ANEMIA AND ASSOCIATED FACTORS AMONG DIABETIC PATIENTS ATTENDING JIMMA UNIVERSITY SPECIALIZED HOSPITAL, SOUTHWEST ETHIOPIA

BY:

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A THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE AND PATHOLOGY, COLLEGE OF MEDICAL SCIENCES, JIMMA UNIVERSITY FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR MASTER OF SCIENCE DEGREE IN CLINICAL LABORATORY SCIENCE SPECIALITY IN HEMATOLOGY AND IMMUNOHEMATOLOGY

NOVEMBER 2014

JIMMA ETHIOPIA

JIMMA UNIVERSITY
COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES,
DEPARTMENT OF MEDICAL LABORATORY SCIENCE
AND PATHOLOGY

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NOVEMBER, 2014
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ABSTRACT

Background*: Anemia is a global public health problem and common in diabetic patients. It is potentially contributing to pathogenesis and progression of many diabetes complications mainly cardiovascular disease, diabetic nephropathy and retinopathy. Anemia in diabetic patients is unrecognized and untreated particularly in Ethiopia.*

Objective*: This study was aimed to determine prevalence of anemia and associated factors in diabetic patients attending chronic care center in Jimma University specialized hospital, Southwest Ethiopia.*

Methods*: An institution -based cross-sectional study was conducted from April 20 to May 30, 2014. A total of two hundred and sixty (260) diabetic patients were involved in the study. Four (4) ml of venous blood was collected from each diabetic patient for hematology and chemistry analysis. Blood film was prepared and examined for peripheral blood morphology and hemoparasites particularly; malarial. Stool sample was also screened for presence of intestinal parasite. Data were analyzed with statistical package software for social scientist (SPSS, version 20, IBM Inc Chicago).Descriptive statistics and logistic regressions were carried out to compute the different rate, proportion and relevant association. Variables with P-value <0.05 were considered as statistically significant.*

Results*: Overall, the prevalence of anemia in the study was 23.1%. Among anemic diabetic patients mild and moderate anemia accounted for 96.7% and 3.3%, respectively. Majority (86.7%) of the anemic diabetic patients had normocytic-normochromic anemia. Having complication (AOR = 2.15, 95% CI = 1.09, 4.23), being illiterate (AOR=4.19, 95% CI =1.61, 10.88) and uncontrolled hyperglycemia (AOR = 2.10, 95% CI = 1.05, 4.18) were identified as associated factors of anemia.*

Conclusion*: This study showed that anemia is moderate public health problem in diabetic patients in the study area. Therefore screening for anemia should be part of their routine management.*

ACKNOWLEDGEMENT

First of all, I have to thank for Almighty God. I would like to pass my heartfelt gratitude to my advisors Mr. Lealem Gedefaw and Dr Tilahun Yemane who have important role in this research thesis. I would like to thank Jimma University and ARRA (Administration of Returnees & Refugees affair) for sponsoring me, and also to department of Medical Laboratory Science and Pathology for given me the opportunity to prepare this paper. Thanks are also given to my friends for their additional suggestions and comments. Finally, I want to express appreciation to my respondents for their voluntarily participation.

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ABBREVIATIONS

AOR: Adjusted odds ratio

BMI: Body mass index

CBC: Complete Blood Count

CKD: Chronic kidney disease

COR: Crude odds ratio

dl: decilitre

DM: Diabetes Mellitus

EDTA: Ethylene diamine tetraacetic Acid

EPO: Erythropoietin

ESAs: Erythropoiesis-Stimulating Agents

ESRD: End-stage renal disease

FBS:fasting blood sugar

fl: femtolitre

g:Gram

Hb: Hemoglobin

Hct: Hematocrit

HTN: hypertension

IDF: International Diabetes Federation

JUSH- Jimma University Specialized Hospital

L:Litre

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean corpuscular hemoglobin Concentration

MCV: Mean Corpuscular Volume

ml: milliliter

pg: picogram

RBC: Red Blood Cell

SOP: Standard Operating Procedure

SPSS: Statistical Package Software for Social Scientist

USA:United States of America

UK:United Kingdom

WHO: World Health Organization

CHAPTER ONE: INTRODUCTION

1.1Background

Several hematological changes affecting the red blood cells, white blood cells and platelets are shown to be associated with diabetes mellitus (DM). Some of these are directly related to the diabetic state, while others are simply coincidental with the disease. Anemia, thrombocytosis and leukocytosis are frequent entities among patients with DM. Diabetic patients may suffer from various RBC abnormalities such as bleeding disorders, iron deficiency anemia and anemia of chronic diseases [1,2].

Anemia mostly associated with diabetic kidney disease is proportional to the severity of the renal failure. It is generally normocytic normchromic, but if there is association with iron deficiency and anemia of chronic disease then microcytic and hypochromic red cells may be seen. A higher frequency of macrocytic anemia is reported in some diabetic patients compared to normal population [3, 4-6].

The etiology of anemia in diabetes is multifactorial and includes inflammation, nutritional deficiencies, concomitant autoimmune diseases, drugs, and hormonal changes in kidney disease. Elevated glucose level that reduces red cell production and survival are possible emerging of anemia in DM. The hyperglycemic state and related metabolic changes lead to red cell functional and membrane defects. In diabetic nephropathy, kidneys are unable to produce enough erythropoietin (EPO), a hormone that regulates the production of red blood cells. EPO production is regulated in part by autonomic nervous system; this hormonal production could be prematurely impaired in patients with severe diabetic autonomic neuropathy. When DM affects kidneys, sympathetic denervation of the kidney related to autonomic neuropathy is seen. Autoimmune gastritis present in up to 2% of the general population. Type 1 diabetic patients are at increased risk for autoimmune disorders such as celiac disease that lead to macrocytic anemia (vitamin B_{12} deficiency)[7-12].

Treatment of diabetes mellitus, hypertension and other related conditions may add to the risk of anemia to be seen. Anti-diabetic medication such as thiazolidinediones and metformin (Glucophage) can increase the risk in development of different of anemia. The vitamin B_{12}

intrinsic factor complex uptake by ileal cell membrane receptors is calcium-dependent affected by metformin; because it can interfere with vitamin B_{12} absorption, and may lead to a vitamin B_{12} deficiency. Drug for hypertension are considered to suppress bone marrow erythropoiesis [13-15].

Like the normal population nutritional deficiencies such as low levels of iron, folic acid or vitamin B_{12} are possible factors of anemia development. Malaria and intestinal helminthic (hook worm) infections are also associated with anemia development. Phenolic compounds found in tea, coffee, red wine, some leafy vegetables, nuts and legumes are responsible for the inhibition of iron absorption. It appears to be the galloyl group in these compounds that is responsible for the inhibitory effects [11,16].

1.2 Statement of the problem

Anemia is becoming a greater global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. Globally, anemia affects 1.62 billion people, which corresponds to 24.8% of the population, About 1 million deaths a year globally 3/4th occur in Africa and South-East Asia [1,17].International diabetes federation (IDF) predicted the increase of DM cases from 382 million in 2013 to 592 million by 2035. In 2004, about 3.4 million people died from consequences of high fasting blood sugar and 80% of diabetes deaths occur in low and middle-income countries [18,19].

Diabetes mellitus is a public health problem in western countries occurs in up to 25% outpatients and in Caribbean diabetic patients up to 45% in tertiary referral clinics have anemia [20, 21]. Prevalence of anemia patients varies in different ethnic groups, ranging from 23% in tertiary referral clinics in Australia to 39% in Far East Asia [6, 22]. In Europe finding indicates about one in ten of diabetic patients are estimated to be anemic which was lower than other regions of the world [23, 24]. While reports in Africa range 19% to 37% of anemic diabetic patients from Ethiopia to Egypt depending on pathological condition of the study participants [7, 9].

The growing problem of hematological diseases and DM are indicative of globally increasing trend of non-communicable diseases [25].Anemic diabetic patients are more likely to die earlier than those who have diabetes mellitus but not anemia, because it is associated with an increased risk of chronic diabetic chronic complications such as nephropathy, retinopathy and macrovascular disease. And also be significant in determining the outcome of heart failure and hypoxia-induced organ damage in DM [12, 14, 26].Anemia is important predictor of quality of life, and contributes to cardiovascular morbidity and mortality in patients with DM. It causes hypoxia induced diseases, including angina, cardiac failure and claudication, worsening of exercise tolerance, lethargy and erectile dysfunction [27, 28,29].

As a precursor in correction of anemia, it is necessary to establish the predictors of anemia in patients with DM. Understanding the associated factors by which this occurs may provide the opportunity to develop therapeutic options that may improve patient outcomes [7].

Early detection of anemia prompts to referral for optimal treatment. Many issues including prevalence, pathophysiology and the consequences of anemia in diabetic patients are remaining unsettled. Currently anemia is often only treated in the late stages of CKD or after the initiation of renal replacement therapy. Thus, anemia in DM is often unnoticed and lacks appropriate treatment. The optimal level of hemoglobin for greatest clinical benefit is unclear. Lack of data for early diagnosis in developing countries is significant shortcoming so diabetic patients are unrecognized and untreated for anemia particularly in Ethiopia [6, 25, 21].

And American diabetes association recommends annual anemia screening for standard care of diabetic patients [30].Hematological parameters (anemia) regardless of the mechanisms; are potentially important biological markers of cardio metabolic risk [23,31].Diagnostic laboratories in developing countries and elsewhere should include CBC in routine laboratory investigations in the management of diabetic patients. Clinical information obtained from laboratory tests such prevalence and associated factor play a key role in the diagnosis and management of diabetic patients [21].

