ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

PREVALENCE OF ANEMIA AND ASSOCIATED FACTORS AMONG TYPE 2 ADULT DIABETIC PATIENTS ATTENDING AMBO TOWN GOVERNMENT HOSPITALS IN WEST SHEWA, ETHIOPIA

BY
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A RESEARCH THESIS TO BE SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCES, COLLEGE OF HEALTH SCIENCE, ADDIS ABABA UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS IN CLINICAL LABORATORY SCIENCES (HEMATOLOGY AND IMMUNO-HEMATOLOGY)

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Research project Submission Form

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Full Title of the Project	Prevalence of anemia and associated factors among type two Adult diabetic Mellitus patients attending Ambo town government hospitals in west shewa, Ethiopia.
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Duration of the project	September---October 2020
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ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared by Jafero Oljira

Prevalence of anemia and associated factors among type 2 Adult diabetic patients attending Ambo town government Hospitals in West Shewa, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and immuno-hematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External Examiner_____Signature _____ Date _____

Internal Examiner_____ Signature _____ Date _____

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Chairman of the Department of Graduate Program Coordinator

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Lists of Abbreviations and Acronyms

ACE	Angiotension Converting Enzyme
AOR	Adjusted Odds Ratio
BMI	Body Mass Index
BP	Blood Pressure
CKD	Chronic Kidney Disease
DM	Diabetes Mellitus
EDTA	Ethylenediamine tetra acetic acid
eGFR	Estimated Glomerular Filtration Rate
Hgb	Hemoglobin
IL	Interleukin
K/DOQI	Kidney disease outcomes quality initiatives
KG	Kilogram
MDRD	Modification of diet in renal disease
Nacl	Sodium Chloride
QC	Quality Control
SOP	Standard Operating Procedure
SST	Serum Separation Tube
T2DM	Type two Diabetes Mellitus
WHO	World health organization

Abstract

Background: Anemia is the most common blood disorder and common complications of Diabetes mellitus. It is also a global public health problem affecting both developing and developed countries with major consequences for human health, social and economic development. In Ethiopia, chronic illnesses are tremendously rising with their complications, but very little research has been conducted, particularly on anemia among type two diabetic Mellitus patients**.** Therefore, this study aimed at assessing the prevalence of anemia and associated factors among type 2 Adult diabetic mellitus patients attending Ambo town government hospitals in west shewa, Ethiopia.

Method: A Hospital-based cross-sectional study was conducted on type two Adult diabetic Mellitus patients for a routine checkup from September - October 2020 at Ambo town government hospitals by using a simple random sampling technique. Data were collected by face-to-face interviews, administered questionnaires, and other biological samples such as blood and urine from 356 patients. Data was entered to EPI-info and exported to the SPSS software package for further analysis, bivariate and multivariate logistic regression models were fitted; crude odds ratio and adjusted odds ratio with 95% confidence interval were computed. In all cases, P-value less than 0.05 is taken as statistically significant.

Results: The study showed that 27.5 % of participants were anemic. Out of anemic type two diabetic Mellitus patients, 92 (92.9%) and 7 (7.1%) had mild and moderate anemia, respectively. Having nephropathy (AOR = 6.2, CI: 1.82, 21.16), being male (AOR = 4.32, CI: 1.09, 517.03), duration of diabetes for 5 years and greater (AOR = 4.42, CI: 1.27, 15.45) and having high serum creatinine (AOR=10.07, CI: 2.89, 35.2) were all significantly associated with anemia.

Conclusion and Recommendation: The magnitude of anemia was 27.5% among type two diabetic Mellitus patients in the study area. Therefore, routine screening of anemia for all type two diabetic Mellitus patients aiding in early identification and improved management of diabetes will lead to improved quality of life in this patient population.

Key Words:-Anemia, Diabetes, Renal Disease

1. Introduction

1.1. Background

Anemia is the most common blood disorder and a common finding in patients with diabetes. It is also considered a key indicator of chronic kidney disease and an important cardiovascular risk factor. Previous studies have shown that the incidence of anemia in diabetic patients is mostly associated with the presence of renal insufficiency. Thus, patients with diabetes have a greater degree of anemia for their level of renal impairment than non-diabetic patients presenting with other causes of renal failure [1].

Diabetes mellitus (DM) is classified as Type-1 diabetes mellitus (T1DM) that results from autoimmune destruction of insulin-producing beta cells of the pancreas. It is also called Juvenile diabetes or insulin-dependent diabetes. Type-2 diabetes mellitus (T2DM) results from insulin resistance, a condition in which cells fail to properly use insulin. It is seen mostly in adults and is also called non-insulin-dependent diabetes. Gestational diabetes mellitus develop during pregnancy that improves or disappears after delivery but 20% - 50% of cases can develop into type-2 diabetes mellitus later in life [2].

There are factors suggested as the reason for the earlier onset of anemia in patients with diabetes, include severe symptomatic autonomic neuropathy, causing efferent sympathetic denervation of the kidney and loss of appropriate erythropoietin; damage to the renal interstitium; systemic inflammation; and inhibition of erythropoietin release. Despite the plethora of reports on the presence of anemia in diabetic patients with renal insufficiency, limited study exists on the incidence of anemia in diabetics before the evidence of renal impairment. This may explain why most diabetic patients with normal renal function are rarely tested for anemia [1, 2].

Diabetes mellitus is a common metabolic disease. Anemia is more frequent and more severe at any level of glomerular filtration rate (GFR) in diabetics compared to non-diabetic patients [3]. Anemia also develops earlier and is more severe in patients with diabetes than in patients with renal impairment from other causes [4]. Diabetes is also the leading cause of chronic kidney disease (CKD) and is associated with excessive cardiovascular morbidity and mortality. Anemia is common among those with diabetes and CKD and greatly contributes to patient outcomes [5].

Anemia is a common complication of diabetes mellitus and is usually related to renal failure. The rate of anemia is higher in patients with diabetic nephropathy than in patients with a non-diabetic renal disease with similar levels of renal function. Anemia is an independent risk factor for the development and progression of cardiovascular disease and chronic renal disease and may also contribute to the development and progression of diabetic retinopathy and other diabetic complications [6].

Diabetes mellitus is the single most common cause of end-stage renal disease and consequently the most common cause of renal anemia. Patients with diabetes mellitus are also twice as likely to have anemia as those with renal impairment from other causes. Furthermore, declining hemoglobin levels may be observed before changes in renal function [7].

Anemia is an important component of diabetic nephropathy. It results from diminished erythropoietin production and to a lesser degree due to the increased excretion of erythropoietin in urine. However, erythropoietin responsiveness remains the same in these patients. Anemia in diabetes has complex and multifactorial etiology. Erythrocyte half-life is itself abnormal in diabetic patients due to several pathologies that have an impact on erythrocyte viability, such as increased osmotic stress on erythrocytes. This leads to an overall short life span of erythrocytes in diabetics further contributing to anemia. Patients with diabetes may be more vulnerable to the effects of anemia due to co-existent significant cardiovascular disease and hypoxia-induced organ damage. Hemoglobin levels may be linked to the risk of cardiovascular events, hospitalization, and increased mortality [8].

Anemia in DM patients is a common and often neglected and untreated complication of diabetes, which may have a negative consequence on the development and progression of other diabetes-related macro-vascular and micro-vascular complications which can further enhance anemia progression, making the vicious cycle. Growing evidence indicates that anemia in T2DM patients is a strong and independent indicator of increased risk for diabetes-related macro-vascular and micro-vascular complications. It causes early occurrence and rapid progression of complications like diabetic nephropathy, diabetic retinopathy, diabetic neuropathy, end-stage renal diseases, ischemic heart disease, and non-healing diabetic foot ulcer [9]. It is also a common condition, but it often goes unrecognized and not treated. Its symptoms are vague and easily mistaken for symptoms of other serious or chronic diseases. But even mild anemia can

significantly lower one's quality of life, and untreated anemia can have serious long-term health effects [10].

Anemia is defined as a decrease in the concentration of circulating red blood cells or in the hemoglobin concentration and a concomitant impaired capacity to transport oxygen. It has multiple precipitating factors that can occur in isolation but more frequently occur. Anemia is a common blood disorder. It is an increasingly recognized entity in patients with diabetes mellitus. It is a clinical condition characterized by a reduction in hemoglobin concentration of blood below the normal level (as defined by WHO criteria <13 g/dl for men and <12 g/dl for women) for the age, sex, physiological problem, and altitude [11]. Anemia is considered as a key indicator of chronic kidney disease, eye disease and an important cardiovascular risk factor therefore diabetic anemic patient's life span is less as compare to non-anemic diabetic patients [2].

1.2. Statement of the Problem

Anemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. It occurs at all stages of the life cycle [11] and affects nearly two billion (27%) people worldwide [12].

Anemia is a common complication of diabetes mellitus, and the risk of anemia in diabetic patients is estimated to be two to three times higher than that of patients without diabetes [13]. Evidence indicates that the incidence and prevalence of anemia in patients with diabetes is typically associated with erythropoietin deficiency due to concomitant renal disease [14, 15]. The risk of developing anemia in patients with diabetes and renal disease is significantly greater, and is often more severe, and occurs earlier than in non-diabetic patients with renal diseases [6, 16]. Recent studies have demonstrated the unfavorable influence of anemia on the progression of renal disease, cost of managing the disease, quality of life and cardiovascular disease, and all-cause mortality in diabetic patients who also have renal disease and the combination of anemia and kidney disease in diabetics identifies a group with adverse outcomes [17–19]. There are, however, other factors that could contribute to the development of anemia in diabetes, including

chronic inflammation, oxidative stress, autonomic neuropathy, nutritional deficiencies (iron, folate,andvitaminB12), autoimmune diseases, drugs, and advanced glycation [14,20].

Serious complications of early-onset anemia in diabetic patients include severe symptomatic neuropathy leading to efferent sympathetic denervation of the kidney and possible damage to the renal interstitial and inability to produce appropriate erythropoietin, systemic inflammation, and inhibition of erythropoietin release [1, 21].

Globally, the prevalence of concurrent anemia and diabetes mellitus (both type1 and type2) ranges from 14% to 45% in various ethnic populations worldwide [14]. The magnitude of anemia among T2DM patients varies among studies and regions, ranging from 7.7% in the United States of America (USA) to 67% in India [22, 23, 24, 25, 26, and 27].

Anemia is a common and often unrecognized complication of diabetes associated with all-cause and cardiovascular disease mortality [28, 29]. Anemia is known to contribute significantly to the development of micro and macro vascular complications of diabetes, which has a negative impact on the quality of life of the patients and health care costs of the disease [30]. Similarly, in patients with diabetes, anemia is an independent risk factor for cardiovascular events and is associated with a rapid decline of renal function and increased need for renal replacement therapy, which is often unavailable or unaffordable in most developing countries like Africa [31, 32].

