mentzer index, we introduced a new CBC index among infants at the age of 1 year in Palestine. These lab references could aid in differentiating IDA and thalassemia among the population and improve initial diagnosis screenings. The Mentzer index calculation for the whole population did not necessarily include cases of IDA, and therefore, it is recommended to comprise microcytic and hypochromic anemia indices prior to performing the Mentzer index.

1. Introduction

Worldwide, anemia is a major public health problem and affects up to one-half of children younger than five years [1–5]. Anemia in childhood is defined as a hemoglobin (HGB) concentration below cutoff levels established by the World Health Organization (WHO) less than 11 g/dl in children aged 6–59 months [6]. Microcytic iron deficiency anemia (IDA) is a common cause of childhood anemia, whereas macrocytic anemia is rare in children. IDA in infants between 6 and 12 months can be caused by getting less than the recommended daily amounts of iron. The recommended daily amounts of iron will depend on the child age and sex. From birth to six months, the recommended daily amounts of iron uptake in milligram (mg) is 0.27, and this amount is increased to 11 [7]. A diet that does not have enough iron is the most common cause. During periods of rapid growth, even more iron is needed [8]. Iron deficiency

can be grouped into three categories according to severity: (1) biochemical iron deficiency with normal erythropoiesis, (2) biochemical iron deficiency plus iron-limited erythropoiesis but without anemia, and (3) biochemical iron deficiency with IDA9. A low serum ferritin and a low serum iron can identify biochemical iron deficiency. Iron-limited erythropoiesis can be recognized by a fall in both reticulocyte HGB content and mean corpuscular volume, without a fall in HGB or hematocrit (HCT) [9, 10].

Most infants and children with mild anemia do not exhibit overt clinical signs and symptoms. Initial evaluation should include a thorough history, such as questions to determine prematurity, low birth weight, diet, chronic diseases, family history of anemia, and ethnic background [11, 12]. A complete blood count is the most common initial diagnostic test used to evaluate for anemia, and it allows differentiating microcytic, normocytic, and macrocytic anemia based on the mean corpuscular volume [13]. In the current study, we assessed for the prevalence of IDA among children visiting mother care centers at one year of age using the Mentzer index.

2. Patients and Methods

2.1. Sampling. In this study, medical records of 3262 infants, aged 12 months, were obtained from seven mother centers in the West Bank (Palestine) provinces of (Nablus, Qalqilya, Tulkarem, Jenin, Ramallah, Bethlehem, and Hebron). Data were screened for IDA between the years of 2018 and 2020. 466 cases were randomly obtained retrospectively from each province. The data were collected through a systematic sample by each year and included RBC count, HGB, HCT, MCV, body weight, and type of delivery. The ethical committee of institutional review board (IRB) provided approval. Data obtained from each patient were summarized at the centers and were handled discretely. Names of the patients were kept anonymous.

2.2. The Mentzer Index. The Mentzer index was used in differentiating IDA from beta thalassemia [14]. The index depends on two parameters included from the complete blood count. If the quotient of the mean corpuscular volume (MCV, in fL) divided by the red blood cell count (RBC, in millions per microliter) is shown to be less than 13, it is likely that the patient could be thalassemic, while if the result is greater than 13, then the patient is most probably with IDA.

2.3. Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) Calculations. MCH and MCHC were calculated from existing data for additional evaluation of IDA as hypochromic anemia: MCH = (hematocrit) HCT (%) × 10/RBC count (10–12/L) and MCHC = Hb (g/dL) × 100/HCT (g/dL).

2.4. Statistical Analysis. Data are shown as means ± SEM, unless stated otherwise. Statistical differences were analyzed between the IDA and thalassemia populations of the CBC indices using either the 2-tailed unpaired Student *t*-test (for comparison between two groups) or one-way analysis of variance (one-way ANOVA with Newman-Keuls posttests among multiple groups) using GraphPad Prism 5.0 (GraphPad software, La Jolla, CA). The *t*-test of p value below 0.05 was considered significant.

3. Results

3.1. Sample Characterization. Blood testing for infants agedone year is a mandatory procedure performed at the Ministry of Health (MOH). In the current study, we aimed to assess the overall prevalence of anemia in our sample population. Infants' data were stratified according to HGB levels; HGB levels under 11 g/dl were considered anemia (10 to 10.9 g/dl as mild anemia, 7 to 9.9 g/dl as moderate anemia, and less than 7 g/dl reflected severe anemia). Infants with HGB levels above 11 g/dl were nonanemic. Figure 1(a) displays that 29.1% of the infants were anemic (HGB less than 11 g/dl): 21.1% had mild anemia, 7.6% had moderate anemia, while 0.2% had severe anemia. Figure 1(b) displays the gender of the population among anemic infants, showing that 52% were females while 48% were males. Next, correlation of anemia severities with the total body weight ± SD of the infants was performed. Figure 1(c) shows inverse correlation between body weight and anemia severity among infants; significant results of $p < 0.05$ were obtained between the groups. Furthermore, attempt to relate type of birth recorded in infants' files with anemia was studied. Many studies demonstrated an association between cesarean delivery and anemia in infants and children [15]. In this study, the association of type of birth (normal vs. cesarean (C-section)) with the prevalence of anemia was assessed. Figure 1(d) shows distribution of mothers' type of birth. Normal birth type was 77% and showed to be dominating the distribution among the nonanemic population (data not shown). Moreover, normal birth type distributions were reduced in the anemic population to 62% in favor of the C-section types. Average of HGB level in the C-section type was 10.2 ± 0.11 g/dl as compared to 10.4 ± 0.18 g/dl in the normal birth types (Figure 1(e), $p = 0.012$).Additional CBC indices were included for better assessing anemia among infants' population. Table 1 shows infants' population grouped according to normal and low levels of MCV, MCH, and MCHC. Of the population, 38.6%, 48.6%, and 18.2% had low MCV, MCH, and MCHC, respectively.

3.2. Prevalence of IDA and Thalassemia by Mentzer Index. In an attempt to calculate IDA prevalence in our all-infants population, the Mentzer index was applied; the index was used in mother centers of the MOH of Palestine, as indicated in methods. Surprisingly, around 90% of the total population exhibit a Mentzer index of >13 while 10% showed <13, indicating the overall prevalence of IDA and thalassemia, respectively (Table 2). In the USA, recent surveys documented that the prevalence of IDA in children aged one to five years is estimated to be 1% to 2%14. Therefore, obtained data revealed a high prevalence of IDA among Palestinian

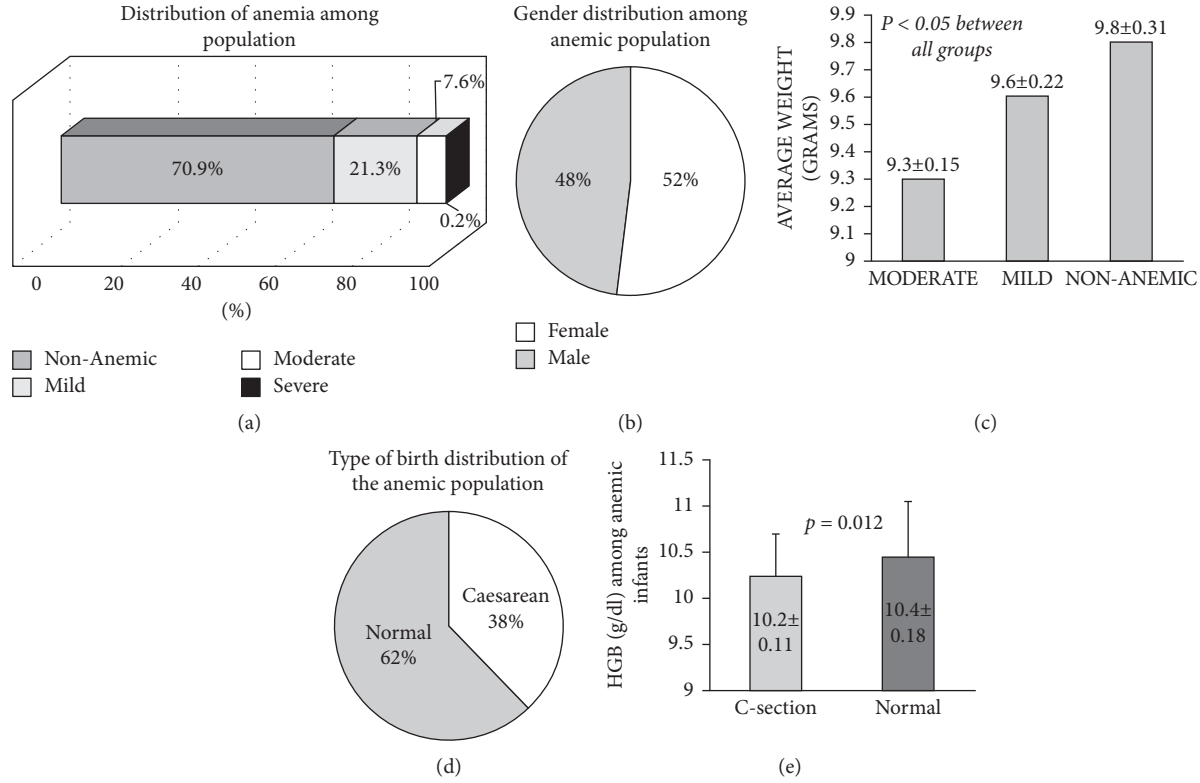

Figure 1: Demographic data of the infants: (a) Distribution of anemia among population according to WHO classification. (b) Gender distribution among anemic population. (c) Average weight of infants according to anemia severities. (d) Type of birth distribution of the anemic population. (e) Averages of HGB among anemic infants showing statistically significant results.

Table 1: Infant population stratified according to CBC indices of MCV, MCH and MCHC.

Values (%)	MCV (72.7–86.5 fl)	MCH (24.1–29.4 pg)	MCHC (32.4–35.3 (g/dl)
Low	38.6	48.6	18.2
Normal	61.4	51.4	81.2

Table 2: Mentzer index in anemia and non-anemic populations. Mentzer index among all population, anemic population, non-anemic population and microcytic and hypochromic population.

Mentzer index	All population (%)	Anemic population (Hb < 11 g/dl)	N (%)on-anemic population (Hb > 11 g/dl)	Microcytic hypochromic population (MCV < 72.7 fl, MCH < 25 pg, MCHC < 32 g/dl)
>13	90	82.3	93.1	5.90
<13	10	16.8	6.9	3.20

infants' population. In order to better interpret these data, the Mentzer index was calculated in the anemic vs. non-anemic population. Table 2 displays the anemic population demonstrating 83.2% of the population with Mentzer index >13 while 16.8% had Mentzer index <13. Moreover, 93.1% of the nonanemic population showed a Mentzer index of >13 and 6.9% had Mentzer index <13 (Table 2).

The above results reveal a high prevalence of IDA and thalassemia cases among the whole population whether they are anemic or nonanemic. In addition, these data indicate that HGB above 11 g/dl does not necessarily exclude cases IDA or thalassemia as indicated by Mentzer index. Data showed a high IDA prevalence among Palestinian infants with the use of Mentzer index. Moreover, the Mentzer index used by MOH mother centers showed surprising data of 16.8% of thalassemia infants, of which infants could be either carrier or diseased. These results, altogether, could raise concerns on using the Mentzer index as a sole indicator for initial screening and emphasize the need to include additional CBC indices that might help improving screening outcome.

3.3. Prevalence of IDA and Thalassemia by Using "Corrected Mentzer Index" and a New Generated CBC Reference Range. The classical diagnosis of IDA and thalassemia should include CBC indices of MCV, MCH, and MCHC. Therefore, from the infants' records, we were able to calculate MCH [HCT (%) × 10/RBC count (10–12/L)] and MCHC [Hb (g/dL) × 100/HCT

Table 3: CBC indices among infants of IDA and thalassemia calculated by Mentzer index and applied on the classified microcytic and hypochromic anemia.

CBC indices	Thalassemia	IDA	P value
RBC count (10^6/ml)	5.42 ± 0.46	4.9 ± 0.2	$P = 0.0001$
HGB (g/dl)	9.5 ± 1.5	10.2 ± 0.7	$p = 0.03$
HCT (%)	33.1 ± 3.3	33.7 ± 1.8	$P = ns$
MCV (fl)	61.1 ± 4.1	69.01 ± 2.2	$P = 0.02$
MCH (pg)	18.1 ± 2.8	20.7 ± 1.4	$P = 0.04$
MCHC (g/dl)	29.7 ± 2.2	30.2 ± 1.7	$P = ns$

(%)]. IDA and thalassemia both are classified as microcytic hypochromic anemia [16] with HGB, MCV, MCH, and MCHC relatively low in favor of thalassemia. Therefore, we reapplied a "corrected Mentzer index" and included infants exhibiting "microcytic hypochromic anemia" using following cutoff indices: HGB less than 11 g/dl (normal values 11–14.1 g/dl), MCV less than 72 fl (normal values 72–84 fl), MCH less than 25 (normal values 25–29 pg), and MCHC less than 32 (32–36 g/dl). Table 3 displays CBC indices of the microcytic and hypochromic infants' population following the "corrected Mentzer index." Calculated results of the "corrected Mentzer index" of the prevalence of IDA and thalassemia are summarized in Table 2 showing 5.90% and 3.20 %, respectively. Later on, CBC indices of RBC count, HCT, MCV, MCH, and MCHC were assessed in both infants' populations. Table 3 summarizes averages of CBC indices in infants at the age of 1 year with microcytic and hypochromic anemia according to the "corrected Mentzer index". Following the use of "corrected Mentzer index," newly generated data were obtained and this included, for the first time, a new CBC reference range differentiating IDA and thalassemia that could be a useful initial screening for assessing anemia among infants at age 1 year.

4. Discussion

Anemia is a global public health concern [1–5]. The American Academy of Pediatrics (AAP) and the World Health Organization (WHO) recommend universal screening for anemia at one year of age 14. The recommended daily amount of iron for infants from birth to 6 months is 0.27 mg, and these requirements increase to 11 mg from the age of 7 months to 1 year [7]. Infants between 6 and 12 months demonstrated increased risk for iron deficiency, especially if they are fed with breast milk or using milk formula that is not fortified with iron. The iron that full-term infants have stored in their bodies is used up in the first 4 to 6 months of life. Breastfed babies who do not get enough iron should be given iron drops prescribed by their doctor [6].