Although there is a clear rationale for correcting anemia in people with DM, it remains to be established whether this will lead to improved outcomes. There is dearth information regarding anemia among the diabetic patients in Ethiopia. In Jimma there are about 2000 diabetic patients attending follow up in Jimma University Specialized Hospital (JUSH) but their status for anemia is not known yet .Therefore this study was aimed to determine the prevalence and its associated factors among diabetic patients

1.3 Significance of the study

Research findings are vital for understanding the existing problems and to design strategies of solving problems. Raised awareness of the prevalence (magnitude) and associated factors of anemia in DM together with appropriate treatment, may contribute significantly to improving long-term outcomes and control of premature death. This also provides evidence in support of using low cost, readily available, routinely collected hematological parameter for the early detection of individuals at risk for complication. So, the study finding will be expected to assist for policy makers, diabetic clinic and diabetic association.

In addition, finding of this study will also contribute and add basic knowledge for currently on going researches on seeking for association between anemia and DM.

CHAPTER TWO: LITRATURE REVIEW

2.1 Anemia in diabetes mellitus

A comparative study (2008) in Caribbeans the high prevalence of anemia was identified in diabetic patients previously shown to have a high prevalence of the metabolic syndrome. While male non-diabetic subjects had significantly higher RBC, Hb and Hct values than non-diabetic female subjects ($p < 0.001$), the RBC and Hct values were similar in male and female diabetic patients .Anemia was a concomitant condition among such patients, with prevalence up to 45% in Caribbean diabetic patients [21].

The study on Chinese diabetic patients' in 2013 identified prevalence of anemia among diabetic patients was 22.8%. Most of anemic patients were normocytic (61%), while fewer were microcytic (27%) and macrocytic (12%)[27]. Based on pattern of hematological disorders studies in Bangladesh 53.41% of anemic patients were collectively with iron deficiency anemia and anemia of chronic diseases Iron deficiency anemia and anemia of chronic diseases were reported among diabetic patients [5].

Based on National Studies of hematological parameters on Saudi-Arabian diabetic patients in the overall groups the mean Hb, Hct, MCV and MCHC values were reduced significantly. Over all one fourth (25%) of the patients had Hb level in the anemic range. The hypochromic microcytic anemia possibly resulting from iron deficiency was the most frequent. At all types of DM lower levels of Hct and Hb were apparent. In each of the diabetic groups were significantly lower compared to the control [2].

In Egypt cross-sectional study among DM patients 37% were reported anemic [9]. And similar research from Ethiopia noted that prevalence of anemia was 19%. These studies indicate that low Hb levels in such patients may increase risk for progression of kidney disease cardiovascular morbidity and other complication [7]. But it was stressed anemia remains under recognized and under-treated.

2.2 Anemia and associated factors in diabetes mellitus

A number of studies have reported significant associations of diabetic and routinely measured hematological parameters. Many factors have been suggested as the reason for the earlier onset of anemia in patients with DM, including severe symptomatic autonomic neuropathy, causing efferent sympathetic denervation of the kidney [32], and loss of appropriate EPO production; systemic inflammation, hyperglycemia [25], drugs and inhibition of EPO release [33].

Researchers indicated that diabetic patients should be strictly monitored for anemia for proper management of diabetic patients in related to complication. DM complications exacerbate many to a higher degree of anemia in patients than in DM patients. [20] Finding in USA found that patients with DM and CKD had the highest risk of anemia (odds ratio 1.73, 95% CI 1.63-1.83). This factor significantly increases the odds of anemia, with greatest risk for patients with DM and CKD [28].

Anemia represents a significant burden in patients with DM. Patients at greatest risk could be identified by the presence of complication. A cross-sectional survey done in Australia in 2003, reported patients with DM have anemia warranting evaluation as 23% of study participants had unrecognized anemia. This prevalence is two to three times higher patients with DM complication than iron stores and other disorders in the general population [6].

Another report in Australia 14% of DM patients had anemia. Patients with DM complication were more than six times more likely to have anemia than that of without complication. Anemia also develops earlier in patients with renal impairment than other causes. It is recognized that reduced Hb levels, even to a limited degree, identify patients at increased risk of progressive renal disease. The levels of anemia found in type 1 diabetic patients were similar to the levels of type 2 diabetic patients. Patients with anemia were more likely to have retinopathy and macrovascular complications than non-anemic patients [44].

Anemia is more prevalent in persons with DM associated with chronic kidney disease when compared to persons without DM. Descriptive analytical cross-sectional study carried out in India indicated that anemia was present in 37% of diabetic patients, 17% in diabetic patients with

chronic kidney disease and 3% in patients with only chronic kidney disease. Anemia was significantly higher in patients with DM with chronic kidney disease. Therefore anemia may be particularly harmful in individuals with DM and CKD. Correction of anemia has a significant role in prevention of other diabetic complications [22].

Based on the retrospective study in china greater decrease in Hb levels was noted in patients with renal DM complication. Anemia was a well-known complication of CKD and recognized as an early component of diabetic nephropathy. In addition, chronic anemia also regarded as a associated factor for CVD outcomes in DM. The main cause of anemia was due to chronic diseases including hypertension (HT), and CKD, and less often they have iron deficiency or vitamin B_{12} deficiency [27].

Anemia can develop in the absence of overt nephropathy. According study in 2005 in UK identified 17.8% male patients and 11.8% female patients were classified as anemic. Over all prevalence was 10.6%.It is therefore an important observation that the development of anemia in DM may predate any abnormality in renal function [24]. Similar finding was reported in from Irish journals [23].

UK prospective study group recognized that outpatients taking biguanides developed vitamin B_{12} deficiency .In 71 metformin taking patients, 4 had developed anemia of vitamin B_{12} deficiency. An observational study on the effect of metformin on vitamin B_{12} status and peripheral neuropathy have identified comparable finding [13, 34].

Anemia in DM is associated with increased cardiovascular morbidity and mortality, hypertension, retinopathy, neuropathy and foot ulcers [35]. Finding of French study, in selected populations identified by screening programs, prevalence of anemia was 30% in diabetic patients with stage III CKD, over twice the rate observed in patients without DM (14%). DM with normocytic anemia had an increased risk of retinopathy and more often exhibit severe lesions. In a cross-sectional survey involving 1,691 diabetic patients, a two-fold increased risk of retinopathy was reported in patients with Hb less than 12 g/dl [29].

A Comparative study in Israel in 2006 showed lower Hb levels and a high rate of anemia in diabetic patients even before complication was detected. Mean Hb levels were lower in subjects with DM than in those without DM (14.2 g/dl vs. 14.7 g/dl respectively; $p < 0.001$). Anemia was observed in 10.8% subjects in the diabetic group, and in only 2.7% in the non-diabetic group ($p < 0.001$). After adjustment for age, gender, history of gastrointestinal tract diseases and renal function, DM duration remained a significant determinant of anemia with an AOR of 2.15[33].

Poor glycemic control and older age are associated with the incidence of anemia in diabetic patients with normal renal function. Comparative cross sectional study from Nigeria identified high incidence of anemia (15.3%) was observed in diabetics without renal insufficiency. The odds of developing anemia was higher in patients with poorly controlled DM compared to those with controlled ones and in patients of age ≥ 60 years compared to those of age < 60 years . The odds of anemia was similar in males and females ($p = 0.26$) Significantly higher Hct value was noted in diabetic males patients with controlled DM compared to females and those with poorly controlled DM respectively. On the other hand, mean Hb indicated no gender differences and Hct indicated no age differences [36].

In centenarians from northern Georgia Clinically defined anemia was present in greater than 60% of the centenarians in the lowest BMI grouping. BMI ≤ 20 was a risk factor for anemia. Accordingly, low BMI had been identified as an independent correlate or risk factor for anemia in some previous studies in older adults and clinical populations. Nonetheless, as anemia is associated with increased mortality in acute and chronic disease states, particularly in those underweight. [37]Obesity has been reported to be associated with anemia in adults in some countries which may be due to up-regulated hepcidin expression thereby hampering iron absorption. In one study in Chinese population, women with overweight/obesity were less likely to be anemic as compared to normal weight women [38].

The inhibitory effect of drinking tea or coffee on iron absorption was first identified in a study that used test meals fed under experimental conditions. Wide varieties of studies with different designs, from different countries in different age and gender groups, have been conducted. Results from these studies are conflicting; some have found a higher risk of anemia among tea

drinkers compared to non tea drinkers while others have shown no such association, both in children and adults.Indoing so the studies concluded that tea and coffee does have an inhibiting effect on iron absorption [39].

The hyperglycemic state and the metabolic changes resulting from DM may lead to red cell functional defects. According to case control study conducted in Nigeria ,anemia was associated with raised blood sugar levels among patients with type 1 DM .The mean Hct value was significantly lower among subjects with type 1 diabetics (39.0 ± 2.19) compared to the non-diabetic controls (40.80 ± 2.24), p=0.003 [25]. There were no statistically significant differences between hematological values based on the gender of diabetic subjects .Another similar study identified other associated factors that significantly increase the odds of anemia were lower educational level, hypertension and macrovascular complication [28].

According the finding of study in Egypt renal complication was significant associated factor for anemia in DM [9]. Similarly in Ethiopia an institutional -based cross-sectional study was conducted in to assess the association of anemia and renal function test among diabetic patients .Anemia was a statistically significant association between anemia and diabetic nephropathy with AOR of 8.58 and CI (10.21, 49.94). The study revealed that anemia is significant association in DM type with AOR of 4.17 (95% CI = 2.58, 8.56) and also with duration of DM.The strong link between the kidney disease and anemia in DM probably reflect the unique vulnerability of the renal microcirculation damage in DM [7].

Reviewed articles similarly showed that several factors contribute to the increased prevalence of anemia in DM, kidney problems appears to be the dominant factor. There is dearth information regarding anemia among the diabetic patients in Ethiopia. As far my knowledge is concerned there is no published data on anemia and its risk factors in diabetic patients in Jimma zone south Ethiopia. This study is, therefore, aimed to analyze anemia and associated factors among diabetic patient attending in JUSH, Southwest Ethiopia.

