HARAMAYA UNIVERSITY
Directorate of Graduate Studies

MAGNITUDE AND ASSOCIATED FACTORS OF IRON DEFICIENCY ANEMIA AMONG ADULT DIABETIC PATIENTSATTENDING GELEMSO GENERAL HOSPITAL, EASTERN ETHIOPIA

MPH THESIS

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Magnitudeand Associated factors of Iron Deficiency Anemia among Adult Diabetic patientsAttending Gelemso General Hospital, Eastern Ethiopia

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APPROVAL SHEET

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I hereby certify that I have read and evaluated this Thesis entitled Magnitude and Associated factors of Iron deficiency Anemia among Adult Diabetic patientsattending Gelemso General Hospital, Eastern Ethiopia prepared under my guidance by **Teshome Tujuba**. I recommended that it be submitted as fulfilling the thesis requirement.

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As a member of the Board of Examinners of the (_____), Thesis Open Defence Examination, I certify that I have read and evaluated the Thesis prepared by **Teshome Tujuba** and examined the candidate. I recommend that the thesis be accepted as fulfilling the Thesis requirements for the degree of Masters of Public Health.

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Final approval and acceptance of the Thesis is contigent upon the submission of its final copy to the Council of Graduate Study (CGS) through the candidate's department or school graduate committee (DGC or SGC)

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ABBREVIATIONS AND ACRONYMS

BMI	Body Mass Index
BP	Blood Pressure
CKD	Chronic Kidney Disease
DM	Diabetes Mellitus
eGFR	estimated Glomular Filtration Rate
EPO	Erythropotin
EDTA	Ethylenediamine tetra-acetic acid
FBS	Fasting Blood Sugar
GFR	Glomular Filtration Rate
Hb	Hemoglobin
IDA	Iron Deficiency Anemia
RBC	Red Blood Cell
RBS	Random Blood Sugar
SPSS	Statistical Package for Social Science
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

ABSTRACT

Background: Anemia is a common complication of diabetes mellitus (DM), intensifying morbidity and mortality. Despite this fact, most of diabetic patients in our community are rarely tested for anemia and little is known about its magnitude and associated factors.

Objective:the aim of this study was toassess the magnitude and associated factors of iron deficiency anemia among adult diabetic patients attending Gelemso General Hospital, West Hararghe, Oromia regional state, Eastern Ethiopia, fromMay 20 to August10, 2020.

Methods:A quantitative cross-sectional study was conducted among 325 randomly selected adult diabetes mellitus patients. A pretested and structured questionnaire wasused to collect information on socio-demographics, nutritional andbehavioral factors.The data werecoded and entered into Epidata version 3.1 and exported to Statistical Package for Social Science(SPSS)version 22for analysis. Data were described using frequency, percentage, tables and figures. Binary logistic regression analysis was performed to identify factors associated with anemia. Strength of association was measured using odds ratio and 95% confidence interval, and P-value less than 0.05 considered as statistically significant.

Result: The magnitude of anemia among the study participants was 30.2%(95% confidence interval (CI): 25%-35%). The magnitude was higher in males than females (36% vs 20.5%). Male gender (Adjusted Odds Ratio (AOR) = 2.2, 95% CI: 1.2, 4.1), DM duration of five years and above(AOR= 2.3, 95% CI: 1.3, 4.2), taking oral hypoglycemic agent (AOR= 4.5, 95%CI: 1.5, 13.3), patients with type IIDM (AOR = 6.0, 95% CI: 1.9, 19.5)and havingdiabetic complication (AOR=2.7, 95% CI: 1.5, 4.8) were significantly associated with anemia.

Conclusion: Anemia was a moderate public heath problem among adult DM patients in the current study. Patients with DM complication, being male in gender, oral medications, type II DM and longer duration of DM were likely to have anemia. Therefore, routine screening and proper treatment for anemia in all diabetic patients in health institutions aid in improving their quality of life. Regular monitoring and proper managementof various complications of DMalso helps in reducing the occurrence of anemiain diabetic patients.

Keywords: Anemia, Adult, Diabetes Mellitus, Prevalence, Gelemso, Ethiopia

1. INTRODUCTION

1.1. Background

Anemia is a reduction of the total circulating red blood mass below normal limit(Sharif et al., 2014). According to World Health Organization (WHO), it is defined as hemoglobin (Hb) level less than 13g/dl for males and less than 12 g/dl for females(Abate et al., 2013). Anemia reduces oxygen-carrying capacity of blood, leading tissue hypoxia(Panda and Ambade, 2018),which results in inadequate to meet the physiologic needs of the human body (AlDallal and Jena, 2018).

Diabetes mellitus (DM) is a common metabolic disease(Rathod et al., 2016). Diabetes mellitus is classified as type-1 diabetes mellitus (Juvenile diabetes or insulin dependent diabetes) that results from autoimmune destruction of insulin producing beta cells of pancreas and type-2 diabetes mellitus (non insulin dependent diabetes) that results from insulin resistance, a condition in which cells fail to properly use insulin(Sharif et al., 2014).

Anemia is a common condition in patients with diabetes(Gulati and Agrawal, 2016), which is attributable to the relative decrease in erythropoietin(EPO) production by the kidneys, absolute or functional iron deficiency and shortened red cell surviva(Kuo et al., 2016).The most common type of anemia in diabetic patients isiron deficiency anemia (IDA)(Antwi-Bafour et al., 2016) which accounts for half of anemia(Nasli-Esfahani et al., 2017).Anemia is generally associated with the severity of renal insufficiency(Loutradis et al., 2016).

The cause of anemia in diabetes is multifactorial and includes inflammation, drugs, nutritional deficiency, kidney disease and concomitant autoimmune disorders(Rajagopal et al., 2018).Blood loss resulting from menstruation, parasitic infestation such as Hookworm, Ascaris, schistosomiasis and Malaria, Cancer, Tuberculosis, and HIV can also contribute to the burden of anemia(Antwi-Bafour et al., 2016).

Anemiacauses physical and mental impairments in diabetic patients such as malaise, fatigue, weakness, dyspnea, impaired cognition and other symptoms(Mehdi and Toto, 2009).It is an independent predictor of adverse outcomes such as poor quality of life, decreased physical

function, impaired cognition, mortality, increased disability and hospitalization(Trevest et al., 2014).

Anemia is one of the world's most common preventable conditions, yet it is often overlooked,especially in people with diabetes mellitus(Singh et al., 2009). Currently, screening for anemia among diabetic patients at the public health care settings is not standard of practice. Due to financial limitation, asymptomatic patients and those without overt bleeding problems and alarming symptoms would be normally prescribed with iron supplements. If there is no improvement with this therapy, they will be subjected to further investigations to ascertain the etiology of the anemia(Idris et al., 2018).

1.2. Statement of the Problem

Anemia is a major public health problem affecting both developing and developed countries, with major consequence on human health as well as social and economic development(Mehdi and Toto, 2009). Globally,it is estimated that 1.62 billion people are anemic, corresponding to 24.8% of the global population(Antwi-Bafour et al., 2016).

Anemia is a common complication in patients with diabetes mellitus (DM), particularly in those with overt nephropathy or renal impairment and is estimated to be nearly two to three times higher than that ofpatients without diabetes, putting these patients at greater risk of complications associated with dysfunctional glomerular filtration rate and iron storage (He et al., 2015). The magnitudeof anemia in diabetic patients was reported as 14-48% worldwide (Baisakhiya et al., 2017).

Anemia in patients with diabetes mostly develops in relation to progression of chronic kidney disease (CKD) and is more severe than those who have no diabetes(Sudchada et al., 2013). Additionally, the risk of anemia is higher in people with diabetic nephropathy compared with people with nephropathy from other causes and is associated with a more rapid decline in the GFR(Al-Salman, 2015).

Anemia is associated with an increased risk of micro-vascular complication such as nephropathy, retinopathy, neuropathy, impaired wound healing, and macro-vascular disease(Thambiah et al.,

2015), which has a negative impact on the quality of life and additional burden on the health of the patients(Al-Salman, 2015).

Despite these facts, anemia is unrecognized and untreated in 25% of the diabetic patients(Al-Salman, 2015), because both share similar symptoms such as lethargy, pale skin, chest pain, irritability, numbness or coldness in the hands and feet, tachycardia, shortness of breath and headache(Sharif et al., 2014).Therefore, early recognition and treatment of anemia in the diabetic population decreases morbidity and mortality and leads to better quality of life of diabetic patients(Baisakhiya et al., 2017).

Although it is known that anemia is common in patients with diabetes, only few studies have been conducted regarding the magnitude and factors associated with anemia among diabetic patients in Ethiopia.Hence, the present study aimed to assess the magnitudeof anemia and associated factors among adult diabetic patients attending Gelemso General Hospital, West Haraghe, EasternEthiopia.

1.3. Significance of the Study

This study attempted to provide information on the magnitude and factors associated with anemia amongadult diabetic patients for health facilities in West Hararghe, and its findings will be useful for West Hararghe Zone health office and Gelemso Hospital. Besides, it serves as a baseline information for further researchers in this area.

1.4. Objective

1.4.1. General Objective

- To assess the magnitude and associated factors of iron deficiency anemia among adult diabetic patients attending Gelemso General Hospital, Eastern Ethiopia, from May 20 to August 10, 2020.

1.4.2. Specific Objectives

- To determine the magnitude of anemia among diabetic patients.
- To identify factors associated with anemia among diabetic patients.

2. LITERATURE REVIEW

2.1.Magnitude ofAnemia amongAdult Diabetic Patients

Based on the cross sectional studies conducted at health facilities have shown that anemia magnitude was 31.7% among 808 Malaysian adult patients with DM and CKD (Idris et al., 2018), 30.4% among 94 Iranian diabetic patients (Hosseini et al., 2014) and 29.7%among 19059 diabetic patients inKuwait(AlDallal and Jena, 2018) in 2013, 2012 and 2017 respectively.

In 2011, studydone in China among 6325 Chinese type 2 diabetic patients showed anemia with magnitude of 22.8% (Chen et al., 2013).Another studies conducted among 354 type 2 diabetic patients in Australia reported magnitude of anemia was 24% (Wee and Anpalahan, 2019)and 18% in India among 54 diabetic patients(Panda and Ambade, 2018) in 2019 and 2017 respectively.

Studies conducted among 247diabetic patients in Thailand showed higher magnitude of anemia with 49.4% in 2010 (Sudchada et al., 2013) and 55.5% among 227 patients with diabetes in Saudi Arabia(Al-Salman, 2015) in 2015. In Pakistan, a study conducted among 200 diabetic patients in 2014 showed 63% of patients with diabetes had anemia(Sharif et al., 2014).