Most infants and children with mild anemia do not exhibit overt clinical signs and symptoms [17]; this could mask initial diagnosis, thereby, preventing anemia consequences if not treated. Prevalence of IDA among the infants' populations could be attributed to inadequate dietary iron intake, feeding problems and noncompliance to treatment with no-follow up program, all of which demand an effective assessments strategy for minimizing IDA cases [1, 18]. In Palestine, mother centers and 1-year-old infants are screened for IDA and thalassemia through the Mentzer index. The Mentzer index was used in differentiating IDA from beta thalassemia [14]. The principle involved is as follows: in iron deficiency, the marrow cannot produce as many RBCs and they are small (microcytic), so both the RBC count and the MCV will be low, and as a result, the index will be greater than 13. Conversely, in thalassemia, which is a disorder of globin synthesis, the number of RBCs produced is normal, but the cells are smaller and more fragile [6, 19]. Hence, the RBC count is normal, but the MCV is low, so the index will be less than 13. Therefore, by using this index, the study aimed to evaluate the prevalence of IDA among children visiting mother care centers at one year of age in 7 major centers all across Palestine (West Bank and excluding Gaza strip). In these centers, gender of infants was recorded together with the type of birth of mothers (natural or C-section). Initial evaluation of family history, questions to determine prematurity, low birth weight, diet, chronic diseases, family history of anemia, and ethnic background was neither included nor registered. Concerning CBC indices, RBC, HGB, HCT, and MCV were obtained. Further calculations were made to calculate the MCH and MCHC, as indicated in the methods section.

Because of high prevalence of IDA and thalassemia among the anemic population of HGB levels above 11 g/dl, using the Mentzer index for the whole population could lack accuracy and might raise some concerns particularly in having false cases. Applying the Mentzer index among the nonanemic infants' population indicated existence of IDA and thalassemia cases that clearly reflects its noneffectiveness in assessing IDA and thalassemia among the general population. Therefore, attempts were made to reapply the Mentzer index on the infants' population exhibiting anemia of microcytic (low MCV) and hypochromic (low MCH and MCHC through manual calculations) indices (corrected Mentzer index). In Palestine, the prevalence of IDA is 5.9%, and this is 5-times higher than that in 1-year-old infants in the USA [13]. More than 40 mathematical indices have been proposed in the hematological literature for discriminating between IDA and thalassemia traits in subjects with microcytic RBCs. None of these discriminant indices is 100% sensitive and specific, and the ranking of the discriminant indices is not consistent [20]. The Mentzer index was used by mother centers of the MOH in Palestine, and the aim of this brief report was to better assess IDA between our populations.

5. Limitations and Conclusions

Although the current study showed no documented follow-up program for infants, where mothers of infants are asked to visit a family doctor for further evaluation and iron therapy, no evidence of visiting a family doctor or hematologist is known. Therefore, this report recommends a policy for follow-up to infants showing IDA and thalassemia through performing hemoglobin electrophoresis, blood film, and serum iron profile, including ferritin. Moreover, the study difficulty was gaining access to a list of a larger

population, time, costs, and that bias can still occur under certain circumstances, especially that more data collection was influenced with COVID-19 epidemic restrictions. The study introduced new CBC indices among infants at the age of 1 year in Palestine that could be used as reference ranges to better identify/differentiate IDA and thalassemia among the population.

Abbreviations

IDA: Iron deficiency anemia
HGB: Hemoglobin
MCV: Mean corpuscular volume
MCHC: Mean corpuscular hemoglobin concentration
MCH: Mean corpuscular hemoglobin
WHO: World Health Organization

Data Availability

The descriptive statistics data used to support the findings of this study are included in the article. Data of the findings for this study are available from the corresponding author upon request.

Conflicts of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Research Article

Donor Blood Procurement, Safety, and Clinical Utilization: A Study of Blood Transfusion Services in a Tertiary Care Hospital in Nigeria

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Background. Donated blood is an essential component of the management of many diseases, and hospital-based blood banks in Nigeria are saddled with the responsibility of provision of safe blood and coordination of its appropriate utilization for patient care. *Objective*. This study reviewed the extent to which the hospital blood transfusion service ensures adequate safe blood supply and utilization. *Materials/Methods*. This was a retrospective study of 2 years record of the blood bank service of Alex Ekwueme Federal University Teaching. Methods of donor blood procurement, transfusion transmissible infection status, the pattern of blood, and blood component usage across the hospital's clinical departments were evaluated. Statistical analysis was conducted using IBM SPSS, and data were presented as percentages. Fisher's tests were used to test significance, and p value <0.05 is significant. *Results*. The highest proportion of donors was male family replacement donors aged 26–35 years (3634 (39.68%)) while total voluntary donors were 315 (2.65%). Hepatitis B had the highest seroprevalence 267 (2.22%) among blood-borne diseases screened. National Blood Transfusion Service (NBTS) supplied only 3 (0.03%) of total blood units used. The accident and emergency department had the highest proportion of persons who utilized whole blood; 4568 (99.96%). *Conclusion*. The hospital blood bank relies heavily on family replacement donors with little or no assistance from the National Blood Transfusion Service. Family replacement donors have the highest risk of TTIs, and hepatitis B infection has the highest prevalence. The high cost of blood component therapy increases the need for whole blood.

1. Introduction

Blood transfusion is the most commonly performed procedure in healthcare facilities [1–3]. Demand for blood is high; blood donation rates are very low, especially in low- and middle-income countries in Africa. Transfusion medicine has evolved from a laboratory service to clinical care with a focus on blood safety and appropriate clinical use of blood. The imperative goal is to improve clinical outcomes and patient safety. However, despite compelling evidence and ongoing WHO policy drive, Blood transfusion safety in Nigeria has been challenged by a shortage of voluntary donors, poor infrastructure, high cost of blood components, and high prevalence of transfusion transmissible infections (TTIs) [4].

Transfused blood is a known risk for transmission of infectious diseases including hepatitis B, C, and HIV. The prevalence of hepatitis B infection among blood donors in

Nigeria has been variable. A study reported the prevalence of HBV, HCV, syphilis, and HIV to be 4.1%, 3.6%, 3.1%, and 4.2% among donors while another study reported rates as high as 17% for hepatitis B seropositivity and as low as 0.45% for HIV [5–9].

Donorship rates for voluntarily donated blood are very low in Nigeria. The National Blood Transfusion Service, which is the agency responsible for safe blood supply in Nigeria is unable to meet more than 75% of the blood needs of the populace [10].

The effectiveness and safety of hospital blood transfusion services in our country with a low human development index are critical to healthcare delivery. This study seeks to define the extent to which the hospital blood transfusion service ensures adequate safe blood supply and utilization, at a tertiary hospital in southeast Nigeria.

1.1. Subjects, Materials, and Methods. This was a retrospective study conducted at the Department of Haematology at the Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State (AEFUTHA). Ethical approval was obtained from the Ethical and Research Committee of AEFUTHA (REC protocol no. 19/04/2021–10/06/2021). Records from the blood transfusion unit and hospital ward blood transfusion register from January 1, 2019, to December 31, 2020, were collated and analyzed to identify the methods of donor blood donation, demographic data (age and sex) of blood donors during the specified study period, the various categories of blood donors, the prevalence of TTI markers, and pattern of blood and blood component usage across the hospital's clinical departments.

No autologous donation was done during the study period. The sources of blood procurement are paid blood donors, voluntary donors, and family replacement donors. A paid blood donor offers a unit of blood for a pecuniary benefit by a contracted hospital vendor. A replacement blood donor is a family member or relative of a patient, donating a unit of blood to be used for a specific patient, while a voluntary blood donor is a member of the society who donates his or her blood without any inducement for use by a recipient not known to him or her. Commercially donated blood is supplied to the hospital blood bank by contracted vendors while blood from voluntary donors and replacement donors are collected in the hospital donor clinic after packed cell volume, blood group, and other eligibility criteria are determined. Thereafter, sera samples are tested for hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (HCV), human immunodeficiency virus (HIV) 1/2, and *Treponema pallidum* using commercially available immunochromatic based rapid kits. A single red line at position *C* (control) on the strip indicates a valid control. A test is positive if two transverse bands (T = test and C = control) are seen and negative when only the one band at control (*C*) is seen. Those who test positive are disqualified. Blood (450 ml) is usually collected into a bag containing 63 ml citrate phosphate dextrose with adenine (CPDA) using the standard guidelines for routine phlebotomy and stored at 2–8°C as whole blood or separated into components (fresh plasma, red cell concentrate, or platelet-rich plasma) using the cold centrifuge machine. Donors for platelet concentrate have their predonation platelet count measured (predonation platelet count of at least 200,00/μl required) and blood group determined since platelet concentrate transfusion is blood group-specific and prepared only on demand due to cost. The Haemonetics MCS apheresis machine is used for platelet concentrate harvest from the donors. They are stored in the platelet agitator. Whole blood supplied by vendors (paid donors) are also tested for, hepatitis B, C, *Treponema pallidium*, and HIV 1 and 2 after supply and if found to be negative for all fours tests, they are then stored in the blood bank refrigerator for use.

Statistical analysis was conducted using IBM SPSS, and data were presented as percentages. Fisher's tests used to test significance, and p value <0.05 is significant.

2. Results

Table 1 shows the age and sex distribution of persons who donated blood during the study period. The majority of blood donors were males (12,268 (97.11%)) amongst which those aged 26 to 35 years were the highest proportion (4,729 (38.47%)).

Table 2 shows the age and sex distribution of study donors across the categories of blood donation. The highest proportion of donors were male family replacement donors (3,634 (39.68%)) and female replacement donors (143 (43.86%)) aged 26 to 35 years. Voluntary donors contributed 315 (2.65%) of all the categories of blood donors.

Family replacement donors had the highest number of rejection/deferral 358 (59.6%) while low haemoglobin was the commonest reason for rejection/deferral 444 (73.8%).

Table 3 shows the distribution of blood groups across persons who donated blood during the study period. The majority of donors were O Rh-positive (8,739 (73.1%)) followed by A Rh-positive (1,411 (11.8%)). Only one person had AB Rh-negative blood group.

Table 4 shows the seroprevalence of blood-borne diseases among blood donors screened during the study period. Hepatitis B had the highest seroprevalence (267 (2.22%)) among blood-borne disease screened for p value <0.001. HIV had the lowest seroprevalence (101 (0.82%)). Amongst the different categories of donors, the hepatitis B, C, HIV, and syphilis seroprevalence was highest among family replacement donors (2.52%, 2.18%, 0.95%, and 2.22%, respectively) while voluntary donors had the lowest. The relative risk of infection transmission was 2.56 and 1.68 for HIV and hepatitis B infections, respectively.

Table 5 shows source/site of blood donation (hospital, private lab, NBTS, and others).

Hospital screening and blood donation (voluntary, family replacement, and paid) constituted the largest source of blood during the study period (8,655 (74.7%)). Vendor-sourced commercial blood accounted for 2,537 (21.9%). NBTS contributed only 3 (0.03%).

Table 1: Age and sex distribution of persons who donated blood during the study period.

Age groups	Male *n* (%)	Female *n* (%)	Total *n* (%)
18–25 years	3,905 (31.83)	148 (40.66)	4,053 (31.90)
26–35 years	4,729 (38.47)	156 (42.86)	4,885 (38.61)
36–45 years	3,041 (24.79)	48 (13.19)	3,089 (24.60)
46–55 years	552 (4.50)	10 (2.75)	562 (4.54)
56–65 years	38 (0.31)	1 (0.27)	39 (0.31)
>65 years	3 (0.024)	1 (0.27)	4 (0.03)
Total	12,268 (97.11)	364 (2.94)	12,632 (100.0)

Table 2: Age and sex distribution of donors across the categories of blood donation.

Age groups	Voluntary donors		Family replacement donors		Paid donors	
	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)
18–25 years	84 (30.32)	16 (42.11)	2,580 (28.17)	122 (37.42)	1,591 (56.11)	0 (0.0)
26–35 years	113 (40.79)	17 (44.74)	3,634 (39.68)	143 (43.87)	1,036 (36.54)	0 (0.0)
36–45 years	69 (24.91)	4 (10.52)	2,483 (27.11)	46 (14.11)	196 (6.91)	0 (0.0)
46–55 years	10 (3.61)	1 (2.63)	429 (4.68)	13 (3.98)	12 (0.42)	0 (0.0)
56–65 years	0 (0.0)	0 (0.0)	28 (0.30)	1 (0.30)	0 (0.0)	0 (0.0)
>65 years	1 (0.3)	0 (0.0)	2 (0.02)	1 (0.30)	0 (0.0)	0 (0.0)
Total	277 (2.2)	38 (0.3)	9,156 (72.4)	326 (2.7)	2,835 (22.44)	0 (0.0)

Table 3: Distribution of blood group across persons who donated blood during the study period.

Blood group	Frequency	Percentage
A Rh−	38	0.3
A Rh+	1,360	12.1
B Rh−	39	0.3
B Rh+	1,057	9.4
AB Rh−	1	0.0
AB Rh+	56	0.5
O Rh−	403	3.6
O Rh+	8,239	73.6
Total	11,193	

Table 4: Seroprevalence of blood-borne diseases among persons screened during the study period.

TTI	Donor group	Positive (%)	Negative (%)	Total *N* (%)	Chi square	*p* value	Relative risk
HIV	VNRD	1 (0.37)	268 (99.63)	269 (100.0)			1
	FRD	87 (0.95)	9037 (99.05)	9124 (100.0)	4.952	0.084	2.56
	PAID	13 (0.49)	2625 (99.51)	2638 (100.0)			1.33
	Total	101 (0.82)	11930 (99.18)	12031 (100.0)			
HBV	VNRD	4 (1.50)	263 (98.50)	267 (100.0)			1
	FRD	230 (2.52)	8894 (97.48)	9124 (100.0)	14.940	0.001	1.68
	PAID	33 (1.25)	2602 (98.75)	2635 (100.0)			0.84
	Total	267 (2.22)	11759 (97.78)	12026 (100.0)			
HCV	VNRD	8 (3.0)	259 (97.0)	267 (100.0)			1
	FRD	199 (2.18)	8925 (97.82)	9124 (100.0)	9.9392	0.009	0.73
	PAID	33 (1.26)	2595 (98.74)	2628 (100.0)			0.42
	Total	240 (2.00)	11779 (98.0)	12019 (100.0)			
Syphilis	VNRD	5 (1.87)	262 (98.13)	267 (100.0)			1
	FRD	203 (2.22)	8921 (97.78)	9124 (100.0)	19.473	0.00006	1.19
	PAID	22 (0.84)	2598 (99.16)	2620 (100.0)			0.45
	Total	230 (1.91)	11781 (98.09)	12011			

Table 6 shows the pattern of usage of blood and blood products across clinical departments.