The cause of anemia in diabetes is multifactorial and includes nutritional deficiencies, inflammation, concomitant autoimmune diseases, advanced age; lower Body Mass Index (BMI), longer duration of diabetes, peripheral vascular disease, specific medications, and hormonal changes in addition to kidney disease [33–36]. Various studies revealed the development of anemia in T2DM patients is significantly associated with the sex [2, 37, 38], age [33, 37, 39], marital status [33], educational status [39], BMI, hypertension, hematological diseases [40], glycemic control, gastrointestinal disorders, and chronic kidney diseases [8]. The duration of diabetes [37] and micro vascular complications of diabetes such as diabetic nephropathy, neuropathy, and retinopathy [37, 41, 42, 39, 43, 44, and 45] have all been found to be significantly associated with anemia in T2DM patients

Although many types of research were done on the prevalence of anemia among T2DM, anemia in T2DM remains unrecognized and untreated in 25% of the diabetic patients [46, 33] because both share similar symptoms such as lethargy, pale skin, chest pain, irritability, numbness/coldness in the hands and feet, tachycardia, shortness of breath and headache [35]. Our country, Ethiopia is one of the developing countries where both anemia and diabetes mellitus are major public health issues [47, 48] with an increased incidence of DM, [7] it becomes mandatory to be aware of such co-morbidities at the earliest.

Current guidelines on the management of diabetes do not recommend routine screening for anemia. Although reports on the prevalence and predictors of anemia in diabetic patients exist elsewhere, such information is very rare in sub-Saharan Africa where other potential contributory factors like infectious and genetic diseases as well as nutritional deficiencies are very frequent and likely to worsen the burden of anemia. Therefore, this study will aim to incorporate anemia screening in the Guideline on the management of diabetes and to determine the prevalence of anemia and its associated factors among patients with type 2 Adult diabetic mellitus patients attending Ambo town government Hospitals, West Shewa, Ethiopia.

1.3. Significance of the Study

In Ethiopia, only a few studies have been carried out to investigate the prevalence of anemia and associated factors among type two diabetic patients. So conducting such kind of research work provides a lot of benefits for Researchers as literature for further study on this area, for policymakers and Health care providers.

It will also create awareness concerning the anemia among type 2 diabetic patients for health care providers and policymakers. The anemic individual will get early treatment so it is possible to minimize the complications of the disease. Additionally, it is also important to design prevention and control strategies. This information will be useful in establishing the clinical relevance and need of incorporating anemia screening into the routine assessment of diabetic complications to improve diabetes care.

2. Literature Review

2.1. Prevalence of anemia among Type Two Diabetic Patients

A Descriptive and Analytical study done in Brazil from January 2010 to January 2013 by Berberi J.et.al with a total sample of 146 type 2 diabetic patients reported 50 of the study participants had anemia, accounting for 34.2% [40]. Additionally, a retrospective study was done in China from January 2010 to December 2011 by Chen CXR., Li YC.et.al 6325 Chinese type 2 diabetic patients indicated, 441 (22.8%) were found to have anemia [49].

Another cross-sectional study involved 808 adult patients with type 2 DM and chronic kidney disease who were recruited via systematic sampling from 20 public primary care clinics in Peninsular Malaysia from October to December 2015 by Idris I.et.al indicated 31.7% prevalence of anemia [25]. A similar study done in Pakistan in 2014 by Sharif A.et.al by recruiting 200 type-2 diabetic patients reported 126 (63%) of the participants were anemic [2].

There is also a cross-sectional study conducted in the outpatient endocrinology clinic at Baqiyatallah University of Medical Sciences Hospital, Tehran, Iran by Hosseini M.S. et. al from February 2011 to February 2012 on a total of 93 patients revealed that the prevalence of anemia was 30.4% [42]. Additionally, there was a study conducted at the Gayatri Hospital, Gandhinagar, in India on 200 adult patients with age more than 30 years attending the outdoor patient department (OPD) or admitted in the ward as an indoor patient department (IPD) of Gayatri Hospital on 2016. The study revealed that the prevalence of anemia among type two diabetic patients was 18% [26].

Furthermore, a retrospective study which was conducted from January 2016 to December 31, 2017, by Aldallal SM.et.al in Amiri Hospital (Al-Asima Capital area) and polyclinics, Kuwait showed that from 19,059 patients, 5,655 patients were found to be anemic accounting for 29.7% prevalence of anemia [50]. According to a case-control study done in 2016 by Antwi-Bafour S.et.al in Accra Ghana on a total of 100 types two diabetic Patients on the prevalence of anemia among patients with type 2 diabetes indicated a high incidence of anemia among the cases and the prevalence was 84.8 % [51].

Additionally, a cross-sectional study done in Nigeria in 2012 by Adjumo B.et.al on 72 participants shows that 15.3% of the patients had anemia [1]. A similar cross-sectional study was conducted from June to 31st December 2013 by Feteh V.F.et.al to determine the prevalence of anemia and investigate the related factors, in type 2 diabetic patients attending a tertiary health care institution in Cameroon. A total of 636 patients were examined including 263 (prevalence rate 41.4 %) who had anemia [39].

Also, a cross-sectional study was conducted in Harari Region, Eastern Ethiopia from February to March 30, 2019, by Bekele A.et.al.by selecting 374 T2DM patients. This study revealed 34.8% of the participants were anemic [52]. Another cross-sectional study conducted from January to April 2018 by Fisseha T.et.al on 412 diabetic adults at the diabetes clinic of Dessie Referral hospital in Northeast Ethiopia reported 26.7% of anemia prevalence [7]. Additionally, a cross-sectional study was conducted from April 1 to May 30, 2019, by Mitiku M.et.al among 249 T2DM patients at Debre Berhan Referral Hospital in North-East Ethiopia showed that the prevalence of anemia was 20.1% [9].

2.2. Associated Risk Factors of Anemia among Type Two diabetic patients

A Descriptive and Analytical study done in Brazil from January 2010 to January 2013 by Berberi J.et.al with a total sample of 146 types 2 diabetic patients reported that the body mass index, hypertension, hematocrit, red cells and hemoglobin were significantly associated with anemia [40].

A retrospective study was done in China from January 2010 to December 2011 by Chen CXR., Li YC.et.al 6325 Chinese type 2 diabetic patients. In this study, independent predictors for hemoglobin level among diabetic patients were age, gender, serum creatinine level, estimated glomerular filtration rate, hemoglobin A1c, and urine albumin-creatinine ratio ($P<0.001$) [49]. Another cross-sectional study involved 808 adult patients with type 2 DM and chronic kidney disease that were recruited via systematic sampling from 20 public primary care clinics in Peninsular Malaysia from October to December 2015 by Idris I.et.al. Multivariate regression analysis showed that women and those with older age were more likely to have anemia [25].

Another study was done in Pakistan in 2014 by Sharif A.et.al by recruiting 200 type-2 diabetic patients. This study shows a higher incidence and risk of anemia in females (36%) as compared to males (27%) ($P< 0.05$) and in poorly controlled diabetes 49.5% compared to those with controlled diabetes ($p < 0.05$) [2]. Additionally, a retrospective study conducted from January 2016 to December 31, 2017, by ALdallal SM.et.al in Amiri Hospital (Al-Asima Capital area) and polyclinics, Kuwait on 19,059 patients, There was a statistically significant relationship between the prevalence of anemia and gender, i.e. the prevalence of anemia was significantly greater in diabetic females than diabetic males ($P < 0.05$). The prevalence of anemia was significantly greater in poorly controlled diabetics than those with glycemic status under control ($P < 0.05$) [50].

Furthermore, a cross-sectional study done in Nigeria in 2012 by Adjumo B.et.al on 72 participants shows that the odds of developing anemia was higher in patients with poorly controlled diabetes (HbA1c > 7.5%) compared to those with controlled diabetes (≤7.5%) and in patients of age ≥60 yrs compared to those of age < 60 yrs ($p < 0.05$). The odds of anemia were similar in males and females ($p = 0.26$) [1]. Also, a cross-sectional study was conducted from June to 31st December 2013 by Feteh V.F.et.al to determine the prevalence of anemia and investigate the related factors, in type 2 diabetic patients attending a tertiary health care institution in Cameroon. A total of 636 patients were examined. Compared with their non-anemic counterparts, anemic diabetic patients who were older had a longer duration of diabetes, lower eGFR, higher prevalence of proteinuria, and diabetic retinopathy were significantly associated with anemia [39].

Moreover, a cross-sectional study was conducted from January to April 2018 by Fisseha T.et.al on 412 diabetic adults at the diabetes clinic of Dessie Referral hospital in Northeast. In multivariate analysis, older age, presence of hypertension, high systolic BP, serum creatinine, and low GFR were independently associated with greater odds for the presence of anemia [7]. A similar hospital-based cross-sectional study was conducted in Harari Region, Eastern Ethiopia from February to March 30, 2019, by Bekele A.et.al.by selecting 374 T2DM patients. This study showed being male, physical inactivity, having nephropathy, poor glycemic control, a recent history of blood loss, and duration of diabetes for five years and greater were all significantly associated with anemia [52].

2.3. Relationship between Anemia and Type Diabetes

A cross-sectional study done by Trevest K.et.al in the united kingdom states that chronic diseases, such as DM, are accompanied by mild-to-moderate anemia, often called anemia of inflammation or infection or anemia of chronic disease [53]. Andrews and Arredondo determined the presence of anemia in type 2 diabetic patients as well as evaluating the expression of genes related to inflammation and immune response. The results found by the authors demonstrate that diabetic patients with anemia exhibit increased expression of pro-inflammatory cytokines as compared to diabetic patients only. In anemic patients increase in IL-6 production, as well as B cell activity was confirmed which reinforces the association between IL-6 and anti erythropoietic action. Moreover, the diabetic and anemic patients had high levels of C-reactive protein and ferritin ultra-sensible; however, these diabetic and anemic patients had low iron contents, showing that ferritin increases were associated with the chronic inflammatory process present in diabetes [54].

A study done by El-Achkar TM.et.al from August 2000 to December shows that several mechanisms have been proposed for the association between DM and anemia. Autonomic neuropathy can decrease sympathetic stimulation of erythropoietin production through renal denervation. Moreover, the effect of DM on tissues responsible for the synthesis of erythropoietin reduces kidney response to hypoxia. Decrease in androgenic hormones level in DM attenuates stem cells in the bone marrow and reduces erythropoietin synthesis in the kidney [16].

According to Craig KJ.et al, reticulocytes show resistance in their response to increased levels of erythropoietin [21]. Diabetic patients without nephropathy have an appropriate erythropoietin response to hypoxia. An important cause of resistance to erythropoietin is the inflammation accompanied by the rise of cytokines and consequent suppression of erythrocyte stem cell proliferation. This shows that before the development of nephropathy, overt inflammation associated with diabetes may culminate in erythropoietin suboptimal response. As mentioned before, anemia may ensue destruction of peritubular fibroblasts and a decrease in erythropoietin level [42].