Anemia magnitude among diabetic patients varies from one country to another in region of Africa. A cross-sectional study done in Cameroon on 636 diabetic patientsat the Douala General Hospital in 2013 reported magnitude of anemia was found to be 41.4% (Feteh et al., 2016) and 45.2%among 15 DM in Nigeria in 2019 (Awofisoye et al., 2019). A similar study conducted in Libiya among 275 diabetic patients at Murzuk general hospital and Brack general hospital estimated anemia magnitude at 31% and 36% of patients with type 1 DM and type 2 DM, respectively(Almahdi et al., 2016).

According to institutional -based cross-sectional study conducted from February to April 2012 among 384 DM patients in Ethiopia, at Fenote Selam Hospital,west Gojam showed anemia magnitude was 19%(Abate et al., 2013). Study conducted in Ethiopia among 412 diabetics at

Dessie referral hospital from January to April 2018 showed magnitude of anemia was 26%(Fiseha et al., 2019).

2.2.Determinants of Anemia among Adult Diabetic Patients

2.2.1.Socio-demographic Factors

A cross-sectional studyconducted among 808 diabetic patients in Malaysia indicated anemia magnitude was significantly associated with old age (OR= 1.04, 95% CI 1.01 to 1.06) and women (OR=1.57, 95% CI 1.12 to 2.21)(Idris et al., 2018).

A study conducted among 1414 DM patients in India reported the magnitude of anemia was higher in age more than 69 years (OR=2.49, 95% CI 1.44 to 4.30)(Ranil et al., 2010). Other study in China showed old age (OR=1.83, 95%CI 1.522-2.196) and female (OR = 1.05 95% CI 1.045-1.059,) were significantly associated with anemia in diabetic patients (Chen et al., 2013).

A cross-sectional study conducted in Kuwait from January 2016 to December 2017 among a total of 19,059 diabetic patients showed 21.6% of males and 38.5% of females were found to be anemic. There was a significant relation between magnitude of anemia and gender, i.e. the magnitude of anemia was significantly greater in diabetic females than diabetic males ($P < 0.05$) (AlDallal and Jena, 2018). Also, study from Pakistan indicated there was higher incidence and risk of anemia in females (36%) as compared to males (27%) ($p < 0.05$)(Sharif et al., 2014).

The study conducted in Nigeria among 72 DM patients also indicated old age(>60years) was associated with the incidence of anemia in type-2 diabetics (OR= 4.13, 95% CI 1.03 - 16.49) where as sexes were not statistically significant (Awofisoye et al., 2019). Other study from UK reported older age (>60 year) (OR= 4.6, 95% CI 1.9–8.1) associated with occurance of anemia. However, there was no significant difference between sexes (Trevest et al., 2014).

In Ethiopia, hospital-based cross-sectional study was conducted from May to September 2018 among 251 CKD patients indicated anemia was significantly prevalent in rural residence(AOR=2.75, 95% CI 1.34–5.65) (Adera et al., 2019). A similar study conducted in

Ethiopia reported old age >60 years(AOR = 2.41, 95% CI 1.11–5.21) was independently associated with the presence of anemia(Fiseha et al., 2019).

2.2.2.Behavioral and MedicalFactors

According to a study conducted in Greece among 184 diabetic patients in 2015, the advancing stage of CKD was associated with progressively increasing risk for anemia development; *i.e.*, Stage 3a (OR = 6.068, 95%CI: 2.112-17.430), Stage 3b (OR = 7.499, 95% CI: 2.604-21.597), Stage 4 (OR= 12.169, 95%CI: 3.783-39.147) were associated with occurrence of anemia. With regards to other existing comorbidities and smoking no significant correlations were observed(Loutradis et al., 2016).

Another cross-sectional conducted among 200 Type2 DM patients in Pakistan reported the magnitude of anemia in diabetic patients was significantly associated with glycemic control. It revealed that the incidence of anemia were higher in patients with poorly controlled (49.5%) diabetes than controlled diabetes(13.5%) ($p < 0.05$)(Sharif et al., 2014). The same study conducted in Kuwait from January 1, 2016 to December 31, 2017 among a total of 19,059 DM patients reported 27.9% well controlled diabetics; whereas 33.46% patients of poorly controlled diabetic group had anemia. It indicated magnitude of anemia was significantly greater in poorly controlled diabetics than those with glycemic status under control ($P < 0.05$) (AlDallal and Jena, 2018).

Similar cross-sectional study conducted in UK among 142 diabetic patients in 2014 showed that anemia in diabetes was significantly associated with longer duration of diabetes(>15 years) (OR= 2.9, 95% CI 1.2–6.9). Where as number of comorbidities and diabetes control were not significant (Trevest et al., 2014). But, a similar study conducted among 305 DM patients in Iraq at Layla Qasim diabetic center showed a positive correlation of lower GFR and duration of diabetes (>10 years) with anemia (OR= 0.96, 95% CI, 0.92-1.00) and (OR=1.7, 95% CI, 1.20-2.00) respectively (Abdulqadir and Polus, 2014).

A survey study conducted among 247 DM patients in Thailand found that 49.4% of these individuals had anemia. A regression analysis showed significant correlations between anemia

and lower eGFR (p = 0.019)(Sudchada et al., 2013). In contrast to this finding, study of anemia among diabetic patients in Brazil have failed to show any evidence of significant association of lower eGFR as being a risk factor of anemia(Barbieri et al., 2015).

A cross-sectional study performed in Nigeria in 2012 among patients with DM revealed the odds of developing anemia was higher in patients with poorly controlled diabetes, HbA1c > 7.5% compared to those with controlled diabetes ≤7.5% (Adejumo et al., 2012).

The study conducted among 636 diabetic patients in Cameroon in 2016 reported the magnitude of anemia with deteriorating kidney function, although up to 31.9 % of patients with normal kidney function had anemia. Patients with anemia had lower mean eGFR than patients without anemia (72.9 ± 35.7 vs. 89.6 ± 29.5 ml/min/1.73 m^2, p < 0.001), and the Pearson correlation coefficient between the eGFR and the hemoglobin level was 0.29 (p < 0.001). Compared with patients with normal eGFR, patients with CKD (eGFR < 90 ml/min/1.73 m2) were about two times more likely to have anemia. Known duration of diabetes, systolic and diastolic blood pressure levels and serum creatinine were inversely correlated with hemoglobin levels. Lower eGFR (OR= 1.01, 95% CI 1.00–1.02) was independent determinants of anemia(Feteh et al., 2016).

A cross-sectional study conducted among 384 DM patients at Fenote Selam hospital, west Gojam, Ethiopia from February to April 2013 reported that there was a significant association of anemia with type of DM, duration of diabetes and glomerular filtration rate. The study revealed that anemia was significant in type two DM (AOR =4.17, 95% CI = 2.58-8.56) and anemia associated with duration of DM for greater than eleven years(AOR= 7.47, 95% CI 1.51-37.07) more likely to develop anemia than patients with DM for less than five years). Diabetes mellitus patients with an eGFR below 60 ml/min/ 1.73 m^2 and between 60–89 ml/min/1.73m^2have 11 times greater (AOR=11.13,95% CI =2.69-45.94) and 4 times greater (AOR=4.29, 95% CI = 1.14-16.13) risk to be anemic than DM patients with an eGFR above 90 ml/min/ 1.73m^2 respectively (Abate et al., 2013).

Another cross-sectional study conducted among 412 diabetic patients at Dessie Referral hospital in Northeast Ethiopia, from January to April 2018 showed that type 2 diabetes (AOR = 2.40, 95% CI 1.14–5.08); presence of hypertension (AOR = 3.78, 95% CI 1.35–10.57); serum creatinine (AOR = 12.80, 95% CI 3.90– 87.98) and low GFR (AOR = 9.50, 95% CI 4.05–22.28) were independently associated with the presence of anemia(Fiseha et al., 2019).

2.3. Conceptual Framework

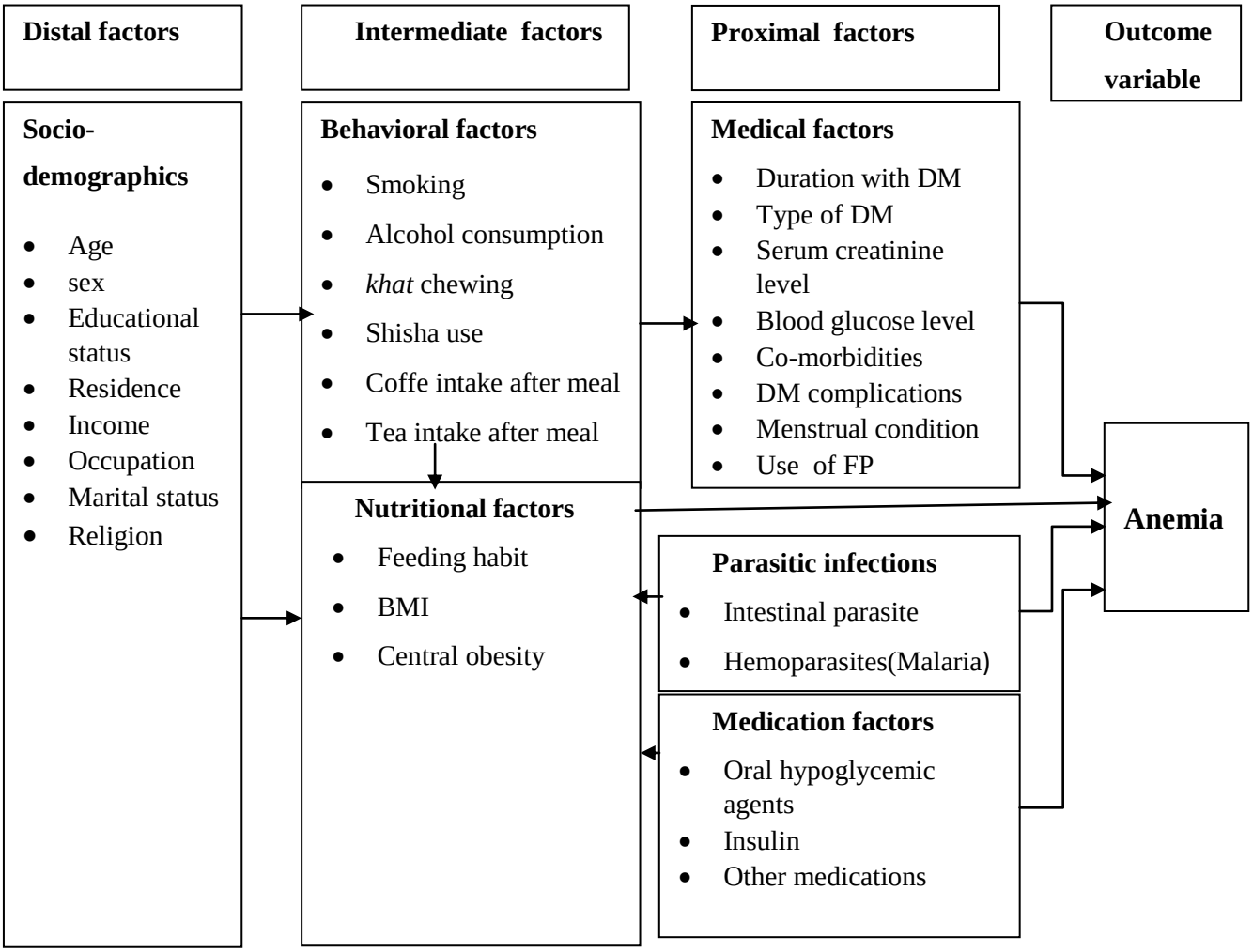

Fig 1: Conceptual framework showing factors associated with anemia among diabetic patients(sources: different literature).

