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Effect of Iron Deficiency Anemia on HbA1c in Diabetic Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia: A case control study, 2016.

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This is to certify that the thesis prepared by Absra Solomon entitled:

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Abbreviation

AAU	Addis Ababa University
CBC	Complete blood count
CDC	Center of Disease Control and prevention
DCCT	Diabetes control& complications Trial
DM	Diabetes mellitus
EDTA	Ethylene diamine tetra acetic acid
EPHI	Ethiopian public health institute
GDM	Gestational diabetes mellitus
GHb	Glycated hemoglobin
HbA1c	Hemoglobin A1C
Hgb	Hemoglobin
HCT	Hematocrit
HPLC	High Performance liquid chromatography
ID	Iron deficiency
IDA	Iron deficiency anemia
IFCC	International Federation of clinical Chemistry
ISO	International standard organization
MODY	Maturity-Onset Diabetes of the Young
PNH	Paroxysmal Nocturnal Hemoglobinuria
RBC	Red blood cells

Rpm	Revolution per minute
SOP	Standard operating procedure
TASH	Tikur Anbessa Specialized Hospital
TTAB	Tetra decyltrimethyl ammonium bromide
TINIA	Turbidimetric inhibition immunoassay
USA	United State of America
WHO	World Health Organization

Abstract

Background: Hemoglobin A1C (HbA1c) is the predominant hemoglobin found in HbA1 fractions. The International Expert Committee recommends A1C assay is accurate, precise measures of chronic glycemic levels thus recommend the use of HbA1c for diagnosing diabetes. On the other hand, the committee stated any condition that changes red cell turnover, such as sickle cell anemia, hemolytic anemia, chronic malaria, major blood loss, or blood transfusions, iron deficiency anemia (IDA), renal failure will lead to spurious A1C results.

Objective: To determine the effect of IDA on HbA1c in diabetic patients in Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Method: A case-control study was conducted to assess the effect of IDA on HbA1c in diabetic patients from April to July 2016. A convenient sampling method was used and 174 diabetic patients (87 IDA and 87 without IDA) were included in the study. CBC (Complete blood count), Serum ferritin were performed by Cell dyn 1800 hematology analyzer, COBAS INTEGRA 400 plus 800 Chemistry analyzer and HbA1c by COBAS C 111 analyzer respectively for the IDA and control group. The data was entered into Excel Spread sheet and exported to Statistical Package for Social Sciences (SPSS) version 21 software systems for analysis. Pearson‟s correlation, chi-square, independent t-test was calculated. The data was presented as mean ± SD. P-value of <0.05 was taken as statistically significant.

Result: Our finding showed that the mean RBC, Hgb, HCT, MCV, MCH, MCHC were lower in IDA group compared to the control group. HbA1c (%) level were significantly lower in IDA group (6.18 ± 1.57) compared with the control group (7.74 ± 1.81) ($p<0.05$). The association between HbA1c and hematological parameters of the IDA patients showed no stastical significance.

Conclusion: HbA1c were significantly lower in IDA group compared to the control group. Therefore monitoring these patients could be misleading hence physician and health care providers should take this into account before making any therapeutic decision.

Keyword: *HbA1c, Iron deficiency anemia, diabetic mellitus*

1. Introduction

1.1 Background

About 97% of hemoglobin of adults is hemoglobin (Hgb) A ($\alpha_2\beta_2$) where Hgb A2 ($\alpha_2\delta_2$) and Hgb F ($\alpha_2\gamma_2$) comprise about 2.5 and 0.5% of the total respectively [1]. The components of Hgb A were identified by charge separation on cation exchange resin and named according to their order of elution: A_0, A1a, A1b, and A1C [2]. HbA_0 is non-glycated hemoglobin, usually synonymous with HbA, HbA1 comprises HbA1a, HbA1b, and HbA1c and total glycated hemoglobin (GHb) comprise HbA1and other hemoglobin-carbohydrate fractions [3]. Hemoglobin A1C (HbA1c) is the predominant hemoglobin found in HbA1 fractions. It constitutes 5% of the total hemoglobin in normal adults and up to 15% in patients with diabetes mellitus [4].