Another cross-sectional study done in Cameroon from June to December 2013 by Feteh V.F shows Hyperglycemia has a direct relationship with the development of an inflammatory condition shown by the increased expression of pro-inflammatory cytokines such as IL-6, TNF-α, and NFκB. Thus, diabetes, as well as hyperglycemia due to its nature, is also an inflammatory disease character. Studies show that the longer the duration of the disease and/or loss of glycemic control, the higher the inflammatory process. The elevation of pro-inflammatory cytokines plays an essential role in insulin resistance and induces the appearance of cardiovascular complications diabetic micro- and macrovascular, kidney disease, and anemia. By increasing especially IL-6, the antierythropoietic effect occurs, since this cytokine changes the sensitivity of progenitors to erythropoietin (erythroid growth factor) and also promotes apoptosis of immature erythrocytes causing a decrease, further, in the number of circulating erythrocytes and consequently causing a reduction of circulating hemoglobin [39].

It should also be noted that, due to the development of diabetes mellitus, nephropathy may arise, which further undermines the renal production of erythropoietin, positively contributing to an increased anemic framework. According to Escorcio et. al [55], approximately 40% of diabetic patients are affected by kidney diseases. The decreased renal function and proinflammatory cytokines are the most important factors in determining the reduction of hemoglobin levels in those patients. The inflammatory situation created by kidney disease also interferes with intestinal iron absorption and mobilization of inventories. Therefore, diabetic patients with kidney disease have the highest risk for developing anemia [39].

A nested case control did by Loutradis C.et.al shows mechanisms promoting anemia in diabetic individuals. Erythropoietin deficiency due to efferent sympathetic denervation of the kidney as a result of diabetic neuropathy, subclinical inflammation leading to functional iron deficiency through increased hepcidin levels, increased non-selective proteinuria excretion resulting in transferrin and erythropoietin loss, increased red blood cell destruction because of disorders in the cellular structure caused by DM and advanced glycation end products (AGEs) possibly decreasing erythrocyte lifespan are among them. Further, increased use of RAAS (Renin-Angiotensin-Aldosterone System)-blockers in diabetic patients, may promote anemia occurrence through inhibition of the physiologic erythropoietic action of angiotensin [56].

3. Objective

3.1. General Objective

- To assess the prevalence of anemia and associated factors among type 2 Adult diabetic patients attending Ambo town government Hospitals in West Shewa, Ethiopia.

3.2. Specific Objective

- To assess the prevalence of Anemia among type 2 Adult diabetic patients attending Ambo town government Hospitals in West Shewa, Ethiopia.
- To determine the associated factors of anemia among type 2 Adult diabetic patients attending Ambo town government Hospitals in West Shewa Ethiopia.

4. Materials and Methods

4.1. Study Area

The study was conducted at Ambo town in government hospitals, west shewa, Ethiopia. Ambo town is located 115km from the capital city of Ethiopia, Addis Ababa. Ambo town has latitude and longitude of 8^{o} 59′N 37 ^{o}S, ′E with an altitude 2101 m above sea level Ambo town has a total population of 96521. Ambo town administration has two hospitals, Ambo General Hospital and Ambo University Referral Hospital. These Hospitals give service to people of Ambo town, surrounding West Shewa zone, and some parts of Horro Guduru Wollega. There are a total of 536 type diabetic patients from these Hospitals. These hospitals also register and treat all diagnosed diabetic patients [57, 58].

4.2. Study Design and Period

A hospital-based cross-sectional study was conducted at Ambo town in government hospitals from September - October 2020 G.C.

4.3. Population

4.3.1. Source Population

The source population includes all type two Adult diabetic patients seeking healthcare services at Ambo town government hospitals.

4.3.2. Study Population

The study population includes all type two Adult diabetic patients seeking healthcare services at Ambo Town Government Hospitals for follow-up from September through October 2020 G.C.

4.4. Inclusion and Exclusion Criteria

4.4.1. Inclusion Criteria

Patients with diagnosed type 2 diabetes, irrespective of gender and aged 18 years or above were included.

4.4.2. Exclusion Criteria

Participants, who are seriously ill, and unable to stand and sit without assistance or support, were excluded from the study because it is difficult to get an appropriate and accurate measurement from these patients.

4.5. Study Variable

4.5.1. Dependent Variables

Prevalence of anemia

4.5.2. Independent Variables

Socio-demographic characteristics: Age, Sex, Marital status, Occupational status, Educational status, and Residence.

Lifestyle characteristics: Cigarette smoking, alcohol consumption, physical activity, Nutritional status (Body Mass Index or BMI).

Clinical conditions: Duration of diabetes(in years), medications taken for diabetes, medications taken for diseases other than diabetes, types of diseases for which other medications taken, Neuropathy, Retinopathy, Nephropathy, history of hypertension, HIV status, history of blood loss, Glycemic control, Systolic blood pressure(mmHg), Diastolic blood pressure(mmHg), Serum creatinine and Urine albumin.

Conceptual Framework of Prevalence of Anemia among Type two Adult diabetic patients and associated factors.

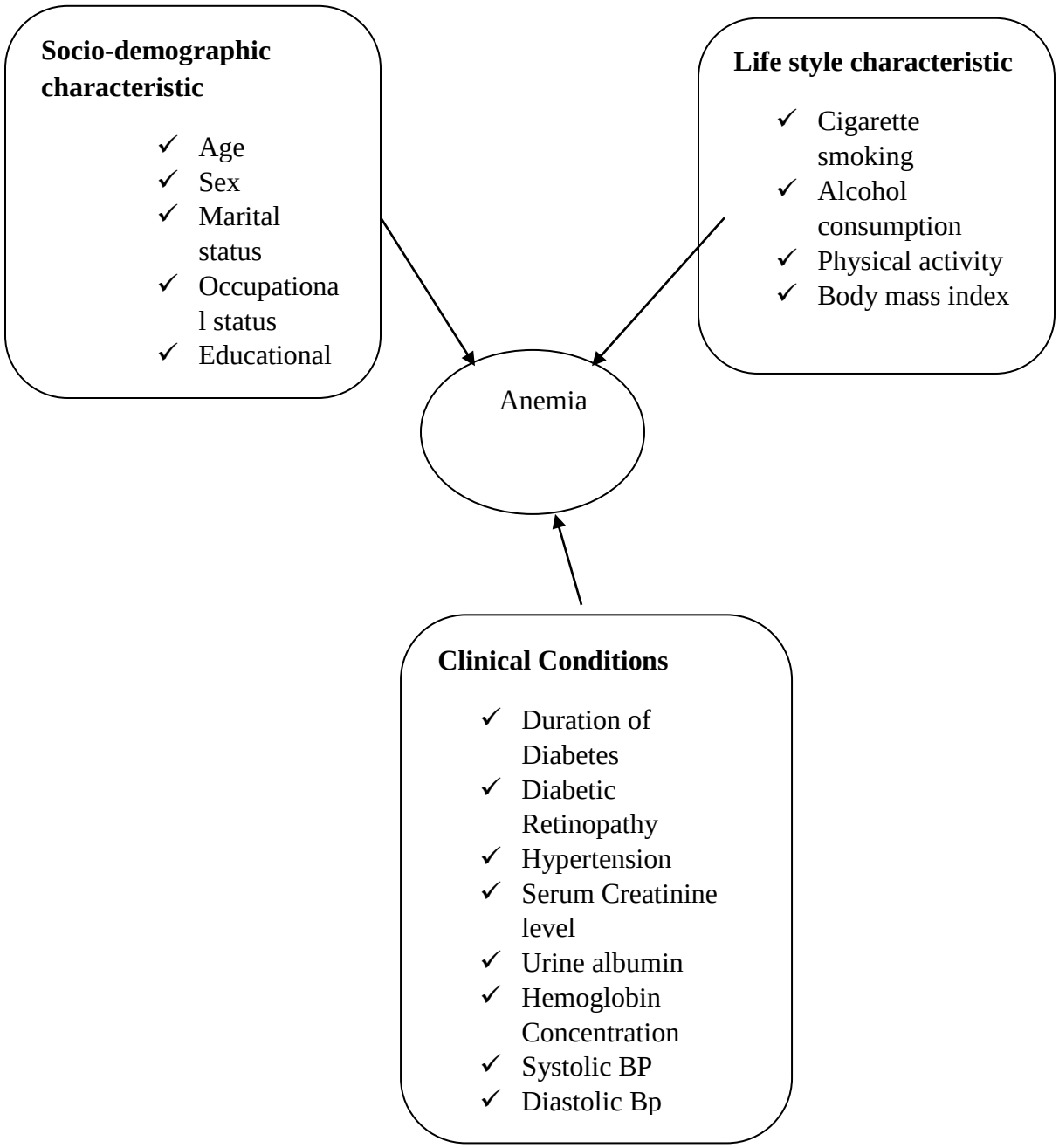

Figure 1. Conceptual Framework developed after reviewing work of different works of literature

4.6. Sample Size Calculation and Sampling Methods

4.6.1. Sample Size Calculation

By Using Single proportion sample size determination, the sample size was estimated assuming a prevalence of anemia of 34.8 % based on a previous study in people with diabetes in Harari Region Eastern Ethiopia [52], a precision of 5 % and a z value of 1.96. By adding 10% for non-respondent a total of 383 participants were required.

$n= (Z1-\alpha_{/2)}^2 \times P\ (1-P)/d^2$

$n= (1.96)^2 \times 0.348 \times 0.652/ (.05)^2 = 348$

By Adding ten present for non-respondents the total sample was

n=348+34.8=383

4.6.2. Sampling Methods

A simple random sampling procedure was used to select the study subject from Ambo town Government Hospitals. Ambo Town has two Hospitals. For this study, both hospitals were selected. The total sample size of 383 was distributed to the hospitals proportionally to their type two diabetic patient's population

To distribute n by the selected hospitals with a proportionate sample from each hospital can be calculated by

ni= (n/N)*Ni

ni = sample size in each hospital

Ni = number of type two diabetic patients in each hospital

N = total type two Diabetic patients

Depending on this:-

The number of type two Diabetic patients in Ambo general hospital is 340

The number of type two Diabetic patients in Ambo University referral hospital is 196

From Ambo General Hospital 383/536*340=243

From Ambo University Referral Hospital 383/536*196=140

So a total of 383 were required from both hospitals.
But, for a different reason, the total sample was 356. The main reasons for the non-response were a failure to follow through, covid 19, and a refusal to participate.