3. MATERIALS AND METHODS

3.1. Study Settingand Period

The studywas conducted at Gelemso General Hospital. Gelemso General Hospital is located in Gelemso town, West Hararghe zone, Oromia regional state, which is found at about 390 km East of Addis Ababa, the capital of Ethiopia. Thehospital is found in Habro district which has an altitude between 1800-2000 meters above sea level(Habro district administration). The hospital serves as referral centre for an estimated of 1.4 million population in the surrounding catchment area. It offers diagnosis and treatment for approximately more than 90,000 patients a year. The hospital has different units and clinics that provide specialized service for patients. The diabetic clinic is among the specialized units in the hospital which offers services for about 660 diabetic patients (Hospital HMIS,2019).The study wasconducted from May 20 to Agust 10, 2020.

3.2. Study Design

A hospital-based quantitative cross-sectional studywas employed.

3.3. Source Population

All diabetic patientswho visitedthehospital.

3.4. Study Population

All adultdiabetic patients who visiteddiabeticfollow up clinicduring the study period and fulfilled the inclusion criteria.

3.5. Eligibility Criteria

3.5.1. Inclusion Criteria

- Patients aged18 years or olderand were receiving pharmacologic treatment.
- Diabetic patients with both types(type1and type2 DM).

3.5.2. Exclusion Criteria

- Transfused patientswithin the last 3 months.
- Patients who were pregnant or had recent delivery or with psychiatric illness.
- Patients in an emergency situation or hospitalized.
- Patients who were on treatment for anemia during data collection.
- Patients with surgical procedures or bleeding due to any reason.

- Patients on treatment for intestinal parasites.

3.6. Sample Size Determination

For specific objective 1: the sample size was determined using a single proportion formula based on the following assumptions:

95% level of confidence,level of statistical significance(Z),5% margin of error and 26% proportion of anemia from a previous study conducted in people with diabetes in Dessie referral Hospital, Northeast of Ethiopia(Fiseha et al., 2019).

The sample size was calculated based on the following formula:

$$\mathbf{n=\frac{(Z\sigma/2)2\ pq}{d^2}}$$

Where; n =calculated sample size,

Z = 95% confidence interval(1.96)

p = magnitude (proportion) of anemia among diabetic patients in Ethiopia

q = 1-p

d = marginal error

n= $\frac{(Z\sigma/2)2\ p\ q}{d^2}$= $(1.96)^2$ .26(1-.26)/0.05^2 = 296.

For specific objective 2:sample size was calculatedusing Epi infoversion7.2.3.1software considering the following assumptions:

80% power of the study, equal unexposed to exposed ratio(1:1), odds ratio, 95% CIand proportions of anemia among diabetic patients.

Table **1**: Sample size calculation for explanatory variables

Explanatory variables	Proportion in exposed	Proportion in unexposed	Odds ratio	Power	Calculated sample size in exposed	Calculated sample size in unexposed	n= n1+n2
DM type	14.5%	4.2%	4.71	80%	138	138	276 (Abate et al., 2013)
Sex	35.5%	19.7%	1.12	80%	137	137	274(Fiseha et al., 2019)
eGFR	59.3%	21.2%	9.5	80%	30	30	60 //
BP	65.50%	12.0%	3.78	80%	16	16	32 //

This provided a sample size of 276.The sample size calculated for magnitude was used for this study as it was greater than the calculated sample size for associated factors. After adding 10% non-response rate, the final sample size was 325.

3.7. Sampling Technique

In the study period, 660 diabetic patients attended diabetic clinic in Gelemso Hospital for their active follow-ups. Sampling frame was made from a register of all diabetic patients registered for services at the hospital. Using a systematic sampling technique, a total of 325 study participants were selected from the sampling frame. The total number of 660 diabetic patients were divided for sample size (325) to obtain sample interval(k^{th} value) which became 2. Hence, every second diabetic patients attended the clinic were enrolled in the study until the calculated sample size was reached.Each study participants was given a unique identification number to avoid repetition.

3.8. Data Collection and Measurement

3.8.1. Data Collectors

Data were collected by two BSc nurses, one laboratory technologist,and supervised by one senior public health professional.

3.8.2. Data collection tools

3.8.2.1. Questionnaire

Socio-demographic characterstics, alcohol consumption, smoking and dietary intake datawere collected using the WHO stepwise approach to surveillance-instrument 3.2 with slight modification(WHO, 2017). Questionnairewas developed for tea intake, coffee intake, shisha use, *khat* chewing, contraceptive use and menstruationfrom different literature(Aynalem and Zeleke, 2018, Seifu et al., 2015, Roba et al., 2019, Tesfaye et al., 2016, Abebe et al., 2015). The questionnaire wasprepared in English and translated to Afan Oromo(local language)and to maintain its consistency back to English by different language experts. Checklists were used to collect clinical datasuch as duration of diabetes, type of medications, type of diabetes and complication of DM from patients' clinical record form.

3.8.2.2. Physical Measurement

Blood pressure was measured using a digital measuring device (Heuer) which was regularly inspected and validated. Before measuring blood pressure (BP), it was made sure the subjects had not consumed any hot beverages, such as tea or coffee, smoked or chewed tobacco and or undertakenvigorous physical activity within the 30 min preceding the interview. Three separate measurementswere obtained on the left arm of the seated subject after rested for at least five minutes in a seated position. The second and third measurement were taken five-to-ten minutes after the first and second measurement respectively. The average of the readings of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken as the BP of the participant. Hypertension was defined as elevated high blood pressure (SBP≥140 or DBP ≥90 mmHg) or a history of treatment with anti-hypertensive agent(Kearney et al., 2005).

Height was measuredusing stadiometer while the participants were in an upright position, without shoes, to the nearest 0.5 cm with participant standing erect against the wall with heels together and touching the wall, and head held in upright position. Weight was measured using

a digital weighing scale. The scale was calibrated to the zero level before each measurement, and was tested for repeatability of the measures. Weight was measured with minimum cloths and without footwear on a standardized weighing machine marked from 0 to 130 kg and was recorded to the nearest 0.5 kg(Kengne et al., 2012).

BMIwas calculated using the formula of weight (in kilogram)divided by height(in meter square)and categorized as underweight (BMI<18.5), normal (18.5 - 24.9), over-weight (25.0 - 29.9) and obese (≥ 30.0)(Barbieri et al., 2015).

Waist circumference was measured using a non-elastic tape measure from the approximate midpoint between the lower margin of the last palpable rib and the top of iliac crest, and was measured to the nearest 0.5cm. Central obesity was defined by waist circumference thresholds≥ 94cm for men and ≥ 80cm for women(Abebe et al., 2015).

3.8.2.3. Laboratory Measurement

About 2mlblood sample was collected from all participants into test tubecontaining tri-potassium ethylenediaminetetraacetate (EDTA K3)anticoagulants by laboratory technologistfor hemoglobin determination. The collected whole blood was mixed properly and analyzed using Sysmex XN-550 hematology analyzer.Altitude adjusted hemoglobinconcentration was obtained by subtracting WHO recommended adjustment values, 0.8 g/dl from all respondentsand for smoking status by subtracting 0.03g/dl. Anemia was defined as hemoglobin level <12g/dl for women and <13g/dl for males.

Five mlvenous blood was collected into test tube without anticoagulantfor creatinine and urea determination. The whole blood without anticoagulant wasallowed to clot for 20 to 30 minutes and centrifuged at 3000 rpm for 5 min to separate serum. Then serum creatinine and urea were determined by kinetic alkaline picrate and enzymatic method respectively, using Mindray BS-200E(Shenzhen Mindray Bio-Medical Electronics Co., Ltd, China) clinical chemistry analyzer(Adera et al., 2019). The creatinine crearance was used to estimate GFR using CKD-EPI Cockroft- Gault formula expressed per 1.73 m^2.

Blood glucose wasmeasured in a fasting stateby finger prick using blood glucometer that was calibrated for plasma glucose values(Chiwanga et al., 2016).

Study participants were provided clean wooden applicator stick and clean, dry and leak proof plastic cup labeled with subject's serial number for stool examination. They were instructed to bring about thumb sizeof fresh stool sample. Examination of specimen was done within 30 minutes using wet mount technique to detect helminths (eggs and or larvae). The remaining samples were preserved in 10% of formalin and processed using formalin-ether sedimentation concentration technique to increase the parasite detection rate,and examined using Olympus Microscopy.

The capillary blood sample was collected by finger prick using a sterile lancet for malaria detection. Thick and thin blood filmswere prepared on the same grease- free clean glass, and air dried. The slides were stained with 10% giemsa for about ten minutes and screened for plasmodium species by laboratory technologist. Slideswere considered negative when 100 high power fields was examined under oil immersion objective(Fana et al., 2015).

3.9. Study Variables

3.9.1. Dependent Variables

Magnitude of anemia

3.9.2. Independent Variables

Socio-demographic factors: gender, residence, ethnicity, marital status, educational status, occupational status and religion.

Medical factors:types of DM, duration with DM, serum creatinine level,urea level, eGFR, blood pressure level, blood glucose level, medication type, intestinal parasitic infections, malaria, menstrual condition and family planning use.

Behavioral factors: cigarette smoking, alcohol consumption, tea consumption after meal, coffee consumption after meal**,** *khat* and shisha use.

Nutritional factors:dietary habit(vegetables and fruits intake), egg and meat intake, BMI and central obesity.

3.10. Operational Definitions

Anemia: wasdefined as hemoglobin levels<12g/dlforfemales and <13g/dl for males in patients aged 18 years of age and above(Loutradis et al., 2016).

Blood glucose control:variable that was coded as controlled or uncontrolled. Blood glucose was defined as uncontrolled if fasting blood glucose (FBG) levelwas <80 or >130mg/dland was controlled when fasting glucose value was between 80 and 130mg/dl(Kassahun et al., 2016).

Hypertension: was defined as systolic BP level of ≥140 mmHg and diastolic BP level of ≥90mmHg orpreviously diagnosed as hypertensive by any health professional(Singh et al., 2017)

Central obesity: Central obesity was defined by waist circumference thresholds(≥ 94cm for men and ≥ 80cm for women(Abebe et al., 2015).

Body mass index: was defined as weight (in kilogram) divided by height (in square metre) and was categorised as underweight(< 18.5 kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2) and obese(> 30.0 kg/m^2)(Owolabi et al., 2017).

Former smoker: individual who reported smoked cigarettes in his or her lifetime but who had quit smoking at the time of data collection.