A total of 11,581 blood units were used during the period under study. The accident and emergency department had the highest proportion of persons who utilized total whole blood (4,568; 99.96%) while internal medicine used the highest number of platelets and fresh frozen plasma with haematology department (28; 2.29%

Table 5: Site of blood donation/procurement.

Source of blood	Frequency	Percentage
Hospital blood bank (VNR and FR)	8,655	74.7
Paid	2,923	25.2
NBTS*	3	0.03
Total	11,581	100.0

*National Blood Transfusion Service.

Table 6: Pattern of usage of blood and blood components across clinical departments.

Blood component	O & G (%)	Paed (%)	Surgery (%)	A & E (%)	Medicine (%)	Haematology (%)
Whole blood	2,479 (99.67)	481 (94.31)	1,041 (98.49)	4,568 (99.96)	1,150 (94.10)	1,710 (98.55)
Red cell concentrate	1 (0.04)	7 (1.37)	4 (0.37)	1 (0.02)	32 (2.61)	3 (0.17)
Platelets	0 (0.00)	9 (1.76)	1 (0.09)	1 (0.02)	28 (2.29)	12 (0.69)
Fresh Plasma	7 (0.28)	13 (2.54)	11 (1.04)	0 (0.00)	12 (0.98)	10 (0.57)
Total	2,487 (21.47)	510 (4.40)	1,057 (9.12)	4,570 (39.46)	1,222 (10.55)	1,735 (14.98)

and 12; 0.98%) and (12; 0.69% and 10; 0.57%), respectively.

3. Discussion

Voluntary nonremunerated blood ensures safety, quality, availability, and accessibility of blood transfusion. Our study revealed that family replacement and commercial donors were the major sources of blood during the study period with voluntary unpaid donors contributing only 2.65%. These results are comparable to those obtained in other reports, where family replacement donors and commercial donors made up 99% [11] and 95.3% [12], respectively of donated blood in the hospital blood bank. Family replacement donors are not the best source of blood since these donors are usually under pressure to give blood to save a loved one even when they are not eligible on account of being potential transmitters of TTIs or their health is at risk. A more appalling twist to family replacement blood donation is the fear that many of the so-called family replacement donors may not be true relatives but commercial donors co-opted to act as family replacements [13]. The lack of effective community blood drive programmes could account for the low level of voluntarily donated blood and a functional donor clinic can facilitate the conversion of the huge family replacement donor base to voluntary donors. Misconceptions, lack of information, and a high rate of unemployment have also encouraged commercial blood donation to thrive [14, 15].

Demographic information of blood donors is important for drafting and monitoring recruitment strategies [16]. In Africa, males constitute the majority of blood donors with women constituting less than 30% of the donor population [17]. In keeping with our study also, females accounted for only 3.99% of blood donors in our blood bank and the majority were family replacement donors. Our findings differ starkly from those obtained from studies in developed countries [18, 19] where females accounted for as high as 40% to 55% of the blood donor population despite the prevailing barriers including pregnancy, breastfeeding, menstrual blood loss, low blood haemoglobin concentration, and greater susceptibility to vasovagal reactions. Lack of access to education, cultural beliefs, and economic deprivation are further barriers to female participation in blood donation in our locality. Deferral rates due to anaemia are high in females, especially in developing countries [20]. In our environment, a high incidence of malarial infections in the general population and iron deficiency anaemia is notable [21].

Transfusion transmissible infections pose the greatest threats to blood transfusion safety [22]. Our study revealed that the prevalence of HIV, hepatitis C, and syphilis infections in our family replacement donors was higher than that of donations in low-income countries as reported by the WHO [3]. Even though the prevalence for hepatitis B among the different categories of donors was slightly lower than reported by the WHO, family replacement donors has the highest prevalence rate of 2.52 with a 1.68 risk of being infected. A similar prevalence of hepatitis B infection has been found in another study [23]. The commercial and voluntary donors had lower seroactivity and relative risk of transmission of blood-borne diseases. Family replacement donors may have a higher rate of seroactivity of the TTIs since they constitute the greater proportion of total donors assessed. They are usually under immense pressure to donate blood to save the life of someone known to them in emergent situations and are likely to evade divulging information connected to a risky lifestyle that might lead to denying them eligibility to donate. This is at variance with voluntary donors who tend to be repeat donors for altruistic reasons and so are aware and maintain a lifestyle that makes them always eligible. The low prevalence of TTIs among commercial donors may be due to their understanding that their source of income from blood donation may be cut off if they engage in high-risk behaviors that may make them ineligible to donate blood in the future. This disturbing concept has arisen due to poverty and unemployment and requires urgent steps to curb it including proper education and job creation.

Blood group and rhesus typing are routinely determined in blood donors as they are clinically important in haemolytic transfusion reactions The predominant blood type in our study was type O and the least common was type AB,

consistent with other studies in Nigeria [24]. In Eastern and Southern African countries, blood group O dominated the populace, while in Pakistan and some regions in India on the Asian continent, blood group B dominated [25–27]. In addition to being the most common blood group in our population, the high rate of utilization of blood group O is seen where there is an inadequate supply of donor blood, particularly in emergencies when it is commonly used as universal donor units for transfusion to A, B, and AB recipients, This is predicated on the premise that blood group O red cells lack A and B antigens on their cell membrane surfaces, despite the risk of the presence of anti-A and anti-B haemolysins in blood type O donors [28].

Contrary to the report by Enosolease et al. [12], our study revealed that blood utilization was more than supplied by the blood bank. This shortage necessitated patients resorting to blood procurement from peripheral laboratories when the hospital blood bank had none. In concordance to the study by Okocha et al. [11], the Accident and Emergency department had the highest rate of utilization of whole blood followed by the obstetrics and gynaecology department. The high blood use by the accident and emergency department is probably a result of the increased requirement of whole blood for emergent resuscitation due to blood loss. The rising incidence of road traffic accidents in our environment is culpable.

More than 98% of blood used during the study period was whole blood. This contributed to increasing our blood needs. The use of whole blood has continued in resource-limited low- and medium-income countries despite the benefits of component therapy. Component therapy allows several patients to benefit from one unit of donated whole blood thereby maximising its use. Most times fresh whole blood transfusion is a quick and cheap fix for patients requiring platelets or coagulation factors only. This is associated with alloimmunization, overload, and poor treatment outcomes. Transfusion of whole blood rather than the indicated component is a failure of stewardship of the scarce blood resource. From our study, the internal medicine department and the haematology unit had the highest utilization of blood components such as packed red cells and platelets possibly due to the chronicity of diseases that affect distinct blood cell lines and so afford time for the patient to gather funds to procure the required blood component. Also, most haematological premalignant and malignant diseases are associated with dangerously low blood counts that require component therapy to support treatment. The low use of blood component therapy is due to the high cost and lack of infrastructure in our environment [12].

Hospital-based blood donation by family replacement and voluntary donors made up the largest contribution to donor blood used in the hospital during the study period while the National Blood Transfusion Service (NBTS) provided only three units of blood. NBTS is saddled with the responsibility to provide safe, quality blood and blood components in a cost-effective manner and distribution to hospitals throughout the country. Currently, Nigeria needs an average of 1.8 million pints of blood annually to meet the blood transfusion needs of 200 million Nigerians, but only 500,000 units are collected annually by the NBTS, amounting to only 27.7% and leaving a deficit of 73.3%.10 Problems faced by the NBTS include inadequate policy enforcement and funding shortfalls which impact its ability to enlighten more people and increase donor recruitment. The funding challenge hinders activities such as media outreach, advocacy, and public awareness campaigns down to the community level which would have tackled the deeply rooted cultural myths and misconceptions on voluntary blood donation in the country. The gap created by the nonfunctional NBTS is filled by vendor-sourced blood which contributed about one-fifth of the total blood used during the study period reflecting the huge dependence on commercially sourced blood in addition to those who disguise as family replacement donors.

More action is urgently needed from policymakers and stakeholders in strengthening the capacity of the National Blood Transfusion Service to ensure that more units of voluntarily donated blood are supplied to hospitals. Donor education of the populace especially targeting the youths is key to inculcating the right concept and culture of VNR blood donation. Hospital donor clinics to aggressively drive community outreaches, donor drive, and design programmes to encourage family replacement donors to become voluntary donors. Government and hospital managers should gear efforts toward the provision of equipment for component preparation and making it affordable. Also, TTI screening must step up to better and more sensitive screening techniques considering the large volume of blood required by patients.

3.1. Study Limitation. This is a retrospective study, and donors/recipients with incomplete data from the blood register were removed from the analysis. The data of most recipients' prehaemoglobin levels and diagnosis were missing and could be included as part of the study.

4. Conclusion

Blood donation deficit has been demonstrated in this study, and hospital transfusion services are still largely dependent on commercial donors and family replacement donors as the predominant source of blood and blood products utilized by the hospital. The utilization of whole blood was increased due to the nonaffordability of blood components. Moving forward, the National blood transfusion service must become proactive in using results of donor behaviour studies and implementation of actionable policies to improve safe blood supplies to the hospitals. Donor education of the populace especially targeting the youths is key to inculcating the right concept and culture of VNR blood donation. There is a great need for mass mobilization and retention of VNRD through effective evidence-based educational, cultural religious, and gender-based peculiarity intervention programmes and incentives. Hospital blood banks should have dedicated units saddled with this primary responsibility. Government and hospital managers should gear efforts toward the provision of equipment for component

preparation and making it affordable. Also, TTI screening must step up to better and more sensitive screening techniques considering the large volume of blood required by patients and prevalence of TTIs.

Data Availability

The data set will be provided by the corresponding author on request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Elucidating the Correlation of D-Dimer Levels with COVID-19 Severity: A Scoping Review

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Aims. The review explores the findings of previous studies to elucidate the association between levels of D-dimer and COVID-19 severity and prognosis. In addition, we assessed the efficiency of anticoagulant therapies in reducing COVID-19 severity and improving the prognosis of the patients. *Materials and Methods*. A comprehensive literature review was conducted using MEDLINE/PubMed databases, Scopus, and Web of Science with the help of keywords "COVID-19," "D-Dimer," "Thrombosis," "Fibrin network," "Anticoagulant therapy," "Inflammation," and "disease severity." Based on all these articles and clinical experience, a scoping review was constructed and the full texts of the articles that were retrieved were accessed. *Results*. A D-dimer is a complex protein molecule that is formed during plasmin-mediated degradation of the fibrin network. Thus, it serves as a marker of thrombotic activity. On the other hand, in addition to severe respiratory distress and reduction in pulmonary gas exchange, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) also triggers prothrombotic changes in the infected individuals. The levels of D-dimer have been postulated to be positively associated with the degree of disease severity among COVID-19 patients. *Conclusions*. It has been postulated that D-dimer could potentially be used as a biomarker to predict the prognosis and outcome of COVID-19 patients at the time of admission to hospitals and facilitate more personalized and efficient clinical management that could significantly reduce the mortality rate of such patients and allow more rapid recovery.

1. Introduction

The first case of coronavirus disease 2019 (COVID-19) was reported in December 2019 in the Wuhan province of China. After that, it quickly spread across 200 countries within a span of a few months. Global research efforts quickly identified the etiological agent of this disease to be a novel coronavirus. This novel coronavirus exhibited approximately 80% homology to SARS-CoV, which had earlier spread during 2002–2003 and was associated with acute respiratory distress syndrome (ARDS) and a high mortality rate [1]. The novel coronavirus was hence named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2 or 2019-nCoV). Although this virus entered the human population via zoonotic transmission, its spread is mainly attributed to human-to-human transmission [2]. The World Health Organization (WHO) declared this outbreak a Public Health Emergency of International Concern by 30th January 2020. Later, this outbreak was declared a global pandemic by 12th March 2020 [3].

The novel coronavirus primarily transmits via respiratory droplets. The angiotensin-converting enzyme 2 receptor present on the cell surfaces plays a key role in the invasion of the novel coronavirus [4]. Most of the COVID-19 patients are usually asymptomatic and do not require hospitalization. However, sometimes, after an incubation of 2–14 days, COVID-19 symptoms may emerge, including shortness of breath, fever, coughing, and pneumonia [5]. Previous studies have reported that some cases exhibit the development of severe pneumonia, leading to hypoxia and respiratory dysfunction. The most severe cases (less than 5% of the symptomatic cases) exhibit ARDS and multiple organ failure, along with several laboratory abnormalities, such as leukopenia, thrombocytopenia, hypercoagulative state, leukopenia, and elevated levels of D-dimer, which often warrant admission to the intensive care unit (ICU) [6, 7]. This review focuses on the potential of D-dimer as a biomarker to predict the disease severity of COVID-19 patients and their outcome. We also assessed the efficiency of anticoagulant therapies among such patients to reduce their disease severity and improve their prognosis.

2. Materials and Methods

A comprehensive literature review was conducted using MEDLINE/PubMed databases, Scopus, and Web of Science with the scope of the searches confined to English language sources. There was no time restriction specified since the items on COVID-19 continue to accumulate on a regular basis. Priority was given to articles with a stronger evidence base, even though all types of articles were examined. The following keywords were employed: "COVID-19," "D-Dimer," "Thrombosis," "Fibrin network," "Anticoagulant therapy," "Inflammation," and "disease severity." In general, we followed the guidelines for producing scoping reviews that were provided to us.

3. Result

3.1. D-Dimer Structure and Formation. A D-dimer is a complex protein molecule that is generated during plasmin-mediated cross-linked fibrin degradation. Figure 1 depicts the process of D-dimer formation in the form of a schematic illustration. As the D-dimer formation commences, the fibrin molecules are formed after the thrombin-mediated cleavage of fibrinogen, a soluble glycoprotein that is found in the plasma. Thrombin cleaves the polymerization site of a fibrinogen molecule, thereby exposing the site to bind with other fibrinogen or fibrin molecules. In this manner, several such cleaved fibrin molecules bind together in an overlapping fashion to form protofibrils [8]. The thrombin molecules remain bound to the fibrin molecules during their polymerization. At this point, thrombin simultaneously activates the fibrinogen-bound plasma factor XIII. The complex of the thrombin molecules, plasma factor XIII, and fibrin polymers together triggers the formation of factor XIIIa [9]. Plasma factor XIIIa is instrumental in the cross-linking of the fibrin molecules. In the next step, plasminogen interacts with the fibrin molecules, which leads to the formation of plasmin.