From Ambo General Hospital is 226

From Ambo University Referral Hospital are 130

4.7. Data Collection and Laboratory Measurement

Data were collected by using a semi-structured questionnaire. Four data collectors (two nurses and two laboratory professionals) were collect the data. The collected information includes Socio-demographic characteristics, clinical characteristics, anthropometric measurements, and laboratory analysis. Socio-demographic data and clinical characteristics like duration of DM were collected using an interview guide; whereas the presence of diabetes-related complications like; retinopathy, neuropathy, nephropathy, and other complication; history of hypertension and current diabetic medications were collected from reviewing of patient's medical records. The Duration of Diabetes is categorized as less than five years and greater than or equal to five years [52]. Four consecutive fasting blood glucose measurements, including measurement at the time of the data collection period, were also recorded from the patient's medical records for calculating the mean blood glucose level. Anthropometric measurements such as weight (kg) and height (m) were measured according to WHO recommendations. The body mass index (BMI) was computed as weight in kilograms divided by the square of the height in meters (kg/m2). The BMI of the participants were classified as: underweight less than 18.5 kg/m2, normal (18.5–24.9 kg/m2), overweight (25–29.9 kg/m2), and obese (≥ 30 kg/m2) [9]. Blood pressure (BP) was measured by using a manual sphygmomanometer after 10 mins of rest in a sitting position Hypertension was defined as Systolic Blood Pressure (SBP) ≥140 mmHg and/or Diastolic Blood Pressure (DBP) ≥90 mmHg [10]. For laboratory data, from each participant, five mL of venous blood was collected under aseptic conditions by venous puncture from the vein using a disposable syringe for hemoglobin determination, and serum creatinine analysis. Hemoglobin (Hgb) values were calculated by using a hematology analyzer, mission hemoglobin meter. Serum creatinine was analyzed by BS-200 Analyzer chemistry analyzer computed and as mg/dl. Serum creatinine values were considered abnormal if values of serum creatinine analysis were > 1.2

mg/dl [59]. Good glycemic control: an average of four consecutive fasting blood glucose measurements was ≤ 130 mg/dl and Poor glycemic control: an average of four consecutive fasting blood glucose measurements was >130 mg/d [10]. Urine samples were collected from the participating patients to detect the presence of albumin which indicates abnormal and the absence of albumin indicates that normal by using dipsticks [7].

4.7.1. Laboratory Analysis

Laboratory Analysis for Creatinine

Purpose

- Creatinine is a kidney function test, providing a rough approximation of glomerular filtration.

Method

- Modified Jaffe method.

Reagent and equipment

- BS-200 Analyzers
- Micropipette and tips
- Test tube rack
- NaCl solution 9 g/L
- Reagent
- Calibrator and controls

R_1	Sodium hydroxide	0.38 mmol/L
R_2	Picric acid	15 mmol/L

Preparation of working reagent

- R1 and R1 are ready to use.

Storage and Stability

- Stable up to expiry date indicated on the label, when stored unopened at 2ºc - 8ºc and protected from the light.
- Once opened the reagent is stable for 14 days when refrigerated on the analyzer or refrigerator.
- Contamination of the reagent must be avoided. Do not freeze the reagents.

Principle

Adding sodium hydroxide to sample

Mixing and incubate at 37 ^{o}c for one minute

Then add picric acid mix thoroughly at 37 ^{o}c for 30 seconds and then read the absorbance change value.

Creatinine + Picric acid ⟷ Creatinine – Picric acid complex

Creatinine forms in alkaline solution an orange-red colored complex with picric acid.

The absorbance of this complex is proportional to the creatinine concentration in the sample.

Laboratory Analysis for Hemoglobin

Mission Hemoglobin Meter

Principle

The Mission Hemoglobin (Hb) Testing System utilizes quantitative reflectance photometry for the measurement of hemoglobin. The test cartridge includes a mesh-covered sample reaction zone. The specimen is applied to the center of the reaction zone and the mesh functions to separate the sample evenly on the entire reaction zone. The reagents on the reaction zone function to hemolyze red blood cells and release the hemoglobin. The hemoglobin is converted to methemoglobin to generate a color change. The meter reads the intensity of the reflected light at 525 nm every second until the endpoint of the reaction is detected. The light intensity at the endpoint is directly proportional to the hemoglobin concentration.

Laboratory Analysis for Albumin

Dipstick

Specimen

After collecting mid-stream /randomly voided urine of 10ml, it should be examined within one hour.

Principle

Urine is a physiological fluid of widely varying composition formed by the kidney from the blood. The principal constituent of urine is water (92 - 99% in normal urine).

Thousands of compounds have been identified in normal urine and the vast majority is derived from the blood. The major urine components are creatinine, urea, uric acid, sodium, potassium,

chloride, calcium, magnesium, phosphates, sulfates, and ammonia. A considerable portion of the urine solids is waste products that have been filtered through the kidneys and are being excreted from the body. Some of the constituents are considered to be regulatory substances, which may be either retained or excreted by the kidney. Urine is a principal pathway for the excretion of body waste and homeostatic regulations of body water and body electrolytes. The chemical principles of the reagent pads on the strips are:

Albumin - This test is based on the protein-error-of-indicators principle. At a constant buffered pH, the development of any green color is due to the presence of protein. Colors range from yellow for "Negative" through yellow-green and green to green-blue for "Positive" reactions

4.8. Data Quality Assurance

The principal investigator supervised the data collection process. The training was given to the data collectors. The completeness of data collected was checked every day by the principal investigator. The reliability of the study findings is guaranteed by implementing Quality control (QC) measures throughout the whole process of the laboratory works. All materials, equipment, and procedures were adequately controlled.

Pre-analytical:- in this phase, the principal investigator checked the sample quality and its accuracy by checking the patient request with full information and proper labeling, handling, and transportation of the sample. Based on sample acceptance and rejection criteria the sample was evaluated for clot, hemolysis, and lipemic. Any sample with that problem was rejected and also patient request that is not properly filled with necessary patient information and miss-match of sample in identification was rejected.

Analytical phase: - in this phase, the patient specimen was prepared for testing and ends when the test result is interpreted and verified. Doing this the specimen was rechecked for its correct labeling and identifications again by the personnel who analyze the sample. After checking the sample accuracy the personnel checked the machine and by doing daily, weekly, or as-needed maintenance. After doing the maintenance the personnel processed the daily QC.

Post-analytical phase: - Post-analytical quality assurance was insured by using appropriate result formats and registrations.

4.9. Data Analysis and Interpretation

The data was entered into "Epi info version 7" and was exported to SPSS version 23 statistical software for analysis and the results were explained by percentages, tables, and frequencies. Chi-squared (x^2) test was used for comparison of categorical variables. Multivariate logistic regression analysis was conducted. The crudes odds ratio was computed (COR) and the corresponding adjusted odds ratios (AOR) and 95% confidence intervals (CI) were used to identify factors independently associated with anemia. In all cases, P-value less than 0.05 is taken as statistically significant.

4.10. Operational Definition

Anemia is defined according to the World Health Organization (WHO) criteria: Hgb concentration <13 g/dl for males and < 12 g/dl for females [5]. It was further classified into mild anemia (female: 11–11.9 g/dl; male: 11–12.9 g/dl), moderate anemia (8–10.9 g/dl) and severe anemia (< 8 g/ dl) [9].

4.11. Ethical Considerations

Ethical clearance was obtained from the Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences, Department Research and Ethical Review Committee (DRERC). Permission was obtained from administrators of hospitals involved. The study aim, benefit, risk, and right for withdrawal any time from the study was explained to the study participants, and informed verbal, as well as written consent was obtained from each study participant. Physicians were informed about anemic and renal disease patients for proper management. Samples were coded and confidentiality of patient data was maintained throughout the study. Type two diabetic patients who are mild anemic 7.1 % were got treatment.

4.12. Dissemination of the Result

This study upon completion could serve as reference material to researchers and experts. To reach these bodies the finalized paper will be submitted to Addis Ababa University, College of Health Sciences, and Department of Medical Laboratory Sciences so it can serve as a reference in the library. In addition, a copy of this material will be given to the Ambo university Referral hospital and Ambo general hospital. The result will also be disseminated through publication in peer-reviewed local and international journals and through presenting it in relevant workshops and seminars.

5. Results

5.1. Socio-demographic characteristics of the participants

A total of 356 Type 2 diabetic patients took part in the study, with a response rate of 92.9 %. The main reasons for the non-response were a failure to follow through, covid 19, and a refusal to participate. Males made up 195(54.8%) out of the participants. The average age of the participants was 48.25 years (SD = 11.401), with the dominant 128(36%) age group was 40-49 years. Out of the total participants 281(78.9%) were married and 129(36.2%) government employers. There were 144(40.4%) participants from the total sample having an educational level college and above and 293(82.3%) were living in the urban area (Table 1).

Table 1. Socio-demographic characteristics of T2DM patients attending government hospitals in Ambo town, West Shewa, Ethiopia, from September to October 2020.

Variables	Categories	Frequency	Percentage
Age	30-39	82	23
	40-49	128	36
	50-59	78	29.9
	≥ 60	68	19.1
Sex	Male	195	54.8
	Female	161	45.2
Marital status	Single	23	6.5
	Married	281	78.9
	Divorced	42	11.8
	Widowed	10	2.8
Occupational status	Farmer	32	9
	Housewife	56	11.7
	Merchant	104	29.2
	Government employer	129	36.2
	Other*	35	9.8
Educational status	Unable to read and write	9	2.5
	Able to read and write	52	14.6
	Primary level(1-8)	77	21.6
	Secondary Level(9-12)	74	20.8
	Collage and above	144	40.4
Residence	Urban	293	82.3
	Rural	63	17.7

*daily laborers, Non- governmental organization, Driver

5.2. Lifestyle of the participants

In this study, 82 (23%) of the participants had smoked cigarettes at least once in their lives, and 41 (11.5%) of the participants were active smokers. Similarly, 191 (53.7%) of the study subjects had used alcohol at least once in their lives, and 49 (13.8%) of the participants were drinking alcohol currently. Most of the participants 18(36.7%) were drinking two times per week. Of the total study participants, 166(46.6%) were engaged in physical activity, and walking 134(80.7%) is the major physical activity used by the participants. The majority of the study subjects were overweight 149 (41.9%) (Table 2).

Table 2 . Lifestyle characteristics of T2DM patients attending government hospitals in Ambo town, west shewa, Ethiopia, from September to October 2020 (n=356).

Variables	categories	Frequency	Percentage
Ever smoked cigarette	Yes	82	23
	No	274	77
Smoking Currently	Yes	41	11.5
	No	41	11.5
Amount of cigarette smoking per day (n=41)	1/4 packet	23	6.5
	1/2 packet	13	3.7
	> 1 packet	5	1.4
Ever consumed alcohol	Yes	191	53.7
	No	165	46.3
Drinking alcohol currently	Yes	49	13.8
	No	142	39.9
Frequency of current alcohol drinking (per week)n=49	once	17	34.7
	two times	18	36.7
	≥ three times	14	28.6
Amount of drinking alcohol in typical days in a bottle (n=49)	1-2	17	14.7
	3-4	20	40.8
	≥ 5	12	24.5
Engaged in Physical activity(n=356)	Yes	166	46.6
	No	190	53.4
Frequency of physical activity (n=162)	brisk walking	134	80.7
	running	72	43.4
	swimming	47	28.3
Body mass index	underweight	11	3.1
	normal	144	40.4
	overweight	149	41.9
	obese	52	14.6

5.3. Clinical conditions, complications, and co-morbidities of the participants

The study showed that the duration of diabetes in most 217 (61%) of the participants was ≥5 years. All the patients were taking drugs for the treatment of their diabetes. Metformin was the most commonly used drug. Among the patients, 173(48.6%) taking it. Besides this, 166(46.6 %) of the patients were taking medication for the management of diseases other than diabetes, and

100(60.2 %) of the patients were taking antihypertensive drugs. Regarding the diabetes-related complications, 33(9.3%), 69(19.4%), 159(44.7%), 47(13.2%), 22(6.2%), 105(29.5%), and 14(4.4%) of the patients had neuropathy, retinopathy, nephropathy, macro-vascular complication, foot sores, history of hypertension and a history of HIV/AIDS respectively. In addition, 57(16%) of the patients have a history of blood loss. Of the study participants, 229(64.3%) of the patients had poor glycemic control. Similarly, 105 (29.5%) had a systolic blood pressure of ≥140mmHg, and 33(9.3%) had a diastolic blood pressure of ≥ 90mmHg. On the other hand, 147 (41.3%) of the participants had positive urine albumin, and 117 (32.9%) of the participants had higher or abnormal serum creatinine (Table 3).