Current smoker: individual who reported smoking at the time of data collection.

Current alcohol user: individual who were alcohol drinker during the time of study.

3.11. Data Processing and Analysis

All data from questionnaire were compiled and checked for clarity and completeness,and coded. The coded data were entered into Epidata version 3.1 and exported to SPSS version 22.0 software package for cleaning and analysis. Descriptive analysis wasperformed and the results were presented by tables and figures. Bivariate analysis was run using logistic regression to identify variables for multivariable analysis. Variables with p-value ≤ 0.25 in bivariate logistic regression wereconsidered as candidates for multivariable logistic regression. Multivariable logistic regression was performed using enter method to identify factors independently associated with dependent variable. Multivariable analysis wasused to adjust the effects of potential confounding variables and showed us independent effect of each

independent variables. The strength of association was measured using odds ratio and 95% confidence interval, and P-value ≤ 0.05 considered as statistically significant.

3.12. Data Quality Assurance

Data collectors and supervisor receiveda one day training on data collection instrument and data collection process. Thirty questinnaires were pre-tested before the actual data collection among diabetic patients at Chiro Hospitalto check where the instructions and language of the tool were clear, understandable and time taken for questionnaire. Systematic sampling technique and multivariable analysis wereused to prevent selection bias and variable confounder effects respectively. Besides, physical measurements wererecorded twice in order to minimize observer error in measurements and records.Furthermore**,** standardoperational procedures were followed during all laboratory sample collection, storage, analytical process and recording.

3.13. Ethical Consideration

Ethical clearance wasobtained fromInstitutional Health Research Ethics Review Committee (IHRERC)of the College of Health and Medical Sciences, Haramaya University. Official letter of support was written from the college to the Gelemso Hospital. Then, informed voluntary written and signed consent was obtained from the head of the hospital and each participant. Participant who was not willing to participate in the study was not forced to participate. They were also informed that all data obtained from them were kept confidential by using codes instead of any personal identifiers and were meant only for the purpose of the study.

3.14. Information Dissemination

Final written report will be submitted to Haramaya University, College of Health and Medical Sciences and presented during mock defence and final defence for the partial fulfillment of Masters Degree in Public Health. Subsequently, an attempt will be made to present the findings on different review meetings, seminars and workshops. Moreover, effort will be made to publish on health journals.

4. RESULTS

4.1. Socio-demographic characteristics

In this study**,** a total of 325 DM patients were consentedfor the study with the response rate of 100%. From the total participants; 62.5%patients were malesand the median age of participants was 40 years (Interquartile Range(IQR)= 20 years old). Most of the respondents, 76% were married and 77.8% were Muslims by religion. Two hundred four (62.8%) participants were living in rural setting and 85.5% wereOromo ethnic. About sixty-two percent of the participants had never attended formal education and 52.6% were farmers (**Table2**).

Table 2:Socio-demographic characteristics ofadult DM patients attendingGelemso General Hospital, Eastern Ethiopia,2020 (n= 325).

Variables	Frequency	Percent (%)
Gender		
Male	203	62.5
Female	122	37.5
Residence		
Urban	121	37.2
Rural	204	62.8
Age in years		
18-25	56	17.2
26-40	128	39.4
41-55	77	23.7
≥56	64	19.7
Educational status		
No formal education	201	61.8
Primary	79	24.3
Secondary	24	7.4
College and above	21	6.5
Ethnicity		
Oromo	279	85.8
Amhara	40	12.3
Other[a]	6	1.8
Marital status		
Single	51	15.7
Married	247	76.0
Divorced /widowed	27	8.3

Table 2 Continued

Occupational status		
Governmental employee	21	6.5
Self-employed	93	28.6
Farmer	171	52.6
Student	17	5.2
Retired	5	1.5
Unemployed	18	5.5

[a] = Guraghe, Somali

4.2. Behavioral and nutritional characteristics

The study showed that 22.8% of the participants had smoked cigarette at least once in their lifetime and 4.0 % were current smokers. Besides, 3.7%and 64.3% of the study participants were current alcohol drinkers and khat chewers respectively. More than one third, 68.3% of the study subjects were taking coffee always after meal. Concerning the nutritional status, 10.2% of the study participants were underweight and 28.3% were overweight. Two hundred fifty-six (78.8%) and two hundred fifty-three (77.8) study participants ate fruits and vegetables once a week during regular week day respectively (**Table 3**).

Table 3: Behavioral and nutritional characteristics of adult DM patients attendingGelemso General Hospital, Eastern Ethiopia, 2020 (n=325).

Variables	Frequency	Percentage
Current Khat chewing		
Yes	209	64.3
No	116	35.7
Current smoker		
Yes	13	4.0
No	312	96
Former smoker		
Yes	74	22.8
No	251	77.2
Current alcohol user		
Yes	12	3.7
No	313	96.3

Table 3 Continued

Coffee use after meal		
Yes	222	68.3
No	103	31.7
Tea use after meal		
Yes	38	11.7
No	313	88.3
Fruits consumption per week		
Less than 5 times	313	96.3
5 or more times	12	3.7
Vegetables consumption per week		
Less than 5 times	310	95.4
5 or more times	15	4.6
Meat consumption per week		
Less than 5 times	312	96.0
5 or more times	13	4.0
Egg consumption per week		
Less than 5 times	311	95.7
5 or more times	14	4.4
BMI in kg/m^2		
<18.5	33	10.2
18.5-24.9	200	61.5
≥25	92	28.3
Central obesity		
Yes	164	50.5
No	161	49.5

4.3. Clinical characteristics of DM patients

More than half (52.3%) of participants were type II DM and the mean duration of DM was 4.5 (SD±4.0) years. Around 29% of the participants have co-morbidity and 44.3% were taking oral hypoglycemic agents (Glibenclamide and/or Metformin).Three forthof the study participants have uncontrolled blood glucose (**Table 4**).

Table 4: Clinical characteristics of adult DM patients attendingGelemso General Hospital, Eastern Ethiopia, 2020 (n= 325)

Variables	Frequency	Percentage
Type of DM		
Type 1	155	47.7
Type 2	170	52.3
Duration of DM		
<5 years	205	63.1
≥5 years	120	36.9
Medication taken for diabetes		
Oral hypoglycemic agents	144	44.3
Insulin use	172	52.9
Both	9	2.8
Medication taken for other diseases		
Yes	93	28.6
No	232	71.4
DM complications		
Yes	86	26.5
No	239	73.3
Co-morbidity		
Yes	94	28.9
No	231	71.1
Blood glucose level		
Controlled	82	25.2
Uncontrolled	243	74.8
Hypertension		
Yes	72	22.2
No	253	77.8
Hypertension (n= 72)		
Controlled	40	55.6
Uncontrolled	32	44.4
Stool examination		
Pos	4	1.2
Neg	321	98.8

4.4. Magnitude and severity of anemia

The magnitude of anemia among the study participants was 30.2% (95% CI: 25-35%). The mean of hemoglobin was 13.2±2.3g/dl (13.4±2.3g/dl in males and 12.9±1.7g/dl in females). The magnitude of anemia was higher in males (36.0%) than females (20.5%) with statistically significant difference (t= 8.7 and p = 0.003). In addition, the magnitude was higher in patients with DM complication (47%) (**Fig 2**). Regarding severity of anemia, the highest percentage was mild anemia 64 (20%), followed by moderate 26 (8%) and severe anemia 8 (3%) (**Fig 3**).

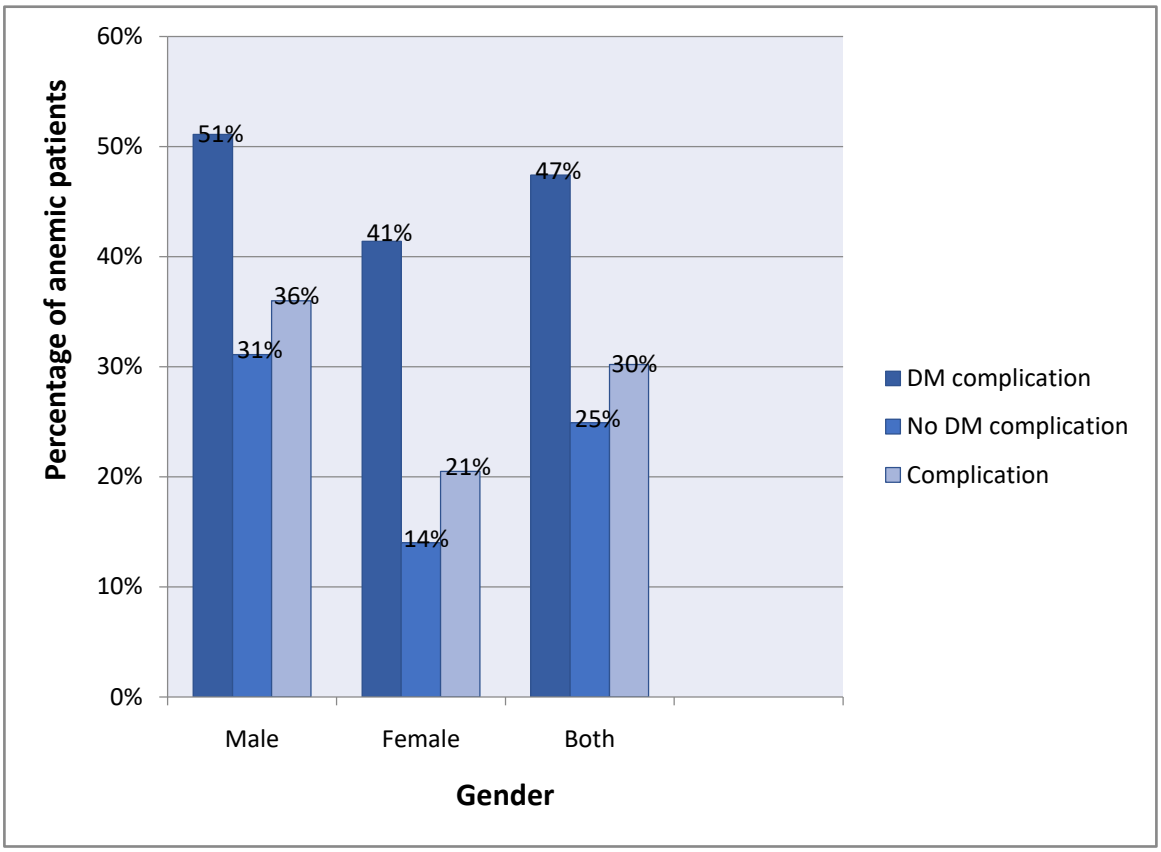

Fig 2: Gender and DM complicationdistribution among anemicadult DM patients attending Gelemso General Hospital, Eastern Ethiopia, 2020