The generated plasmin molecules, in turn, bind with the fibrin molecules and mediate degradation of the bound fibrin into products with different molecular weights, commonly known as fibrinogen degradation products (FDPs). Plasmin also mediates terminal degradation of the cross-linked fibrin molecules into soluble fragments that contain DDE fragments. A DDE fragment, simply put, is a D-dimer molecule that is noncovalently bound to fragment E. The plasmin molecule further breaks down the DDE fragment into DD and E fragments. The D-dimer (DD fragment + E fragment) is a soluble complex and circulates in the plasma until it is eliminated by the reticuloendothelial and renal pathways [9]. It is noteworthy that the half-life of D-dimer that circulates in the plasma is 8 h and can be detected in the blood only 2 h after the formation of a thrombus [10]. The formation of D-dimers only occurs during the generation and degradation of the cross-linked fibrin molecules, which takes place during coagulation and fibrinolytic events. Therefore, the D-dimer molecules serve as a direct marker of these events as well as an indirect marker of thrombotic activity.

There are three major steps of D-dimer formation:

(i) Fibrin monomers are generated after the degradation of a fibrinogen molecule by thrombin. The generated fibrin monomers then bind to other fibrin or fibrinogen molecules, which results in the formation of protofibrils. The dotted lines between the D-E domain and the D domain depict the noncovalent interactions that aid in maintaining the structural integrity of the protofibrils.

(ii) Simultaneously, thrombin activates the formation of plasma factor XIIIa that binds to the D-domains of the fibrin polymers via covalent interactions.

(iii) Then, fibrin degradation products (FDPs) are formed after the disrupting action of plasmin on multiple sites of fibrin, which, in turn, exposes the D-dimer antigen epitope. These FDPs are then further degraded, resulting in the formation of a terminal DDE complex [9].

3.2. Cross-Link between Thrombosis and Fibrinolytic Pathways. The fibrinolytic system is responsible for the prevention of the formation of fibrin thrombi. Such thrombi are mainly composed of fibrin polymers and, under normal circumstances, they are disrupted by the fibrinolytic system as soon as they are generated. Currently, such FDPs are the most widely used biomarkers of thrombosis. Several modulators play a key role in the promotion (such as tissue plasminogen activator and TPA) or prevention (such as thrombin activatable fibrinolysis inhibitor) of fibrin degradation. As abovementioned, the DDE fragment (D-dimer) only generates when plasma factor XIII degrades the fibrin polymers. Thus, this fragment holds great potential as a biomarker of fibrin degradation and coagulatory pathways and, in turn, thrombosis [11].

3.3. COVID-19 and Thrombosis. Since its emergence, several investigators around the globe have published a plethora of studies on the epidemiology of COVID-19. Although its

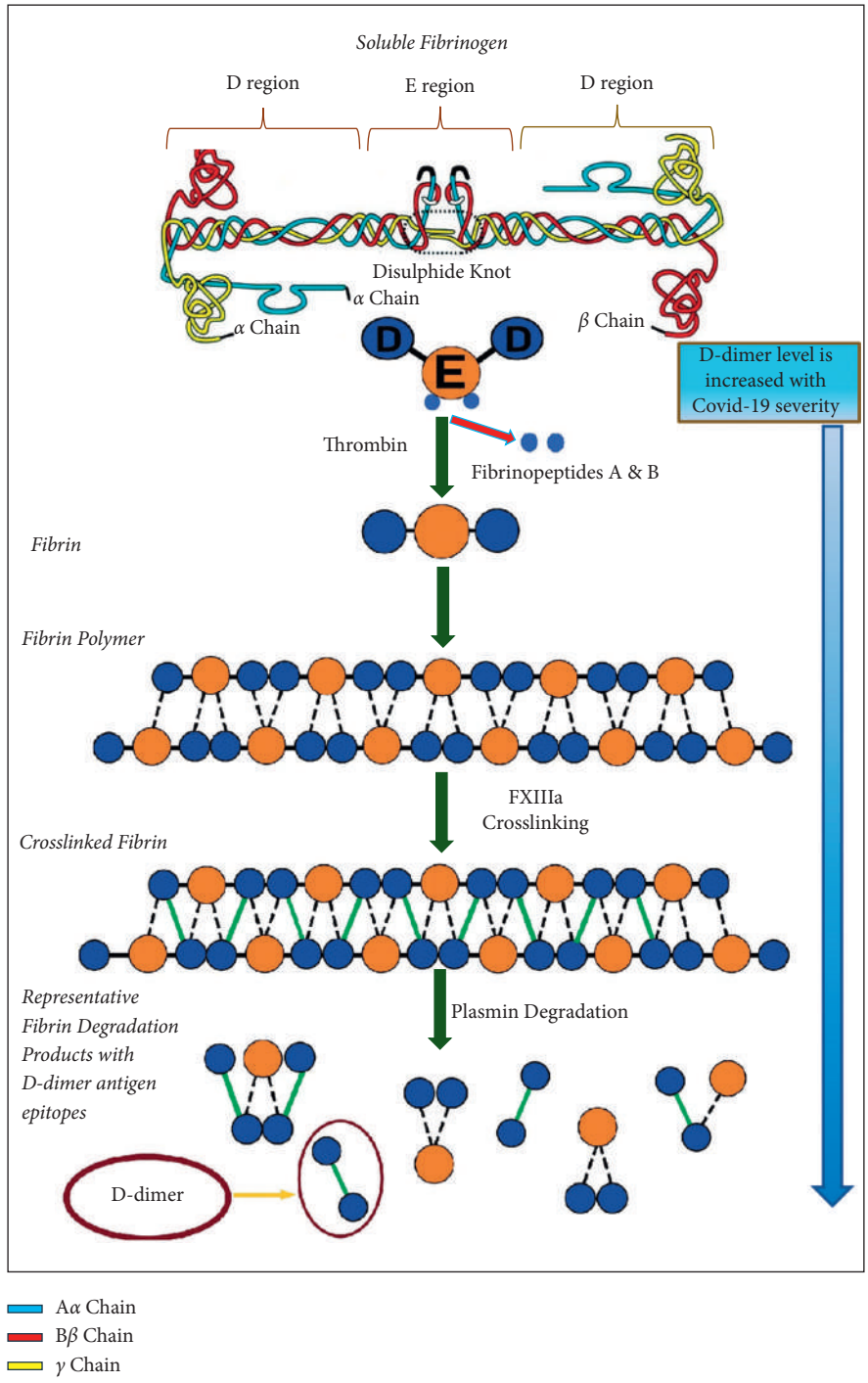

FIGURE 1: Schematic illustration of Fibrin structures of human (A) fibrinogen and (B) fibrin fibers. The fibrin structure was constructed by performing a best fit of each end of fibrinogen to each D region of the D dimer. Fibrinogen is clotted by thrombin, and the fibrin monomers that are produced polymerize spontaneously in a half-staggered format into protofibrils. The fibrin network is enhanced by factor XIIIa, which crosslinks adjacent monomers. Plasminogen activation is enhanced with fibrin formation, and the resultant plasmin digests the individual fibers. Plasmin cleavage between the D and E domains yields (DD)E, and the noncovalent complex of D-dimer (DD) and fragment E. D-dimer level is increased with COVID-19 severity.

transmittance rate is extremely high, it has a very low mortality rate of 2.3% [12]. A higher incidence of COVID-19 has been reported among individuals aged more than 65 years and less than 18 years, with a higher sequential organ failure assessment (SOFA) score, male gender, and several comorbidities, including diabetes, hypertension, and coronary heart disease [3]. In addition, several studies have also shown that a D-dimer level of more than 1 μg/mL to be a potential risk factor of COVID-19 [13]. The formation of thrombi has been reported in both the venules as well as the arterioles of COVID-19

patients at the time of hospitalization. It has been postulated that this observation could be attributed to the risk factors that contributed to the aggravation of COVID-19 in an individual, such as obesity, pregnancy, and comorbidities like diabetes mellitus, which often themselves participate in and trigger the formation of clots in the bloodstream [14, 15]. The formation of such thrombi and the resultant angiogenesis often give rise to impaired microcirculation in COVID-19 patients [16]. Previous studies on COVID-19 patients have shown that the novel coronavirus is often responsible for endothelial injury and cell membrane destruction. This, in turn, reduces the fibrinolytic activity of endothelial cells, which promotes the formation of thrombi [17].

It is noteworthy that the proinflammatory cytokines are involved in both inflammatory and coagulatory processes. Previous studies have shown that severe infection of novel coronavirus triggers severe inflammatory reactions, as indicated by the significant upregulation of proinflammatory cytokines [18]. The cytokine upregulation and coagulopathy observed in cases of severe novel coronavirus infection have been attributed to acute sepsis [19]. Furthermore, severe novel coronavirus infection often predisposes infected individuals to sepsis-induced coagulopathy and disseminated intravascular coagulation [20]. Recently, Maier et al. reported a significant association between novel coronavirus infection and an increase in the viscosity of the plasma of the infected individual [21]. Such an increase in plasma viscosity has been reported to be associated with an increase in SOFA scores. This finding indicated that hyperviscosity of the plasma could trigger both endothelial dysfunction as well as thrombosis. Blood viscosity is also affected by fibrinogen levels [22]. Previously, it has been reported that the fluidic state of the plasma is maintained only till thrombin is able to cleave about 25% to 30% of the plasma fibrinogen. This, in turn, facilitates efficient polymerization of fibrin monomers and promotes the activation of plasma factor XIII by thrombin [9]. It is demonstrated that COVID-19 patients also exhibited high plasma levels of fibrinogen [22].

4. Discussion

4.1. Association between Levels of D-Dimer and COVID-19. As stated above, the levels of D-dimer directly correlate with the rate of formation and degradation of plasmin. Hence, any pathological condition that upregulates the rate of plasmin generation and degradation would also increase the levels of D-dimer. Thus, the pathologies that promote chronic inflammation, such as rheumatoid arthritis, asthma, and cancer, also lead to an increase in the levels of D-dimer. It follows that infection of a novel coronavirus, which leads to upregulated inflammatory reactions among individuals, would also increase the levels of D-dimer. This is evident by the findings of several previous studies that showed that levels of D-dimer were significantly higher in COVID-19 patients, especially those who were either severely ill or had deceased [23, 24]. Some investigators have postulated that the upregulated levels of D-dimer in individuals with severe novel coronavirus infection might be associated with severe illness, higher rates of thrombotic activity, and higher mortality rates of such patients [14, 25].

In 2020, Guan et al. presented the results of a large retrospective study that indicated the correlation between abnormal levels of D-dimer and disease severity of the COVID-19 patients for the first time [26]. Setting a cut-off point of a D-dimer level of more than 0.5 mg/L, they reported that a significantly higher proportion of novel coronavirus infected individuals with severe illness exhibited abnormally high levels of D-dimer than those with only mild or moderate illness ($p = 0.002$) [26]. Furthermore, Tang et al. reported that COVID-19 patients with a severe level of illness exhibited approximately 3.5 times higher levels of D-dimer compared to patients with only mild or moderate levels of illness [27]. Their results were corroborated by Wang et al., who reported approximately 2.5- and 5-times higher levels of D-dimer, respectively, in COVID-19 patients with a severe level of illness compared to patients with only mild or moderate levels of illness [23, 24]. In line with these findings, Wang et al. also found that the levels of D-dimer in COVID-19 patients with severe illness were more than two times lower compared to the levels of D-dimer of deceased COVID-19 patients. Their results were supported by Tang et al., who demonstrated that COVID-19 patients with severe illness exhibited around four- and nine-times lower levels of D-dimer, respectively, compared to the levels of D-dimer of deceased COVID-19 patients [13, 27].

In a recent study, Lippi and Favaloro observed that the levels of D-dimer of the COVID-19 patients with a mild or moderate level of illness, that is, those who did not require ICU admission, were significantly lower than the levels of D-dimer of the patients with a severe level of illness, that is, those who required ICU admission [28]. In another study, Yao et al. demonstrated that COVID-19 patients who were categorized as suffering from a severe level of illness on the basis of their oxygenation index, lung CT scans, and corresponding clinical guidelines exhibited a significant association between their disease severity and levels of D-dimer [18]. Bilaloglu et al. conducted a multicentric study to examine the upregulation of levels of D-dimer in individuals who were hospitalized due to COVID-19 [29]. Their results indicated that, among the recruited patients, around 76% of the patients exhibited abnormally high levels of D-dimer during admission to the hospital, whereas around 86% of the patients exhibited abnormally high levels of D-dimer at any time during their hospitalization. Petrilli et al. reported that, at the time of admission to the hospital, the COVID-19 patients with abnormal levels of D-dimer exhibited poorer outcomes [30]. Around 43%, 20%, and 45% of the patients suffered from acute kidney injury, thrombosis, and critical illness, respectively. In addition, they conducted a multivariate analysis to identify the underlying factors that affected the outcomes of such patients and found that the levels of D-dimer were independently correlated with the patient's outcome. On the contrary, patients with normal levels of D-dimer exhibited higher odds of recovering without developing severe illness.

The novel coronavirus infection leads to upregulation of the inflammatory pathways. According to the results of several previous studies, the abnormal increment in the levels of D-dimer under inflammatory conditions indicates that the upregulation of inflammatory reactions and proinflammatory agents might be associated with the

induction of the coagulatory pathways [31]. Hence, it follows that the coagulatory events in the COVID-19 patients might be triggered by the upregulated inflammatory reactions. This postulate was further strengthened by the findings of Bilian et al., who reported a significant correlation of the levels of D-dimer with the levels of hsCRP, a marker of inflammation, in COVID-19 patients [32]. In another study, Chen et al. showed how the levels of D-dimer could potentially be used as a marker to predict the inhospital mortality rate of COVID-19 patients [33]. Based on their results, they were able to determine a cut-off D-dimer value of more than 2.14 mg/L to predict the outcome of COVID-19 patients at the time of admission to the hospital. They reported that this cut-off value could be efficiently used to predict the rate of inhospital mortality with 88.2% sensitivity and 71.3% specificity. On the other hand, in their recent review, Zheng et al. reported that the levels of D-dimer of more than 0.5 mg/L indicated abnormally high blood coagulability of COVID-19 patients and were significantly correlated with poor outcomes of such patients [34]. Table 1 enlists some of the retrospective studies that demonstrated a significant correlation between levels of D-dimer and disease severity among COVID-19 patients [13, 27, 35–43].