Table 3. Clinical conditions, complications, and co-morbidities characteristics of T2DM patients attending government hospital in Ambo town, west shewa, Ethiopia, from September to October 2020 (n=356).

Variables	Categories	Frequency	Percentage
Duration of diabetes	< 5	139	39
	≥ 5	217	61
Medication taken for diabetes	Metformin	173	48.6
	Metformin +glebinclamide	90	25.3
	Insulin	56	15.7
	Other	37	10.4
Medication taken other than diabetes	Yes	166	46.6
	No	190	53.4
Medication taken for disease other than diabetes(n=166)	ACEI inhibitor	37	22.3
	Beta-blockers	46	27.7
	Omeprazole	32	19.3
	Others	51	30.7
Types of disease for which other medication taken	Hypertension	100	60.2
	Heart failure	21	12.7
	Gastritis	33	19.9
	HIV/AIDS	6	3.6
	Others	6	3.6
Neuropathy	Yes	33	9.3
	No	323	90.7
Retinopathy	Yes	69	19.4
	No	287	80.6
Nephropathy	Yes	159	44.7
	No	197	55.3
Macrovascular complication	Yes	47	13.2
	No	309	86.8
Foot sores	Yes	22	6.2
	No	334	93.8
History of Hypertension	Yes	105	29.5
	No	251	70.5
Ever tested for HIV/AIDS	Yes	316	88.8
	No	40	11.2
HIV status of the patient (n=316)	Positive	14	4.4
	Negative	302	95.6

Variables	Categories	Frequency	Percentage
History of blood loss	Yes	57	16
	No	299	84
Glycemic control	Good	127	35.7
	Poor	229	64.3
Systolic blood pressure (mmHg)	< 140	251	70.5
	≥ 140	105	29.5
Diastolic blood pressure(mmHg)	< 90	323	90.7
	≥ 90	33	9.3
Albumin	Positive	147	41.3
	Negative	209	58.7
Serum Creatinine	Normal	239	67.1
	Higher	117	32.9
Hemoglobin(g/dl)	Male < 13 g/dl	79	22.2
	Male ≥ 13g/dl	116	32.6
	Female < 12 g/dl	19	5.3
	Female ≥ 12g/dl	142	39.9

* Glimepiride, Galvusmet,

** Amoxicillin, Ciprofloxacin, nifidipine, lamuvudine, Efaverenz and Tenofovir

*** Pneumonia, Urinary tract infection, Typhoid fever

5.4. Magnitude of anemia and associated factors

The results of the hemoglobin test showed that 98 (27.5%) of the participants were anemic (Table 3). Males (78.6%) had a higher proportion of anemia than females (22.4%). Furthermore, it was greatest in patients aged 50 years and above (64.3%) and lowest in those aged less than 50 years (35.7%). Out of anemic T2DM patients, 91 (92.9%) and 7 (7.1%) had mild and moderate anemia, respectively. To classify variables linked to anemia, bivariate and multivariate analyses were used in binary logistic regression. As a result, having nephropathy, being male, having diabetes for five years or longer, and having a high serum creatinine level were identified as associated factors for anemia in T2DM patients. Male patients were 4.32 times more likely to have anemia than females (AOR = 4.32, CI: 1.09, 517.03). The occurrence of anemia was 6.2 times higher among patients who had nephropathy (AOR = 6.2, CI: 1.82, 21.16). Patients with high serum creatinine (AOR=10.07, CI: 2.89, 35.2) and patients having diabetes for five years and above (AOR = 4.42, CI: 1.27, 15.45) were significantly associated with anemia (Table 4).

Table 4. Factors Associated with anemia among T2DM patients attending government hospital in Ambo town, west shewa, Ethiopia, from September to October 2020 (n=356)

Variables	Categories	Crude OR (95% CI)	P-value	Adjusted OR (95%)	P-Value
Sex	Male	4.35(2.53-7.47)	0.00	4.32(1.09-17.03)	0.04
	Female	1		1	
Age	30-39	1		1	
	40-49	0.19(0.82-2.0.42)	0.00	1.09(0.17-6.91)	0.92
	50-59	10.32(0.17-0.63)	0.01	3.34(0.51-21.91)	0.21
	≥ 60	1.04(0.54-2.00)	0.9	3.50(0.51-23.80)	0.2
Medication taken for a disease other than diabetes	ACE inhibitor	1		1	
	Beta blockers	2.67(1.09-6.62)	0.31	4.98(0.98-25.50)	0.05
	Omeprazole	0.39(0.14-1.09)	0.08	0.78(0.14-4.25)	0.77
	Others	0.40(0.16-0.99)	0.05	0.51(0.11-2.30)	0.34
Nephropathy	Yes	8.55(4.88-14.90)	0.00	6.2(1.82-21.16)	0.01
	No	1		1	
History of Hypertension	Yes	5.32(3.21-8.81)	0.00	3.33(0.44-25.31)	0.11
	No	1		1	
Duration of diabetes	< 5	1		1	
	≥ 5	4.47(2.59-8.413)	0.00	4.42(1.27-15.45)	0.02
Body mass index	Underweight	0.81(0.22-3.01)	0.76	0.48(0.04-6.09)	0.57
	Overweight	0.28(0.15-0.55)	0.00	1.69(0.22-12.68)	0.61
	Obese	0.68(0.16-0.55)	0.00	1.45(0.24-10.68)	0.48
	Normal	1		1	
Systolic blood pressure	< 140	1		1	
	≥140	0.17(0.12-0.29)	0.00	0.27(0.04-1.75)	0.17
Diastolic blood pressure	< 90	1		1	
	≥ 90	4.08(1.5-11.03)	0.01	0.67(0.11-4.20)	0.67
Serum creatinine	Normal	1		1	
	Abnormal	7.28(4.36-12.18)	0.00	10.07(2.89-35.2)	0.00

6. Discussion

In this Hospital-based cross-sectional study, the prevalence of anemia and its associated factors among T2DM patients at Ambo Town government hospital, West Shewa Ethiopia, has been assessed. As a result of the hemoglobin test, 27.5 percent of the participants in the study were anemic. Out of anemic T2DM patients, 54 (87%) and 8 (13%) had mild and moderate anemia, respectively. Being male, diabetic nephropathy, High serum creatinine, and duration of diabetes Greater than or equal to five years were identified as associated factors of anemia among T2DM patients.

Anemia is a common finding in patients with diabetes and harms the patient's sense of wellbeing; it also impairs the ability to work and reduces the quality of life. Out of the total participants in this study, 27.5% of the diabetes patients had anemia. This finding is similar to a previous study done in Iran, which showed a 30.4% prevalence of anemia among diabetes patients [42]. This is also consistent with a study conducted in China and Kuwait which is 22.8% [41], 29.7% [60] respectively showed the prevalence of anemia among diabetes patients.

However, it was higher than other study conducted from various regions of the world, in Iran (19.6%) [45], India (12.3%) [37], Nigeria (15.3%) [1], Debre-Berhan (20.1%) [9], Kuwait (13%) [61] and Gondar (8.6%) [10]. These differences might be due to socio-demography, Feeding Habits, duration of DM, and age of the study participant.

On the other hand, the magnitude of anemia in this study was lower compared to the findings of other studies conducted in Brazil (34.2%) [40], Sub-Saharan Africa (Cameroon) (41.1%) [39], Pakistan (63%) [8], Harari Region (34.8%) [52], Ghana (84.8%) [51], Egypt (63%) [62], And United Kingdom (59%) [53]. These differences might be due to socio-demographic, feeding habits, lifestyle, measurements, duration of DM, age of the study participants, and altitude could all contribute to the variations.

Gender was shown to be significantly associated with anemia in the current study. Male patients were 4.32 times more likely to have anemia than females. Similar findings have been reported in Harari [52], Gonder [10], and the USA [16]. However, another analysis conducted in Pakistan

[2] and India contradicted this finding [37]. The possible explanation could relate to co-morbidities such as history of hypertension and nephropathy.

Even though there is no significant association between anemia and age among type two diabetes, this study showed the prevalence of anemia is greater in older age. This is consistent with other related studies done at the diabetes clinic of Dessie Referral hospital in Northeast Ethiopia [7], Amri Hospital and polyclinics, Kuwait [50], Debre Berhan Referral Hospital (DBRH), North-East, Ethiopia [9], and Australia [63]. This result was anticipated since aging is related to decreased hemoglobin levels and an increase of anemia irrespective of health status [64 and 65]. It also may be related to deficiencies of vitamins such as folate, bone marrow abnormality, and a higher number of co-morbidities, which are common in the elder [63].

In this study, anemia is more prevalent in hypertensive patients, while no significant association between anemia and hypertension by multivariate logistic regression. The main cause could be diabetic patients, the risk of renal impairment, thus increasing the subsequent development of anemia. In addition, nutritional deficiencies especially iron deficiency and chronic inflammation can be the cause [7]. This finding is consistent with a cross-sectional study performed in Ethiopia [7 and 10].

Although drug therapy which is taken for a disease other than diabetes such as, ACE inhibitors and Beta are associated with anemia [7] there is no significant association between anemias and thus drugs in this study. A Descriptive and analytical study done in Brazil [40] showed that there is a significant association between body mass index and anemia this is inconsistent with this study reveals there is no significant association between anemia and body mass index. Another study was done in China [49] and Ethiopia [7] showed that there is a significant association between anemia and high serum creatinine level which contradicts this study.

Anemia was 6.2 times more common in patients with nephropathy, which is consistent with research from Malaysia [43], Iran [42], and Harari [52]. Erythropoietin is a hormone made by the kidney and if the kidney is not working properly, there may not be sufficient hormone produced [52]. Anemia is a well-known complication of diabetes-related chronic kidney disease (CKD) and related to the degree of renal impairment, mainly due to impaired production of erythropoietin by peritubular fibroblast of the kidney [44]. The finding in this study further

supports this principle, as the results showed a gradual increase in the prevalence of anemia with a progressive reduction in renal function. There are many potential mechanisms, by which anemia can exist in patients with reduced renal function tests. Damage to erythropoietin-producing cells through either fibrosis or chronic inflammatory activities; tubulointerstitial changes; and autonomic neuropathy, which prevents anemia detection by peritubular fibroblasts of the kidney, is the possible mechanism [10, 56, and 66].