Glyco-hemoglobin is formed by the non-enzymatic glycation of the N-terminal valine on the β chain of Hgb in a two-step Maillard reaction. First, glucose forms a labile and readily reversible aldamine (Schiff base) with the N-terminal valine on the β chain. The aldamine then undergoes an Amadori rearrangement to form a stable ketoamine [2]. Hgb A to HbA1c conversion takes place during the entire life span of the red blood cell, so the HbA1c concentration is higher in old red cells than in new red cells. The rate of this reaction is faster in diabetics because of the higher prevailing glucose concentration, resulting in a higher concentration of HbA1c [5].

The first description of HbA1c has been variously accredited to Kunkel or Huisman, but Rahbar, in 1969, is generally credited with the recognition of HbA1c as abnormal in diabetes. He noted “unusual hemoglobin” in diabetes. Glycated hemoglobin is also known as glycol-hemoglobin, glycosylated hemoglobin or as hemoglobin A1C, HbA1c, A1C, or Hb1c [6].Red blood cells (RBC) are freely permeable to the plasma glucose molecules, and hemoglobin is practically exposed to the same glucose concentrations as plasma [7].Therefore, the HbA1c level is directly proportional to average blood glucose concentration over the previous 4 weeks to 3 months or the average lifespan of the erythrocyte[8]. Fitzgibbons *et al* study showed minor hemoglobin components Al_{a+b} and AlC increase with erythrocyte age [9].

Glycated hemoglobin provides an accurate and objective measure to access the glycaemic control and also to diagnose new Diabetes mellitus (DM) [10].On the other hand, assessing the

average plasma glucose can also be taken as a drawback because it does not give an indication of the stability of glycaemic control [11]. The higher the level of blood sugar, the more sugar attaches to hemoglobin and the higher the percent of hemoglobin which is glycosylated (HbA1c) [12].

The International Expert Committee recommends A1C assay is an accurate, precise measure of chronic glycemic levels and correlates well with the risk of diabetes complications thus recommend the use of HbA1c for diagnosing diabetes [13]. There are a number of methods available to estimate glycated hemoglobin like immunoturbidimetric, ion exchange high-performance liquid chromatography (HPLC), boronate affinity, and enzymatic method [14]. A1C level of ≥6.5% is sufficiently sensitive and specific to identify individuals who are at risk for developing retinopathy and who should be diagnosed as diabetic [15].

DM is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [16]. Diabetes can be classified into the four general categories; Type 1 diabetes, type 2 diabetes, gestational diabetes mellitus (GDM) and the specific types of diabetes due to other causes like monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis), and drug or chemical-induced diabetes [17].

HbA1c may be affected by a variety of genetic, physiological, hematological and illness-related factors [2].Falsely elevated HbA1c concentrations encountered when there is increased circulating erythrocyte life span (decreased red cell clearance) or impaired reticulocyte production. The longer the erythrocyte circulation time, the more glycated its hemoglobin becomes and this result in the formation of higher HbA1c levels. Alcoholism, iron deficiency, renal failure, and hyperbilirubinaemia and also in pre-menopausal women HbA1c level increase [8, 18, 19]. On the other hand, falsely decreased HbA1c level is seen in conditions with a reduced erythrocyte life span (increased hemoglobin turnover) or where a large number of reticulocytes are produced. This condition includes acute or chronic blood loss, sickle cell anemia, thalassaemias, glucose-6-phosphate dehydrogenase (G6PDH) deficiency, hemolytic

anemia, aplastic anemia, splenectomy and paroxysmal nocturnal hemoglobinuria (PNH) [2, 18, 20]

In addition, English *et al* conclude that abnormalities of erythrocyte indices are a considerable confounder in the analysis of HbA1c in diabetic patient. They suggested if abnormalities of erythrocyte indices or anemia are identified, consider correction of the abnormality before using HbA1c for diagnosis or monitoring [21].