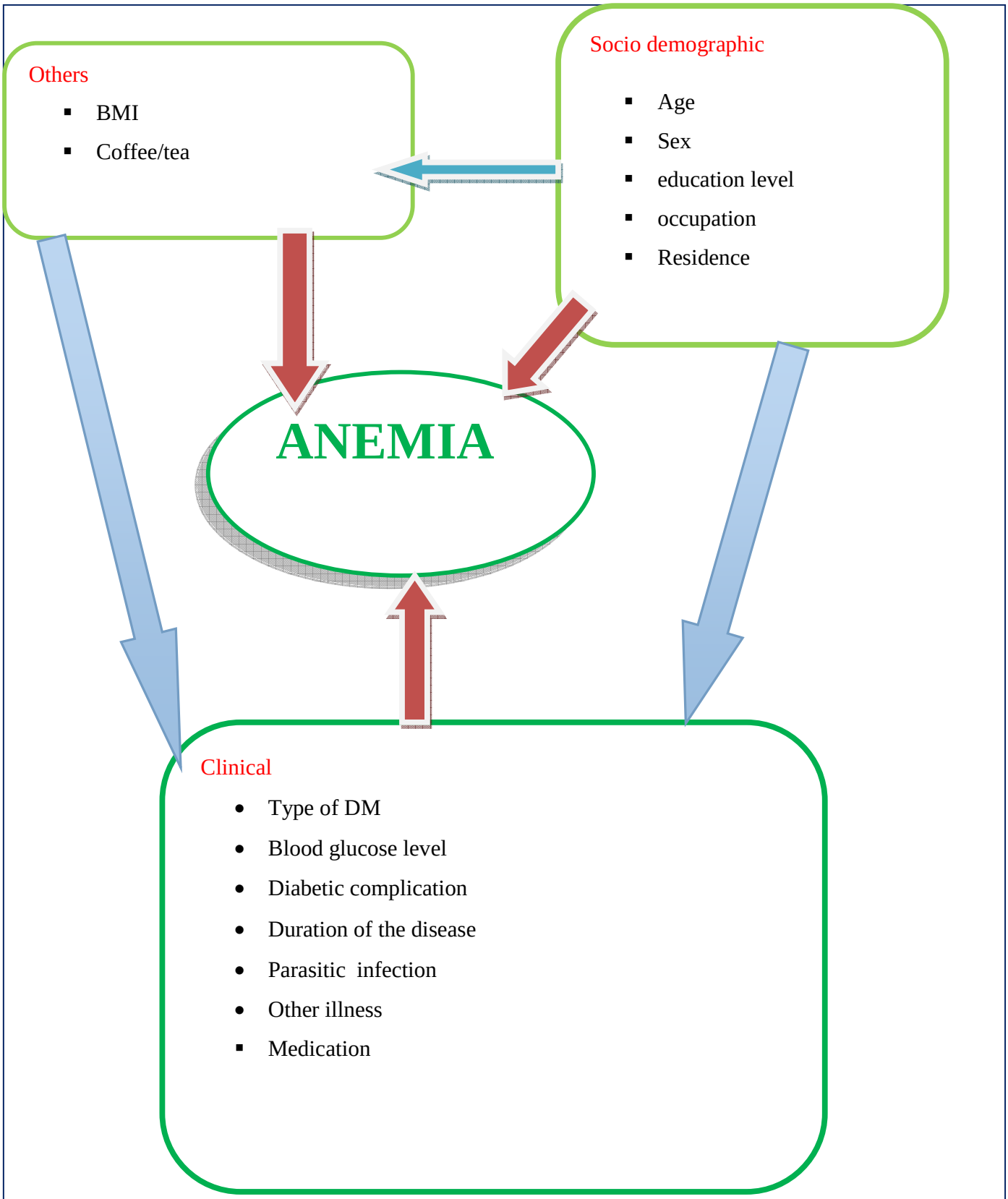

Figure -1: Conceptual frame work to determine anemia and associated factors in diabetic patients attending JUSH, Southwest Ethiopia, April 20 to May 30, 2014

CHAPTER THREE: OBJECTIVES

3.1General objective

To determine anemia and associated factors among diabetic patients at chronic care center in JUSH Southwest Ethiopia

3.2 Specific objectives

- To determine the prevalence of anemia among diabetes patients in JUSH Southwest Ethiopia ,2014
- To determine the morphological type of anemia among diabetic patients in JUSH Southwest Ethiopia ,2014
- To identify associated factors of anemia among diabetic patients in JUSH Southwest Ethiopia ,2014

CHAPTER FOUR: METHODS

4.1 Study setting

This study was conducted from April 20 to May 30, 2014 in JUSH. JUSH is located in Jimma Town which is located 346 km Southwest of Addis Ababa with in latitude and longitude of 7°40′N and 36°50′E, respectively. It has an altitude of 1780 meters above sea level with annual temperature of 25 -30^{0}C and annual rain fall of 1200 -2000 mm. The Hospital is the only teaching hospital in Jimma Zone of Oromia region, Southwest of Ethiopia. Currently as ownership to Jimma University it provides services to residents of Jimma and neighboring zones for approximately 9,000 inpatient and 80,000 outpatient attendances a year coming from the catchment population of about 15 million people.JUSH serve for 2,000 diabetic patients. The monthly diabetic follow-up clinic gives service to 70-90 patients per day [18].

4.2 Study design

Institution based cross-sectional study design was conducted

4.3 Population

4.3.1 Source population

Diabetic patients attending out patient in chronic care center JUSH

4.3.2 Study participant

- All diabetic patients who came to chronic care center for follow up during the study period.

4.4 Eligibility criteria

4.4.1 Inclusion criteria

- All adult diabetic patients attending JUSH and willing to participate
- DM patients with complete medical records.

4.4.2 Exclusion criteria

- Pregnant diabetic patients
- Received treatment for anemia for the last 4 months

4.5 Sample size and Sampling technique

4. 5.1 Sample size

The sample size was determined using a single population proportion formula as follows

$$n = \frac{(Z\alpha/2)^2 P (1-q)}{d^2}$$

Where:

n = sample size

$Z_{\alpha/2}$= the standard normal variable at 95% CI (1.96)

P= the prevalence of anemia for the population (19%) =0.19[16]

D=margin of error (0.05)

$$n = \frac{(1.96)^2 .0.19(1-0.19)}{(0.05)^2}$$

n=236 +10% (non response rate) =260

4.5.2 Sampling technique

All DM patients attending JUSH during study period were included consecutively.

4.6 Study variables

4.6.1Dependent variable

- Prevalence of anemia
- Type of anemia

4.6.2 Independent variables

Socio demographic variables

- Age
- Sex
- Educational level
- Occupation
- Residence

Clinical variable

- Type of diabetic
- Blood glucose level
- Diabetic complication
- Duration of the DM
- Treatment for DM or other diseases.
- Parasite infection
- Other clinical illness

Others variables

- BMI
- Habit of drinking Tea/coffee cups before or after eating food.

4.7 Data Collection

4.7 .2 Socio-demographic and clinical data

First personal information covers the socio-demographic data of patients including gender, age, residence; education and occupation were collected through face to face questionnaire guided interview by clinical nurses at the chronic care centre in parallel with specimen collection. Then, clinical history of diabetic patients including: type of diabetic, complication, treatment, and duration of the disease were obtained from their medical records using questionnaire.

Weight and height was be recorded by locally made stadiometres with a sliding headpiece, and portable mechanical analog scales. Height was measured to the nearest 0.1 cm and weight to the nearest 0.1 kg. Each subject weighed with minimum clothing and no foot wear. The scales were be carefully handled and periodically calibrated by placing standard calibration weights of 2 kg iron bars on the scale to ascertain accuracy and measurements were taken twice; when necessary any discrepancies were resolved by a third measurement. Mean average values used for data analysis ,Body mass index (BMI) was calculated as weight (kg) divided by height (m^2) .This used to explain nutrition status of patient and to categorize as underweight (BMI <18.50 $kg/m^{2)}$ normal weight (18.50-24.99 kg/m^2), overweight (25.00-29.99kg/m^2) and obese ($\geq$30.00kg/m^2)[40] .

4.7.2 Laboratory data

4.7.2.1Specimen collection and processing

Four (4) ml of venous whole blood collected by syringe in the morning was dispensed in to two EDTA test tubes. One for hematological measurements and the other 2ml was centrifuged to separate plasma for glucose determination. The sample was transported to hospital laboratory within 30 minutes of collection kept ambient temperature. Subjects were also given a dry clean container and instructed to collect about 5g of stool for intestinal parasite examination. It was transported with in ice box to the laboratory.

4.7.2.2 Specimen analysis

The EDTA blood was used for the determination of complete blood count (CBC). CBC parameters particularly red cell parameter such as red blood cell count (RBC),Hemoglobin (Hb),hematocrit(Hct),mean cell volume(MCV),mean cell hemoglobin(MCH), mean cell hemoglobin concentration(MCHC) values were determined by CELL DYNE 1800 CBC analyzer*(abottdiag, USA).* Hb level was used to determine prevalence and severity of anemia. Peripheral thin blood film fixed with methanol and stained with Wright stain was used for RBC morphological examination. Morphological type of anemia was assessed based on the result of RBC morphology plus red cell indices. While the thick blood film stained with Geimsa was used to observe malaria parasite under oil immersion field microscope.

Adjusted Hb values were calculated against altitudes for Jimma town 1780 meter above sea level using the equation below.

$$Hb_{adjusted} = Hb_{see\ level} - 0.32 \times (\text{altitude in mts} \times .0033) + 0.22 \times (\text{altitude in mts} \times .0033)^2 \quad \text{.....[41]}$$

Stool specimens were examined microscopically for the presence of parasite eggs, cysts and trophozoites using direct saline thin smear and formol-ether concentration methods.