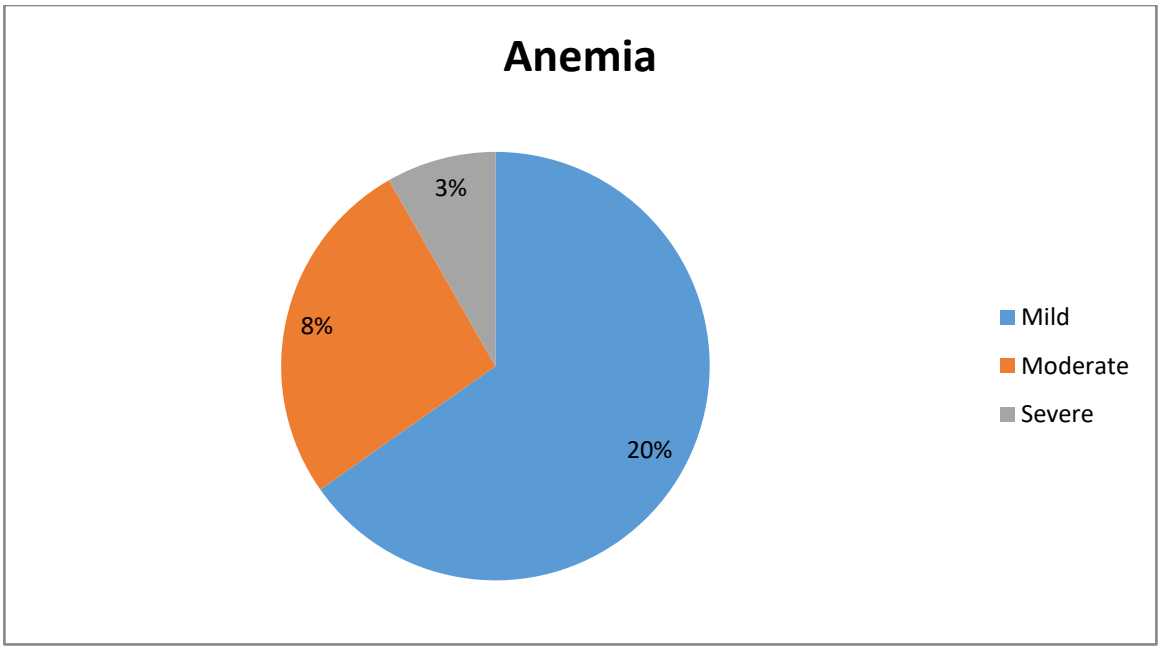

Fig 3: Pie chart percentage of severity of anemia among adult DM patients attending Gelemso General Hospital, Eastern Ethiopia, 2020

4.5. Factors associated with anemia among adult DM patients

Bivariate and multivariable logistic regression analysis were conducted to assess the association between anemia and related factors. In bivariate analysis, age, gender, former smoker, coffee intake after a meal, type of DM, DM medication type, co-morbidities,diabetic complications and duration of DM were significant variables at p-value < 0.25 and recruited for multivariate binary logistic regression.

In multivariable logistic regression, type II DM, male gender, oral hypoglycemic agent, duration of DM of 5 and more years, andpresence of DM complicationswere remained associated with anemia. Type II DM patients were 6 times (AOR= 6.0, 95% CI: 1.9, 19.5)more likely to be anemic than type I DM. Male patients were 2.2 times(AOR= 2.2, 95% CI: 1.2, 4.1) more likely to have anemia than females.Odds of anemia was 4.5 times(AOR= 4.5, 95% CI: 1.5, 13.3)higher among patients taking oral hypoglycemic agent compared to insulin. The occurrence of anemia was 2.3 times (AOR= 2.3, 95% CI: 1.3, 4.2) higher among patients with longer duration of DM(5 years and above). Besides, the occurrence of anemia was 2.7 times(AOR= 2.7, 95% CI: 1.5, 4.8) higher among patients having DM complications compared to their counterparts(**Table 5**).

Table 5: Factors associated with iron deficiencyanemia among adult DM patients attending Gelemso General Hospital, Eastern Ethiopia, 2020 (n=325)

Variables	Anemia		COR (95% CI)	AOR (95% CI)
	Yes (n=98)	No (n=227)		
Gender				
Male	73 (36.0)	130 (64.0)	2.2 (1.3, 3.7)*	2.2 (1.2, 4.1)**
Female	25 (20.5)	97 (79.5)	1	1
Age in years				
18-25	12 (21.8)	43 (78.2)	1	1
26-40	33 (26.6)	91 (73.4)	1.3 (0.7, 3.1)	1.0 (0.4, 2.4)
41-55	25 (30.5)	57 (69.5)	1.6 (0.6, 3.0)	0.3 (0.1, 1.1)
>55	28 (43.8)	36 (56.3)	2.8 (1.2, 6.3)*	0.5 (0.1, 1.8)
Former smoker				
Yes	28 (37.8)	46 (62.2)	1.6 (0.9, 2.7)	1.1 (0.6, 2.3)
No	70 (27.9)	181 (72.1)	1	1
Coffee intake after a meal				
Yes	73 (32.9)	149 (67.1)	1.5 (0.9, 2.6)	1.4 (0.8, 2.6)
No	25 (24.3)	78 (75.7)	1	1
Type of DM				
Type I	33 (21.3)	122 (78.7)	1	1
Type II	65 (38.5)	105 (61.8)	2.3 (1.4, 3.8)*	6.0 (1.8, 19.5)**
Medication for DM				
Oral hypoglycemic agents	55 (38.2)	89 (61.8)	1.9 (1.2, 3.1)*	4.5(1.5, 13.3)**
Insulin	42 (24.4)	130 (75.6)	1	1
Duration of DM				
<5 years	50 (24.4)	155 (75.6)	1	1
≥5 years	48 (40.0)	72 (60.0)	2.1 (1.3, 3.4)*	2.1 (1.2, 3.9)**
Presence Co-morbidity				
Yes	11 (78.3)	4 (26.7)	1.5 (0.9, 2.5)	1.0 (0.5, 1.8)
No	87 (28.1)	223 (71.9)	1	1
DM complication				
Yes	18 (78.3)	5 (21.7)	3.1 (1.9, 5.2)*	2.7 (1.5, 4.8)**
No	80 (26.5)	222 (73.5)	1	1

* Statistically significant at p-value = 0.05-0.01

** Statistically significant at p-value <0.001

5. DISCUSSION

This studyassessed the magnitude and factors associated withanemiaamong DM patients attending Gelemso Hospital. The magnitude of anemia among study participants was 30.2%, described as a moderate public health problem. Being male in gender, type of DM, duration of DM, oral hypoglycemic medicationsand DM complication were identified as factors aggravated the occurrence of anemia among adult DM patients.

The magnitude of anemia in this study was higher than results of studies conducted in Ethiopia, Fenote Selam Hospital (19%) (Fiseha et al., 2019), China (22.8%)(Chen et al., 2013), Australia (24%)(Wee and Anpalahan, 2019) and India(18%) (Panda and Ambade, 2018). However, the magnitude of anemia in this study was lower compared to studies conducted in Thailand(49.4%)(Sudchada et al., 2013), Saudi Arabia (55.5%)(Al-Salman, 2015) andCameroon (41.4%)(Feteh et al., 2016). Thisdiscrepancy might be due to variations in the age ofthe study population.For example, unlike the current study which was conducted among adults above 18 years-old, a study in Thailand included adults ≥60 years old, whereas the other study conducted in Cameroon included adults ≥50 years old.This could be due to decreased renal function, inflammation, bone marrow suppression and nutritional deficiency in older people(Trevest et al., 2014).

In this study, anemia was more common in males thanfemales counterparts. This finding isin contrast to studies conducted in Malaysia(Idris et al., 2018) and China(Chen et al., 2013).Both studies reported the occurrence of anemia is more likely in diabetic females than diabetic males. The reasons for the discrepancies may be due tohigh khat chewing amongmale in the current study, which leads to the loss of appetite. The other possible reason might be due to high coffee and tea consumption among male, which inhibit absorption of iron from intestine.

In current study, participants with type II DM were more likely to develop anemia compared to type I DM, which is in line with the previous study conducted in Ethiopia (Abate et al., 2013) and Libya (Almahdi et al., 2016). This might be due to the fact that patients with type 2 DM pass through a period of pre-diabetes and may experience renal impairment at the time of diagnosis, thus exposing patients to anemia(Fiseha et al., 2019).

Patients with longer duration of DM (five years and more)were more likely to have anemia than those with DM less than five years in the current study. This finding goes in line with studies conducted inEthiopia, Fenote Selam hospital(Abate et al., 2013), Iraq (Abdulqadir and Polus, 2014)and UK (Trevest et al., 2014).This might be due to longer exposure to hyperglycemia. Physiologically explained, hyperglycemia in diabetes leads to increased inflammatory cytokines. These cytokines have an anti-erythropoietic effect, change the sensitivity of progenitors to erythropoietin and promote apoptosis of immature erythrocytes causing a decrease in the number of circulating erythrocytes and consequently causing a reduction of circulating hemoglobin (Barbieri et al., 2015).

In current study, anemia was more commonamong patients with DM complications, the finding is consistent with the previous study conducted in China(He et al., 2015).DM complications cause prominent damage to the cells and vascular architecture of the renal interstitium, systemic inflammation and the induction of inhibitors of erythropoietin release have all been suggested as contributing to anemia in diabetes(Thomas et al., 2004).

In this study, the odds of anemia werehigher amongpatientstakingoral hypoglycemic medicationcompaired to insulin. Similar to this finding, a study conducted in India reported that DM patients taking oral medication have a higher chance of developing anemia (Panda and Ambade, 2018). The reason might be due to the metformin, associated malabsorption that leads to vitamin B12 deficiency,which potentially results in megaloblastic anemia:-as a result of impairment of purine and thymidylate syntheses, hampered DNA synthesis and erythroblast apoptosis causing ineffective erythropoiesis(Singh et al., 2009).

5.1. Strengths and limitation

The strengthen of this study was thatit was one of few studies conducted in Ethiopia where chronic diseases like DM becoming more common and it was a laboratory based. On the other hand, since this study was a cross-sectional design, it doesn’t allow for causal inferences to be made. There is a possibility of social desirability bias in substance use.

6.CONCLUSION AND RECOMMENDATION

6.1. Conclusion

Anemia was a moderate public heath problem among adult DM patients in the current study.Being male, having diabetic complications, patients with type II DM, longer duration of DM and oral hypoglycemic medicationswere identified as factorsaggravated the occurrence of anemia amongadult diabetic patients.

6.2. Recommendation

The finding of this study showed a moderate public health problem among diabetic patients in the study settingwhich can affect their quality of life.Therefore, based on this study finding, I suggest the following recommendations:-

Federal and Regional Health offices

- Health system needs to develop policy and guidelines which can increase the reach of relevant screening and diagnostic services of anemia for diabetic patients.

Health Professionals

- Health professionals should conduct routine screening and proper treatment for anemia in all diabetic patients in health institutions which can improve their quality of life.
- In addition to screening, regular monitoring and proper management of DM complication also helpsin reducing the occurrence of anemia diabetic patients.
- Health professionals should assess Vitamin B12 level in patients taking oral medications, particularly metformins.