4.2. Future Implications: Scope of Anticoagulation Therapy. Considering the COVID-19 pandemic, identifying therapies that improve outcomes is crucial. The findings presented in this review indicate that D-dimer could prove to be a potential biomarker to assess the disease severity level of COVID-19 patients and predict their outcome. This postulate is further supported by the analysis of Sakka et al., who suggested that levels of D-dimer could be helpful in categorizing the COVID-19 patients in terms of their disease severity levels at the time of admission to the hospital itself. They further reported that such categorization of the patients could facilitate personalized and more efficient clinical management in a timely manner based on their disease severity level [44]. Furthermore, several studies have shown that novel coronavirus infections lead to a significant increase in the formation of thrombi in the vascular system of COVID-19 patients. These findings indicate that apart from being a marker of elevated coagulation activity and prothrombosis in COVID-19 patients, D-dimer could also be involved in the pathogenesis of the disease [7]. Owing to the significant association of levels of D-dimer with prognosis and outcome of COVID-19 patients, the latest guidelines of the International Federation of Clinical Chemistry and Laboratory Medicine suggest that physicians must consider examination of levels of D-dimer in individuals suffering from COVID-19 [18, 45]. Recently, Long et al. also warranted the need to modify the thromboprophylaxis protocol for COVID-19 patients who are hospitalized [46]. In another study, Barnes et al. suggested that the modified prophylaxis protocol of COVID-19 patients must include anticoagulant therapies commencing immediately after admission to the hospital to reduce the rate of thrombi formation in such patients, provided such therapies do not lead to the risk of bleeding among the patients [47]. In this direction, Spyropoulos et al. further suggested that the application of any such modified prophylaxis protocol should be followed by an examination of a modified venous thromboembolism risk score to assess whether the patients are benefitting from the modified protocol [48].

Currently, the COVID-19 patients are managed using pharmacological DVT prophylaxis. However, this management approach is not specific to COVID-19 patients [49]. Acutely ill or hospitalized COVID-19 patients, including those receiving critical care, were found to have high rates of venous thromboembolism (VTE). In these patients, the best thromboprophylaxis strategy is still uncertain [50]. Recently, the American Society of Hematology (ASH) guideline panel suggests using prophylactic-intensity over intermediate-intensity or therapeutic intensity anticoagulation for patients with COVID-19–related acute illness who do not have suspected or confirmed VTE [51]. Conte G et al. thought that recommendations for using pharmacological DVT prophylaxis are mostly based on the results of research with medical patients who did not have COVID-19. Furthermore, it is unclear whether antithrombotic prophylaxis in COVID-19 patients should be guided by risk assessment models (as was the traditional approach in nonsurgical hospitalized patients prior to the present epidemic), D-dimer values, or clinical judgment alone [52]. In a multicenter, randomized, controlled trial including patients hospitalized with confirmed COVID-19 and elevated D-dimer concentration, a 30-day course of therapeutic anticoagulation with rivaroxaban at 20 mg daily (and enoxaparin 1 mg/kg twice daily for clinically unstable patients) did not result in better clinical outcomes when compared with in-hospital prophylactic anticoagulation with heparin. Therapeutic anticoagulation for 30 days with rivaroxaban or enoxaparin led to a higher incidence of major or clinically relevant nonmajor bleeding than did inhospital prophylactic anticoagulation [53]. According to Al-Ani et al., the following anticoagulants hold great potential in reducing the frequency of microthrombi formation in COVID-19 patients [54].

(1) Heparin: In their recent retrospective review, Tang et al. assessed the effect of heparin therapy on the mortality rate of COVID-19 patients. They reported that, overall, there was no significant difference in the mortality rates of COVID-19 patients who either received or did not receive heparin. However, the COVID-19 patients with abnormally high levels of D-dimer who received heparin exhibited significantly lower mortality rates than the COVID-19 patients with abnormally high levels of D-dimer but who did not receive heparin. The former result could be attributed to the fact that other clinical settings and treatment modalities received by the patients were not revealed, impacting the mortality of the COVID-19 patients [55, 56].

(2) TPA: Although to date very few studies have assessed the effects of TPA administration on mortality rates of COVID-19 patients, their results have been positive. These studies have reported significant improvement in the ventilator parameters and

Table 1: A list of some retrospective studies showing the significance of the association of D-dimer levels with disease severity among the COVID-19 patients [13, 27, 35, 36, 37, 38, 39, 40, 41, 42, 43].

	Study population	Observations
Han et al. 2020	Control vs. COVID-19 patients	COVID-19 patients exhibited significantly higher levels of both D-dimer and fibrinogen
Zhou et al. 2020	COVID-19 survivors vs. nonsurvivors	Patients with D-dimer level >1 μg/mL exhibited significantly (18 times) higher mortality than those with D-dimer level <1 μg/mL ($p = 0.0033$)
Cui et al. 2020	COVID-19 patients with VTE vs. those without VTE	A D-dimer level cut-off value of 1.5 μg/mL could be used to predict VTE
Tang et al. 2020	COVID-19 survivors vs. nonsurvivors	About 28.36% and 85.7% of the COVID-19 patients with DIC exhibited fibrinogen level <1 g/L and D-dimer level >3 mg/dL, respectively
Qui et al. 2020	Mild COVID-19 vs. moderate COVID-19; all pediatric patients	Mild COVID-19 patients exhibited significantly lower levels of D-dimer compared to moderate COVID-19 patients
Liu et al. 2020	Mild COVID-19 vs. severe COVID-19	Mild COVID-19 patients exhibited significantly lower levels of D-dimer compared to severe COVID-19 patients
Zhang et al. 2020	Mild or moderate COVID-19 vs. severe COVID-19	Mild/moderate COVID-19 patients exhibited significantly lower levels of D-dimer compared to severe COVID-19 patients
Chen et al. 2020	Moderate COVID-19 vs. severe COVID-19	Moderate COVID-19 patients exhibited significantly lower levels of D-dimer compared to severe COVID-19 patients
Wu et al. 2020	COVID-19 patients with ARDS vs. those without ARDS	Risk of ARDS in COVID-19 patients directly correlated with the levels of D-dimer ($p < 0.001$)
Zhou et al. 2020	Patients with no aggravated COVID-19 vs. Patients with aggravated COVID-19	The pathological progression of COVID-19 was not impacted by the levels of D-dimer
Wu et al. 2020	COVID-19 survivors vs. nonsurvivors	Higher levels of D-dimer significantly correlated with higher mortality risk among COVID-19 patients who presented with ARDS ($p = 0.002$)
Tang et al. 2020	COVID-19 survivors vs. nonsurvivors	(i) Multivariate analysis revealed the level of D-dimer to be independently associated with 28-day mortality (ii) Among the COVID-19 patients with levels of D-dimer >3.0 μg/mL, those who did not receive heparin exhibited a significantly higher 28-day mortality rate compared to those who received heparin ($p = 0.017$)
Yin et al. 2020	Individuals suffering from both severe pneumonia and COVID-19 vs. individuals suffering from severe pneumonia but not COVID-19	Among the COVID-19 patients with levels of D-dimer > 3.0 μg/mL, those who did not receive heparin exhibited a significantly higher mortality rate compared to those who received heparin ($p = 0.017$)
Zhang et al. 2020	COVID-19 survivors vs. nonsurvivors	(i) Among the patients with levels of D-dimer of more than 1 mg/L, about 81% of patients exhibited severe illness, and around 72% of patients reached the composite endpoints, that is, admission to ICU or death (ii) Higher levels of D-dimer correlated significantly with the severity of pneumonia among the COVID-19 patients and the risk of the patient reaching the composite endpoints, that is, admission to ICU or death ($p < 0.001$)

VTE, venous thromboembolism; DIC, disseminated intravascular coagulation; ARDS, acute respiratory distress syndrome.

oxygenation index of severely ill COVID-19 patients after TPA administration [55].

(3) Direct oral anticoagulants (DOACs): This category of anticoagulants seems to be effective in COVID-19 patients based on the findings of Testa et al. who reported a marked increment in the plasma levels of DOAC in COVID-19 patients who received antiviral medications [57].

A recent study published by Spyropoulos et al. reported that the use of therapeutic-dose low molecular weight heparin (LMWH) for thromboprophylaxis in high-risk inpatients with COVID-19 decreased thromboembolism and mortality in their trial. High-dose anticoagulant therapy altered the course of illness in patients who were not in the intensive care unit but had a very high risk of thromboembolism and mortality (36.1% incidence in the standard dose group) [58].

5. Conclusion

In conclusion, it is clearly evident that levels of D-dimer are directly associated with the disease severity among COVID-19 patients. Novel coronavirus infections promote

inflammatory and coagulation reactions, leading to an increase in thrombotic event rates. Currently, the evaluation of levels of D-dimer has not been adopted in the routine laboratory assessment of COVID-19 patients. Laboratory testing for D-dimer and proinflammatory cytokines could help to categorize the COVID-19 patients based on the severity of their illness. This could, in turn, be helpful in adequate and more efficient management of such individuals. We also recommend that future investigators should focus on conducting more comprehensive and multicentric prospective studies to elucidate further the association of levels of D-dimer with the levels of proinflammatory cytokines and with the thrombotic pathways, especially in COVID-19 individuals. Finally, we propose that physicians should consider using anticoagulant therapies to counter the upregulated thrombotic activity in COVID-19 patients.

Data Availability

The data that support the findings of this review are available in the reference section.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors made substantial contributions to conception and design and/or writing. They participated in drafting the review and revising it critically for important intellectual content, and they gave final approval of the version to be submitted and any revised version.

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Research Article

Pediatric Sickle Cell Disease in Sudan: Complications and Management

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Background. Sickle cell disease (SCD) is a life-threatening genetic disorder due to the formation of sickle hemoglobin molecule (HbS) that polymerizes in hypoxic conditions leading to SCD-related complications. Different approaches have been used in the management of SCD including symptomatic management, supportive management, and preventive management. *Objectives*. To assess the management of SCD in pediatric patients in Gaafar Ibnauf Referral Hospital in Khartoum locality, Sudan. *Method*. A descriptive, retrospective, hospital-based study was conducted in Gaafar Ibnauf Hospital using a data collection sheet. The study included all medical files of pediatric patients with SCD attending the hospital during the period from the first of April 2018 to the first of July 2018. The data were analyzed using descriptive statistics and the chi-square test. $P < 0.05$ was considered statistically significant. *Results*. Out of 207 pediatric patients, 53.1% were females (mean age of 7.5 ± 3.1 years), with a 1.1 : 1 female:male ratio and low socioeconomic status. Only 4.3% of participants had health insurance. The Messeryia tribe in western Sudan had the highest prevalence of the disease among the Sudanese tribes (11.1%). Vaso-occlusive crisis (33.3%), infections (13.5%), and neurological complications (10.6%) were the most frequent complications reported during routine visits. After initiation of management, only 3.4% of pediatric patients had hemolytic crises, and 1.4% of the anemic patients had splenomegaly. 100% of patients received folic acid, 73.9% used hydroxyurea, and 69.6% underwent blood transfusion for the management of SCD. Prophylactic penicillin was prescribed for 15% of patients, and 41.1% were immunized with pneumococcal vaccine (PPSV23). Most patients had been scheduled for planned follow-up visits every 3–6 months (93.2%). Hydroxyurea and blood transfusion significantly reduced fever and vaso-occlusive crisis. *Conclusion*. The SCD treatment protocol in Gaafar Ibnauf Children's Hospital, involving preventive and symptomatic therapy, is consistent with the internationally implemented protocols for SCD management. However, immunization and prophylactic penicillin approaches are deficient.

1. Introduction

Sickle cell disease (SCD) is a life-threatening genetic disorder due to the formation of sickle hemoglobin molecule (HbS) with low affinity to oxygen. In the presence of hypoxia, HbS polymerizes and ultimately results in sickled red blood cells that rupture leading to hemolytic anemia. Moreover, the sickled cell also results in vascular occlusion causing tissue infarction, organ damage, and pain [1].

SCD affects many people throughout the globe, particularly those descending from Sub-Saharan Africa, the Middle East, and South Asia. In general, SCD exists in the malarial regions of tropical areas. However, migration from a malarial area increases the number of children with SCD in Europe and North America [2–4]. In the United States, SCD affects 1 in 500 African Americans. It is estimated that the population of sickle cell disease is approximately 4.4 million people, whereas 43 million are estimated to have sickle cell trait [5]. In Sudan, sickle cell disease is a major health problem in certain parts of the country, particularly the western region. The HbS allele frequently exists among the Misseriya tribe, and it is estimated to range from 18.2% in Kordofan to 30.4% in Darfur [6, 7].

The main complications of SCD include pain syndromes, hemolytic anemia, and organ damage/failure [8]. The incidence of complications varies with age from infancy through adult life. Recurrent pain episodes, the hallmark of the disease, result from acute painful vaso-occlusive crises (VOCs) when the sickled cells block the blood vessels. The pain is usually nociceptive in nature, which varies from patient to patient. It could be acute or chronic, somatic or visceral, unilateral or bilateral, localized or diffuse. Generally, pain affects long bones, joints, and the back; however, the scalp, face, jaw, abdomen, and pelvis may be involved [8]. In addition, bacterial infections and anemia are common complications in children with SCD [9,10]. Acute chest syndrome (ACS), splenic sequestration, and multiorgan damage are considered life-threatening conditions that require immediate hospitalization. Cardiopulmonary complications, including cardiomyopathy, pulmonary hypertension, and sudden cardiac death, are the most common causes of morbidity and mortality. Children with SCD are at high risk for developing thrombosis and stroke. SCD is now recognized as a systemic disease, which causes widespread tissue/organ injury, including inflammatory dysfunction and coagulation abnormalities. All children with SCD should be screened annually with transcranial Doppler ultrasonography (TCD). It is highly recommended for children with SCD from 2–16 years of age [8, 11–13].

The management of SCD and its complications requires major approaches, including supportive management, symptomatic treatment, as well as preventative measures. The ultimate goal of treatment is to alleviate the symptoms and to maintain a good quality of life [8, 14].

A published guideline [15] strongly recommends daily oral prophylactic penicillin for children with SCD up to the age of 5 years to reduce the risk of infections. In addition, immunization especially pneumococcal vaccination, was strongly recommended by SCD treatment guidelines [15,16]. The American Society of Hematology (ASH) guideline recommends opioids for the treatment of acute pain associated with vaso-occlusive crisis. For adults who have SCD-related chronic pain, the ASH guideline suggests the use of nonopioid analgesics such as NSAIDs, duloxetine, gabapentinoids, or tricyclic antidepressants, as options for pain management [16]. Blood transfusion reduces the level of HbS, thereby it is used to treat acute symptomatic anemia as well as treating and preventing many SCD complications [15]. To reduce the risk of stroke, especially in children with abnormal transcranial Doppler velocity, regular blood transfusion for at least one year is recommended to reduce HbS levels below 30% and to maintain Hb levels >9.0 g/dL. Assessment of iron overload is required to initiate iron chelators when indicated. Moreover, the ASH guideline suggests hydroxyurea therapy for children (ages 2–16 years) who live in low-middle-income areas (where regular blood transfusions and chelation therapies are not available or affordable). Hydroxyurea therapy decreases SCD-related complications (such as VOCs, ACS, hospitalization, and mortality rate) by increasing fetal hemoglobin (HbF) [15, 17].

