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Magnitude and Associated Factors of Anemia Among Type 2 Diabetes Mellitus Patients at Mizan Tepi University Teaching Hospital, South West Ethiopia

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Abstract

Background: Anemia among diabetic patients is a common public health problem that occurs as a complication and has a major impact on the overall health and survival. Although it is a major public health problem, there is limited research done on determinant factors.

Objective: This study was aimed to determine the magnitude and associated factors of anaemia among Type 2 Diabetic adult patients at Mizan Tepi University Teaching Hospital, southwest Ethiopia.

Methods: A hospital-based cross-sectional study was conducted on 272 adult Type 2 diabetic patients at Mizan Tepi University Teaching Hospital from September to December 2023. Data on socio-demographic, dietary, and clinical characteristics were collected using structured, pre-tested questionnaires. About 5 ml of venous blood was drawn from each participant into an EDTA test tube and analysed by Symiens ADVIA hematology analyzer for hemoglobin and erythrocytes. Blood film and stool examination were performed. Data was entered into EpiData version 4.6 and analysed by STATA statistical software version 14. Descriptive statistics and measures of association were computed for associated factors by multivariate logistic regression. The association was interpreted as a p-value < 0.05 to measure the association.

Results: The overall magnitude of anemia was 40.35 % (95 % CI 35.25, 46.15). Determinant factors that showed significant association with anemia, was duration of diabetes mellitus greater than five years (2.14(95 % CI, 1.85, 2.27), Malaria infection (4.5(95 % CI, 4.30, 4.72)), birth interval greater than two years (1.45 (95 % CI, 1.25, 1.64)), third trimester gestational age (1.73 (95 % CI, 1.18, 2.31)), being overweight 3.0 (95 % CI, 2.52, 3.49)) and underweight 2.14 (95 % CI, 1.65,2.45).

Conclusion and Recommendation: Anemia was a major public health problem among diabetes mellitus patients and showed a significant association with duration of diabetes mellitus greater than five years, malaria infection, birth interval greater than two years, third-trimester gestational age, being over and underweight. So, we recommend that diabetes mellitus patients should have their hemoglobin checked with continuous nutritional and lifestyle modification, contraceptives to increase birth interval, and use a vegetable-based diet.

keywords: Type 2 DM, anemia, low hemoglobin, red cell indices, infection

Introduction

Diabetes Mellitus (DM) is a metabolic disease characterized by persistently high blood sugar levels and abnormalities in protein, lipid, and carbohydrate metabolism brought on by abnormal insulin function or secretion [1]. The global burden of DM among adults has dramatically risen from 108 million in 1980 to 463 million, with 4.2 million deaths in 2019 [2]. Over the last 20 years, the number of persons with DM has doubled, with 80 % of those affected living in low- and middle-income nations, and 592million is estimated by 2035 [3]. Type 2 diabetes (T2DM) is linked to a variety of metabolic, cellular, and hematological abnormalities, all of which can contribute to vascular problems. T2DM is a part of the metabolic syndrome that comprises dyslipidaemia, obesity, hypertension, and derangement in hematological parameters. Changes in the function, shape, and metabolism of red blood cells (RBCs) are all seen in T2DM patients. When compared to non-diabetic individuals, these changes may emerge as immunological and coagulation difficulties, basically anemia characterized by a

decrease in RBC count, hemoglobin (Hgb), and hematocrit (HCT) levels [4]. The poor metabolic glycaemic control and lifestyle alteration results in derangement of total RBC, Hgb, variation in red cell indices, and RDW of T2DM patients, which can be commonly detected in diabetic patients. Obesity, dyslipidaemia, and major organ destruction are strongly associated with diabetic framework and significantly contribute to increasing the risk of cardiovascular diseases. The RBC count decrement is common in increased diabetic complications due to renal damage. For this reason, the routine RBC, Hgb, HCT, and red cell indices checking of patients with T2DM is important to prevent complications associated with aberrations in hematological values due to defect on iron absorption secondary to inflammation related to increased liver protein hepcidin and macrophagic lactoferrin [5]. Among hematological abnormalities, the most common one is anemia, which results from RBC membrane viscosity that could be common due to an increase in hyperglycaemia. They are more vulnerable to anemia due to increased risk of diabetic complications, including nephropathy, organ damage, and micro vascular problems. RBC breakdown in T2DM patients due to metabolic abnormality results in decreased Hgb, RBC, MCV, MCHC, MCH, and RDW. Especially, Vitamin B12 deficiency anemia is the most common in type 2 DM patients due to inappropriate consumption of metformin, and anemia of chronic disease is also common due to increased acute phase reactant proteins such as lactoferrin and hepcidin [6], [7]. The hematological abnormality that needs more attention for understanding prognosis, including RBC, Hgb, and hematocrit decrement, with high RDW, as predictors of inflammation and disease burden in T2DM. Because diabetes is complicated by accelerated atherosclerosis, and platelet activation plays a key role in inflammation, the RBC destruction becomes increased due to blood cell blockage, which contributes to the development of hemolytic anemia and or anemia of chronic disease in a patient with T2DM [8]. Even though the global study on anemia among diabetic patients has been intensively studied, there was no published study on anemia among DM patients in the study area, and there is limited information present concerning red cell indices variation among type 2 DM patients and its importance for the prediction of prognosis of disease and burden. Different studies have shown variation in the magnitude of anemia, but the limited information published concerning this has led to controversy in the literature. Additionally, red cell indices are not routinely tested for diabetic individuals, even though they are commonly affected [9]. In Ethiopia, diabetic patients rarely undergo regular assessments of red blood cell count, hemoglobin levels, and red cell indices, and studies on anemia among this group are limited. Determining the magnitude and associated factors of anemia is essential for preventing diabetes-related complications. Therefore, this study aims to assess the prevalence and determinants of anemia among individuals with type 2 diabetes mellitus attending Mizan Tepi University Teaching Hospital, Southwest Ethiopia.