Plasma was separated and used for glucose measurement. Plasma glucose was analyzed by chemistry analyzer (ECHO LINEAR, ITALY). Glucose was determined after enzymatic oxidation in the presence of glucose oxidase at $37^{O}C$ for 10 minutes. The formed hydrogen

peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to red-violet quinoemine dye as indicator. The intensity of the color was measured at wave length of 500 nm. This instrument was fully automated. After sufficient amount (50µl) of sample was loaded on sample rack, analyzed and calculated result .Then result displayed on the screen was be recorded. This fasting glucose level discussed its possible association for anemia (See Annex 1)

4.8 Statistical analysis

A statistical analysis was performed with SPSS version 20(IBM Inc, Chicago). Descriptive statistic such as mean, standard deviation and frequency was used to explain and characterize socio-demographic, clinical history and laboratory data. A logistic regression also carried out to identify indicators of anemia in DM. Odd ratio was applied to analyze the degree of association of independent variables to anemia. All socio-demographic and clinical variables that showed significant associations at P-value < 0.25 with anemia in bivariate analysis were selected and entered for multivariate logistic regression analysis to identify the most important predictors of anemia. Independent variables with P-value <0.05 were considered statistically significant. Results were presented in the form of tables and figures.

4.9 Quality assurance

In order to obtain reliable and valid data in this study standard operating procedure (SOP) was followed. Pre-test was done in 5% of the total sample size. Training was given for the data collectors to ensure the quality of data. Manufacturer Instructions were followed to maintain equipment performance and reagent expired date. Control reagents were used to check the accuracy and precision of the results for all tests. Besides, the collected data was checked for completeness and internal consistency.

4.10 Ethical clearance

Ethical clearance was obtained from the Ethical Review Committee of College of Public Health and Medical Science, Jimma University. Permission was asked from medical director of the hospital and head of referral clinics. The purposes and the importance of the study were explained & written informed consent was secured from each participant. Confidentiality was maintained at all levels of the study. Participants' involvement in the study was on voluntary basis; those who were unwilling to participate in the study & those who wish to quit their

participation at any stage were informed to do so without any restriction. Patients with abnormal results were referred to their physicians for management.

4.11 Plan for dissemination of findings

The finding of the study was presented and submitted to Medical Laboratory science and pathology department to be used as reference. It will also be submitted to the hospital also for intervention. Researchers do effort to present the paper in seminars or to publish in health journal

4.12. Operational definitions

- Diabetic patients: was defined as adult (>15 years) patients who were recognized as diabetics and attending follow up in chronic care center JUSH [1].
- Body mass index (BMI) was calculated as weight (kg) divided by height^2 (m^2) and can categorize as underweight (BMI <18.50 $kg/m^{2)}$ normal weight (18.5-24.99kg/m^2), overweight (25-29.99kg/m^2) and obese (≥30kg/m^2) [39].
- Anemia was defined as blood Hb concentration <12g/dl and <13g/dl for female and male, respectively. The severity of anemia was classified into three stages: mild (11g/dl -11.9g/dl), moderate (8g/dl -10.9g/dl), or severe (Hb< 8g/dl) [1, 40].
- Based on red cell morphology anemia was classified as microcytic-hypochromic (MCV<80fl, MCH<27pg, macrocytic (MCV>100fl) and normocytic-normchromic (80 fl<MCV<100fl,27pg<MCH<32pg) [1,6].
- DM complication :any acute or chronic complication that have identified in diabetic patients[4]
- Diabetic Nephropathy: was progressive chronic kidney disease developed in diabetic and obtained from patient medical records [4].
- Uncontrolled blood glucose level: high blood glucose which is unable to control and FBS >150 mg/dl with diabetic medication [36].

CHAPTER FIVE: RESULTS

5.1 Description of sociodemographic characterstics of study participants

A total of 260 DM patients (61.5% male and 38.5% female) had participated in the study. The mean age of participants was 51.4 ± 1.48 years and majority of them 121(46.5%) were within the age group of 46-60 years old. From the total of DM patients, 157 (60.4%) were rural residents. Educational level of the study participants varies from illiterate to higher education and 69 (26.9%) were illiterate. Their mean BMI was 23.6 ± 3.6 and the majority 158 (60.8%) were in the range of 18.50-24.99 kg/m^2 (Table 1).

5.2 Description of clinical data

The distribution of type one and type two DM among study participants was 74(28.5%) and 186 (71.5%), respectively. The majority 109 (41.9%) of patients, had been DM for less than 5 years. Diabetic patient with complication were 123(47.3%), of whom 48(39.0%) were DM patients with renal complication. The mean FBS of participants was 182.7 ± 90.2 which ranges 52 to 460 mg/dl and 167(64.2%) were above 150 mg/dl (table 1).

Out of 260 study participant examined for intestinal parasites 40(15.3%) were positive for at least one parasite. Total of seven species were identified. Majority were *Ascaris lumbricoid* 20 (50%) which is followed by *Giardia lamblia* 10(25%), *Trichuris trichiura* 6 (15%) and others (*Entamoeba histolytica, Tinea saginata, Shistosoma mansoni and hook worm*) 4 (10%). Double infections of *Trichuris trichiura with Ascaris lumbricoid* were observed in three diabetic patients. All DM patients were examined that they were negative for malaria.

Table- 1: Socio-demographic and clinical characteristic of diabetic patients attending JUSH, Southwest Ethiopia, April 20 to May 30, 2014.

Variables		Frequency	
		No	(%)
Age	15-30	30	11.5
	31-45	46	17.7
	46-60	121	46.5
	>60	63	24.3
Sex	Male	160	61.5
	Female	100	38.5
Address	Urban	103	39.6
	Rural	157	60.4
Occupation	Student	11	4.2
	Farmers	73	28.1
	House wife	56	21.5
	G.employee	68	26.2
	Merchants	34	13.1
	Others*	18	6.9
Education	Illiterate	69	26.5
	Read & write	27	10.4
	Primary	61	23.5
	Secondary	43	16.5
	Post secondary	60	23.1
Tea/Coffee	Yes	182	70.0
	No	78	30.0
BMI*	Under weight	14	5.4
	Normal weight	158	60.7
	Over weight	74	28.5
	Obese	14	5.4

Variables		Frequency	
		No	%
DM* type	Type 1	74	28.5
	Type 2	186	71.5
DM duration	<5 year	109	41.9
	5-9 year	89	34.3
	>9 year	62	23.8
Medication type	Metroformn	89	34.2
	Insulin	171	65.8
Diabetic complication	Yes	123	47.3
	No	137	52.7
Complication type	Nephropathy	48	39.0
	Hypertension	40	32.5
	Others **	35	28.5
Intestinal parasite	Positive	40	15.3
	Negative	220	84.7
Other illness	Yes	4	1.5
	No	256	98.5
Fasting Blood sugar	<150 mg/dl	93	35.7%
	≥150 mg/dl	167	64.3%

Other=driver, guard, daily labor, maid wife. Others**= cardio vascular diseases, neuropathy, retinopathy, diabetic kitoacodosis (DKA), hyperosmolar hyperglycemic state(HHS) ,hypoglycemia skin infection, foot ulcer, impotence, DM=diabetes Mellitus, BMI=body mass index*

5.3 Prevalence and morphological type of anemia

Participant's Hb level was used to determine the prevalence of anemia. The overall prevalence of anemia in this study was 60(23.1%). Among the anemic DM patients mild and moderate anemia was reported as 58(96.6%) and 2 (3.4%) respectively. Most (70%) of anemic diabetic patients were above mean age (51 ±1.48 year). Prevalence of anemia was reported as 29.0% in females DM patients and 19.4% in males. Thirty nine (31.7%) of participants with DM complication were identified as anemic. Among 167 patients with uncontrolled FBS, 50 (30.0%) were reported anemic (Table 2).

Table- 2: Prevalence of anemia with socio-demographic and clinical characteristics among diabetic patients attending JUSH, Southwest Ethiopia, April 20 to May 30, 2014

Variables	Category	Non-Anemic		Anemic		Total	P value
		No	-(%)	No	(%)		
Age	≤ 51years	108	(85.7)	18	(14.3)	126	(1)
	>51years	92	(68.6)	42	(31.4)	134	0.001*
Sex	Male	129	(80.7)	31	(19.3)	160	0.28
	Female	71	(71.0)	29	(29.0)	100	(1)
Address	Urban	74	(71.9)	29	(28.1)	103	(1)
	Rural	126	(80.3)	31	(19.7)	157	0.27
Occupation	Student	11	(100)	0	(0)	11	0.999
	Farmer	53	(72.7)	20	(27.3)	73	0.354
	House wife	37	(66.1)	19	(33.9)	56	0.273
	G.employee	57	(83.9)	11	(16.1)	68	0.960
	Merchants	27	(79.5)	7	(20.5)	34	0.733
	Others	15	(83.4)	3	(16.6)	18	(1)
Education	Illiterate	42	(60.9)	27	(39.1)	69	0.001*
	Read and write	18	(66.7)	9	(33.3)	27	0.020*
	Primary	53	(86.9)	8	(13.1)	61	0.809
	Secondary	34	(79.0)	9	(20.9)	43	0.26
	Post sec and above	53	(88.9)	7	(11.6)	60	(1)
Coffee /tea	Yes	137	(75.3)	45	(24.7)	182	0.336
	No	63	(80.8)	15	(19.2)	78	(1)
BMI	Under weight	9	(64.3)	5	(35.7)	14	0.253
	Normal weight	116	(73.5)	42	(26.5)	158	0.323
	Over weight	63	(85.2)	11	(14.8)	74	0.955
	Obese	12	(85.8)	2	(14.2)	14	(1)
Type of diabetics	Type 1	58	(78.4)	16	(21.6)	74	0.725
	Type 2	142	(76.4)	44	(23.6)	186	(1)
Duration of diabetic	<5 years	91	(83.5)	18	(16.5)	109	0.215*
	5-9 years	68	(76.5)	21	(23.5)	89	(1)
	>9 years	41	(66.2)	21	(33.8)	62	0.215*
Diabetic complication	Yes	84	(68.3)	39	(31.7)	123	0.002*
	No	116	(84.9)	21	(15.1)	137	(1)
Type of medication	Metroformin	69	(77.5)	20	(22.5)	89	0.867
	Insulin	131	(76.6)	40	(23.4)	171	(1)
Type of complication	CKD	30	(62.5)	18	(37.5)	48	0.277
	Hypertension	30	(76.2)	10	(23.8)	40	0.945
	Others	24	(75.6)	11	(24.4)	35	(1)
Intestinal parasite	positive	28	(70.8)	12	(29.2)	40	0.307
	Negative	171	(78.1)	48	(21.9)	220	(1)
Others illness	Yes	3	(75.0)	1	(25.0)	4	0.927
	No	197	(76.9)	59	(23.1)	256	(1)
Fasting blood sugar	<150 mg/dl	83	(89.3)	10	(10.7)	93	(1)
	≥150 mg/dl	117	(70.0)	50	(30.0)	167	0.094