The duration of DM is one of the factors linked to the prevalence of anemia in this study. It was observed that a positive relationship between the duration of DM and anemia with a higher chance in patients with ≥ 5 years. Compared with patients with < 5 years duration of DM, the odds ratio of developing anemia in individuals with ≥5 years was 4.42 times. This finding is in agreement with the previous studies in Harari Region [52] and Iran [42]. The reason for this increased chance of anemia development with increasing duration of DM may be due to the chronic effects of hyperglycemia. Diabetes-related chronic hyperglycemia can cause a chronic hypoxic milieu in the renal interstitium and disturbance of the interstitial organization of vascular architecture, atypical cell growth, and collagen proliferation in tubular cells and peritubular fibroblasts, which cause the impaired synthesis of erythropoietin by the peritubular fibroblasts [48 and 59].In addition, in patients with prolonged hyperglycemic conditions, the erythrocyte precursors cells in the bone marrow might be exposed to prolonged direct glucose toxicity leading to disturbances in the erythrocyte production [60].

The multivariate analysis of this study showed that having high serum creatinine is independently associated with greater odds for the presence of anemia. A patient with high serum creatinine was 10.07 times more likely to develop anemia and this is consistent with other related studies done in northeast Ethiopia and Ghana [10, 51]. Elevated creatinine level signifies impaired kidney function or kidney disease. As the kidneys become impaired for any reason, the creatinine level in the blood will rise due to poor clearance of creatinine by the kidneys. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys. Anemia due to renal insufficiency is primarily a result of reduced secretion of EPO by the failing kidneys, and anemia subsequently occurs when creatinine clearance is less than 50 mL/minute. This is observed earlier in patients with diabetes with renal insufficiency or disease [51, 67]. The high incidence of anemia may also be due to other risk factors related to DM. Several studies

have reported factors that increase the risk of anemia, which include; damage to renal interstitium due to chronic hyperglycemia and consequent formation of advanced glycation end products by increased reactive oxygen species, and systemic inflammation as well as reduced androgen levels induced by diabetes [51, 67, and 68].

7. Limitations

The study lacks control groups and did not assess the causes of anemia in diabetic patients; due to the nature of the study design, cross-sectional studies. The other limitation in this study is, morphological classification of anemia was not done due to budget problems.

8. Conclusions

The study showed that 27.5 % of participants were anemic. In this study, anemia is more common in diabetes mellitus particularly in diabetic male patients, and is a frequent complication of diabetic nephropathy. Out of anemic T2DM patients, 92.9% and 7.1% had mild and moderate anemia, respectively. Having nephropathy, being male, duration of diabetes for 5 years and greater, having high serum creatinine were all significantly associated with anemia.

9. Recommendation

The results suggest all Health institutions or diabetic clinics must incorporate routine screening for anemia in all type two adult diabetic patients mainly for patients with these identified risk factors to facilitate early detection and management of anemia among T2DM and consequently improve the overall care of these patients. Keeping diabetes under control and proper investigations to identify anemia in diabetic patients at an early stage can reduce the severity of the complications caused due to anemia in a diabetic population. Diabetic males and diabetic patients with nephropathy are the most vulnerable group to anemia, thus care should be taken in terms of their nutrition and supplements Further studies are needed because of the cross-sectional nature of the study, where a better relationship between anemia and different potential factors affecting it progressively cannot be well established, so a longitudinal study is needed to assess the relationship over time and to elaborate the mechanisms of anemia in patients with diabetes.

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10. Annex

Annex I. Information sheet

Title of Study: "Prevalence of Anemia and Associated Factors Among type two Diabetic Patients Attending Ambo Town Government Hospitals in West Shewa, Ethiopia."

Investigator: the research investigator is Jafero Oljira

Rights to refuse or stop participation at any time: the study participant can refuse or stop their participation in the study if he/she feel anything which harm the participant

Risks and benefits of participating in the study: since the data collectors are experienced there is no such risk that may harm the study participants. Depending on the findings of the study, the study participants will be recommended to receive appropriately tailored therapy in consultation with the treating physician(s).

Purpose of the study: To assess the prevalence of anemia and associated risk factors among type 2 diabetic patients attending Ambo town government Hospitals in West Shewa, Ethiopia.

Place of Study: this research work will be conducted in Ambo Town Government Hospitals.

Duration of Study: the duration of this study will be from September – October 2020 G.C.

Confidentiality and privacy concerns: all the information regarding the study participant will not disclosed to anyone unless the study participant allowed i.e. your confidentiality will be kept whatever your responses are towards the study questions.

Who to contact if you have questions: - Jafero Oljira

Tel: +251-916-605704 E-mail address: Jaferool12@gmail.com

Annex II. Consent Form English Version

Hello! Good Morning! My name is Jafero Oljira. I'm studying for my second degree at Addis Ababa University. Now, I'm conducting research on the Prevalence of Anemia and Associated Factors Among type two Diabetic Mellitus Patients Attending Ambo Town Government Hospitals. I need you to give me some information and samples like; urine and blood.

I would like to assure you that the study is confidential. I will not keep a record of your name and address. Your participation in the study will be based on your willingness. You have the right to skip any question that you do not want to answer. But, your correct answer to each question can make the study valuable. For your participation**,** the process of the study has no payment or special benefit. I can assure you that the study also has no physical or psychological trauma as well as a political problem, but participation in the study by giving your correct answer can play a great role in the success of the study and also it will provide great input to bring change in the quality of health. Therefore, you are kindly requested to respond genuinely and voluntarily with patience. To fill these questions, it may take 30 minutes.

Are you willing to participate in the study?

1. Yes
2. No

If not agree go to next home

Name:_____Signature_____Date _____Time___

Principal investigator address: _____

Responses of the interviewee: (to be filled at end of interview)**: (**1) Completed (2) Refused (3) Partially completed

Checked by**:** Supervisor Name_____Signature_____Date_____

Annex III. English Version of the Data Collection tool (questionnaire and checklist)

Anemia and associated factors among type-II diabetes mellitus patients attending Ambo town government Hospitals of West Shoa, Oromia, Ethiopia, 2019

Date_____ Service area _____

Code _____ Interviewer name _____

S.No.	Variable	Response	Skip
Part I: Socio-demographic characteristics			
101	Age	(in completed year)	
102	Sex	1. Male 2. Female	
103	Marital status	1. Single 2. Married 3. Divorced 4. Widowed 5. Separated 6. Others (specify) _____	
104	Educational status	1. Unable to read and write 2. Able to read and write 3. Primary level (1-8) 4. Secondary level (9-12) 5. College and above	
105	Occupation	1. Farmer 2. Housewife 3. Merchant 4. Governmental employer 5. Others (specify) _____	
106	Residence	1. Urban 2. Rural	

Part II: Lifestyle			
201	Have you ever smoked a cigarette?	1. Yes 2. No	If no, skip to 204
202	If yes, are you smoking cigarettes currently?	1 Yes 2 No	If no, skip to 204
203	How many packets do you smoke daily?	¼ packet ½ packet 1 packet >1 packets	
204	Have you ever drunk alcohol?	1. Yes 2. No	If no, skip to 208
205	If yes, are you drinking alcohol currently?	1. Yes 2. No	If no, skip to 208
206	How many times do you drink alcohol in a week?	1. Every day a week 2. Once a week 3. Twice a week 4. Three times a week	

		5. Four times a week 6. 5-6 times a week	
207	How many bottles/cups are you drinking on typical days?	1. 1-2 2. 3-4 3. 5-6 4. >6	
208	Do you usually do some physical activity for at least 30 minutes?	1. Yes 2. No	If no, skip to 301
209	If yes, how many times a week?	1. Once a week 2. Twice a week 3. Three times a week 4. Four times a week 5. Five times a week or more	
210	What types of exercise are you doing (more than one answer is possible)?	1. Brisk walking (outside or inside on a treadmill) 2. Jogging/Running 3. Dancing 4. Swimming 5. others (specify) _____	

Part IV: Complication, comorbidity, and glycemic control-related variables				
301	How long have you had diabetes?	Months _____ Years _____		
302	Are you taking medication for diabetes?	1. Yes 2. No		If no, skip to 404
303	If yes, which drugs/ medications are you taking? (more than one answer is possible)	1 Metformin 2Metformin+Glibenclamide 3 Insulin 4 Others(specify)_____		
304	Do you take any medications other than diabetes mellitus drugs?	1 Yes 2 No		If no, skip to 409
305	If yes, for which disease?	Specify _____		
306	What types of drugs are you taking?	Specify _____		
307	Do you have any loss of sensation in your feet or toes including burning, tingling, or numbness (neuropathy)?	1 Yes 2 No		
308	Do you have a history of the problem with eyes (retinopathy) told by health professionals?	1 Yes 2 No		
309	Do you have a history of kidney problems (nephropathy)?	1 Yes 2 No		
310	Do you have any history of heart problems and blocked arteries in the	1 Yes 2 No		

	legs (Marco-vascular complications)			
311	Do you have a history of foot sores that do not heal?	1 Yes 2 No		
312	Have you ever been told by a doctor or nurse that you have high blood pressure?	1 Yes 2 No		
313	Have you ever been tested for HIV/AIDS?	1 Yes 2 No		If no, skip to 417
314	What is your result?	1 Positive 2 Negative		
315	Do you have a history of any blood loss for the last 3 months?	1 Yes 2 No		If no, skip to 420
316	If yes, did you take medication for blood loss?	1 yes 2 No		If no, skip to 420
317	What type of medication?	Specify _____		
318	Have you ever been told by a doctor or nurse that you have kidney problem for the last 3 months (CKD)?	1 Yes 2 No		
319	Do you know your most recent blood glucose result measured after no caloric intake for at least 8 hours (FBS)?	If yes, mg/dl If no, refer card		
320	Do you know your most recent HbA1C (glycosylated hemoglobin) result?	If yes (in %) If no, refer card		
321	Creatinine result	_____mg/dl		
322	Albumin result	Positive or negative		
Part V: Anthropometric, blood pressure, and hemoglobin data				
401	Weight	_____(in kg)		
402	Height	_____(in meter)		
403	Waist circumference	_____(in cm)		
404	Blood pressure	_____mm/Hg		

Annex IV. Amharic Version of the Data Collection tool (questionnaire and checklist)

ደም አነሰ እና ከደም አነሰ ጋር የተገናኙ በሁለተኛዉ አይነት ሰኳር በሽታኞች አምቦ ከተማ በመንግስት ሆስፒታሎች የሚከታተሉት ላይ ጥናት ማድረግ ነው።