Methods

Study area

The study was carried out at the Mizan Tepi University Teaching Hospital (MTUTH), which is 588 kilometres away from Addis Ababa capital of Ethiopia, and situated in the Mizan Aman town, Bench Sheko zone, Southwest Ethiopia, and is bounded by Gambella in the southwest, Omo zone in the south southwest, Sheka zone and Kafa zones in the north and northeast, and Sudan in the south. The hospital has about 106 beds. The total staff number is about 226, including physicians, nurses, midwives, and medical laboratory professionals. MTUTH hospital provides health services to over two million people who are around and in the town.

Study design and period

A cross-sectional study was carried out to determine the magnitude of anemia and associated factors among adult patients with type 2 DM at MTUTH in Southwest Ethiopia from September to December, 2023

Source population

All type 2 diabetic adults attending (on follow-up) at MTUTH, South West Ethiopia, 2023

Study population

All selected type 2 diabetic adults attending (on follow-up) at MTUTH, south west Ethiopia, during a time of data collection were used as the study population. Exclusion and Inclusion criteria

Inclusion and Exclusion Criteria

Inclusion criteria

All adults aged 18 years and above with confirmed type 2 diabetes mellitus who were on follow-up at MTUTH, Southwest Ethiopia.

Exclusion criteria

Individuals who were seriously ill or unable to participate and or unable to give blood samples due to critical health conditions were excluded.

Sample size determination

The required sample size for this study is determined using a single population proportion formula and by considering the following assumptions. The study conducted on Debre Birhan to show the magnitude of hematological abnormality gives anemia at 23.1 % [10]. The sample size can be calculated by using a 23.1 % proportion with a 95% confidence interval and 5% marginal error. The sample size (n) was determined using the following statistical formula.

$$n = \frac{z^2_{\alpha/2}\, p(1-p)}{d^2} \tag{1}$$

$$= \frac{(1.96)^2 \cdot 0.231(1-0.231)}{(0.05)^2} \tag{2}$$

$$= 273 \tag{3}$$

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Where n = sample size, Z = Z statistics for a level of confidence P = expected prevalence or proportion, d = margin of error between the sample and the population

Sampling technique

A systematic random sampling technique was used to select each study participant from the three-month average diabetic population (N=720) attending the follow-up. The interval of sample selection can be calculated by dividing the total estimated population within three months on follow-up (N) by sample size (n) (k=N/n=720/273=2.64 =3). The study subjects were selected every three intervals of Type 2 diabetic individuals who were attending for their follow-up. The data were also obtained from the records of diabetic patients at the hospitals over the last three months.

Study variables

Dependent variables:

• Presence of Anemia.

Independent variables:

Socio-demographic characteristics

• Age, sex, marital status, residency, occupation, and family size

Clinical variables

• Infections (Mycobacterium tuberculosis, malaria parasite, and intestinal parasite),

• Pregnancy, Recent blood loss, abortion history, Hypertension, and BMI

• Duration of diabetes and Types of medication

• Nutritional characteristics: - Eating meat and vegetables per week, drinking Coffee and tea

OPERATIONAL DEFINITIONS

Confirmed type 2 DM is a condition in which fasting plasma glucose $\geq$ 126 mg/dL, 2-hour postprandial glucose $\geq$ 200 mg/dL (11.1 mmol/L), glycated hemoglobin (HbA1c) $\geq$ 6.5% (48 mmol/mol), or random plasma glucose $\geq$ 200 mg/dL with classic symptoms.

Anemia is a condition in which the adjusted amount of Hgb is less than 12 g / dl for females and less than 13 g [11].

Mild anemia when Hgb level is between 11-12.9 g/dl for men, 11-11.9g/dl for non-pregnant women, and 10-10.9 g/dl for pregnant women [12], [13].