Other=driver, guard, labor, maid wife. Others**= cardio vascular diseases, neuropathy, retinopathy, diabetic kitoacodosis (DKA), hyperosmolar hyperglycemic state (HHS), hypoglycemia, skin infection, foot ulcer, impotence, DM=diabetes Mellitus, BMI=body mass index*

Among the 60 diabetic patients who were anemic, majority were normocytic-normochromic type (figure 2).This morphological classification didn't show gender difference. Regarding age, majorities were normocytic anemia in all age groups. While few macrocytic were identified in older (above mean) age and microcytic anemic individual in young age. Although microcytic in type 1 DM and macrocytic anemia in type 2 DM were reported, normocytic type of anemia was dominant in both groups. From the 20 anemic DM patients taking metroformin only two were macrocytic while others were normocytic type of anemia.

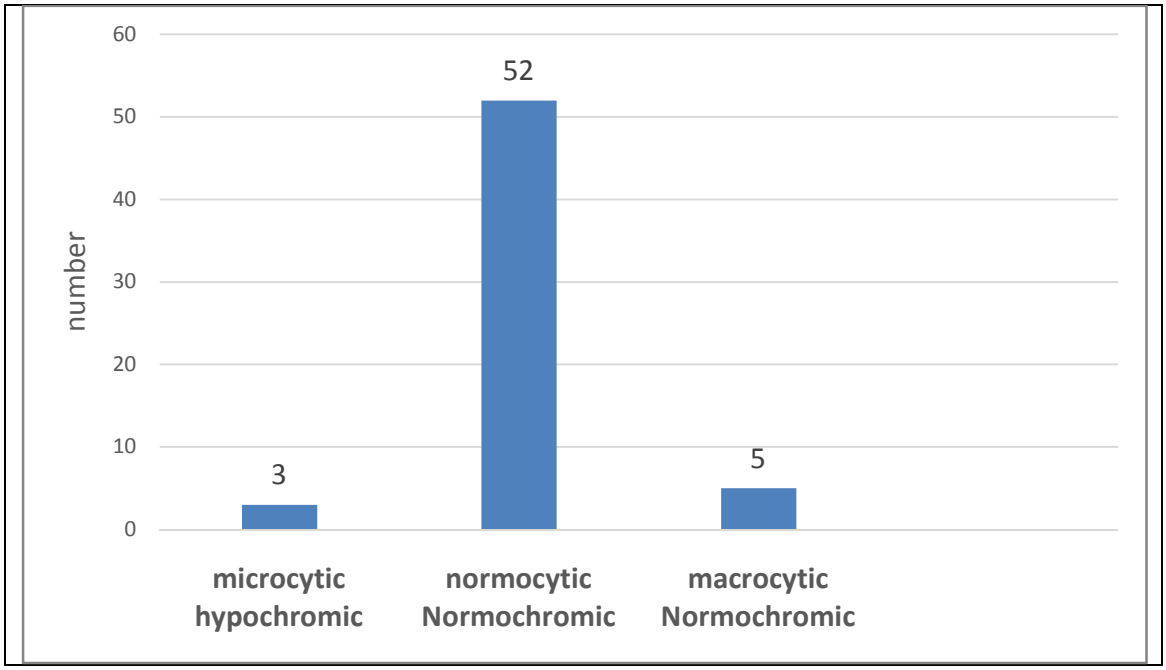

Figure- 2: Morphological type of anemia among DM patients attending JUSH, Southwest Ethiopia, April 20 to May 30, 2014

5.3 Risk factors of anemia among DM patients

During bivariate analysis, variable found to be significantly associated with anemia were: age of the patients, educational level, DM complication, duration with DM and FBS level. According multivariate analysis from the above listed variables only educational status, DM complication and FBS were found to be significantly associated with anemia at p value of < 0.05. Diabetic patients with DM complication were 2 times (AOR = 2.15, 95% CI = 1.09, 4.23) more likely to be anemic than DM without complication. Illiterate DM patients were 4 (AOR=4.19, 95% CI =1.63, 10.88) times more likely to be anemic than DM patients with education level post secondary. While DM patients with uncontrolled hyperglycemia were 2 times more likely (AOR = 2.10, 95% CI = 1.05, 4.18) to be anemic than those of controlled glycemic level (Table 3).

Table- 3: Association of anemia with socio-demographic and clinical variable among diabetic patients attending JUSH, Southwest Ethiopia, April 20 to May 30 ,2014

Variables	Category	COR*	(95%CI)	P-value	AOR* *(95%CI)	P-value
Age	=<51 years	(1)		0.001*	(1)	
	>51 years	2.74	(1.47,5.08)		1.79 (0.77 ,4.15)	0.172
Sex	Male	(1)				
	Female	1.70	(0.95,3.05)	0.88		
Address	Urban	(1)				
	Rural	1.62	(.351,1.124)	0.277		
Education	Illiterate	4.86	(1.93,12.27)	0.001*	4.19 (1.61,10.88)	0.003**
	Read &write	3.78	(1.23,11.63)	0.020*	3.88 (1.18,12.67)	0.025**
	Primary	1.14	(0.38,3.37)	0.809	1.08 (0.35,3.30)	0.886
	Secondary	2.00	(0.68,5.88)	0.26	2.06 (0.67,6.28)	0.203
	Post sec	(1)			(1)	
Occupation	Student	.00	.00 .	0.999		
	Farmer	1.88	(0.49,3.22)	0.354		
	House wife	1.56	(0.66,2.97)	0.273		
	G.employee	.96	(0.23,3.90)	0.960		
	Merchants	1.29	(0.29,2.76)	0.733		
	Others*	(1)				
Coffee & tea	Yes	1.38	(0.71,2.65)	0.336		
	No	(1)				
BMI*	Under weight	1.33	(0.52,1.27)	0.253		
	Normal weight	1.17	(0.46,1.11)	0.323		
	Over weight	1.04	(0.20,1.33)	0.955		
	Obese	(1)				
DM* Type	Type 1	1.12	(0.58,2.14)	0.725		
	Type 2	(1)				
DM duration	<5 years	Ref(1)			(1)	
	5-9 years	1.56	(0.77,3.15)	0.215*	1.08 (0.50,2.31)	0.837
	>9 years	(1)	(1.24,5.37)	0.011*	1.66 (0.74,3.71)	0.217
Type of medication	Metroformin	0.94	(0.51,1.74)	0.867		
	Insulin	(1)				
DM complication	Yes	2.56	(1.40,4.67)	0.002*	2.15 (1.09,4.23)	0.026**
	No	(1)			(1)	
Type of complication	CKD	1.85	(0.75,2.54)	0.277		
	Hypertension	0.96	(0.36,2.58)	0.945		
	Others**	(1)				
Intestinal parasite	Positive	0.67	(0.32,1.42)	0.307		
	Negative	(1)				
Others illness	Yes	1.11	(0.11,10.90)	0.927		
	No	(1)				
Fasting blood sugar	<150 mg/dl	(1)1)			(1)	
	≥150 mg/dl	1.68	(0.91,3.08)	0.094*	2.10(1.05,4.18)	0.03**

COR = Crude odds ratio, AOR**= Adjusted Odd Ratio, CI = Confidence Interval. Other*=driver, guard, labor, maid wife. Others**= cardio vascular diseases, neuropathy, retinopathy, diabetic kitoacodosis (DKA), hyperosmolar hyperglycemic state (HHS), hypoglycemia, skin infection, foot ulcer, impotence, DM=diabetes Mellitus, BMI=body mass index*

CHAPTER SIX: DISCUSSION

Facility based cross sectional study was conducted to investigate the prevalence and associated factors of anemia in diabetic patient attending JUSH, JimmaTown. The overall prevalence of anemia was 23.1% suggesting that anemia is a public health problem in the area. Among the anemic DM patients 96.6 % were reported as mild anemia. And morphologically, most (86.7%) of anemic patients had normocytic–normochromic anemia. Diabetic complication, educational level and uncontrolled hyperglycemic were identified the strongest predictor of anemia.

Globally, according IDF reports type 2 DM making up about 90% of the cases [19]. In this study Type 2 DM were 71.5% of the study participant. The finding from Northwest Ethiopia reported that type one and type two DM were equally distributed among participants. While the Study from Australia identified that 80% of DM patients were type 2 [6, 7]. This difference might be due to socio-economic, epidemiological and methodological.

The prevalence of anemia in this study was comparable with the studies done in Saudi-Arabia (25%) [2], Australia (23%) [6], Northwest Ethiopia (19%) [7] and china (22.8%) [27]. But lower than study conducted in Bangladesh (54.3%)[5], Egypt (39%) [9], Caribbean (45%) [21] and India (37%) [22].This variation could be due to difference in life style, age and pathological condition of study population. For example in Egypt these patients were (from referral nephrology centre) with established renal complications and this complication lowers RBC production due to decrease in EPO production. Anemia is also long term effect of low iron intake and other micronutrient deficiencies .High prevalence of anemia was reported in Bangladesh diabetic patients suffering from various hematological diseases [5]. This higher prevalence of anemia in adolescent could be described by the fast growth and increased requirement for iron during their development in addition to the disease state[17].