የተቋሙ ሰም የመጠይቁ መለያ ቁጥር _____

መጠይቁ የተካሄደበት ቀን _____ መጠይቁን የሞላው ሰው ሰም _____

ተ.ቁጥር	ጥያቄ	ምላሽ	ወዴ ለላ ጥያቄ መሄድ
ክፍል አንድ፡ ማህበራዊ የሰነህዝብ አወቃቀር መጠይቅ			
101	ዕድሜ?	አመት	
102	ፆታ	1 ወንድ 2 ሴት	
103	የጋብቻ ሁኔታ?	1 ያላገባ/ች 2 ያገባ/ች 3 የሞተበት/ባት 4 የተለያዩ	
104	የትምህርት ደረጃዎ እስከምን ድረስ ነው?	1 ያልተማረ/ች 2 ያልተማረ/ች ግን መጻፍ እና ማንበበ የሚችል 3 አንደኛ ደረጃ የተማረ/ች(1-8) 4 ሁለተኛ ደረጃ የተማረ/ች （9-12） 5 ኮሌጅና ከዚህያ በላይ የተማረ/ች	
105	ሰራዎት ምንድነው?	1 ገበሬ 2 የቤት እመቤት 3 ነጋዴ 4 የመንግስት ሰራተኛ 5 ለላ ካለ ይግለፁ_____	
106	የሚኖሩበት ቦታ?	1 ከተማ 2 ገጠር	
ክፍል ሁለት፡ ከኑሮ ሁኔታ ጋር የተገናኙ መጠይቅ			
201	ሲጋራ አጭሶ ያውቃሉ?	1 አዎ 2 አይ	አይ ከሆነ ወደ 204 ይሂዱ
202	መልሶዎ አዎ ከሆነ አሁነ ያጨሳሉ?	1 አዎ 2 አይ	አይ ከሆነ ወደ 204 ይሂዱ
203	አዎ ከሆነ በቀን ምን ያክል ፓኮት ያጨሳሉ?	1 ¼ ፓኮት 2 ½ ፓኮት 3 1 ፓኮት 4 ≥ 1 ፓኮት	
204	የአልኮለ መጠጥ ጠጥቶዎ ያውቃሉ?	1 አዎ 2 አይ	አይ ከሆነ ወደ 208 ይሂዱ
205	አዎ ከሆነ አሁነ የአልኮለ መጠጥ ይጠጣሉ?	1 አዎ 2 አይ	አይ ከሆነ ወደ 208 ይሂዱ
206	አዎ ከሆነ በሳምንት ምን ያህል ጊዜ የአልኮለ መጠጥ ይጠጣሉ?	1 በየቀኑ 2 በሳምንት አንዴ 3 በሳምንት ሁለቴ 4 በሳምንት ሶስቴ	

		5 በሳምንት አራቴ 6 በሳምንት 5_6 ጊዜ	
207	የአልኮለ መጠጥ በምጠጡበት ቀን ምን ያህል ጠርሙሰ/ብርጭቆ ይጠጣሉ?	1 1-2 2 3-4 3 4-5 4 ≥ 6	
208	ቢያንሰ ለሰላሳ ደቂቃ ያክል የሰውነት እንቅሰቃሴ አድርጎ ያውቃሉ?	1 አዎ 2 አይ	አይ ከሆነ ወደ 301 ይሂዱ
209	አዎ ከሆነ በሳምንት ሰንት ጊዜ ያደረጋሉ?	1 በሳምንት አንዴ 2 በሳምንት ሁለቴ 3 በሳምንት ሶስቴ 4 በሳምንት አራቴ 5 በሳምንት አምሰት ጊዜና ከዚያ በላይ	
210	ምን ዓይነት እንቅሰቃሴ ነው የሚያደረጉት (ከአንድ መለሰ በላይ ይቻላል)?	1 የእግር ጉዞ ማድረግ (ደረጃ መውጣትና መውረድ) 2 ሩጫ 3 መደነሰ 4 መዋኘት 4ለላ ካለ ይግለፁ_____	

ክፍል አራት፡ የሰኳር በሽታ ተጓዳኝ እና ከሰኳር መጠን ቁጥጥር ጋር የተገናኙ መጠይቅ				
301	በደምዎ ውሰጥ ሰኳር መኖኑሩን ያውቁት መቼ ነበር?	_____ ወር _____ ዓመት		
302	ለሰኳርዎ መድሃኒት የወሰዱ ነው?	1 አዎ 2 አይ		መለሶ አይ ከሆነ ወደ 404 ይህዱ
303	አዎ ከሆነ ምን አይነት መድሃኒት የወሰዱ ነው (ከአንድ መለሰ በላይ ይቻላል)?	1 ሜትፎርምን 2 ሜትፎርምን + ግልብንክላማይዲ 3 እኒሱሊን (insulin) 4 ለላ ካለ ይግለፁ_____		
304	ከሰኳር ህመም መድሃኒት በተጨማረ ልላ መድሃኒት ይወሰዳሉ?	1 አዎ 2 አይ		አይ ከሆነ ወደ 407 ይህዱ
305	አዎ ከሆነ ለየትኛው በሽታ ነው የሚወሰዱት?	የበሽታውን ሰም ይግለፁ_____		
306	ምን ዓይነት መድሃኒት ነው የሚወሰዱት?	የመድሃኒት ሰም ይግለፁ_____		
307	ሰውነዎት ላይ ወይንም እግሮዎት/እጆዎት ላይ የማቃጠል እና የመደነዘዝ ሰሜት አለ?	1 አዎ 2 አይ		
308	ከዚህ በፊት በጤና ባላሙያ/በለላ ሰው የዓይን ችግር/ህመም አለቦዎት ተበሎ ተነግሮዎት ያውቅ ነበር?	1 አዎ 2 አይ		
309	ከዚህ በፊት የኩላሊት ህመም/እንፈክሺን አለ ተበሎ ተነግሮዎት ያውቅ ነበር?	1 አዎ 2 አይ		

310	ከዚህ በፊት በጤና ባላሙያ የልብ ህመም ፤የደም ሰር ችግር እና የተገናኘ ህመም አለቦዎት ተበሎ ተነግሮዎት ያውቃል?	1 አዎ 2 አይ		
311	እግሮዎት ላይ ሳይድን የቆየ ቁስል ነበር?	1 አዎ 2 አይ		
312	ከዚህ በፊት በጤና ባላሙያ ደም ግፊት አለቦዎት ተበሎ ተነግሮዎት/ መድሃኒት ተሰጥቶዎት ያውቃል?	1 አዎ 2 አይ		
313	ከዚህ በፊት ለኤች አይቭ ተመርምረዎ/ አለቦዎት ተበሎ ተነግሮዎት ያውቃሉ?	1 አዎ 2 አይ		መለሶ አይ ከሆነ ወደ 417 ይሀዱ
414	አዎ ከሆነ ውጤቱ ምን ነበር?	1 ፖሰትቭ 2 ነገትቭ		
315	ባለፈው ሶስት ወር ውስጥ የደም መፍሰስ ህመም/ ችግር አጋጥሞዎት ነበር?	1 አዎ 2 አይ		አይ ከሆነ ወደ 420 ይሀዱ
316	አዎ ከሆነ ለደም መፍሰስ ህመምዎ መድሃኒት የወሰዱት አለ?	1 አዎ 2 አይ		አይ ከሆነ ወደ 420 ይሀዱ
317	ምን ዓይነት መድሃኒት ነው የወሰዱት?	የመድሃኒት ሰም ይግለፀ_____		
318	ከዚህ በፊት በጤና ባላሙያ ሶስት ወር/ከሶስት ወር በላይ የቆየ የኩላሊት ህመም አለቦዎት ተበሎ ተነግሮዎት ነበር?	1 አዎ 2 አይ		
319	በቅርብ ጊዜ ምግብ ከበሉ ከሰምንት ሰዓት በኋላ የተሰራውን የደም ሰኳርዎን መጠን ያውቃሉ (FBS)?	1 አዎ ሚሌግራም/ደሲሊትር 2 አይ ከሆነ የበሽተኛው ካርድ ይመለከቱ		
320	ሄሞግሎብን/የደም ናሙና ውጤት	_____ግራም/ደሲሊትር		
321	ከራትንን ውጤት	_____mmHg		

ክፍል አምሰት፡ የአንትሮፖሜትር እና የደም ናሙና ውጤት				
401	ክብደት	_____ ኪሎ ግራም		
402	ቁመት	_____ ሰንትሜትር		
403	የወገብ ዙረያ ልኬት	_____ ሰንትሜትር		
404	የደም ልኬት (BP)	_____mmHg		

Annex V. Consent form Afaan Oromoo Version

Heloo! Akkam bultan/ooltan?Maqaan koo Jaafaroo Oljirraan jedhama.

Ani Digirii Lammaffaa Universitii Addis Ababaatti barachaan jira. Har'a kanan dhufe qorannoo waayee Hanqina dhiigaa fi rakkoolee isaan walqabatan dhukkubsattoota sukkaara gosa 2ffaa qaban kanneen hospitaalota magaala Amboo keessatti hordoffiirra jiran qorachuuf qophaayerratti gaaffileewwan isin gaafachuufi akkasumas Samuuda Kan akka Fincaanii Fi dhiiga Fudhachuufi.

Icciitiin keessan qaama biraatt idabarfamee kan hin himamne ta'uu akka beektan durseen sin hubachiisa. Qorannoo kana irratti maqaa keessan heeruun hin barbaachisu.Hirmaannaan keessanis fedha keessan irratti kan hundaa'edha. Gaaffii deebisuu hin barbaannee irra darbuu ni dandeessu. Garuu deebiin isin laattan qorannoo kanaaf murteessaadha.Waan qorannoo kanarratti hirmaattaniif kanfaltii yookaan faayidaa addaa argattan hin jiru.Qorannichi dhiibbaa qaamaa yookaan dhimma siyaasaarraa bilisaa fi yaadni keessan garuu rakkoowwan qorannoon kun furuuf godhamu irratti jijjiirama fiduuf murteessaadha. Kanaaf fedhakeessaniin daqiiqaa 30 keessatti yaada keessannuuf gumaachuu dandeessu.

Qorannoo kana irratti hirmaachuuf fedhiini qabduu?

1. eeyyee
2. fedha hin qabu

Yoo fedha hin qabu ta'e garanama isa itti aanuutti darbi

Mallattoo koo_____Guyyaa_____Sa'aa____

Odeeffannoo fuunaanaa: Odeeffannoon armaan olii akka laatameef mallattoo kootiinan mirkaneessa.