Moderate anemia when Hgb level is between 8-10.9 g/dl for men and non-pregnant women, and 7-9.9g/dl for pregnant women [12], [13].

Severe anemia when Hgb level is less than 8 g/dl for men, and non-pregnant women, and below 7 g/dl for pregnant women [12], [13].

Data collection procedure

Sociodemographic and clinical data collection

The data was collected by semi-structured questionnaires for socio-demographic information via face-to-face interviews. The clinical data such as BMI, duration of DM, Diabetes-related micro vascular complications, history of hypertension, and medication, were extracted manually or by computer from participants' records, and pregnancy was screened by human chorionic gonadotropin hormone (HCG) strip (Annex I, II).

Sample collection procedures and hematological analysis

Sample collection procedures

About 5ml of venous blood for hematological investigation was drawn from the study participant by an appropriate syringe with a needle for hematological analysis, blood film preparation, and in an EDTA anticoagulated test tube. From the collected blood sample, hematological analysis was performed, and then a blood film was prepared from the remnant sample (Annex V). A pea-sized stool sample was collected by a stool cup for parasitological examination by stool microscopy and sedimentation technique.

Hematological analysis

The hematological analysis was performed on a blood sample in an EDTA anti-coagulated test tube to confirm the presence of hematological abnormality by following the procedures. Hgb measurement, RBC count, and RBC indices such as MCV, MCH, and MCHC were performed by using the automated hematology analyzer Symiens ADVIA. RBC count on whole blood by impedance principle, and hemoglobin determination was done by spectrophotometric principles [14]. The blood sample, which is suspended in diluent, passes through the apparatus, causing direct current resistance. A change in blood cell size is detected as the electrical pulse, and blood cell count is measured by counting pulses (Annex V).

Blood film morphological examination

A thin blood smear was prepared by wedge method by putting a drop of blood from the remnant of blood used for complete blood count on the slide about 1-2cm from the end of the slide and making a smear by another smooth-edged slide as spreader at an approximate angle of 30o on three-four (¾) of the length of the slide. The prepared smear was stained by undiluted Wright stain, which was done by placing the air-dried smear film side up on a staining rack and flooding the Wright stain on it. The smear was covered with undiluted stain, left for 1 minute, washed, then dried and examined by using oil immersion (100 x) on the microscope. In the blood film, the presence of abnormal morphologies such as schistocytes, spherocyte, bite cells, and nucleated RBCs evidences haemolysis, and in the meantime a cross-check for morphology with analyzer result was performed (Annex V).

Stool examination for intestinal parasite detection

Stool examination was performed by direct saline wet mount of each stool sample, and for motile intestinal parasites, the formol-ether concentration procedure was performed. A drop was placed on a labelled, clean, dry 76 mm x 26 mm slide and covered with a 20 mm x 20 mm cover glass. The preparation was examined with a Primo Star light microscope.

Data quality management

Quality for Socio-demographic information

The data collectors received training to reduce technical and

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observer bias in order to guarantee the quality and reliability of the data. Before being utilized for actual data collection, the questionnaires were translated into the local tongue and pretested for validity and reliability on a random sample of patients from Mizan Health Center.

Quality control for hematology analyzer

Quality control for working equipment and reagents was ensured using standard controls as well as standard operational procedures, and for the Siemens ADVIA hematology analyzer, normal background reading. Also, the analyzer was checked daily by low, normal, and high controls for Hgb. The result of every test was properly recorded.

Quality control for microscopy

The microscope was cleaned daily for quality examination of blood smear morphology and reticulocyte count. A microscopic smear review was performed to check RBC morphology and to see the distribution. If there is doubt in the result or for an extremely out-of-range reticulocyte count, 500 cells are counted on slides for backup purposes of the analyzer. The result should agree within the range of $\pm 15\%$ then the sample should be rechecked if it does not match.

Data management and analysis

Data entry was entered into Epic-data version 4.6 (Epidata, Inc., Redwood City, CA, United States), and analysis was done by using STATA (Statistical Software for statistics and data science) statistical software version 14 developed by StataCorp for data. Every day, the collected data was checked for completeness and accuracy by the principal investigator. During the entry of data, it was cross-checked to ensure the right data was entered and cleaned for accuracy. Descriptive statistics such as frequency, charts, tables, and percentages were used to summarize the data. The multivariate logistic regression model was fitted to determine the associations of independent variables with outcome variables. The measure of association variable was analyzed by a multivariable logistic regression model, and those variables that have a P-value less than or equal to 0.25 were included in the multivariable logistic regression model to control the confounding factors. Then, a multivariable logistic regression was computed for selected variables, and the significance of the association was determined and interpreted. Both Crude odds ratio (COR) and adjusted odds ratio (AOR) with their corresponding 95% confidence interval (CI) were used to see the strength of association between dependent and independent variables. A p-value < 0.05 in a multivariable logistic regression model was considered statistically significant. The results were presented in words and tables. Based on the study results, conclusions and recommendations were drawn.