However anemia prevalence of this study was greater than the findings in Ireland (13%) [23], UK (10.6%) [24] and Israel (10.8%) [33].This variation might be due to low socioeconomic status and lower nutritional status in this study area than those reported from elsewhere. In addition to ethnicity, accessibility to health information & health care services plays a part in the liability to anemia [7].

Prevalence of anemic diabetic patients was 29.0% in females and 19.4% males in this study. This variation could be due to females might had lower access to health services and some women were excluded as being pregnant from this study population .But study from UK found, male (17.8%) and female (11.8%) were anemic patients [24]. This inconsistence within female prevalence might be due to ethnical difference in addition to variation in accessibility to health information & health care services.

Anemia of diabetic patients was generally normocytic-normochromic [3].Similarly among diabetic patients of this study who were anemic; most of them were normocytic-normochromic type .This result supports the reports of china [27] Ireland [23]. But hypochromic- microcytic anemia was the most frequent in Saudi-Arabia [2]. This variation could be due to difference in life style and age (children were included) of study population. Hypochromic- microcytic anemia is common in long term effect of low iron intake and other micronutrient deficiencies. Iron requirement is lower in adults than in children [17].

The finding of this study noted that anemia was significantly associated with DM complication .DM patients with complication were 2 times (AOR = 2.15 CI =1.09, 4.23) more likely to be anemic than DM patients without complication. This result was in agreement with studies in Northwest Ethiopia[7], Egypt [9], china [27], Australia[32], Caribeans [21] India[22] and Israel [33]. These strong links between the DM complication and anemia in DM probably reflect the unique vulnerability of microcirculation to damage in DM [6].

Prevalence of DM complication in the study was 47.3%, of these patients 20.7% had CKD. Renal impairment in DM didn't show significant association with anemia in this study. However several researches reported that there was significantly an increase odd of anemia, with greatest risk for patients with DM and CKD [7,9, 32, 22]. This inconsistence might be due difference in method of evaluation of renal impairment. These studies determined CKD based on laboratory result like glomerular filtration rate (GFR) and identified significant differences among the different stages of CKD.

The result of our study showed that anemia was also significantly associated with educational status. Illiterates DM patients were 4 times (AOR =4.19, 95% CI =1.63, and 10.88) more likely to develop anemia than of post secondary. The high prevalence in illiterates might be due to the fact that most of them residing in rural areas so might not have adequate information about nutrition and other factors that could cause anemia. This result supports finding of USA [30].

On this finding DM patients with uncontrolled blood glucose level were 2 (AOR = 2.10, 95% CI = 1.05, 4.18) times more likely to be anemic than good blood glucose control. This is attributed to the, decreased RBC survival, early development of end-organ damage, and late presentation for medical care in patients with poorly controlled DM. This finding was consistent with studies conducted in Egypt [9] and Nigeria [25, 36].

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Although it was previously believed that decline in Hb levels might be a normal consequence of aging, evidence has accumulated that anemia does reflect poor health and increased vulnerability to adverse outcomes in older person [36]. This study also showed that the prevalence of anemia increases with older ages and duration of DM; however these factors were failed to show significant association with anemia. The study in Israel indicated that age and DM duration had a significant association with anemia in DM patients [33]. Ethnical and life style differences may cause for these variations.

As strength of this study, it is one of few investigations in developing countries where chronic diseases such as DM is becoming more prevalent .It reminds or create awareness on the unrecognized and undiagnosed complication of DM that is anemia .we have tried to analyzes many variables.

Because of the cross-sectional nature of our study, therefore no causal relationship could be established.HbA1C and Renal function were not done due financial limitation .This research was also unable to assess nutritional, alcohol status of participant due to lack of guidelines or standards against anemia. This study is institutional based and used small sample size so was not possible to generalize to the whole population of Jimma zone as well as Ethiopia.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

7.1 Conclusion

This study showed that anemia was moderate public health problem in DM patients in the area. Among the anemic DM patients mild and moderate anemia was reported as 96.6% and 3.4% respectively. Morphologically majority of anemic Diabetic patients were normocytic-normochromic while the rest were macrocytic-normochromic and microcytic- hypochromic anemia. The multivariate logistic regression identified that illiterate DM patient; poor glycemic control and DM complication were independent predictors of anemia. Lack of education and proper management impose an individual health to acute and chronic complications of DM. If anemia left undiagnosed and untreated, it affects the quality of patient's life and has fatal consequences.

7.2 Recommendation:

Based on the above concluding ideas, this high prevalence of anemia in DM recommends screening should be included as routine works for early detection of anemia for these highly risk groups. Health policy makers and health providers need to consider patient education that covers all aspects of anemia management which is relevant to a person's clinical and psychological needs, and adaptable to their educational and cultural background. Therefore, further longitudinal studies are needed to explore more on the causes of anemia in diabetic patients for possible intervention.

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ANNEXES:

Annex 1: Standard operating procedures (SOP)

A.Standard operating procedures for CELL DYNE 1800

Purpose

the purpose is to evaluate anemia, leukemia, reaction to inflammation and infections, peripheral blood cellular characteristics, polycythemia, hemolytic disease of the new born, inherited disorders of red cells, white cells, and platelets; manage chemotherapy decisions; determine qualitative and quantitative variations in white cell numbers and morphology, morphology of red cells and platelet evaluation.

Principle

The CELDYN 1800 uses two independent measurement methods; they are:

Electrical impedance method for determining WBC, RBC, and PLT data

Modified Methemoglobin Method for determining HB

During each instrument cycle, the sample is aspirated, diluted, and mixed before each parameter is measured.

Specimen Required

Collected in EDTA anticoagulant. Follow the manufacturer's guidelines regarding collection and stability.

Mixed well before processing.

Fresh whole blood specimens are recommended (process within eight hours after collection). A minimum of 50µL must be collected for micro-collection specimens. This ensures an adequate amount of blood for the 30 µL aspiration.

Supplies and Materials required

CELL-DYN 1800

Waste reservoir

CELL-DYN 1800 Reagents

CELL-DYN 1800 Controls

Biosafety materials

EDTA (K3) VACUTAINER Blood collection tube (Lavender top, liquid EDTA).

Calibrator

Reagents

Cyanide-Free Diff Lyse Reagent, Detergent, Diluents and Enzymatic Cleaner. Reagent must be stored at room temperature except Enzymatic Cleaner which should be stored between 2 and 8 degree centigrade. Do not use reagents that have been frozen.

Procedure

Entering and Running Patient Specimen

Note: prior to running patient specimens, perform daily start-up procedures

When the ready message is displayed on the run screen, the instrument is ready to run specimens.

Entering specimen

From run screen, press [specimen type]

In the specimen type screen, press [patient specimen]

The cursor is placed in the <next Id #> entry field. Use the alphanumeric keys on the pc keyboard to enter a specimen Id of up to 16 characters.

Running patient specimen

To run patient specimens, proceed as follows:

With the cap tightly secured on the specimen tube, slowly invert the tube 10 to 15 times.

Remove the cap from the pre-mixed specimen tube.

Place the tube under the aspiration probe and raise tube so that the end of the probe is deeply immersed in the specimen.

Press the touch plate to aspirate the run.

When the sample has been aspirated from the tube, the probe will move up through the wash block. Remove the specimen tube and replace the cap.

After the cycle is completed, run results are displayed on screen and the aspiration probe moves into position to accept a new specimen. The current run data is saved to the Data Log.

If Automatic Graphics printout has been specified in the setup menu, a report is printed according to the parameters selected during the setup procedure.

If Automatic Graphics printout has not been specified in the setup menu, press [print report] to obtain a copy of the results.

Note: if a system has been idle for 15 minutes or more, a normal background should be run immediately prior to running patient specimens.

Quality Control

Quality control checks should be performed daily according to the laboratory's protocol. Commercial controls materials should be properly warmed and mixed according to the manufacturers' recommendations patient controls should be handled according to the laboratory's protocol.

B.Procedure for peripheral blood Examination

1) Smear cells on slide and allow to air dry.
2) Fix slides for 30 seconds in absolute methanol.
3) Add approximately 0.5 ml of Wright's- stain and allow staining for 2 minutes.
4) Dilute with tap water, pH 6.6, (for full development of staining).
5) Allow to stain for an additional 3 minutes and wash with water.
6) thick blood film after dried directly stain with Geimsa (1:9) for 10 minutes

7) Allow to air dry by tilting the slide before examination.
8) Observe under microscope (oil immersion)
9) thin blood film for RBC morphology and thick film for hemoparasite

C.Standard operating procedures glucose test (GOD-PAP METHOD)

After at least 10 hours of overnight 2 ml fasting venous blood sample will be drawn by senior medical laboratory technologist using syring**,** from consent subjects after adequate disinfection of the area. Then the specimen will be centrifuged and the plasma will be separated for analysis within 30 minutes.

Glucose will be determined after enzymatic oxidation in the presence of glucose oxidase (EC 1.1.3.4) at 37^{O}C for 10 minutes. The formed hydrogen peroxide reacts under catalysis of peroxidase, POD (EC 1.11.1.7) with phenol and 4-aminophenazone to red-violet quinoemine dye as indicator. The intensity of the pink color will be measured.

$$\text{Glucose} + O_2 + H_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + 4AP + \text{phenol} \xrightarrow{POD} 4H_2O + 4\,(\text{p-benzoquinoneimine})\,\text{phenazone}$$

Contents

RGT 4X100 ML

Phosphate buffer

4-aminophenazone

Phenol

Glucose oxidase

Peroxidase

Mutarotase

Stabilizers

STD 3ml standard

Glucose 100mg/dl

Reagent preparation

RGT and STD are ready for use.