SCD is a life-threatening disorder that is associated with acute and chronic complications interfering with daily activities and the quality of life of patients. In Sudan, SCD mainly affects children who continue to suffer from repetitive pain crises and frequent severe complications, and ultimately leads to early death. Therefore, specialized medical care focusing on prevention and regular assessment of disease management is urgently needed to reduce morbid events as well as the mortality rate. This study aimed to assess the management of sickle cell disease in pediatric patients at Gaafar Ibnauf specialized pediatric hospital in Sudan in light of international guidelines.

2. Methodology

2.1. Study Design and Setting. A descriptive, retrospective, hospital-based study was carried out in Gaafar Ibnauf Pediatric Hospital, Khartoum state, Sudan. Gaafar Ibnauf Hospital is the biggest referral specialized pediatric hospital in Sudan, receiving patients from all over the country. It encompasses nine units of different specialties in pediatrics (cardiology, neurology, gastroenterology, respiratory, hematology, and nursery).

2.2. Study Population. Study populations consisted of pediatric patients with SCD attending Gaafar Ibnauf Hospital and outpatient's clinic. All patients' files during the period (1 April 2018–1 July 2018) were manually screened.

2.2.1. Inclusion Criteria. Medical files of pediatric patients with SCD attending Gaafar Ibnauf Hospital from April to July 2018 were included in this study.

2.2.2. Exclusion Criteria. Incomplete patients records were excluded from the study.

2.3. Sampling Technique and Sample Size. A total coverage sampling technique was used in the current study. Two hundred and seven medical files of pediatric patients with SCD were selected based on the inclusion and exclusion criteria.

2.4. Data Collection Tool. The data were collected using a data collection sheet constructed by the researchers based on the study objectives. The data collection sheet consists of three parts. The first part covered the patients demographic data. The second part listed the common complications of SCD, and the third part covered diagnostic tests, management of the disease, and therapeutic monitoring throughout the scheduled follow-up visits.

2.5. Statistical Analysis. The data was analyzed using Statistical Package for Social Science Software (SPSS, version 20) and Microsoft Excel. For numerical data, the mean and standard deviation were calculated. For categorical data, descriptive statistics such as frequency and percentage were used to summarize the results. The Chi-square test was used

to describe the association between variables. *P*value <0.05 was considered statistically significant.

2.6. Ethical Considerations. The ethical approval was obtained from the Research Board at the University of Khartoum, Faculty of Pharmacy (Research Ethics Committee, No. 59-5-3–2018). In addition, ethical clearance has been obtained from the Research Department, the Ministry of Health, Sudan. Permission to perform the study was obtained from the general director of the hospital. To ensure confidentiality of the patients' information, coded data collection sheets were used.

3. Results

3.1. Sociodemographic Characteristics of Patients. The study included 207 patients, with a mean age of 7.5 ± 3.1 years. More than half of patients (53.1%) were females, with a 1.1 : 1 female:male ratio and low socioeconomic status (53.6%). Only 4.3% had health insurance. Two-thirds of patients (66.2%) had no family history of sickle cell disease (Table 1).

3.2. Distribution of Sickle Cell Disease among Different Tribes in Sudan. Messeryia (11.1%) and Selehab (8.2%) tribes had the highest rate of SCD among the Sudanese tribes (Table 2).

3.3. Diagnostic Laboratory Tests for Sickle Cell Disease and Routine Laboratory Monitoring for Patients with Sickle Cell Disease. The major laboratory test that had been carried out for SCD diagnosis in the participants was hemoglobin electrophoresis (96.1%). All patients had been routinely monitored for hematological problems using a complete blood count test. 37.7% and 7.7% of patients had been monitored for liver and renal function, respectively. Transcranial Doppler ultrasonography (TCD) or pulmonary function tests were not routinely requested for the patients at each follow-up visit (Table 3).

3.4. Complications of Sickle Cell Disease among Participants. One-third of patients (33.3%) experienced vaso-occlusive crisis/pain, including 1% of patients having acute chest syndrome (ACS). 13.5% of patients had developed infections, with 9.6% infected with pneumococcal and meningococcal bacteria (respiratory tract infections, meningitis, and sepsis), whereas 3.9% had malaria infection. 4.3% of patients had experienced GIT symptoms. 11.1% and 10.6% of patients had experienced fever and neurological complications (such as hemorrhagic or ischemic stroke and headache). Anemia (hemolytic crises) had been encountered in 3.4% of patients, and 1.4% of the anemic patients had splenomegaly. Hepatomegaly/jaundice has been observed in 3.4% of patients (Figure 1).

3.5. Scheduled Follow-Up Visits in Patients with SCD. The majority of patients (93.2%) had been scheduled for follow-up visits every 3–6 months (Figure 2).

Table 1: Patient's socio-demographic characteristics ($N = 207$).

Demographic Data	Mean	Std. deviation (%)
Age	7.5	3.1
Gender	Frequency	Percent
Male	97	46.9
Female	110	53.1
Socioeconomic status		
Low	111	53.6
Moderate	80	38.6
Unknown	16	7.7
Health insurance		
Yes	9	4.3
No	198	95.7
Family history of sickle cell disease		
Yes	64	30.9
No	137	66.2
Unknown	6	2.9

Table 2: Rate of sickle cell disease in the Sudanese tribes.

Sudanese tribes	Frequency	Percent
Messeryia	23	11.1
Selehab	17	8.2
Barno	14	6.8
Fallata	13	6.2
Bargo	13	6.2
Four	7	3.4
Rezegat	6	2.9
Rashyda	6	2.9
Hosa	6	2.9
Noba	5	2.4
Jammoeia	5	2.4
Zagawa	4	1.9
Benihalba	4	1.9
Bedireia	4	1.9
Taayisha	3	1.4
Omtenger	3	1.4
Rofaien	2	1
Masalti	2	1
Kanania	2	1
Hawazma	2	1
Gazami	2	1
Deedab	2	1
Dago	2	1
Berti	2	1
Baggara	2	1
Gaaline	2	1
Nemawia	1	0.5
Edasha	1	0.5
Danjo	1	0.5
Notrecorded	51	24.6

3.6. Management of Patients with Sickle Cell Disease. This study showed that folic acid had been prescribed for all patients (100%), and 73.9% of patients had been treated with hydroxyurea therapy. The starting dose of hydroxyurea was 10 mg/kg/day and then the dose was escalated to 15 m/kg/day up to 35 mg/kg/day. The majority of patients received 15 mg/kg/day. Only 15% of patients received prophylactic penicillin with the majority of them (87.1%) receiving amoxicillin. More than half of patients (58.9%) were unvaccinated, and 41.1% of

Table 3: Diagnostic laboratory tests and routine laboratory monitoring for patients with sickle cell disease.

Test	Frequency	Percent
Diagnostic laboratory tests		
Sickling test	4	1.9
Solubility test	3	1.5
Haemoglobin electrophoresis	199	96.1
HPLC	0	0.0
Isoelectric focusing test	0	0.0
Not reported	1	0.5
Routine laboratory tests		
CBC with Reticulocyte Count	207	100.0
Liver Function Test (LFT)	78	37.7
Renal Function Test (RFT)	16	7.7
EKG and Echocardiogram	7	3.4
Abdominal Ultrasound	4	1.9
Ophthalmology Test	2	1.0
Pulmonary Function Test	0	0.0
Transcranial Doppler Ultrasonography	0	0.0

patients had been immunized with pneumococcal vaccine. All children younger than 2 years received 3 doses of the 13-valent pneumococcal conjugate vaccine (PCV13). Children 2–5 years old were administered 1–2 doses of PCV13 if they were unvaccinated or had received incomplete doses of PCV13. After completion of the PCV13 vaccine series, children aged ≥2 years received 1 dose of the 23-valent pneumococcal polysaccharide vaccine (PPSV23), with a booster dose 5 years later. More than two-thirds of patients (69.6%) were subjected to blood transfusions, with 13.9% ($n = 20$) of them undergoing chronic blood transfusions monthly for 3 years (Table 4).

3.7. Association between SCD Complications and Treatment Modalities. The use of hydroxyurea for the management of SCD was significantly associated with a low risk of fever ($P = 0.045$). A significant reduction of vaso-occlusive crisis/pain ($P = 0.04$) has been observed after blood transfusion (Table 5).

4. Discussion

Sickle cell disease (SCD) is an inherited blood disorder associated with acute and chronic complications and early death [18]. In Africa, thousands of children are born with SCD, and 90% of them die before the age of 5 years [3]. To date, there are no established programs in Africa for screening and clinical interventions for SCD management. Screening, pneumococcal prophylaxis, affordable treatment options, and caregivers' education effectively reduce child mortality due to SCD [19]. Providing optimal and comprehensive care to individuals with SCD can be challenging. Thus, implementing guidelines that provide evidence-based recommendations for the management of this life-threatening condition is of crucial importance and helps healthcare professionals to improve their practice [15]. This study aimed to assess the management protocol used for SCD in Gaafar Ibnauf Hospital in Sudan.

In this study, the participants were young children with a mean age of 7.5 ± 3.1 years. Most patients were females with low socioeconomic status. The results showed that 11.1% of the cases were from the Messeryia tribe in western Sudan. As reported in the literature, the prevalence of SCD in Sudan ranges from 2 to 30.4%, with a higher prevalence among tribes in western Sudan [6, 7, 20]. This could be due to migration from West African countries where the high prevalence of the disease is well documented [21].

In routine laboratory monitoring, all patients have been routinely monitored for complete blood counts, and unfortunately, transcranial Doppler ultrasonography (TCD) is not a routine practice at each follow-up visit. According to published guidelines, annual screening of stroke using transcranial Doppler ultrasonography is recommended for all children with sickle cell disease [15]. However, in Gaafar Ibnauf Hospital, TCD is only requested when neurological symptoms appear, and this practice could be due to the low socioeconomic status of the patients, unavailability, and unaffordability of the test.

Vaso-occlusive crises (VOCs), infections, fever, and neurological complications are the most serious complications that are reported during routine visits. Pneumococcal and meningococcal infections are the most commonly encountered complications in pediatric SCD. Hemorrhagic or ischemic stroke and headache are the most frequently encountered neurological complications in the current study. This finding is consistent with a previous study in Africa which demonstrated stroke as a common neurological complication of SCD [22]. In Africa, some complications such as silent brain infarcts, peripheral neuropathies, neurocognitive deficits, encephalopathy, or moyamoya disease are often underestimated because of the unavailability and unaffordability of diagnostic tests such as neuroimaging, transcranial Doppler ultrasonography, electroencephalogram (EEG), and neuropsychological evaluation [22]. Acute chest syndrome is a life-threatening complication, but it is not common in this study.

Anemia (hemolytic crises; Hb < 10 mg/dl) is one of the least complicated conditions in this study. This shows the effectiveness of the implemented treatment protocol in reducing the risk of anemia. The low incidence of hemolytic crises among the study participants reflects the adherence of physicians to the hospital treatment protocol regarding folic acid prescription for all patients and routine monitoring of hematological parameters. In this study, malaria infection and splenomegaly have been encountered in a few patients. According to the literature, splenomegaly is attributed to recurrent infections with *Plasmodium* species and is frequently reported in children with SCD (HbSS) in malaria-endemic countries [23]. These contradictory results seemed to be attributed to the early development of autosplenectomy in most children attending the hospital. Repeated attacks of VOCs cause autosplenectomy, rendering our patients more vulnerable to systemic infections. Autosplenectomy occurs because caregivers do not seek medical help at the earliest sign of the disease and most children start medications, especially hydroxyurea, at a later age (more than 9 months) after the onset of complications. This practice among patients could be related to a lack of good health education, low socioeconomic status, and health insurance coverage [24]. Moreover, most

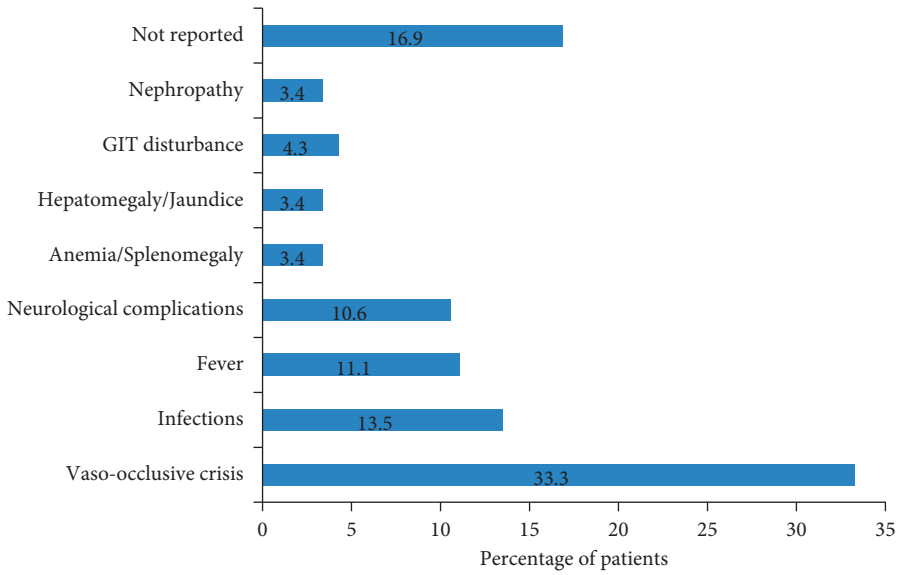

Figure 1: Complications of sickle cell disease among participants.

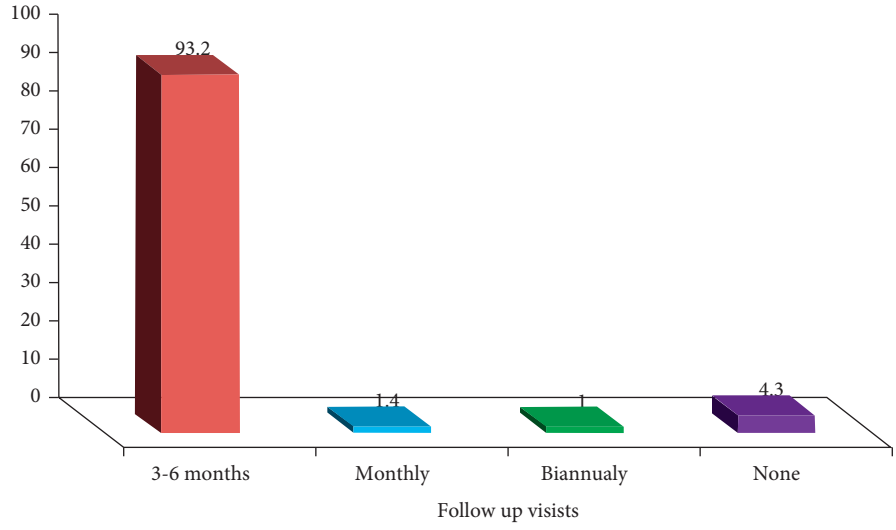

Figure 2: Distribution of patients according to the follow up visits.