Results

A total of 273 study participants took part in this investigation. Women made up 183 (56.6%) of the study population and 194 (60%) of the residents; the remaining individuals were urban and rural, respectively (Table 1).A total of 273 study participants took part in this investigation. Women made up 183 (56.6%) of the study population and 194 (60%) of the residents; the remaining individuals were urban and rural, respectively (Table 1).

Intestinal parasite distribution among DM patients

The burden of intestinal parasites among DM patients was 33.70 % (92 out of 273). The most common parasite was A. lumbricoides (21.32 %), followed by S. mansoon (15.73%) (Figure 1).

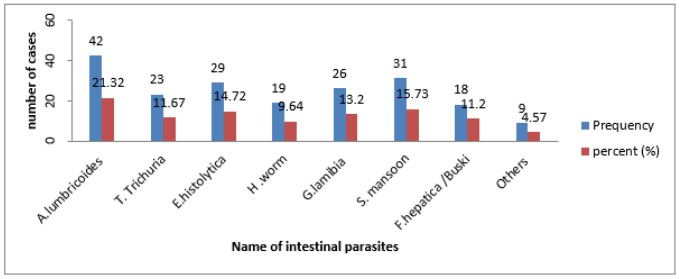


Fig. 1. Intestinal parasite distribution of participants for Magnitude and associated factors of anemia among Type 2 DM patients at MTUTH

Red cell indices parameters

The erythrocyte count, Hgb, and HCT showed a normal distribution, whereas the red cell indices were not normally distributed. The Hgb levels of the study participants ranged from 6.6 to 21 g/dL, with a mean of 13.9 ± 3.71 g/dL.

Magnitude of Anemia among type 2 Diabetic patients

The overall magnitude of anemia was 40.35 % (95 % CI 35.25, 46.15). Among patients with anemia, 44.55 %, 39.99 % and 15.46%, respectively, were mild, moderate, and severe anemia. On the morphology and red indices, the majority (57%) of the anemic individuals had microcytic hypochromic anemia (Figure 2).

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TABLE 1
SOCIO–DEMOGRAPHIC AND CLINICAL FACTORS OF STUDY PARTICIPANTS FOR MAGNITUDE AND ASSOCIATED FACTORS OF ANEMIA AMONG TYPE 2 DIABETIC PATIENTS AT MTUTH FROM SEPTEMBER TO DECEMBER 2023, SOUTH WEST ETHIOPIA

Variable	Response	Frequency (N)	Percentage (%)
5*Age	18–27	52	19.04
	28–36	47	17.20
	37–45	35	12.82
	45–65	72	26.40
	> 65	67	24.54
2*Sex	Male	118	43.34
	Female	155	56.66
2*Residency	Urban	164	60.00
	Rural	109	40.00
4*Marital status	Single	24	8.80
	Married	194	71.01
	Divorced	46	16.85
	Widowed	9	3.34
2*Occupation	Farmer	63	23.07
	Housewife	100	36.63
	Merchant	47	17.20
	Government employee	41	15.03
	Others	22	8.07
4*Educational status	No formal education	72	26.37
	Primary school	88	32.23
	Secondary school	74	27.11
	University/college	39	14.29
3*BMI	Normal	211	77.29
	Underweight	33	12.09
	Overweight	29	10.62
2*Intestinal parasite infection	Yes	92	33.89
	No	181	66.11
3*Family history of DM	Yes	103	37.73
	No	127	46.52
	Unknown	43	15.75
2*Malaria infection	Yes	222	81.50
	No	51	18.50
2*Pregnancy	Yes	57	36.60
	No	98	63.40
3*Gestational age	1st trimester	23	40.35
	2nd trimester	9	15.80
	3rd trimester	25	43.85
2*Autoimmune disease history	Yes	34	12.40
	No	239	87.60
2*TB infection	Yes	28	10.20
	No	245	89.80
2*History of abortion	Yes	25	15.85
	No	130	84.15
2*Abortion frequency	Once	14	55.17
	Twice	11	44.83
3*Type of DM medication	OHA	115	42.10
	Insulin	73	26.62
	Combination	85	31.28
2*Cigarette smoking	Yes	31	11.36
	No	242	88.64
2*Hypertension history	Yes	123	45.05
	No	150	54.95
2*History of blood loss	Yes	47	17.11
	No	226	82.89
3*Meat consumption	At least once per week	76	27.86
	Less often per week	166	60.80
	Never (¿1 year)	31	11.34
2*Green leafy vegetables	At least per day	117	42.86
	Less often per day	156	57.14
2*Alcohol consumption	Yes	126	46.15
	No	147	53.85
2*Coffee/tea after diet	Yes	195	71.43
	No	78	28.57