Researchers

- Further researchesshould be conductedto know reasons for high magnitude of anemia among malesin this study area.
- More researches should be done regarding the magnitude of anemia among diabetic patients.

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8. APPENDICES

Appendix A. Information Sheet and Informed Voluntary ConsentForm for Hospital Head

My name is (_____). I am working as data and sample collector for the study being conducted in this institution by Teshome Tujubawho is studying for his Master's degree at Haramaya University, College of health and Medical science. I kindly request you to lend me your attention to explain you about the study and your institution being selected as the study setting.

1. The project title:

Magnitude and associated factors of iron deficiencyanemia among adult diabetic patients attendingGelemsoGeneral Hospital, West Haraghe, Oromia regional state, Eastern Ethiopia, 2020

2. Purpose of the study:

The findings of this study can be of a paramount importance for the Hospitalto plan intervention programs in early screening, diagnosis and treatment of anemia in diabetic patients. Moreover, the aim of this study is to write a thesis as a partial requirement for the fulfillment of a Master's Program in public health.

3. Procedure and duration:

I will be interviewing the participants using a questionnaire to provide me with pertinent data that is helpful for the study. There are 32questions to answer where I will fill the questionnaire by interviewing them. The interview will take about 20 minutes, so I kindly request you to allowme to conduct the study in this hospital.

4. Risk and Benefits:

The risk of being participating in this study is very minimal, but only taking few minutes from participants' time. There would not be any direct payment for participating in this study. But the findings from this research may reveal important information for the local health planners.

5. Confidentiality:

The information participants will provide us will be confidential. There will be no

information that will identify them in particular. The findings of the study will be general for the study community and will not reflect anything particular of individual persons or housing. The questionnaire will be coded to exclude showing names. No reference will be made in oral or written reports that could link participants to the research.

6. Rights:

Participation for this study is fully voluntary. You have the right to declare to allow or not the study to be conducted. If you decide to allow, you have the right to stop the study at any time and this will not label you for any loss of benefits which you otherwise are entitled.

7. Contact address:

If there are any questions or enquires any time about the study or the procedures, please contact: Teshome Tujuba, Phone: 0913950019, Email: tujubateshome08@yahoo.com

Contact address of the responsible Institutional Health Research Ethics Review Committee (IHRERC) at office phone 0254662011 or P.O.Box 235, Harar, Ethiopia].

8. Declaration of informed voluntary consent:

I have read the participant information sheet. I have clearly understood the purpose of the research, the procedures, the risks and benefits, issues of confidentiality, the rights of participating and the contact address for any queries. I have been given the opportunity to ask questions for things that may have been unclear. I am informed that the Hospital has the right to stop this study at any time from being conducted if any misdeeds and unethical procedures are observed during the data collection process in the Hospital's premises. Therefore, I declare my voluntary consent on behalf of Gelemso General Hospital management to allow this study to be conducted in the Hospital with my initials (signature).

Name and Signature of Head of the Hospital:_____,_____ Date _____

Name and Signature of Data Collector:_____, _____Date _____

Appendix B.Participant Information Sheet and Informed Voluntary Consent Form

My name is (_____). I am working as a data and sample collector for the study being conducted in this hospital by Teshome Tujubawho is studying for his/her Master's degree at Haramaya University, the College of Health and Medical Sciences. I kindly request you to lend me your attention to explain you about the study and being selected as the study participant.

1. The study/project title:

Magnitude and associated factors of iron deficiency anemia among adult diabetic patients attending Gelemso General Hospital, West Haraghe, Oromia regional state, Eastrn Ethiopia, 2020

2. Purpose/aim of the study:

The findings of this study can be of a paramount importance for the district health office to plan in early screening, diagnosis and treatment of anemia in diabetic patients. Moreover, the aim of this study is to write a thesis as a partial requirement for the fulfillment of a Master's Program in public health.

3. Procedure and duration:

I will be interviewing you using a questionnaire to provide me with pertinent data that is helpful for the study. There are 32 questions to answer where I will fill the questionnaire by interviewing you. The interview will take about 20 minutes, so I kindly request you to spare me this time for the interview.

4. Risks and benefits:

The risk of being participating in this study is very minimal, but only taking few minutes from your time. There would not be any direct payment for participating in this study. But the findings from this research may reveal important information for the local health planners.

5. Confidentiality:

The information you will provide us will be confidential. There will be no information that will identify you in particular. The findings of the study will be general for the study community and will not reflect anything particular of individual persons or housing. The questionnaire will be coded to exclude showing names. No reference will be made in oral or

written reports that could link participants to the research.

6. Rights:

Participation for this study is fully voluntary. You have the right to declare to participate or not in this study. If you decide to participate, you have the right to withdraw from the study at any time and this will not label you for any loss of benefits which you otherwise are entitled. You do not have to answer any question that you do not want to answer.

7. Contact address:

If there are any questions or enquires any time about the study or the procedures, please contact: 0913950019

Contact address of the responsible Institutional Health Research Ethics Review Committee (IHRERC) at office phone 0254662011 or P.O.Box 235, Harar, Ethiopia.

8. Declaration of informed voluntary consent:

I have read/ was read to me the participant information sheet. I have clearly understood the purpose of the research, the procedures, the risks and benefits, issues of confidentiality, the rights of participating and the contact address for any queries. I have been given the opportunity to ask questions for things that may have been unclear. I was informed that I have the right to withdraw from the study at any time or not to answer any question that I do not want. Therefore, I declare my voluntary consent to participate in this study with my initials (signature).

Name and signature of participant: _____, _____Date _____

Name and signature of Data Collector:_____, _____ Date _____

Appendix C. Translated Participant Information Sheet and Informed Consent Form in Afan Oromo

Odeeffannoo fi guca walii galtee hirmaattota qorannoof filatamaniif

Akkam jirtu? Maqaan koo_____Jedhama. Ani qorannoo obbo Tashoomaa Tuujubaa barumsa Digirii lammaffaf Yuuniversiitii Haramayaa, Damee Fayyaa Hawwaasaatiin haalduree guutuuf hojjechuuf karoorfamee irratti odeefannoo guuruufin hojjedha. Kanaaf isinis qorannoo kana irratti akka hirmaattaniif waan filatamtaniif,waa'ee qorannoo kanaa akkan isinitti himuuf xiyyeeffannaan akka na dhaggeefattan kabajaanin isin gaafadha..

1. Mata dureen qorannon kana

Tatamsa'ina hir'ina dhiigaa fi rakkoolee isaan wal qabatan dhukkubsattoota dhibee sukkaraa hospitaala Galamsootti hordoffiirrajiran 2020

2. Barbaachisummaa qorannoo kana

Qorannoon kun tatamsa'ina hanqina dhiigaafi wantoota ka'umsa isaaf sababa ta'an addaan baasuuf yoo ta'u, bu'aan qorannoo kana ogeessota fayyaatiif, waajjira fayyaa godiinaatiif fi biiroo fayyaa naannootiif, tajaajila qorannoo dhukkuba kana yeroon akka godhamuu fi tajaajila godhamuu qabu murteessuuf akka ka'umsaatti ni fayyada. Kana malees, qorannoon kun barumsa fayyaa hawwasummaandigirii lammaffaa akkanargadhuuf na gargaara.

3. Adeemsaa fi turtii qorannoo kanaa

Gaaffiilee qorannoo kanaf qophaa'an waanan isin gaafadhuuf, odeefannoo isiin nuuf laaattan fi dhiigni qorannoo kanaaf oolu 7ml kan hin caalle fuudhamu galma ga'iinsa qorannoo kanaaf iddoo olaanaa waan qabuuf deeggarsi keessan barabaachisaadha. Gaaffilee qopha'aniif deebii sirri akka nuuf laattaanii fi yeroo gabaabaa daqiiqa 20 hin caallee aarsaa akka gootan isin gaafadha.

4. Miidhaa fi Bu`aa qorannoo kanaa

Qorannoo kana keessatti hirmaachun miidhaan isinirra gahuu danda'u xiqqaadha. Yeroo gabaabaa fi dhiiga qorannoof oolu mililitra 7 hin caalle qofaadha. Yeroo saamudni dhiiga fudhamu rakkoo uumamuuf, ogeessi fayyaa garagaarsa isiniif kenuu danda'an qophaa'uu isaanii isiniif ibsina. Dhibeen Hirina dhiiga qoricha nama fayyisu ni qaba. Bu'aan qorannoo kee hirina dhiiga yoo agarsiise, hakiimotni yaala siif barbachisu waan siif godhaniif bu'aa

qaba. Qorannoo kanarratti hirmachuu keessaniif kanfaltiin isin argattan hin jiru. Garuu bu'aan qorannoo kanaa isiniif bu'aa ni qaba yoo ta'e, yaalii siif taasifamuuf gargaarsi nama qorannoo kana raawwatuun siif godhama.

5. Icciitii

Odeeffannoon ati nuuf kennitu icitiin isaa ni eegama. Akkasumas bu`aan qorannoo akka waliigalaatti kan ibsamu malee, yaadi nama dhuunfaan kan ibsinu miti. Gaaffii gaafatamtu irratti maqaan kee kooddiin kan ibsamu ta`a.

6. Mirga

Qorannoo kana irratti hirmaachuun fedhakee irratti hundaa`a. Hirmaachuu fi hirmaachuu dhiisuuf mirga guutuu qabda. Yoo hirmaattes gaaffii deebisuu fedha hin qabne dhiisuu fi yeroo barbaddetti qorannicha keessa bahuu ni dandeessa. Qorannicha keessaa bahuu keetiif bu'aan ati dhabdu hin jiru.

7. Teessoo (Contact address)

Gaaffii yoo qabaattan: Email: tujubateshome08@yahoo.com ykn lakk. Bilbila: 0913950019 fayyadamuun nu qunnamu dandeessu.Akkasumas dhaabbata seera qorannoo fayyaa irratti Xiinxala gaggeessan (IHRERC) Univarsiiti Haramayaa: Bilb.0254 660708,Saanduqa Poostaa 235, Harar, Kanaan Yaada keessan dhiyeessuu ni dandeessu.

8. Labsii Walii galtee Hirmaannaa fedhii irratti Hundaa'e

Waa`ee qorannoo kanaa ilaalchisee kaaayyoo, faayidaa, adeemsa,miidhaa,iccitii, mirgaa hirmaachuu fi dirqamni kiyya odeeffannoo waraqaa kanarra jiru irraa dubbisee/naaf dubbifamee naaf galee jira. Waan naaf hin galle gaafachuuf carraan naaf laatamee jira. Qorannoo kana keessaa yeroon barbaade bahuu akkan danda'uu fi gaaffin deebisuu hin barbaannee dhiisuuf mirga akkan qabu natti himameera. Kanaafuu fedhii kiyyaan qorannoo kana irratti hirmachuuf mallattoo kiyyaanin mirkaneessa.