Table 4: Management of patients with sickle cell disease ($N = 207$).

Management	Frequency	Percent
Folic acid	207	100.0
Hydroxyurea	153	73.9
Supplements	95	45.9
Antibiotics for infections	15	7.2
Analgesics	10	4.8
Prophylactic penicillins ($N = 31$)	31	15.0
Amoxicillin	27	87.1
Penicillin VK	4	12.9
None	176	85.0
Vaccination		
Pneumococcal vaccine	85	41.1
None	122	58.9
Blood transfusion		
Yes	144	69.6
No	63	30.4

Table 5: Association between complications of SCD and the treatment modalities.

Complications	Hydroxyurea ($N = 153$)		*P value*
	Yes	No	
Fever	13 (8.5%)	140 (91.5%)	0.045*
Vas-occlusive crisis	54 (35.3%)	99 (64.7%)	0.30
	Blood transfusion ($N = 145$)		
Vas-occlusive crisis	42 (29%)	103 (71%)	0.04*

*$P \leq 0.05$

patients are from rural areas in western Sudan where there is a lack of health facilities. The low rate of malaria infection could be attributed to less exposure of patients to malaria infection by using preventive and control measures of malaria such as mosquito nets and repellents. Moreover, inaccessibility of screening tests and underreporting of

complications may lead to underestimation of patients with splenomegaly and malaria.

In this study, most patients were unvaccinated and did not receive prophylactic penicillin. The high incidence of infections observed among participants in the current study may be explained by the failure in implementing an effective immunization protocol and the physician's nonadherence to guidelines, regarding antibiotic prophylaxis for children with SCD. Initiation of penicillin prophylaxis up to the age of 5 years, in addition to vaccination, is recommended to reduce the risk of developing serious bacterial infections [14, 15, 25]. Low prescription rates of prophylactic penicillin may be attributed to fear from the emergence of penicillin resistance, noncompliance of physicians to treatment guidelines, and nonadherence to medications in SCD patients [15, 26, 27]. Oral amoxicillin was the most frequently used prophylactic penicillin among the participants. Children with SCD in this study received only pneumococcal vaccine which was given according to clinical practice guidelines of pneumococcal immunization. Children younger than 2 years should receive 4 doses of the 13-valent pneumococcal conjugate vaccine (PCV13). Before starting the 23-valent pneumococcal polysaccharide vaccine (PPSV23), children 2–5 years old (unvaccinated or having received incomplete doses of PCV13) should be vaccinated with 1–2 doses of PCV13. After completion of the PCV13 vaccine series, children aged 2–18 years should receive 1 dose of PPSV23, with a booster dose 5 years later [28]. As indicated in SCD treatment protocols, immunizations should include all children's routine vaccines with the addition of the flu vaccine, pneumococcal vaccine, and meningococcal vaccine [14].

In this study, hydroxyurea was prescribed to most participants, reflecting the physician's adherence to SCD treatment guidelines regarding this medication. The starting dose of hydroxyurea was 10 mg/kg/day and escalated to a maximum tolerated dose (maximum dose is 35 mg/kg/day). The majority of patients received hydroxyurea at a dose of 15 mg/kg/day. Although oral L-glutamine was approved by the FDA in 2017 to reduce the acute complications of SCD in adult and pediatric patients older than 5 years [14,29], it has not yet been used or even registered in Sudan.

In this study, more than two-thirds of patients received blood transfusions, with 13.9% of them undergoing chronic blood transfusions every month for 3 years to prevent primary or secondary stroke. Chronic transfusion is used when unremitting reduction of HbS (less than 30%) is required for stroke prevention [15]. The majority of the patients in this study received episodic transfusions (periodic transfusions) for acute chest syndrome, acute anemia, hepatic sequestration, progressive intrahepatic cholestasis, priapism, and sepsis. Simple blood transfusion was frequently used in comparison with exchange blood transfusion.

In this study, few patients received analgesics for the management of painful crises. The SCD treatment guidelines strongly recommend immediate initiation of nonopioid and opioid analgesics for the management of pain associated with VOC, based on the level of patient-reported pain [15]. It appeared that most patients had mild pain, and the main barrier of the frequent prescription of NSADs is the side effects of these medications.

The scheduled follow-up visits are necessary for effective disease control. In this study, most patients had been scheduled for planned follow-up visits every 3–6 months. It has been observed from the medical history of patients that VOC episodes and hospitalizations usually occur before the three-month check-up period. Therefore, a three-month check-up period is rather long for monitoring the therapeutic outcomes of children with SCD. As an observation, some patients are unable to adhere to their follow-up visit, and this could be due to a lack of awareness of patients or their caregivers about the benefit of follow-up visits. In addition, financial issues may be a reason for nonadherence to follow-up visits since most patients are of low socioeconomic status and without health insurance.

This study revealed that hydroxyurea and blood transfusion for the management of SCD significantly reduced fever (*P: 0.04*) and VOCs (*P: 0.04*), respectively. However, patients taking hydroxyurea appeared to be healthier with less frequent VOCs and other complications than those not taking hydroxyurea. As reported in the literature, both hydroxyurea and blood transfusion are used to reduce SCD-related complications such as recurrent vaso-occlusive crisis and fever [29]. Insignificant effects of hydroxyurea in reducing VOCs could be attributed to the use of relatively low doses of hydroxyurea (15 m/kg/day) in most patients or patients' nonadherence to hydroxyurea therapy. Hydroxyurea requires a monitoring protocol to ensure the highest benefits and safety of the therapy. Low doses of hydroxyurea have minimal effect in fetal hemoglobin production that mitigates tendencies for red blood cell sickling and VOC [15, 29]. A previous study demonstrated that hydroxyurea at a dose of approximately 30 mg/kg/day significantly reduces sickle cell-related adverse events (such as VOC) when compared to hydroxyurea at a dose of 20 mg/kg/day [30].

5. Limitations

The study is a single-center study with a relatively small sample size that may affect the generalizability of the results. In addition, some medical files had been excluded because of poor documentation.

6. Conclusion

The prevalence of SCD in Sudan is higher in the Messeryia tribe, originating from western Sudan. The treatment protocol at Gaafar Ibnauf Hospital-Sudan includes hydroxyurea, analgesics, folic acid, and blood transfusions. This treatment protocol, involving preventive and symptomatic therapy, is consistent with the international implemented protocols for the management of SCD in children. However, immunization and prophylactic penicillin approaches are deficient. The use of hydroxyurea and blood transfusion for children with SCD significantly reduces fever and vaso-occlusive crisis, respectively. Unfortunately, recently approved drugs, such as L-glutamine, have not yet been used in hospitals.

7. Recommendations

(i) Ongoing communication between healthcare providers and patients or caregivers on the use of hydroxyurea will enable informed joint decision-making and empower patients to initiate hydroxyurea therapy.

(ii) Institution of comprehensive SCD centers focusing on life-long SCD management. Physicians, clinical pharmacists, and other healthcare workers involved in the care of SCD patients should be well trained and acquainted with current knowledge and standard practices in the treatment of SCD to improve treatment outcomes.

(iii) Patients should be counseled on the need for adherence to scheduled vaccinations and ensure that all patients have received all vaccines that have been recommended by SCD treatment protocols.

(iv) Communicate with health policymakers to provide free of charge medicines (hydroxyurea, folic acid, penicillin, and pneumococcal vaccine-23) and offer low-cost and high-quality laboratory tests for patients with SCD to increase the adherence to SCD treatment protocol.

(v) Intensify educational programs to enhance awareness of children and caregivers about the nature of the disease, possible complications that require immediate medical attention, the importance of antibiotic prophylaxis and vaccination to prevent life-threatening pneumococcal infections.

Data Availability

The data supporting this research article are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Meysaa, G.T. participated in the conception, literature review, collection, and analysis of the data. Abdoon, I. contributed to the conception, study design, follow-up, interpretation of data, writing of the original manuscript, and providing critical revision of the manuscript. Osman, B. participated in reviewing, editing, and providing final approval of the manuscript. Abdalla, S.A. contributed to the drafting and proofreading of the manuscript. Mirghani H. M. contributed to reviewing, proofreading, and final approval of the manuscript. The manuscript has been read and approved by all authors.

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Research Article

Neonatal Screening for Sickle Cell Disease in Congo

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Introduction. Sickle cell disease is an autosomal recessive inherited disorder due to the mutation of a gene coding for the globin beta chain. The aim of this study is to update the epidemiological data on hemoglobinoses, in particular sickle cell disease in newborns in Congo. *Materials and Methods*. This was a descriptive cross-sectional study, conducted from October 1, 2019, to March 31, 2020, throughout the Congolese national territory. It involved all full-term newborns, without distinction of nationality, aged 5 days or less, and whose parents consented to participate in the study. The blood samples, taken at the heel and collected on Whatman blotting paper, were analyzed using the HPLC Variant NBS machine. *Results*. In 2897 newborns (NN) screened, hemoglobin abnormalities were found in 603 NN (20.81%). The mean age of these newborns was 1 day (extremes 0 and 5 days). The male-to-female ratio was 1.03. Abnormal hemoglobins were mainly Hb S ($n = 597$ (97.71%)); Hb C ($n = 5$ (0.82%)); and variants ($n = 7$ (1.15%)). The national prevalence of major sickle cell (MSC) syndromes and sickle cell trait was 1.35% and 19.43%, respectively. The prevalence ranged from 1.77% to 2.56% for MSS in four departments and from 20.5% to 25.8% for the sickle cell trait in six other departments. *Conclusion*. Data on homozygous sickle cell disease remain consistent with previous studies. However, further studies should clarify the molecular anomalies of the variants observed in our samples.

1. Introduction

Sickle cell disease is an autosomal recessive inherited disorder linked to the mutation of a gene coding for the beta chain of globin [1–3].

It is the first genetic disease in the world which mainly comprises four (4) primitive foci: African focus, Asian Peninsula, South-East Asia, and an incidental focus in the Balkans.

A distinction is made between heterozygous forms (AS) and homozygous forms (SS) which are part of the major sickle cell syndromes (MSS). The major sickle cell syndromes include the following entities: homozygous abnormal hemoglobin S, hemoglobin S associated with other abnormalities (C, D, and O), and intermediate forms of thalassemia. Multiple studies have been carried out worldwide on the prevalence of this genetic disorder [4–9]. In Africa, studies report a prevalence of 0.8 to 3% of major forms and 7 to 24% of heterozygous forms [4, 5, 10–16]. Studies in Congo have involved local sampling and analysis, most often carried out outside Congo [4, 16]. These analyses placed the frequency of sickle cell trait in Congo between 19 and 24% and the homozygous form around 1% according to neonatal samples [4, 16].

This study, which focuses on neonatal sampling, was conducted entirely in Congo. The samples were taken from newborns in all departments of the Republic of Congo, and the analyses were carried out on site. In order to update the data on sickle cell disease in Congo, we conducted this study to determine the prevalence of sickle cell disease and to

estimate the variants of hemoglobin (Hb) in newborns in each department of Congo.

2. Materials and Methods

This was a cross-sectional descriptive study conducted from 1 October 2019 to 31 March 2020 over the entire national territory of Congo (Figure 1). The Republic of Congo is located in sub-Saharan Africa, in the sickler belt. It is made up of 12 departments (Figure 1) in which samples were collected and sent to the Centre National de Reference de la drepanocytose (CNRDr) for analysis. The CNRDr is a subregional facility, open to the public in 2016, specialised in the management of genetic diseases including sickle cell disease. It comprises care, imaging, and medical biology units.

The target population consists of full-term newborn babies (NBs) born in the Congolese territory. All NBs without distinction of nationality, aged 5 days or less, were included in the study. We did not include all NBs in the posttransfusion period and those in intensive care. Furthermore, only the NBs whose parents had given their informed consent were included. Only those whose parents gave consent were screened. Refusal to screen a newborn was a criterion of exclusion. The recruitment was carried out in a comprehensive way.

Considering the prevalence of the sickle cell trait of 21% [17] and the effect of the sampling design set at 1.5 and the imponderables estimated at 5%, the minimum sample size required for this study is 406. The number 406 corresponds to the minimum of patients needed to carry-on the study. It is not the sample of our study but the minimum required. The minimum sample size for each department (cluster) was calculated by weighting the national minimum baseline sample size (406 NBs) according to the size of the population in the department. By rounding up these numbers, the minimum sample size selected was 412, broken down by department (Table 1)

For each newborn baby, a drop of blood was collected at the heel on Whatman 903TM (903 protein saver card) blotting paper under aseptic conditions. The samples were stored for a maximum of 72 hours in pouches (Multi-Barrier Pouches) with desiccant from GE Healthcare company laboratories. They were analyzed using the cation exchange high-performance liquid chromatography (HPLC) procedure (BioRad Variant NBS). Patient profiles were obtained by ranking hemoglobins in descending order of magnitude (thus, an FSA profile assumes a hemoglobin F concentration greater than hemoglobin S concentration and the latter greater than hemoglobin A concentration). The chromatographic profile of MSS was established in the presence of Hb: FS, FSC, FSE, FSD, FSA, FSE, and FSa.

The data obtained were entered and processed using Microsoft Excel version 17.2 and R 3.6.3 software [18]. Qualitative variables were presented as numbers and percentages. Ethical clearance was obtained from the Comité Ethique de Recherche en Science de la Santé (CERSSA).

3. Results

At the end of this study, we obtained a total of 2,897 newborns, 603 of whom had abnormal hemoglobins, being 20.81%. The mean age of these newborns was one (01) day with extremes ranging from 0 to 5 days and a sex ratio (F/M) of 1.03. They were born at 38 (q1 36–q3 39) weeks of amenorrhea with an average weight of 3114 ± 567 g.

Figure 1 illustrates the prevalence of abnormal hemoglobin in newborns by department.

The abnormal hemoglobins found were Hb S ($n = 597$; 97.71%), Hb C ($n = 5$; 0.82%), Hb D ($n = 1$; 0.16%), Hb E ($n = 1$; 0.16%), Hb Bart ($n = 1$; 0.16%), and unidentified Hb ($n = 7$; 1.15%). The distribution of the different types of abnormal hemoglobin by department is described in Table 2.