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TABLE 2
RED CELL INDICES OF ADULTS WITH T2DM PATIENTS ATTENDING AT MTUTH

Red cell indices	Range	Standard deviation	Median (IQR)
Erythrocyte (x10^{12}/L)	3.5–8.1	5.18 ± 2.1 x10^{9}/L	—
HCT (L/L)	0.28–0.52	0.37 ± 0.25	—
Hemoglobin (g/dL)	6.6–21	13.9 ± 3.71	—
MCV (fL)	56–110	—	76.5 (73.2–86.8)
MCH (pg)	23–34	—	33.76 (21.15–47.75)
MCHC (g/dL)	27–37	—	34.18 (24.00–45.00)
RDW (%)	11–22	—	19 (13–24)

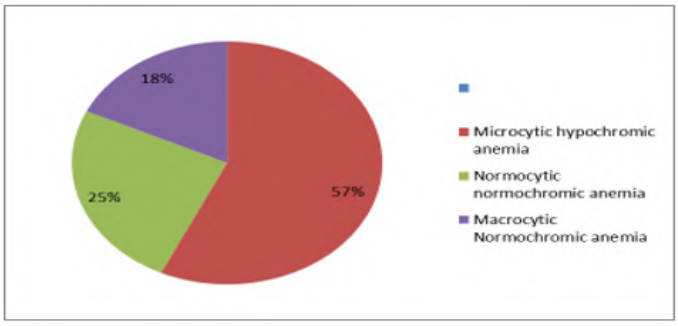


Fig. 2. Anemia distribution based on morphological classification among DM patients.

Factors associated with anemia

To determine the association between anemia, with the independent variable as a bi-variable, a multivariable logistic regression model was used. Based on the analysis, variables with a p-value less than 0.2 in the bivariable logistic regression model were included in the multivariable analysis. Finally, the association was interpreted at a p-value < 0.05 to measure the association. Accordingly, intestinal parasite infection, malaria infection, smoking cigarettes, using green leafy vegetables, birth interval greater than two years, duration of DM greater than five years, third trimester gestational age, being underweight, being overweght, and TB infection showed a significant association with anemia (Table 3).

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TABLE 3
BI-VARIABLE AND MULTI-VARIABLE LOGISTIC REGRESSION MODEL ANALYSIS OF FACTORS ASSOCIATED WITH ANEMIA AMONG PATIENTS WITH TYPE 2 DM AT MTUTH FROM SEPTEMBER TO DECEMBER 2023 (N=273)