Anemia is defined as a decrease in the concentration of circulating red blood cell or in the hemoglobin concentration and a concomitant impaired capacity to transport oxygen [22]. Anemia may be classified according to their physiology or their morphology. The morphological classification is based on red blood cell indices, while the physiological classification is determined based on symptoms and bone marrow response [23]. In blood cell morphology, iron-deficiency anemia (IDA) will manifest as microcytic, hypochromic (small, pale) RBC [24].

IDA is diminished red cell production due to low iron stores in the body. It is the most nutritional disorder worldwide and accounts for half of anemia. IDA can result from inadequate iron intake, decreased iron absorption, increased iron demand and increased iron loss [25]. Patients with diabetes may be more vulnerable to the effects of anemia. The definitive test for diagnosis of IDA is bone marrow aspiration however the procedure is invasive, difficult and expensive. Thus, serum ferritin is found to be the best test for distinguishing those with IDA from those who are not iron deficient. According to WHO criteria, IDA was defined as Hgb< 120 g/l for non-pregnant women (above 15 years of age) and < 130 g/l for men (above 15 years of age), mean cell volume (MCV) < 80 fl, mean cell hemoglobin (MCH) < 27 pg, mean corpuscular hemoglobin concentration (MCHC) < 32% and serum ferritin level < 15 ng/ml was defined as IDA [26].

Since there is no study conducted in Ethiopia on the effect of IDA on HbA1c in diabetic patients, this study focused on the effect of IDA on HbA1c in diabetic patients and also investigates IDA in diabetic patients at Tikur Anbessa Specialized Hospital (TASH). Even though, HbA1c is the most accurate, precise diagnostic tool for diabetic patients there are different factors like IDA which can give spuriously result of HbA1c.

1.2 Statement of the problem

Anemia is one of the most common and widespread disorders in the world, is a public health problem in industrialized and non- industrialized countries. According to McLean *et al* survey which includes 48.8% of global population, the global estimate of prevalence of anemia is 24.8%, affecting mainly preschool-age children and women making it global health problem [22].

Globally, anemia affects 1.62 billion people which correspond to 24.8% of the population [27].IDA in the general population is a common cause of anemia and is prevalent in patients with DM [28].

In India, Makadiya R *et al* studied to determine the prevalence of anemia in type 2 DM and to estimate the HbA1c in type 2 diabetic patient. A total of 100 patients, 44 patients are anemic while from the study females were more prone to anemia than males. HbA1c >6.5% it was taken for diabetics, total of 56 out of 100 are diabetic and among the diabetics 37 had anemia [29].

Furthermore, in Trinidad and Tobago, studies showed that there is a high prevalence of DM. Anemia was more prevalent among diabetic which out of 155 diabetic patients 47.8 female and 45.9 male type 2 diabetic patients had anemia even though the study didn‟t determine the type of anemia, the common one is IDA. Again, the result also revealed diabetic patients with anemia had significantly higher level of glycated hemoglobin 7.7 ± 0.3 in anemic type 2 diabetic and 7.6 ± 0.2 in non-anemic type 2 diabetics [30].

A cross-sectional survey in Iran was done on a total of 1,962 patients with type 2 diabetic in 2011. About 19 .6% of the patients had anemia and conclude that anemia is a highly prevalent finding in Iranian type 2 diabetic patients [31].

Anemia is unrecognized and largely untreated in patients with diabetic in Ethiopia. From a recent study in west Gojam, the distribution of diabetic was 50.3% type 1 and 49.7% type 2 diabetic and out of this 21.9% and 78.1% of anemic DM patients were type 1 and type 2 respectively. Therefore this study implies that anemia is public health problem in DM patients [32].There is no study conducted on the effect of IDA on HbA1c in diabetic patients in Ethiopia. Therefore this study will be helpful in determining the association of IDA with HbA1c and also prevalence of IDA in diabetic patients.

1.3 Significances of the study

Assessing the effect of IDA on HbA1c in diabetic patients has a relevant significance from different perspectives. HbA1c is affected by ethnicity/race [33] and most studies had inconsistent result regarding the concentration of HbA1c in iron deficient diabetic patients which could affect the interpretation of the result and misleads the clinician. Misinterpretation of the concentration of HbA1c in diabetic patients could affect in monitoring the diabetic patients. Therefore, this result will help the patient and also clinician or other health care provider to consider IDA before making any diagnostic or therapeutic decision.