Reagent Stability

The reagents are stable up to the given expiry date when stored at $2\text{-}8^{0}$ c.

When opened contamination must be avoided. RGT is stable for 2 weeks at 15-25 ^{0}c

Specimen

Whole blood, serum, plasma

The glucose is stable for 5 days at 20-25^0 c, if deproteinisation and centrifugation of the whole blood is performed promptly after collection.

The glucose is stable for 24 hours at 2-8 ^{0}c, if serum or plasma is prepared with in 30 minute after collection.

Assay

Wavelength: 500 nm,

Optical path: 1 cm

Temperature: 20-25 ^{0}c or 37 ^{0}c

Measurement: Against reagent blank, only one reagent blank per series is required.

Performance characteristics

Linearity

The test is linear up to glucose concentration of 700mg/dl.

Normal values

Serum, plasma (fasting): 75-115mg/dl

D.Parasitological procedure

A. Direct examination of feacal specimens /wet mount smear preparations

Procedure

1. Place one drop of 0.85% NaCl on the slide.
2. Take a small amount of faecal specimen and thoroughly emulsify the stool in saline
3. Slide a 22mm cover slip at an angle in to the edge of the emulsified faecal drop. Push the cover slip across the drop before allowing it to fall into place.
4. Systematically scan the entire 22mm cover slip with overlapping fields with the 10x Objective.
5. Switch to high dry (40X objective) for more detailed study of any suspect eggs or Protozoa.

B. Formol-Ether Concentration Technique

Procedure

1. Using a stick, emulsifying 2 g of stool in 7 mL of 10% (v/v) formal-saline.
2. the suspension allow to stand for 30min

3. then straine through two layers of gauze into a 15mL conical centrifuge tube
4. Centrifuge at 2000 rpm for 5min.
5. Washing step was repeated until supernatant becomes clear.
6. The sediment resuspended with 10mL of 10% (v/v) formal-saline,
7. Then add 3mL of diethyl ether
8. shaken vigorously for 30 sec and centrifuge at 2000 rpm for 5min
9. Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom. Tap the bottom of the tube to re-suspend and mix the sediment.
10. Transfer the sediment to the slide, and cover with cover glass. To assist the identification of cysts run a drop of (iodine) under the cover glass.
11. Examine the preparation microscopically using the 10x objective with the condenser closed sufficiently to give good contrast. Use 40 x objectives to examine cysts.

Annex 2: Materials

Equipment & consumables materials

Duplicating paper
Pen
Pencil
Marker
CD-RW
Note pad
Binding of the document
Syringe with needle
Slides
EDTA tube
Frosted slides
Gauze and Cotton
Conical test tube
Disposable glove
Pasteur pipette
Celldyne 1800
Mindray 3000BC
Echo linear (chemistry analyser)
Stadiometres
microscope

Reagent and chemicals

CELL DYNE 1800

- Cyanide-Free Diff Lyse Reagent,
- Detergent,
- Diluents
- Enzymatic Cleaner
- Patient sample Controls

Echo linear (chemistry analyser)

- Glucose reagent (**GOD-PAP)**
- Glucose Standard
- Glucose controls

Giemsa stain
Wright stain
Methanol
Bleach
70% alcohol
10%Formaldehyde
Ether
Normal saline
Distilled water

Annex 3. Informed consent

Dear Diabetic patients (participants)

The aim of this study is to assess anemia and associated factor in diabetic patients in JUSH Southwest Ethiopia, 2014. And you are chosen to participate in this study by chance.

This research study will contribute for information on the anemia and associated factors as complication of diabetes mellitus. Presence of anemia among diabetic patient will be recommended for treatment. It is expected to improve the patient status through Clinical information obtained from laboratory tests for complication control and control premature death.

Your responses will be completely confidential. It is your full right to refuse in responding any question or all of the questions. If you don't want to participate you can leave the questionnaire empty. However, your honest answers to these questions will help us in better understanding of hematological abnormalities due to diabetes mellitus. So; we are requesting you to give your honest responses and keep participation. It will take a maximum of 15 minutes to answer these questions. Would you willing to participate please?

If you are pleasurable to participate in the study please visit the next page. For any further question, contact the investigator:

Code of participant: _____ Signature_____ Date: _____
Name of the witness _____Signature_____Date: _____

Habtom Kifleyesus phone no- 0925493361, Email address- habtomkif@yahoo.com

M. Lealem Gedefaw, phone no- 0913024541, Email address- lealew07@yahoo.com

Dr. Tilahun Yemane, phone no- 0917804067, Email address- yemanetilahun@yahoo.com

የመጠይቅ ፈቃድ:የተከበራችሁ የጥናቱ ተሳታፊዎች

የዚህ ጥናት ዋና አላማ በ2006 **ዓ.ም ጅማ ዩኒቨርስት ሰፐሻላይዝድ ሆስፒታል በሚከታተሉ የስኳር ህመምተኞች ተያያዥ የሚከሠት የደም ችግር ደም ማነስ ዓይነቶቹ ለማጥናት ነው ፡፡ እንደሚታወቅ የበሽተኛው ሁኔታ ለመሻሻል ከላባራቶሪ የሚገኝ ውጤት ኮምፕሊኬሽን እና ሞትን**

ለመቆጣጠር የላቀ አስተዋጸኦ አለው፡፡ የህ ጥናት የደም ማነስ መጠን በፐርሰንት ምን ያህል እንደሆነ ፡ ተያያዥ ችግሮች በመለየት የስካር ህመምተኞች ተከክለኛው የሆነ ክትትል እንዲያገኙ ጥናታዊ መርጃ ይሰጣል፡፡ስለዚ ከእናንተ በሚገኘው መረጃ መሰረት በማድረግ በጀማ ዩኒቨርስት ሰፐሻላይዝድ ሆስፒታል በሚከታተሉ የስኳር ህመምተኞች ሁኔታቸው ታውቆ ለፖሊሲ አዘጋጅዎችና ለሌሎችም ለሚመለከትቸው ስለሚረዳ ህመምተኞች ሚስፈልጋቸው የህክምና እርዳታ እንዲያገኙ ነው፡፡የዚ ጥናት ተሳታፊ ደም ማነስ የተገኙበት የስካር በሽተኛ መድሃኒት እንዲያገኝ እናመለክታለን፡፡

በአጋጣሚ እርስዎም በዚህ ጥናት እንዲሳተፉ ተመርጠዋል፡፡ በጥናቱ ውስጥ ለጤና መረጃና የግል ሕይወታቸውን የሚያካትቱ ጥያቄዎች ተካተዋል፡፡ ጥናቱ በትክክል አላማውን እንዲመታ የእርሰዎን ድጋፉ እንጠይቃለን፡፡ የማንኛውም ግለሰብ ሃሳብ ብቻውን ዱፉ እንዲዎጣ አይደረግም:: ሀሳቡ ሙሉ በሙሉ በሚስጥር የተጠበቀ ነው፡፡ በመጠይቁ ያለመሳተፍ በሙሉም ሆነ በከፊል ጥያቄዎችን ያለመመለስ ሙሉ መብት አለዎት፡፡ በጥናቱ መሳተፍ ካልፈለጉ መጠይቁን ክፍት መተው ይችላሉ ነገር ግን እርስዎ ለጥያቄዎቹ የሚሰጡን ትክክለኛ ምላሽ ህመም ሰካር ተያያዥ ምክንያቶችን የሚከሰቱ የጤና ችግሮች በይበልጥ ለመርዳት እና ለማሻሻል ይረዳናል፡፡ ስለዚህ ግልፅ የሆነ ምላሽና ከልብ የመነጨ ተሳትፎዎን እንዲሰጡን በአክብሮት እንጠይቃለን፡፡ ቃለ መጠይቁን ለመሙላት ሊወስድ የሚችለው ጊዜ ቢበዛ 15 ደቂቃ ነው፡፡

ስለጥናቱ ማንኛውንም ጥያቄ ወይም እርስዎ በዚህ ጥናት ውስጥ ለሚኖርዎት ድርሻ፣አሳሳቢ ጉዳይ ወይም ቅሬታ ካለዎት የሚከተሉትን ስልኮች ወይም ኢሜል አድራሻ መጠቀም የጥናቱን ባለቤቶች ማነጋገር ይችላሉ።ለመሳተፍ ፈቃደኛ ነዎት::ለጥናታችን ስኬት ትክክለኛዉን መረጃ እንዲሰጡ በአክብሮት እንጠይቃለን።

ተሳታፊ ፊርማ----------ቀን----------

ታዛቢ/እማኝ----------ፊርማ----------ቀን----------

ሃብቶም ክፍለኢየሱስ፡ ስ ቁ 0925493361 ኢ. ሜይል - habtomkif@yahoo.com

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ዶ/ር ትላሁን የማነ: ስ ቁ 917804067 ኢ. ሜይል - yemanetilahun@yahoo.com

Annex 4: Information Sheet

Introduction

This information sheet is prepared to explain the research project that you are asked to review and approve. The main aim of this research project is to investigate anemia and associated factors among diabetic patients at JUSH. The research includes a final year (clinical laboratory science) CLS graduate student and two senior advisors from Jimma University, College of public health and Medical science, department of medical laboratory science and pathology.

Name of Principal Investigator: HABTOM KIFLEYESUS

Name of Advisors:Mr LEALEM GEDEFAW, and Dr TILAHUN YEMANE .

The investigator is a student of final year Msc in CLS specialization hematology and immune-hematology truck with advisors from medical laboratory science and pathology department.

Purpose:

This study is primarily designed to asses prevalence of anemia and associated factors among diabetes mellitus patients in JUSH and to determine magnitude and risk factors of anemia in diabetic in order to contribute appropriate intervention, control and patient management. Results from this study will contribute to improve the patient life through clinical information obtained from laboratory tests for control of complication and premature death.