Variables	Category	Yes	No	COR (95% CI)	AOR (95% CI)	P-value
5*Age	18–27	16 (30.78)	36 (69.32)	1	1	
	28–36	23 (48.94)	24 (50.14)	2.16 (0.95, 4.90)	1.30 (0.67, 1.86)	
	37–45	19 (54.30)	16 (45.70)	2.67 (1.09, 6.49)	1.72 (0.89, 2.43)	
	45–65	26 (36.12)	46 (63.88)	1.27 (0.59, 2.72)	0.94 (0.56, 1.34)	
	>65	22 (32.84)	45 (67.16)	1.10 (0.50, 2.40)	0.98 (0.54, 1.40)	0.09
2*Residence	Urban	75 (45.73)	89 (54.27)	1	1	
	Rural	41 (37.62)	68 (62.38)	0.72 (0.43, 1.17)	1.58 (0.59, 11.20)	0.84
2*Intestinal parasitic infection	Yes	53 (57.61)	39 (42.39)	2.89 (1.71, 4.84)	2.34 (1.89, 2.72)	0.03
	No	58 (32.05)	123 (67.96)	1	1	
3*Family history of DM	Yes	43 (41.75)	71 (58.25)	1.14 (0.67, 1.93)	1.76 (1.24, 2.25)	0.82
	No	44 (34.66)	83 (65.35)	1	1	
	Unknown	13 (30.24)	30 (69.76)	0.81 (0.38, 1.72)	1.17 (0.53, 1.34)	
2*Duration of DM	<5 years	58 (38.16)	94 (61.84)	1	1	
	>5 years	51 (42.15)	70 (57.85)	1.12 (0.72, 1.93)	2.14 (1.85, 2.27)	0.02
3*Type of medication	Insulin	32 (43.85)	41 (56.15)	1.77 (0.92, 3.40)	2.14 (1.89, 2.45)	0.02
	OHA	48 (41.74)	67 (58.26)	1.62 (0.89, 2.93)	2.10 (0.92, 2.34)	0.32
	Both	26 (30.59)	59 (69.41)	1	1	
2*Malaria infection	Yes	92 (41.45)	130 (58.55)	1.30 (0.69, 4.12)	4.50 (4.30, 4.72)	0.01
	No	18 (35.30)	33 (64.70)	1	1	
2*Cigarette smoking	Yes	15 (48.40)	16 (51.60)	1.58 (0.75, 3.35)	1.95 (1.75, 2.15)	0.006
	No	90 (37.20)	152 (62.80)	1	1	
3*Meat consumption	$\geq$ once/week	16 (21.06)	50 (78.84)	1	1	
	Less often	68 (40.97)	98 (59.03)	2.14 (1.14, 4.12)	0.98 (0.19, 5.00)	
	Never	18 (58.07)	14 (41.93)	4.01 (1.63, 9.85)	3.18 (0.60, 7.24)	0.25
2*Green leafy vegetables	$\geq$ once/day	43 (36.76)	74 (63.24)	1	1	
	Less often	67 (42.95)	89 (57.06)	1.30 (0.79, 2.11)	1.60 (0.84, 1.54)	0.003
2*Birth interval	<2 years	30 (31.92)	64 (68.08)	1.15 (0.58, 2.26)	1.45 (1.25, 1.64)	0.004
	>2 years	20 (29.00)	49 (71.00)	1	1	
2*Pregnancy	Yes	26 (45.62)	31 (54.39)	1.34 (0.71, 2.68)	1.56 (0.95, 1.83)	
	No	37 (31.63)	61 (68.37)	1	1	0.34
3*Gestational age	1st trimester	11 (47.82)	14 (60.87)	1	1	
	2nd trimester	4 (44.44)	5 (55.56)	1.02 (0.22, 4.71)	0.98 (0.88, 1.33)	0.98
	3rd trimester	9 (36.00)	16 (64.00)	0.72 (0.23, 2.23)	1.73 (1.18, 2.31)	0.02
2*History of abortion	Yes	11 (44.00)	14 (56.00)	2.57 (1.07, 6.17)	3.00 (1.13, 4.26)	0.04
	No	36 (27.70)	118 (72.30)	1	1	
2*Alcohol consumption	Yes	60 (47.65)	66 (52.35)	1.76 (1.08, 2.87)	1.87 (0.85, 1.50)	0.02
	No	50 (34.02)	97 (65.98)	1	1	
3*BMI	Normal (18.9–25.9)	57 (27.02)	154 (72.98)	1	1	
	Underweight (<18.9)	16 (48.48)	17 (51.52)	2.54 (1.20, 5.37)	2.14 (1.65, 2.45)	0.02
	Overweight (>25.9)	9 (31.05)	20 (68.95)	1.21 (0.52, 2.82)	3.00 (2.52, 3.49)	0.01
2*TB infection	Yes	13 (46.42)	15 (53.58)	3.06 (1.37, 6.83)	4.00 (2.82, 3.34)	0.04
	No	54 (22.05)	191 (77.96)	1	1	