Maqaa fi Mallattoo gaafatamaa_____,_____, Guyyaa_____

Mallattoo nama odeeffannoo guutee_____,_____,Guyyaa_____

Appendix D. Research Questionnaire

Title:Magnitudeand associated factors of iron deficiency anemia among adult diabetic patients attendingGelemsoGeneralHospital, West Hararghe,Oromia regional state,Eastern Ethiopia, 2020

General Information		
Location and Date	**Response**	**Code**
Facility Name	Gelemso General Hospital	I1
Interviewer ID		I2
Date of completion of the instrument		I3
Time of interview		I4
Name		I5

Participant Identification Number _____

Part I: Demographic information			
Before you begin, I would like to ask you to answer question by encircling the answer or by filling in the space provided.			
S.No.	Questions	Response	Code
101	sex	1. Male 2. Female	C101
102	Where do you live?	1. Urban 2. Rural	C102
103	In which kebele do you live?	Kebele_____	C103
104	How old are you?	_____in years	C104
105	What is your religion?	1. Orthodox 2. Muslims 3. Protestant 4. Others(specify)_____	C105
106	Taking the past year, can you tell me what the average earnings (Birr) of the household have been?	1. Per month ____ or 2. Per year _____	C106
107	What is the highest level of education you have completed?	1. No formal education 2. Primary school completed (grade 1-8) 3. Secondary school completed (grade 9-12) 4. College and above	C107
108	What is yourethnic group?	1. Oromo 2. Amhara 3. Tigre 4. Somale 5. Guraghe 6. Other(specify)_____	C108
109	What is your marital status?	1. Unmarried 2. Married 3. Divorced 4. Widowed	C109

110	Which of the following best describes your mainwork status over the past 12 months?	1. Government employee 2. Non-government 3. Self-employed 4. Farmer 5. Student 6. Homemaker 7. Unemployed	C110
Part II: Behavioral measurements			
Tobacco use			
201	Do you currently smoke any tobacco products, such as cigarettes, cigars or pipes?	1. Yes 2. No If No, go to A17	T201
202	Do you currently smoke tobacco products daily?	1. Yes 2. No	T202
203	If Yes, how many cigarettes do you smoke per a day?	1. <20 cigarettes per a day 2. >20 cigarettes per a day	T203
204	In the past, did you smoke daily?	1. Yes 2. No	T204
205	How old were you when you first started smoking tobacco?	Age (years)_____ 77. Don't know	T205
206	During the past 12 months, have you tried tostop smoking?	1. Yes 2. No	T206
Alcohol consumption			
207	Have you ever consumed any alcohol such as beer, wine, spirits or *other*?	1. Yes 2. No If No, go to B209	A207
208	How many standard drinks containing alcohol do you have on a typical day when you are drinking?	1. 1 to 2 2. 3 to 4 3. 5 to 6 4. 7 to 9 5. 10 and above	A208
Tea consumption			
209	Do you drink tea immediately (within 30 minutes) after meal?	1. Yes 2. No If No, go to K211	B209

210	How often do you drink tea after(within 30 minutes) meal?	1.Every day 2.Weekly 3.Monthly 4. Other(specify)_____	B210
Coffee consumption			
211	Do you drink coffee after meal?	1. Yes 2. No If No, go to J213	K211
212	How often do you drink coffee after meal?	1. Every day 2. Weekly 3. Monthly 4. Other (specify)_____	K212
Chat and shisha use			
213	Do you chew *khat*?	1. Yes 2. No	J213
214	How often do you chew *khat*?	1. Every day 2. Weekly 3. Monthly 4. Other (specify)_____	J214
215	Do you ever use shisha?	1. Yes 2. No If No, go to D301	J215
216	Do you currently use shisha?	1. Yes 2. No	J216
Part III: Diet			
301	In a typical week, on how many days do you eat fruit?	1. Once a week 2. 2-4 times a week 3. 5-6 times a week 4. Nearly every day 5. Never 77. Don't know	D301
302	In a typical week, on how many days do you eat vegetables?	1. Once a week 2. 2-4 times a week 3. 5-6 times a week 4. Nearly every day 5. Never	D302

		77. Don't know	
303	How often do you eat a portion of bread in a typical week?	1. Once a week 2. 2-4 times a week 3. 5-6 times a week 4. Nearly every day 5. Never 77. Don't know	D303
304	How often do you eat meat in a week?	1. Once a week 2. 2-4 times a week 3. 5-6 times a week 4. Nearly every day 5. Never 77. Don't know	D304
305	How often do you eat egg, egg whites or egg substitutes?	1. Once a week 2. 2-4 times a week 3. 5-6 times a week 4. Nearly every day 5. Never 77. Don't know	D305
Part IIII: Family planning use			
401	Do you use contraceptives (for women only)?	1. Yes 2. No	F401
402	During your most recent menstrual period, do you experience a heavy bleeding?	1. Yes 2. No	F402

Additional comment

_____
_____
_____
_____
_____

THANK YOU FOR YOUR HELP

Appendix E. Afan OromoVersion Questionnaire

Mataduree Qorannoo: Tatamsa'ina hir'ina dhiigaa fi rakkoolee isaan wal qabatan dhukkubsattoota dhibee sukkaraa hospitaala Galamsootti hordoffiirrajiran,Harargee Lixaa,Naannoo Oromiyaa, Itoophiyaa,2020.

Odeeffannoo waliigala		
Iddoo fi guyyaa	Deebii	koodii
Maqaa mana yaala	Hospitaala Waliigala Galamsoo	I1
Lakk nama gaafatee		I2
Guyyaa itti xumurame		I3
Sa'aati		I4
Maqaa		I5

Lakkofsa nama gaafatamuu _____

Kutaa I: Odeeffannoo haala hawaasumma			
Osoo hin jalqabi duraa, gaaffin dhiyyataniif filannoo itti maruun, bakka duwwaatti guutun akkaa naaf deebistan kabajaan isin gaafadha**.**			
Lakk.	**Gaaffii**	**Deebii**	**koodii**
101	Saala	1. Dhiira 2. Dubara	C101
102	Eessa jiraata?	1. Magaalaa 2. Baadiyyaa	C102
103	Aradda kam keessa jiraata?	Araddaa_____	C103
104	Umuriin kee meeqa?	waggaa_____	C104
105	Amantii kam hordofta?	1. Ortoodoksii 2. Musiliima 3. Piroteestaanti 4. kan biro(Ibsi)_____	C105
106	Kan waggaa darbee irratti hundaa'uun, galii waggaatti argattan ibsuu dandeessaa?	1. Ji'atti_____ykn 2. Waggaatti_____	C106
107	Sadarkaan barnoota kee hangam?	1. Barumsaa idilee hin qabu 2. Sadarkaa 2ffa xumureera 3. Sadarkaa 2ffa xumureera 4. Koleejii/ Yuunivarsiitii	C107
108	Sabni kee maali?	1. Oromoo 2. Amaara 3. Tigree 4. Sumaalee 5. Guraagee 6. Kan biro(Ibsi)_____	C108
109	Haalli gaa'ilaa akkam?	1. Kan hinfuune 2. Kan fuudhe 3. Kan hiike 4. Kan irraa du'e	C109
110	Baroota darban keessa hojii kee irra caalatti kan ibsu kami?	1. Hojii mootummaa 2. Hojii dhuunfaa 3. Qotee bulaa 4. Barataa	C110

		5. Barataa 6. Hojii manaa 7. Soorama kan bahee 8. Hojii kan hin qabne	
Kutaa II: Safartuu amalaa			
Fayyadam sijaara			
201	Yeroo amma kanati sijaara ni aarsita?	1. Eeyyee 2. LakkiYoo lakki ta'e, gara A207	T201
202	Eeyye yoo ta'e, guyyaa guyyaan sijaara aarsitaa?	1. Eeyyee 2. Lakki	T202
203	Guyyatti lakkofsan hangam aarsita?	1. <20 2. >20	T203
204	Amman dura guyyaa guyyaan aarsaa turte ?	1. Eeyyee 2. Lakki	T204
205	Sijaara umuri meeqan aarsuu calqabdee?	Age (years)_____ 77. Don't know	T205
206	Waggaa darbe keessa sijaara aarsuu dhaabuuf yaaltee?	1. Eeyyee 2. Lakki	T206
Fayyadama alkoolii			
207	Alkoolii dhugdee beekta?	1. Eeyyee 2. Lakki Yoo lakki ta'e, gara B209	A207
208	Torbanitti alkooli meeqaa dhugda?	1. 1 hanga 2 2. 3 hanga 4 3. 5 hanga 6 4. 7 hanga 9 5. 10 fi isaa ol	A208
Fayyadama shaayii			
209	Nyaataan boodaa(daqiiqaa 30 keessatti) shaayii ni dhugdaa?	1. Eeyyee 2. Lakki Yoo lakki ta'e, gara K211	B209
210	Nyaataan booda (daqiiqaa 30 keessatti) shaayii haal meeqa dhugda?	1. Guyyaa guyyaan 2. Torbee torbeen 3. Baatin 4. kan biro (Ibsi)_____	B210
Fayyadam bunaa			

211	Nyaataan boodaa buna ni dhugda?	1. Eeyyee 2. Lakki Yoo lakki ta'e, gara J213	K211
212	Nyaataan booda buna haal meeqa dhugdaa?	1. Guyyaa guyyaan 2. Torbee torbeen 3. Baatin 4. kan biro (Ibsi)_____	K212
Fayyadama jimaa fi shiishaa			
213	Jimaa ni qamaata?	1. Eeyyee 2. Lakki	J213
214	Yoo qamatee, haal meeqa?	1. Guyyaa guyyaan 2. Torbee torbeen 3. Baatin 4. kan biro (Ibsi)_____	J214
215	Shiishaa ni fayyadama turtee?	1. Eeyyee 2. Lakki Yoo lakki ta'e, gara D301	J215
216	Yeroo amma shiishaa fayyadama jirtaa?	1. Eeyyee 2. Lakki	J216
Kutaa III: Safartuu nyaata			
301	Torbanitti fudura haal meeqa nyaataa?	1. Torbanitti haal takka 2. Torbanitti guyyaa 2-4 3. Torbanitti guyyaa 5-6 4. Guyyaa hundaa 5. Gonkumaa 77. Hin beeku	D301
302	Torbanitti kudura haal meeqa nyaataa?	1. Torbanitti haal takka 2. Torbanitti guyyaa 2-4 3. Torbanitti guyyaa 5-6 4. Guyyaa hundaa 5. Gonkumaa 77. Hin beeku	D302