Procedure:

This study uses cross-sectional study design, through face-face interview using structured questionnaire. Permission will be asked from the JUSH chronic care center and medical laboratory science and pathology department Jimma University

Risk and/or Discomfort:

Except small pain to give 4ml blood samples and dedication of time for responding the questioner, there is no any risk or discomfort that you will face by participating in this research. Any personal information registered in registration books will not be copied and transferred to

other bodies. Every piece of information will be kept confidentially using coding system or anomicity.

Benefits:

There may benefit for policy maker and chronic care center specifically to these diabetic patients participating in this research and Ethiopian diabetic association as well. Based on the findings of the research, generally it will help to design effective and appropriate management follow-up for diabetic patients to prevent complication.

Incentives/Payment for Participating:

There was no incentive or payment to be gained by taking part in this project.

Persons to contact:

If you have any question or want to know more information you can contact through the address below the following individuals.

Investigator: HABTOM KIFLEYESUS Tel: 0925493361 Email: habtomkif@yahoo.com

Advisors: 1. Mr. LEALEM GEDEFAW Tel: 0913024541 Email - lealew07@yahoo.com

2. Dr. TILAHUN YEMANE Tel: 0917804067 Email - yemanetilahun@yahoo.com

Medical laboratory science and pathology department

Jimma University

የጥናቱ ተሳታፊዎች መረጃ ቅፅ(የአማርኛ ግልባጭ)

የጥናቱ ርዕስ፡ ጅማ ዩኒቨርስት ሰፐሻላይዝድ ሆስፒታል በሚከታተሉ የስኳር ህመምተኞች ላይ የሚከሠት የደም ማነስ ተያያዥ ችግርች ለማጥናት

የዋና ተመራማሪ ስም:ሃብቶም ክፍለኢየሱስ

የድርጅቱ ስም፡-ጅማ ዩኒቨርስቲ

ድጋፍ ሰጭ ተቋም:

ይህ የመረጃ ቅፅ የተዘጋጀው ከላይ በተጠቀሰው ጥናት ለሚሳተፉ ታዳጊ ተማሪወች ሲሆን በአጠቃላይ በጥናቱ ውስጥ ልናካሂዳቸው ስለፈለግናቸው ጉዳዮች እና ስለጥናቱ ጠቅላላ ማብራርያ ይሰጣል:: በመሆኑበ ጥናቱ የሚሳተፉት በራስዎ ፍላጐትብቻ መሆኑን በትህትና እንገልፃለን፡፡

የጥናቱአላማ

የዚህ ጥናት ዋና አላማ በ2006 ዓ.ም ጀማ ዩኒቨርስት ሰፐሻላይዝድ ሆስፒታል በሚከታተሉ የስኳር ህመምተኞች ላይ የሚከሠት የደም ማነስ ተያያዥ ችግርች ለማጥናት ነው፡፡

በአጋጣሚ እርስዎም በዚህ ጥናት እንዲሳተፉ ተመርጠዋል፡፡የዚህ ጥናት ጥቅም እነዚህ የሚማሩ ወጣት ተማሪዋች የደምማነስ መጠን በፐርሰንት ምን ያህል እንደሆነ ወጣት ተማሪዋች በሚሰጡት ምላሽ መሰረት መረጃዎችን በማሚላት በተገኘው ውጤት ለፖሊሲ አዘጋጅዎችና ለሌሎችም ለሚመለከትቸው ሁሉ ሊረዳ ይችላል በሚል ነዉ፡፡

የጥናቱ ሂደት ዝርዝር

በጥናቱ ለመሳተፍ ከተስማሙ የሚከተሉትን መረጀዎችና ናሙና እንወስዳለን፡

እርሰዎም እንዲሞሉ የተዘጋጀ ጥያቄዎች አሉ 4 ሚሊ ሊትር ያክል
የደምናሙናይወስዳል፡:የሠገራ ናሙና :የተወሰደዉናሙና ኣስፈላጊዉ ምርመራ
ይደረግበታል።

ስጋት ና ጉዳት

ህክምናው የሚያስገድደውን የአሰራር ሂደት ስለምንከተል ሊያጋጥሙ የሚችሉ የህመም ስሜት በጣም አነስተኛ ነዉ :: ቢሆንም የደም ናሙና በሚወስድበት ጊዜ ንሽ የህመም ስሜት ሊያጋጥም ይችላል፡፡ ነገር ግን ይህ ህመም በአጭር ጊዜ ይጠፋል፡፡

ሊያስገኛቸው የሚችሉት ጥቅሞች

በዚህ ጥናት ውስጥ በመሳተፍዎ በጥሬ ገንዘብ የሚደረግ የካሳ ክፍያ አይኖርም:: ነገር ግን የምርመራዉ ዉጤት በወቅቱ የሚሰጥ ሲሆን በምርመራዉ ዉጤት መሰረት ኣስፈላጊዉ የህክምና እርዳታ ይጠቆማል።

የጥናቱ ምስጢራዊነት

ማንኛውም በጥናቱ የሚገኙ መረጃዎች በምስጢር ይጠበቃሉ:: የጥናቱ መረጃዎች በሙሉ የሚቀመጡት ከእርሶስም ጋር ሳይሆን ለጥናቱ ተብሎ በሚሰጠው ስውር ቁጥር ሲሆን ጥናቱን ከሚያስከሂዱት ባለሙያዎች በስተቀር ማንም ሊያውቅ አይችልም፡; የእርስዎን ማንነት በሚገልጥ መልኩ የተዘጋጀውን መረጃ በፊርማዎ የተረጋገጠ ፍቃድ ሳናገኝ ይፋ አናደርግም:፡ ይህ ጥናት ሳይንሳዊ መረጃ እንደመሆኑ መጠን በወረቀት ታትሞ ቢወጣ ወይንም በሚድያ ቢነገር የእርስዎ ስም በምንም መልኩ አይጠቀስም፡:

ያለ መቀበል ወይ ምጥናቱን የማቋረጥ መበት

በዚህ ጥናት ውስጥ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ ፈቃደኝነት ላይ የተመሰረተ ይሆናል:፡ በማንኛውም ጊዜ ይህንን ጥናት የማቋረጥ መብትዎ ሙሉ በሙሉ የተጠበቀ ነው::

በጥናቱ ባለመሳተፍዎ ወይም ከጥናት በመገለልዎ ምክንያት በአሁኑ ወይም የወደፊት የህክምና እርዳታላይ ተፅዕኖ አይኖረውም፡ከዚህ በፊት ሲያገኙ ከነበሩት ጥቅሞች አንዳች ነገር አይጐድልቦትም፡ ጥናቱን የሚያከናውነው አካል ወይም ድጋፍ ሰጭ አካል ከእራስዎ ጥቅም ሲባል በጥናቱ እንዳይሳተፉ ሊከለከል ይችላል፡፡

ጥያቄ ካለወት

ስለጥናቱ ማንኛውንም ጥያቄ ወይም እርስዎ በዚህ ጥናት ውስጥ ለሚኖርዎት ድርሻ፣ አሳሳቢ ጉዳት ወይም ቅሬታ ካለዋት የሚከተሉትን ስልኮች ወይም ኢሜል አድራስ መጠቀም የጥናቱን ባለቤቶች ማነጋገር ይችላለ፡፡

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Annex 5.

I. Questionnaire

1 File number(code) in clinic……

2. Age: …….

3. Sex: M F

4 Location: urban Rural

5. Education: Illiterate

.read and write . Secondary

. Primary .post secondary

6. Occupation: Students Farmers

Housewife G. Employee

Merchants other

7. Drinking Tea/coffee cups before or after eating food

Yes……..

No ……

8. Smoking: Yes No

9. Preganant: Yes No

10. Physical Data: Weight …….. Height ……..

BMI……

11.Type of diabetic: type 1 Type 2

12. Duration of the diseases: < 5 year 5-9 year >9year:

13. Complication: Yes…….. No:

14. Treatment for DM: Metroformin:

Insulin

15. Other illnesses Yes……. No .

II.Laboratory result format

Code no_____

1. Laboratory requesting and recording format for parasitological examination

2. Laboratory data

2.1 Physical examinations

2.1.1 Consistency of the stool …….

2.1.3 If macroscopic worm is present, type of the worm_____

2.2 Microscopic examination

2.2.1 Direct microscopic examination

- No ova or parasite seen
- Types of ova parasite seen……
- Other intestinal protozoa seen ……

2.2.2 Concentration technique

- No ova or parasite seen
- Types of ova parasite seen_____

2. Hematology

CBC for RBC	RESULT	REF.RANGE	
		MALE	FEMALE
RBC	$\times 10^6$/ µl	4.1-5.6$\times 10^6$/ µl	3.8-5.1$\times 10^6$/µl
HCT	%	36-50	34-44
HGB	g/dl	12-17	12-15
MCV	fl	80-98	80-98
MCH	Pg	27-34	27-34
MCHC	g/dl	32-36	32-36
FBS	mg/dl	70-115	

3. Peripheral blood morphology.....

4. Hemoparasite on blood film _____

Annex 6. Declaration sheet

Assurance of principal investigator

The undersigned agreed to accept responsibility for the scientific ethical and technical conduct of the research thesis and for provision of required progress reports as per terms and conditions of the laboratory science and pathology department in effect at the time of grant is forwarded as the result of this application.

Name of the principal investigator: HABTOM KIFLEYESUS

Date._____ Signature _____

Approval of the first advisor

Name: Mr. LEALEM GEDEFAW

Date._____ Signature _____

Approval of the second advisor

Name: Dr. TILAHUN YEMANE

Date._____ Signature _____

Approval of the External examiner

This article has been submitted with my approval as external examiner

Name: Dr. ESAYAS KEBEDE

Date._____ Signature _____

Approval of the internal examiner

This article has been submitted with my approval as internal examiner

Name: Mr. WONDIMAGEGN ADISU

Date._____ Signature _____