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Discussion

Anemia is a major problem among type 2 diabetic individuals as it affects different metabolic activities and changes in the function, shape, and metabolism of RBCs. In type 2 DM patients, the most commonly observed hematological abnormalities were a decrease in RBC count, Hgb, and Hct levels, which defines anemia [15]. The overall prevalence of anemia was 40.35 % (95 % CI 35.25, 46.15). The present study indicated that the overall prevalence of anemia was 40.35 % (95 % CI 35.25, 45.35). The finding this study was comparable with the finding of studies done in Nigeria (45.2) [15], Cameroon (41.4 %) [5], Russia (38.8%) [16], Hapur [India] (40%) [17] and Punjab [Pakistan] (39 %) [18] but lower than study done in Karachi [Pakistan] (63%) [19], Saudi (55.5%) [20], New Delhi [India] [21], Ghana (Ghana) [22] . The lower magnitude in our study compared to others might be due to dietary practices, such as the consumption of iron-rich foods like red teff (bread/injera) and meat, which also contains a high amount of easily absorbable heme iron. The magnitude of anemia in this study is higher than study done in Iran (21.5%) [23], Delhi [India] (16.8%) [24], Gujarat [India] (18%) [25], Ayder [Ethiopia] (17.9%) [10], Debre Tabor [Ethiopia] (29.81) [7], Debre Birhan [Ethiopia] (20.1%) [26], Bale [Ethiopia] (18.1%) [27], [Harar] [Ethiopia] (34.8%) [28], and Finote Selam [Ethiopia] [29]. The possible reason for this higher burden could be the existence of malaria epidemics in this study area, which results in hemolytic anemia that might be immune-mediated or non-immune-mediated (direct destruction of the RBCs by the parasite) by infecting red blood cells of the study population [30]. Among anemic patients 45%, 39% and 26 % of them, respectively, were mild, moderate, and severe anemia. The finding of this study is consistent with the finding of study done in Harar [Ethiopia] which stated that 65%, 26.5% and 8.2% respectively were mild, moderate and severe anemia [31], this finding was also consistent with study done at Tehran [Iran] [32] and Hapur [India] [17]. The finding indicated that Intestinal parasitic infection (2.34 (95 % CI, 1.89, 2.72)), duration of DM greater than five year (2.14(95 % CI,1.85,2.27), Malaria infection (4.5 (95 % CI, 4.30, 4.72)), smoking of cigarette (1.95 (95 % CI,1.75, 2.15)), birth interval greater than two year (1.45 (95 % CI, 1.25, 1.64)), third trimester gestational age (1.73 (95 % CI, 1.18, 2.31)), being underweight (2.14 (95 % CI, 1.65, 2.45)), being overweight (3.0 (95 % CI, 2.52,3.49)) and TB infection 3.0(95 % CI, 2.82,3.34) shown significant association with anemia among DM patients. The results of this study show that patients with diabetes mellitus who have had the disease for more than five years are twice as likely to develop anemia (2.14 (95 % CI, 1.85, 2.27)) as those who have had it for less than five years. The findings of this study were in agreement with studies done in Iraq [33], United kingdom [34], Finote selam [Ethiopia] [29], Ambo [Ethiopia] [35], and Debre Birhan [Ethiopia] [26]. The possible reason might be that in long-term diabetes mellitus, there is formation of inflammation due to this long-term illness, which increases acute phase reactant proteins such as hepcidin, which are important for regulation and increased storage of iron (ferritin). The increment of this protein (hepcidin) results in decreased release of iron from macrophages and enterocytes finally results in anemia (anemia of chronic disease) [36]. As a finding of this study, type 2 diabetic patients with intestinal parasitic infection (2.34 (95 % CI, 1.89, 2.72)), more likely to develop anemia than those individuals without intestinal parasite infection. This study finding was in corroboration with studies done in Debre Tabor [Ethiopia] [7], Debre Birhan [Ethiopia] [26], Laso Liben [Ethiopia] [37], and Benin [Nigeria] [38]. The possible reason for this association might be the parasites, which undergo heart-lung migration, resulting in hemophage or blood loss at the site of attachment in the adult worm, which then increases the burden of anemia. This parasite infection is a common symptom of many helminths, and the most common hookworm infection [6]. The findings of this study indicated that individuals who were infected with the malaria parasite were 4.5 times more likely to develop anemia than uninfected individuals. The finding of this study was supported by the finding of a study done in Baso Liben [Ethiopia] [37] and Najo [Ethiopia] [39]. The possible reason might be that the malaria parasite results in hemolytic anemia, either immune-mediated or non-immune-mediated. During non-immune mediated hemolytic anemia, which can happen when plasmodium species infects RBC and breaks down, resulting in shistocytosis or burr cells, which are unable to carry oxygen and finally cause tissue hypoxia (anemia). In addition to parasitic infection, the presence of antimalarial medication such as artemisinin-based therapy (artesunate) results in anemia, which occurs after 7 to 30 days of initiation and results in post-artesunate or artemisinin delayed haemolysis, as the study area was a malaria-endemic area [40]. This study pointed out that diabetic Patients (women) with a birth interval of less than two years were 1.5 times more likely to develop anemia than those who had a birth interval greater than two years. The finding of this study was supported by a study done in China [41] , and Harar, Ethiopia [42]. The reason for this finding might be that in continuous birth, there might be continuous bleeding (blood loss during delivery), which results in decreased iron stores in women due to the occurrence of frequent delivery, which may need surgery (caesarean section) [43]. According to this study, participants who were overweight ($BMI > 25.9$) are three times more likely to develop anemia than those of normal weight and under individuals. The finding of this study was supported by studies done in Worabe [Ethiopia] [44] and Tehran [Iran] [45]. The possible reason for this variation is increased hepcidin level due to inflammation, which acts as a negative regulator of intestinal iron absorption, inhibiting iron transportation and resulting in anemia of chronic disease [25], [46]. As the finding of this study, pregnant women with type 2 DM in the third trimester of gestational age were 1.73 times more likely to develop anemia than others. This study finding was supported by the findings of studies done in Harar [Ethiopia] [42], Pawi [Ethiopia] [47], and China [41]. The possible reason

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for this decrement was due to physiological haemodilution in pregnancy, especially in the third trimester, which results in alterations of hematological parameters, particularly in a reduction of Hgb concentration [7], [48]. The findings of this study indicated that diabetic patients who had a history of smoking were twice as at risk of anemia as those who had no smoking history. The finding of this study was supported by a study done in India [25]. This may occur because smoking reduces vitamin C levels, which are essential for iron absorption, leading to lower Hgb. While smoking can increase hemoglobin through carbon monoxide induced hypoxia and potentially cause secondary polycythaemia, chronic smoking also triggers oxidative stress, inflammation, and impaired erythropoiesis. Additionally, smoking hampers iron absorption, which can result in deficiencies of folate and vitamin B12 both crucial for red blood cell production. Another contributing factor could be iron deficiency anemia caused by damage to the lining of the stomach and intestines [7]. This study revealed that diabetic patients who were infected with TB are 1.4 times more likely to develop anemia than uninfected patients. The finding of this study is supported by a study done in Quetta, Pakistan [49] and India [50]. The possible reason was that chronic TB infection results in anemia of chronic disease due to inflammation, due to the presence of macrophagic lactoferrin as well as increased hepcidin, and it also affects stem cells, finally resulting in anemia (aplastic anemia) [48]. The findings of this study indicated that diabetic patients who take Insulin were 2.14 times more likely to develop anemia than those who take insulin and a combined dose. This might be due to the presence of adverse effects related to their use, including hypoglycaemia, weight gain, gastrointestinal disturbance, lactic acidosis, and fluid retention, which can cause blood loss and finally result in anemia [46].