Maqaa _____Mallattoo_____

Teessooqorataa:_____

Waliigaltee xumuura gaaffichaa:1. Xumuurame 2.Ni didame 3.Gartokkee deebisan

Maqaa qajeelchaa_____Mallattoo_____Guyyaa_____

Annex VI. Afaan oromo Version of the Data Collection Tool (Questionnaire and checklist)

Hanqinadhiigaa fi rakkooleeisaanwalqabatandhukkubsattootasukkaaraagosa 2ffaa qabankanneen hospitaalota Ambookeessattihordoffiirrajiran, 2018/2019

Guyyaabakkatajaajila _____

Code _____ maqaa itti gaafatamaa_____

Lak	Variables	Deebii	Garagaaffiiitti aanuttidarbi
Kutaa I: Haalahawaasumma fi demografii			
101	Umurii	(Waggaadhaan)	
102	Saala	1 dhiiraa 2 dhalaa	
103	Haalagaa'ilaa	1 hinheerumne 2 fuudhe/heerumte 3 Addaanbahan 4 inni/isheenlubbuunhinjiru 5 gargarbahaniijiru	
104	Sadarkaabarnootaa	1 barreessuu fi dubbisuuhindanda'an 2 dubbisuu fi barreessuunidanda'a 3 Sadarkaa 1ffaa(1-8) 4 Sadarkaa 2ffaa (9-12) 5 12+ (koollejjii/ universitii/	
105	Gosahojii	1 Qoteebulaa 2 Haadhamanaa 3 Daldalaa/ttuu 4 Hojjetaamootummaa 5.kan biroo_____	
106	Bakka jireenyaa	1 magaalaa 2 baadiyyaa	

Kutaa II: Haalaa malajireenyaa			
201	Tamboo ni xuuxxaa?	1 eyyen 2 lakki	Yoo lakki jette 204tti darbi
202	Yoo eeyye jette amma ni xuuxxaa?	1 eyye 2 lakki	Yoolakkijette 204tti darbi
203	Guyyaatti pakeeta meeqa xuuxxa?	1 ¼ pakeetii 2 ½ paakeetii 3 1 paakeetii 4>1 paakeetii	
204	Alkoolii dhugdee in beektaa?	1 eeyyen 2 lakki	Yoolakkijette 208tti darbi
205	Yoo eeyyen jette amma ni dhugdaa?	1 eyyen 2 lakki	Yoo lakki jette 208tti darbi
206	Torban tokko keessatti yeroo meeqa dhugda?	1 torbanitti tokko 2 torbanitti lama 3 torbanitti sadii 4 torbanitti afur 5 guyyaa dhaan	
207	Yeroo dhugdutti qaruura meeqa dhugda?	1 1-2 2 3-4	

		3 5-6 4 >6	
208	Yeroo yerootti hojii qaamaa ni hojjettaa yoo xiqqaate daqiiqaa 30f?	1 eeyyen 2 lakki	Yoo lakki jette 301tti darbi
209	Yoo eeyye jette torbanitti yeroo meeqa hojjetta?	1 torbanitti tokko 2 torbanitti lama 3 torbanitti sadii 4 torbanitti afur 5 torbanitti shanii fi isaa ol	
210	Hojii/gocha/ akkami hojjettaa (deebii tokkoo ol ni danda'ama)?	1 miilaan ykn konkolaataan deddeemuu 2 utaalcha/fiigicha 3 shaggooyyee/qaamaan sirbuu 4 bishaan daakuu 5 kan biroo	

Part IV: miidhaa fi rakkoolee walxaxoo dhukkuba sukkaaraa waliin walqabatan				
301	Dhukkubni sukkaaraa kun erga sirratti mullate hagam ta'e?	_____ji'a _____ (waggaa)		
302	Dawaa dhukkuba sukkaaraa fudhachaa jirtaa?	1 eeyye 2 lakki		Yoo lakki jette 409tti darbi
303	Dawaa fudhachaa jirtu (deebii tokkoo ol ni danda'ama).	1 Metformin 2 Metformin + gliblenclamide 3 Insulinii 4kan biroo_____		
304	Dawaa biraa kan sukkaaraa alatti kan fudhattu ni jira?	1 eeyyen 2 lakki		Yoo lakki jette 409tti darbi
305	Yoo eeyye jette, dawaa maalii?	Adda baasi		
306	gosa dhukkubaa maaliitif?	Adda baasi		
307	Qaama kee irratti miirri dhagahamuu diduu muldhateeraa, miilakee, ykn qubakee irratti kan akka qaama sigubuu, hadooduu?	1 eyyen 2 lakki		
308	Rakkoo ijaa waliin walqabate kan mana yaalaatti sitti himame niqabdaa?	1 eyye 2 lakki		
309	Rakkoo kale waliin walqabatu ni qabdaa?	1 eyye 2 lakki		
310	Rakkoo onnee waliin walqabatan cufamuu ujummoo dhiigaa miilaa ni qabdaa (Marco vascular complications)?	1 eyyen 2 lakki		

311	Kanaan duratti madaa miila keessan irraatti mul`ate siif fayyuu dide ni jiraa?	1 eyyen 2 lakki		
312	Dhiibbaa dhiigaa qabda jedhamee wanti mana yaalaatti sitti himame ykn dawaa dhiibbaa dhiigaa fudhatte ni jiraa?	1 eeyye 2 lakki		
313	HIV/AIDS qoratamtee beektaa?	1 eeyye 2 lakki		Yoo lakki jette 417tti darbi
314	Bu'aan isaahoo?	1 Positive 2 Negative		
315	Baatii sadan darban keessatti qaamni si dhiigee beekaa?	1 eeyye 2 lakki		Yoo lakki jette 420tti darbi
316	Yoo eeyye jette dawaa kanaaf fudhatte ni jirtaa?	1 eeyye 2 lakki		
317	Dawaa gosamaalii?	Addabaasi _____		
318	Dhukkuba rakkoo Kaleen walqabatan kan baatii sadan darban keessatti mana yaalaatti sitti himame ni jiraa?	1 eeyye 2 lakki		
319	Sukkaara dhiiga kee kan yoo xiqqaate saatii 8f osoon nyaatiin yeroodhiyootti siif hojjetame ni beektaa (FBS)?	Yoo eeyye jette, mg/dl Yoo lakki jette,kaardii ilaali		
320	Heemoogloobini iargame	_____(g/dl)		
321	Keratinine	_____ mg/dl		
322	Albumin	Poositivi ykn negatiivii		

Kutaa V: Safartuu Antiropometrii fi bu'aa dhiiga fudhatamee				
401	Hanga ulfaatinaa	_____(Kg dhaan)		
402	Dheerina	_____(Meetraan)		
403	Waist circumference	_____(Cm dhaan)		
404	Dhiibbaa dhiigaa	_____mm/Hg		

Annex VII. Standard Operating Procedure for Creatinine

BS-200 Analyzer

- After collection of Venous Blood Serum will be separated by using SST or EDTA tube.

Principle

Creatinine + Picric acid ⟷ Creatinine – Picric acid complex

Creatinine forms in alkaline solution an orange-red colored complex with picric acid. The absorbance of this complex is proportional to the creatinine concentration in the sample.

Reagent and equipment

BS-200 Analyzers, Micropipette and tips, Test tube rack, NaCl solution 9 g/L, Reagent, R1 Sodium Hydroxide (0.38mmol/L), R2 Picric Acid (15mmol/L), Calibrator and controls

Procedure

	Blank	Sample
Reagent 1	180uL	180uL
Distilled water	18uL	-
Sample	-	18uL
Mix , Incubate for 1 min. at 37^{o}c,then add:		
Reagent 2		
Mix thoroughly at37oc for 30 sec. and then read the absorbance change value over further 2 min.		
ΔA = (ΔA sample) – (ΔA blank)		

Wave length = 510nm

Optical path = 1cm

Temperature = 37^{o}c

Quality Control

At least two level control materials should be analyzed once a day.

These controls should be run with a new calibration, each new reagent cartilage, and after specific maintenance or troubleshooting procedures.

Reference Range

Serum/ plasma 9-2420 µmol/L (0.1-27.4 mg/dL)

Estimation of Glomerural Filtration Rate

Annex VIII. Standard Operating Procedures for Hemoglobin

Mission Hemoglobin Meter

- 10µl of blood is used

Principle

Methemoglobin

Materials

- Hemoglobin meter
- Hemoglobin test strip
- Control test strip
- Code chip
- typed capillary tubes
- Lancets

Procedure

1. Before testing let the controls reach room temperature, between 59-86 °F (15 – 30 °C).
2. Shake the control solution bottle for at least 2 minutes.
3. Turn on the meter.
4. Insert the code chip into the meter and code the meter correctly. Refer to Coding the Meter in the User Manual for details. Compare the code number on the code chip with the code number printed on the test cartridge canister label, and ensure the two numbers are identical to avoid inaccurate results.
5. Remove a test cartridge from the closed canister and use it as soon as possible. Immediately close the canister tightly after removing the required number of test cartridges.
6. Wait for the meter to flash the Test Cartridge Symbol. Insert the test cartridge into the test cartridge channel in the same direction as the arrows on the test cartridge. Ensure that the test cartridge is inserted at the end of the cartridge channel.
7. While the meter is flashing the Blood Drop Symbol, apply one drop of the control solution to the center of the sample well. A line with 3 dashes will appear on the meter to show the test is in progress.
8. Read the results on the screen after 15 seconds. Refer to Testing in the User's Manual for detailed testing procedures

Quality control

Test known specimens or controls at each of the following will be done.

Each new day of testing

A new canister of strips is opened

Test results seem inaccurate

Reference range

For male 13g/dl-17g/dl

For female 12g/dl-15g/dl

Annex IX. Standard Operating Procedures for Albumin Test

Dipstick

Specimen

- After collecting mid-stream /randomly voided urine of 10ml, it should be examined within one hour.

Principle

Protein/ Albumin - This test is based on the protein-error-of-indicators principle. At a constant buffered pH, the development of any green color is due to the presence of protein. Colors range from yellow for "Negative" through yellow-green and green to green-blue for "Positive" reactions

Procedure

1 Thoroughly mix urine specimens by inverting ten times
2 Remove one strip from the vial and replace the cap
3 Hold the strip against the vial to observe the proper reading format.
4 Completely immerse the reagent areas of the strip in the urine specimen and remove immediately. Start the timer and touch (blot) the edge of the strip on an absorbent material to remove the excess urine. This prevents the 'run-off' phenomenon which can lead to erroneous or inaccurate results.
5 Hold the strip in a horizontal position to prevent possible mixing of chemicals from adjacent reagent areas and/or contaminating the hands with urine
6 Compare reagent areas to the corresponding Color Chart on the bottle label at the time specified. Hold strip close to color blocks and match carefully. Avoid laying the strip directly on the Color Chart as this will result in the urine soiling the chart.
7 After dipping the strip, check the pH area. If the color on the pad is not uniform, read the pH reagent area immediately, comparing the darkest color to the appropriate Color Chart
8 Refer to specific manufacturer's instructions on a package insert or vial for permitted reading time. A positive reaction (small or greater) at or less than 2 minutes on the leukocyte test may be regarded as a positive indication of leukocytes in urine. Color changes that occur after 2 minutes are of no diagnostic value.
9 Record results on patient chart.

Annex X: Declaration

Title of Project: Prevalence of Anemia and Associated Factors Among type two Diabetic Mellitus Patients Attending Ambo Town Government Hospitals, West Shewa, Ethiopia.

I, the undersigned, declare that this MSc research project is my original work. It has not been presented for a degree in any other University. False statements could be cause for invalidating this research project and may lead to other administrative or legal action.

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