303	Torbanitti fooni haal meeqa nyaataa?	1. Torbanitti haal takka 2. Torbanitti guyyaa 2-4 3. Torbanitti guyyaa 5-6 4. Guyyaa hundaa 5. Gonkumaa 77. Hin beeku	D303
304	Torbanitti daabboo haal meeqa nyaataa?	1. Torbanitti haal takka 2. Torbanitti guyyaa 2-4 3. Torbanitti guyyaa 5-6 4. Guyyaa hundaa 5. Gonkumaa 77. Hin beeku	D304
305	Torbanitti killee haal meeqa nyaataa?	1. Torbanitti haal takka 2. Torbanitti guyyaa 2-4 3. Torbanitti guyyaa 5-6 4. Guyyaa hundaa 5. Gonkumaa 77. Hin beeku	D305
Kutaa IIII: Fayyadama karoora maati			
401	Karoora maatii ni fayyadamtaa (dhalaaf qofa)?	1. Eeyyee 2. Lakki	F401
402	Yeroo dhiyooti sababa laguun dhiigni sirraa dhangala'ee beekaa?	1. Eeyyee 2. Lakki	F402

Yaada dabalataa yoo qabattan

_____
_____
_____
_____
_____
_____
_____

Gargaarsa keessaniif galatoomaa

Appendix F. Checklist for Physical Measurements

Participant ID_____

Height and Weight		
P01	Height	in Centimeters (cm)_____
P02	Weight	in Kilograms (kg) _____
P03	BMI in Kg/m^2	_____
Waist		
P04	Waist circumference	in Centimeters (cm)_____
Blood Pressure		
P05	Reading 1	Systolic (mmHg)_____Diastolic (mmHg)_____
P06	Reading 2	Systolic (mmHg)_____ Diastolic (mmHg)_____
P07	Reading 3	Systolic (mmHg)_____ Diastolic (mmHg)_____

Appendix G. DM Card Review Report Form

Please fill the following information from patient card
Participant ID_____

Code	Variable	Response			Remark
C01	Year of DM diagnosis				
C02	Type of DM				
C03	Treatment(medication)type	Oral hypoglycemic agent------1 Insulin therapy-----2 Both----------3 No information-------00			
C04	Other medication (please write medication type and dose the patient is using currently e.g metformin 2tab/ day…)	_____			
C05	Do you ever/currently have any known diabetic complication? *YES*…1 *No (* No information) --2 (IF No SKIP TO C007		Yes	No	
		Ophthalmologic			
		Hypoglycemic			
		Renal			
		Neurologic			
		Coma			
		Other(specify)			
C06	Presence of Co-morbidities *Yes*……1 *No (* No information) ...2	Hypertension			
		Cardiovascular disease			
		Other (specify)			

Appendix H.Hemoglobin Determination

Method: SLS detection method

Procedure:

- Label tubes with study participant's name or identification number
- Explain the blood drawing procedures to the paticipants.
- Wear gloves and make patient confortable position
- Prepare vacutainer tube and needle
- Tie tourniquent around the arm of the participant just above the bend in the elbow
- Tell the study participant to clench his/her fist
- Using the tip of index finger examine the phlebotomy site, feel the vein and decide exactly where to place the puncture
- Disinfect the phlebotomy site with alcohol swab or cotton wool soaked in isopropyl alcohol
- Insert the needle directly into the vein and draw peripheral blood of approximately 2 ml in EDTA tube
- Tell the participant to open his/her clenched fist
- Release the tourniquent
- Withdraw the needle from the vein and cover the puncture
- Mix the venous blood with anticoagulant(EDTA)
- Place the sample tube in the sample tube holder
- The sample tube holder retracts into the instrument
- Aspiration and analysis start

Reference: male(13-18mg/dl), female(12-16mg/dl)

Appendix I. Serum Creatinine Determination

Method: kinetic alkaline picrate method

Procedure:

- Label tubes with study participant's name or identification number
- Explain the blood drawing procedures to the client
- Wear gloves and make study participant confortable position
- Prepare vacutainer tube and needle
- Tie tourniquent around the arm of the study participant just above the bend in the elbow
- Tell the study participant to clench his/her fist
- Using the tip of index finger examine the phlebotomy site, feel the vein and decide exactly where to place the puncture
- Disinfect the phlebotomy site with alcohol swab or cotton wool soaked in isopropyl alcohol
- Insert the needle directly into the vein and draw peripheral blood of approximately 5ml venous blood into Gel and Clot activator test tube without anticoagulant.
- Allow the whole blood without anticoagulant to clot for 20 to 30 minutes and then centrifuge at 3000 rpm for 5 min
- Separate the serum from whole blood
- Place the sample tube in the disk that holds sample tube
- Place R1 and R2 of creatinine in reagent disk
- The probe aspirates certain amount of sample(2-45µl) from the designated sample tube or reagents(10-350µl) from reagent bottle, then dispenses them into the designated cuvette on the reaction disk.
- In the reaction disk, sample reacts with reagnt and colorimeteric readings are taken.

Reference: Male(0.6- 1.1), Female(0.5-0.9)

Appendix J.Serum Urea Determination

Method: Enzymatic method

Procedure:

- Label tubes with study participant's name or identification number
- Explain the blood drawing procedures to the client
- Wear gloves and make study participant confortable position
- Prepare vacutainer tube and needle
- Tie tourniquent around the arm of the study participant just above the bend in the elbow
- Tell the study participant to clench his/her fist
- Using the tip of index finger examine the phlebotomy site, feel the vein and decide exactly where to place the puncture
- Disinfect the phlebotomy site with alcohol swab or cotton wool soaked in isopropyl alcohol
- Insert the needle directly into the vein and draw peripheral blood of approximately 5ml venous blood into Gel and Clot activator test tube without anticoagulant.
- Allow the whole blood without anticoagulant to clot for 20 to 30 minutes and then centrifuge at 3000 rpm for 5 min
- Separate the serum from whole blood
- Place the sample tube in the disk that holds sample tube
- Place R1 and R2 of urea in reagent disk
- The probe aspirates certain amount of sample(2-45µl) from the designated sample tube or reagents(10-350µl) from reagent bottle, then dispenses them into the designated cuvette on the reaction disk.
- In the reaction disk, sample reacts with reagnt and colorimeteric readings are taken.

Reference: Male/Female(10-50mg/dl)

Appendix K.Blood Glucose Determination

Method: **Glucometer device**

Procedure:

- Wear gloves
- Request study participant washes/cleanses his/her hands to remove any possible contaminants that affect the test results
- Prepare the equipment accordance with manufacturer's guideline
- Ensure the test strip is correct in date
- Only use a single lancet
- Don't use thumb or index finger
- Don't squeeze the finger- milk the finger instead
- Obtain the blood sample and apply the test strip
- Apply or encourage the study participant to apply gently pressure to stop bleeding

Appendix L. Parasite Detection

Method: **Direct wet mount stool examination**

Procedure:

- Study participants are provided clean wooden applicator stick and clean, dry and leak proof plastic cup labeled with subject's serial number.
- Instruct them to bring 2gm (about thumb size) of fresh stool sample
- Examination of specimen is done within 30 minutes of sample collection
- Check the consistency of (degree of moisture) and write formed, soft loose, or watery
- Obtain a microscope slide and the stool specimen
- Take a small amount of the specimen and place it on a microscope slide
- If the stool is solid, add a drop of saline to the specimen and mix
- Cover the slide with coverslip to keep the organism from moving and prevent the preparation from drying.
- Scan the entry coverslip area using the 10x objective, if something suspicious is seen, a higher magnification may be necessary.

Appendix M. Parasite Detection

Method: **Formalin-Ethyl Acetate Sedimentation Concentration**

Procedure:

- Study participants are provided clean wooden applicator stick and clean, dry and leak proof plastic cup labeled with subject's serial number.
- Instruct him/her to bring 2gm (about thumb size) of fresh stool sample
- Mix the stool specimen well.
- Strain about 5ml of the fecal suspension through wetted cheesecloth-type gauze placed over a disposable paper funnel into a 15 ml conical centrifuge tube.
- Add 0.85% saline or 10% formalin through the debris on the gauze to bring the volume in the centrifuge tube to 15 ml. Distilled water may be used.
- Centrifuge at 500 × *g* for 10 minutes.
- Decant supernatant. Add 10 ml of 10% formalin to the sediment and mix thoroughly with wooden applicator sticks.
- Add 4 ml of ethyl acetate, stopper the tube, and shake vigorously in an inverted position for 30 seconds..
- Centrifuge at 500 × *g* for 10 minutes.
- Free the plug of debris from the top of the tube by ringing the sides with an applicator stick. Decant the top layers of supernatant.
- Use a cotton-tipped applicator to remove debris from sides of the centrifuge tube.
- Add several drops of 10% formalin to resuspend the concentrated specimen.
- Take a small amount of sediment and place on a microscope slide
- Cover with cover slip
- Scan the entire cover slip area using the 10x objective, 40x if something suspicious is seen.

Appendix N. Laboratory procedure for Blood film

Method:Microscopic examination of thick and thin blood films stained with Giemsa stain.

Procedure:

- Wear gloves during the procedure
- Prepare pre-cleaned slides in a clean surface
- Elect the middle finger and clean the area with a alcohol swab and allow to dry.
- Puncture using a sterile lancet.
- Apply gentle pressure to allow blood drop to ooze out and wipe away the first drop of blood
- Apply gentle pressure to the finger and collect a single small drop (~2µl) of blood in the center of the slide for thin film.
- Release the pressure immediately to allow recirculation.
- Apply gentle pressure again to transfer more blood and collect one bigger (~6µl) for thick film
- Make film and allow the blood films to dry quickly.
- Fix the films using absolute methanol
- Staining blood film using giemsa working solution for minimum of 10 minutes
- Remove the slides and clean the back of each slide with dry gauze
- Place a drop of immersion oil on the edge of the middle of the film.
- Scan using low magnification.
- Carefully examine the film using the 100x objective

Appendix O. Laboratory Recording Format

Participant ID_____

Laboratory results		
Code	Procedure type	
L01	Serum creatinine	in mg/dl_____
L02	Serum urea	in mg/dl_____
L03	Hemoglobin	in g/dl_____
L04	FBS/RBS	In mg/dl_____
L05	Parasite species	_____
L06	Hemoparasite species	_____

Appendix P. Curriculum Vitae

1. General information

Name	Teshome Tujuba
Sex	Male
Place	Hararghe
Date of Birth	April, 1987
Nationality	Ethiopian
Mobile	09-13-95-00-19
Email	tujubateshome08@yahoo.com

2. Qualification

BSc degree in Medical Laboratory Technology from Addis Ababa University

3. Work Experience

I have been working in laboratory department for 7 years.

4. Trainings

- TB microscopy, CD4 clinical chemistry and hematology Lab. diagnosis and Laboratory Quality Management System(LQMS) and Gene expert training.

5. Skills

Language skill

Language	Reading	Speaking	Listening	Writing
English	Excellent	Excellent	Excellent	Excellent
Afan Oromo	Excellent	Excellent	Excellent	Excellent
Amharic	Excellent	Good	Excellent	Excellent

6. Reference:

Mr. Ziyad Hussen - Gelemso Hospital Laboratory head

Phone: 09 12-29-66-58

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