Strengths and limitations of the study

Limitations of the study

Since this study is a cross-sectional study design, it may not provide full information on cause and effect or the temporal relationship between cause and effect. It does not allow us to observe causality in the relationship between each studied anemia and its determinant factors, as it is a temporal association. Additionally, we did not classified the subtypes of oral hypoglycaemic agents (OHA) as independent predictors and we did not performed association with dosage and treatment duration

Conlusion and Recommendatiion

The magnitude of anemia was 40.35% which indicates, it is a major public health problem among type 2 Diabetic patients. The determinant factors associated with anemia were intestinal parasitic infection, duration of DM greater than five years, malaria infection, smoking of cigarettes, birth interval greater than two years, third trimester gestational age, being underweight, being overweight, and TB infection.

Recommendatiion

Anemia among DM Patients is a multifactorial problem with different determinant factors, including intestinal parasitic infection, duration of DM greater than five years, malaria infection, smoking of cigarettes, birth interval greater than two years, third trimester gestational age, TB infection, being underweight, and overweight. So, we recommend that DM patients should have their hemoglobin checked with continuous nutritional (use green leafy vegetables) and lifestyle modification, such as not smoking cigarettes, using contraceptives to increase birth interval, and using a vegetable-based diet. Health care workers who are working on chronic disease especial DM patients should consider hematological abnormalities for diabetic patients and perform laboratory investigation of hemoglobin and red cell indices with respective to history of their patients for determinant factors such as intestinal parasitic infection, duration of DM greater than five year, malaria infection, smoking of Cigarette, birth interval greater than two year, third trimester gestational age, being underweight, being overweght, and TB infection. The governmental and non-governmental organizations working on non-communicable diseases, especially DM should consider anemia and the factors that are associated with hematological abnormalities. Finally, we recommend that further multi-institutional studies be done to investigate anemia in a large population to increase the representativeness of the findings.

Abbreviation /Acronomy

AGE: Advanced Glycation End products, **CVD:** Cardiovascular Disease, **FBS:** Fasting Blood Sugar, **HCT:** Hematocrit, **Hgb:** Hemoglobin, **IDF:** International diabetes federation, **MCH:** Mean Cell Hemoglobin , **MCHC:** Mean Cell Hemoglobin Concentration, **MCV:** Mean Cell Volume , **MPV:** Mean Platelet Value, **RBCs:** Red Blood Cell, **RDW:** Red Cell Distribution Width, **OHA:** Oral Hypoglycaemic agents, **ROS:** Reactive Oxygen Species, **DM:** Diabetes Mellitus, **EBV:** Ethiopian Birr virus

Declarations

Availability of Data and Materials

All relevant data are available within the manuscript. In case of need, the data that support the findings of this study are available from the corresponding author on reasonable request.

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Ethical Approval

The study was conducted after ethical clearance was obtained from the Mizan Tepi University, College of Medicine and Health Sciences Ethical Review Committee (Reference number CHS/2968/2023) in accordance with the Helsinki Declaration. After taking permission from hospital management, the data collection was started from volunteer participants after a brief explanation of the procedure and its purposes.

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Participants' permission was taken by informed consent or assent, then sociodemographic data, clinical data, and samples were collected for research. Code rather than names of respondents was used during data collection time. From those participants who have hematological abnormalities were linked to clinicians with their laboratory results for additional diagnosis and treatment of disease Consent to participate: the study was done after consent or permission from study participants.
Consent to publish: Not applicable
Clinical trial: Not applicable

Author's contribution

The manuscript contains all pertinent information. The corresponding author can provide the data supporting the findings of the research upon reasonable request, if necessary. The proposal was written by SS, GM, TD, WA, and AA. SS, GM, TD, FD, WA, and AA written draft of the research and took part in the data gathering and analysis. SS, FD, WA, and AA edited and provided feedback on the article, analysis, and design. In addition to designing and supervising data quality, GM, AA, TD, FD, and SS also took part in the analysis and wrote the manuscript. The manuscript was reviewed, modified, and approved by SS, GM, FD, and AA. SS oversaw the manuscript's composition and conducted the data analysis. The manuscript's final draft was approved by all authors. This article's first author is SS.

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