Among the 2,897 newborns registered, major sickle cell syndrome (MSS) was found in 39 NBs and the sickle cell trait was found in 563, representing national prevalences of 1.35% and 19.43%, respectively (Table 3).

Heterozygous qualitative hemoglobin abnormalities were AS ($n = 551$ (97.87%)) and AC ($n = 5$ (0.89%)).

Table 4 reports the prevalence of the sickle cell trait and MSS phenotypes according to the departments of Congo.

4. Discussion

This study's objective was to update the epidemiological data on sickle cell disease in Congo. The systematic screening approach was favored over the approach targeting NBs from parents with a family history of sickle cell disease, firstly, because healthy AS carriers do not express the disease and are often unaware of their status, which makes it difficult to identify subjects at risk and, secondly, because of the high prevalence of the S gene in the Congo Basin. This screening was carried out using the HPLC variant NBS technique, which has the advantage of being fully automated but also has a high sensitivity to detect normal neonatal hemoglobins (Hb F and Hb A) and the main hemoglobin variants (Hb S, C, E, and D).

In our study, the sex ratio (1.03), in favour of men, was similar to that reported in the literature [5, 13, 14, 19–21]. The average weight of newborns with abnormal hemoglobins was comparable to observations made by Munyanganizi in Rwanda and Kafando in Burkina Faso [10, 19]. It was lower than the one reported by McGann in Angola, which found an average weight of 3.201 ± 526 g [15]. Dietary, environmental, and genetic factors could explain these differences.

The prevalence of abnormal hemoglobin was 20.8%, mainly represented by hemoglobin S (97.71%). The geographical location of the Republic of Congo in the sickler belt and the endemic nature of malaria in Congo could be one of the factors favoring this condition. Indeed, in order to better resist malaria, the populations of this region have undergone mutations that have led to the appearance of hemoglobin S. Subjects carrying this mutation in its heterozygous form present a certain resistance to *Plasmodium*

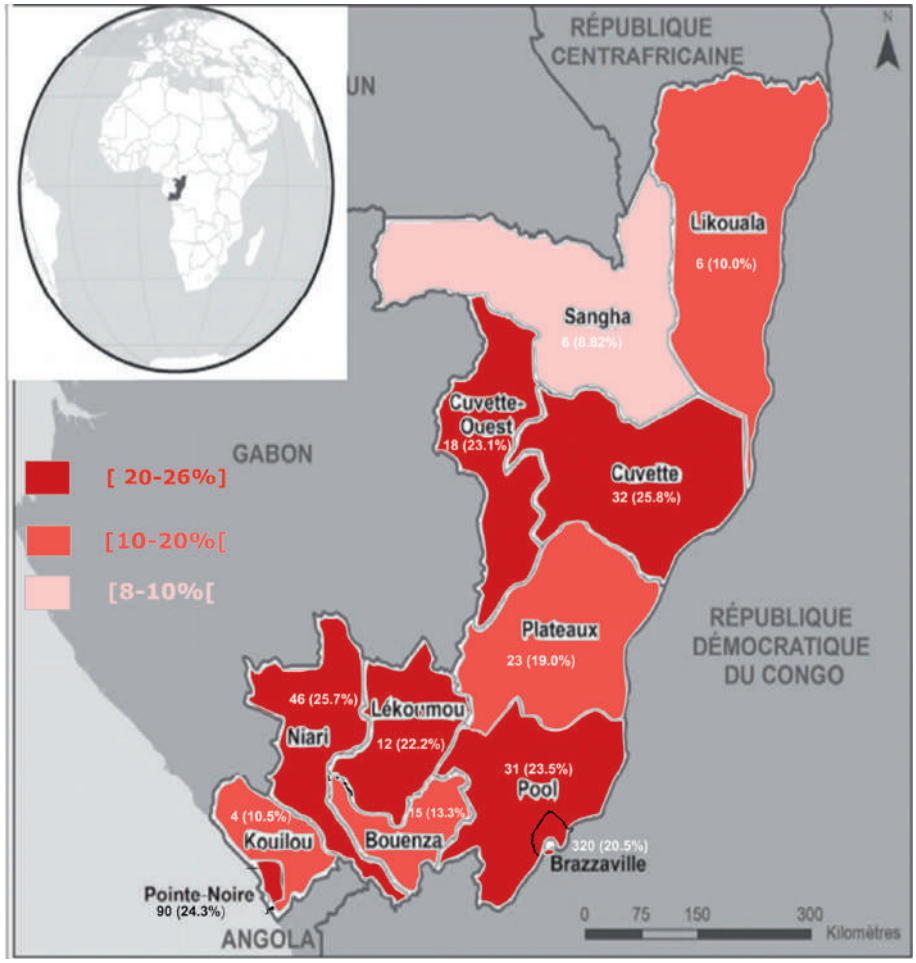

Figure 1: Prevalence of abnormal hemoglobin in newborns in the departments of Congo.

Table 1: Minimum sample size by department.

Department	Population census of 2007	Minimum newborn size to be tested	Number of newborns tested
Brazzaville	1733271	151	1560
Pointe Noire	902828	79	370
Bouenza	390032	34	113
Pool	298580	26	132
Niari	291865	26	179
Plateaux	220328	20	121
Cuvette	196930	18	124
Likouala	194507	17	60
Lekoumou	121639	11	54
Kouilou	116065	11	38
Sangha	108211	10	68
Cuvette-Ouest	92123	9	78
Total	4666379	412	2897

falciparum 22. A selective pressure with the evolution of the disease would have contributed to the increase in its prevalence. Results found in the literature indicate abnormal hemoglobin frequencies ranging from 0.22% to 31% [4, 5, 9, 13, 20, 23–25]. Some of these observations are comparable to ours [4, 5]; on the other hand, others had different results from ours [9, 13, 20, 23–25]. Results comparable to ours can be justified by the fact that these studies were carried out in Central Africa in the Congo Basin, the epicenter of sickle cell disease as in our case [4, 5, 22]. The results differing from ours can be explained by the fact that these screenings were carried out in non-endemic or low-malaria-endemic areas where sickle cell disease cases are mainly imported cases or from families with

Table 2: Distribution of abnormal hemoglobins (Hb) in NBs by department between November 2019 and March 2020.

Department	HbS, *n* (%)	Autre Hb*, *n* (%)	HbC, *n* (%)	HbE, *n* (%)	HbBart, *n* (%)	HbD, *n* (%)
Bouendza	15 (2.5)	2 (28.6)	—	—	—	1 (100)
Brazzaville	314 (52.6)	1 (14.3)	5 (100)	—	1 (100)	—
Cuvette Centrale	32 (5.4)	1 (14.3)	—	—	—	—
Cuvette-Ouest	18 (3.0)	—	—	—	—	—
Kouilou	4 (0.7)	—	—	—	—	—
Lekoumou	12 (2.0)	—	—	—	—	—
Likouala	6 (1.0)	—	—	—	—	—
Niari	46 (7.7)	2 (28.6)	—	—	—	—
Plateau	23 (3.9)	—	—	—	—	—
Pointe Noire	90 (15.1)	—	—	1 (100)	—	—
Pool	31 (5.2)	1 (14.3)	—	—	—	—
Sangha	6 (1.0)	—	—	—	—	—
Total	597 (100)	7(100)	5 (100)	1 (100)	1 (100)	1 (100)

n : number; *: unidentified Hb variant.

Table 3: Prevalence of sickle cell disease among newborns according to the departments of Congo between November 2019 and March 2020.

Department	Total, *n* (%)	Carrier Hb AA, *n* (%)	Carrier Hb AS/AC, *n* (%)	MSS*, *n* (%)
Bouendza	113 (3.90)	98 (86.7)	13 (11.5)	2 (1.77)
Brazzaville	1560 (53.8)	1241 (79.6)	298 (19.1)	21 (1.35)
Cuvette Centrale	124 (4.28)	92 (74.2)	32 (25.8)	—
Cuvette-Ouest	78 (2.69)	60 (76.9)	16 (20.5)	2 (2.56)
Kouilou	38 (1.31)	34 (89.5)	4 (10.5)	—
Lekoumou	54 (1.86)	42 (77.8)	12 (22.2)	—
Likouala	60 (2.07)	54 (90.0)	6 (10.0)	—
Niari	179 (6.18)	133 (74.3)	44 (24.6)	2 (1.12)
Plateau	121 (4.18)	98 (81.0)	22 (18.2)	1 (0.83)
Pointe Noire	370 (12.8)	280 (75.7)	81 (21.9)	9 (2.43)
Pool	132 (4.56)	101 (76.5)	29 (22.0)	2 (1.52)
Sangha	68 (2.35)	62 (91.2)	6 (8.82)	—
Total	2897 (100)	2295 (79.21)	563 (19.43)	39 (1.35)

*MSS: major sickle cell syndrome (FS, FSE, and FSA).

Table 4: Distribution of abnormal Hb chromatographic profiles in newborns according to departments between November 2019 and March 2020.

Department FAS*	Heterozygous profile	MSS profile			
		FAC, *n* (%)	FS, *n* (%)	FSA**, *n* (%)	FSE, *n* (%)
Bouendza	13 (100)	—	1 (50)	1 (50)	—
Brazzaville	293 (98.32)	5 (1.68)	8 (38.1)	13 (61.9)	—
Cuvette Centrale	32 (100)	—	—	—	—
Cuvette-Ouest	16 (100)	—	—	2 (100)	—
Kouilou	4 (100)	—	—	—	—
Lekoumou	12 (100)	—	—	—	—
Likouala	6 (100)	—	—	—	—
Niari	44 (100)	—	1 (50)	1 (50)	—
Plateau	22 (100)	—	—	1 (100)	—
Pointe Noire	81 (100)	—	4 (44.44)	4 (44.44)	1 (11.11)
Pool	29 (100)	—	—	2 (100)	—
Sangha	6 (100)	—	—	—	—
Total	558	5	14	24	1

*6 cases of unidentified Hb variant and 1 Hb FASD; **FSA and FSa profile.

relatives from highly endemic areas. This justifies the need for the establishment of a national program of systematic screening for sickle cell anemia in order to provide early management of newborns living with sickle cell anemia. Moreover, early management makes it possible not only to delay the onset of complications but also to undertake

genetic counseling of the parents of newborn babies living with sickle cell anemia [25–27].

Sickle cell trait prevalences of around 25% were obtained in some departments (Cuvette Centrale, Niari, and Pointe Noire) and 10% in others (Sangha, Likouala, Kouilou, and Bouendza). The low prevalence in these regions can be justified not only by their relatively small numbers compared to those of other departments but also by cultural habits regarding marriage.

The main abnormal Hb encountered was Hb S (97.71%) and C (0.82%). Variant C was found only in NBs with Malian parentage. This observation is similar to that made by some authors [6, 13, 14, 20, 24, 28], but differs from others in West Africa where Hb C predominates in most series 10. Indeed, Hb C is the most common abnormal Hb in West Africa and more particularly in Burkina Faso where it originated [29].

The prevalence of MSS found in our study was higher than that of our Congolese predecessors (1.35% versus 1%). The increase in prevalence is explained by the fact that 2010 study [4] was conducted in only two cities, whereas this study covered the entire country. Furthermore, the MSS profiles observed in our study were FS, FSE, and FSA, whereas they reported only one hemoglobin profile (FS). The type of equipment used in 2010 and the criteria used to define MSS could justify these differences.

In addition, MSS prevalences ranging from 0.06% to 3% have been reported in Europe, South America, Central Africa, and other countries. These variations could be justified not only by the fact that MSS is endemic in some regions while in others, it is mainly imported cases but also by the lack of knowledge of sickle cell disease in regions of high prevalence and the existence of consanguineous marriages [5–7, 9, 11, 12, 14, 15, 21, 23, 24, 28].

Similarly, the prevalence of MSS is heterogeneous within the departments of Congo. It predominates in some departments where its prevalence is higher than that observed at the national level (Table 3). Cultural habits in terms of choice of spouse, the lack of systematization of premarital screening, the mere availability of the Emmel test, and the lack of awareness in some departments could explain this disparity and, hence, the interest in setting up a capacity-building system for awareness raising, screening of couples at risk, and even genetic counseling in order to help reduce the prevalence of sickle cell disease. In addition, it will make it possible to initiate prophylaxis with penicillin therapy, which substantially reduces the morbidity and mortality of NBs [27, 30].

With regard to the sickle cell trait, as in the study by Mpemba et al., its national prevalence is 19.43%. It varies from one department to another (Table 3). In addition, we observed that the department of Cuvette Centrale, although it has a high prevalence of sickle cell trait, has fewer cases of MSS (Table 3). These facts could be justified by the health education of the population in the department. Because Cuvette Centrale is a department with an area of 48,250 km^2 and three general hospitals within a radius of 100 km, consanguineous marriage is prohibited. The prevalence of the sickle cell trait in our study is similar to that of some authors, who reported a prevalence of the sickle cell trait in a range of 16 to 21%. This could be justified by the fact that these countries belong to the Central African zone [4, 5, 14, 15]. In contrast, other authors in West and East Africa reported lower prevalences than ours (5 to 12%). This may be justified by the fact that these areas are far from the epicenter of sickle cell disease, which is in Central Africa [6, 10, 12, 13, 28]. Much lower prevalences (0.08 to 3.8%) are reported in European and American studies [9, 21, 23–25, 31].

In light of our observations, screening for sickle cell disease should also be systematic upstream during the premarital check-up in order to provide couples at risk with appropriate genetic counseling to guide their choice. However, it would be advisable to supplement this study with molecular biology techniques which would provide additional information on the possible presence of other haplotypes in Congo due to migration flows.

5. Conclusions

This work made it possible not only to update data on sickle cell disease in the neonatal period but also to provide epidemiological characteristics within each department of Congo. Prevalence remains stationary for the sickle cell trait, while it is increasing for major sickle cell syndromes. This resumes the frequency of homozygous and heterozygous forms of sickle cell disease in the Republic of Congo. The increase can be explained by the existence of consanguineous marriages and the absence of HbS screening tests in some regions, as well as the use of the Emmel test in other departments of Congo, although this test is less reliable The implementation of a national program leading to systematic neonatal screening will make it possible to identify and provide early care for newborns with homozygous forms of hemoglobin S, which is a guarantee of a better quality of life, by limiting infectious and vaso-occlusive complications. The study was conducted with the aim of making neonatal screening systematic. It is a project that needs funding to be sustainable.

Data Availability

Data supporting the results of this study can be found in the attached document.

Conflicts of Interest

The authors declare no conflicts of interest in the conduct of this study.

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