This study is aimed to show the effect of IDA and HbA1c in diabetic patients. Additionally this study will serve as a starting point for further study since there is no published paper available in Ethiopia.

2. Literature review

Even though HbA1c is recommended diagnostic option for diabetic patients, most studies conflicts with the recommendation based on the result findings. The International Expert Committee stated the common factors that affect HbA1c thus, any condition that changes red cell turnover, such as hemolytic anemia, chronic malaria, major blood loss, or blood transfusions, will lead to spurious A1C results [34].

In Mexico, a study conducted by Francisco *et al* to analyze the advantage and disadvantage of using HbA1c as a diagnostic method for diabetes, in 2010. They concluded that it is insufficient to recommend it as the method of choice for diagnosis and the major arguments was lack of a universal threshold for the diagnosis of diabetes, the cost of the test, abnormal hemoglobin, anemia and iron deficiency and renal failure [35].

In USA, Bleyer *et al* conducted a retrospective study in 2010 on the effect of sickle cell trait on HbA1c and showed sickle cell trait did not significantly affect the HbA1c serum glucose concentration [36] in controversy, Gallagher *et al* state that hemoglobinopathies can influence A1C concentration [2].

Likewise, anemia may either increase or reduce the A1C values due to changes in the half-life of RBC [37].Thus, numerous studies have been done to show the effect of iron deficiency anemia and HbA1c, some of related literatures are reviewed below.

A Cross-sectional study by Ford *et al* in 2011 in United State (US) examined the effect of iron and Hgb status on HbA1c. HbA1c concentration is higher in the presence of iron deficiency since a change in erythrocyte turnover may alter the test result [38].

In contrast, in Brazil Cavagnolli *et al* had done a systematic review with meta-analyses on 4 studies about IDA and/or ID in 2015. The study revealed inconclusive result compared to the available studies on the effects of IDA and/or ID on HbA1c so they recommend further studies to clarify the glycation mechanisms in individuals with IDA and/or ID with and without diabetes [37].

In 2015, Silva *et al* carried out a control-case study in Brazil to investigate the effect of iron deficiency anemia on HbA1c levels in non-diabetic individuals. HbA1c results were higher in

patients with moderate and severe anemia unlike mild anemia thus concludes that IDA affects HbA1c results and the effect is dependent on anemia degree [39].

In 2015, a systematic review has been done in United Kingdom by English *et al* on 12 article that focused on IDA and that increase HbA1c with presence of iron deficiency with or without anemia. The study deduces HbA1c is likely to be affected by ID and IDA with a spurious increase in HbA1c values which may lead to confusion when diagnosing diabetes using HbA1c [19].

In United Kingdom in 1980, Brooks *et al* study revealed HbA1c concentration increase in iron deficient patients and reduced after oral iron treatment from 9.9 ±0.3% to 8.1±0.2%. Thus, HbA1c may be a misleading for diabetic with IDA. As Brooks *et al* suggestion in IDA, the quaternary structure of the Hgb molecule was altered due to absence of iron and thus the glycation occurred more readily in the globin chain[40].According to Sluiter *et al* explanation from Netherlands in 1980 for the increment of HbA1c is that if iron deficiency has been worsening in previous months, the red cell production rate will have fallen, leading to anemia and also higher than normal average age of circulating erythrocytes consequently the HbA1c increase. Therefore, as long as the average age of the red cells is not constant, HbA1c concentration will be affected [41].

Likewise, in 1990, Gram-Hansen *et al* conducted a study to determine the effect of HbA1c in IDA and vitamin B12 deficient patients. Unlike Brook‟s *et al*, the finding reported that no significant difference in both patients but after treatment HbA1c level decrease significantly. The explanation for this was glycosylated hemoglobin is a sensitive marker of the changes in the erythrocyte population that are observed when predominantly immature erythrocytes are being produced [42].

Beside, Shekha *et al* conducted a study to determine whether the HbA1c levels were increased among the anemic patients without diabetes. The finding suggests the mean HbA1c in IDA was higher 9.5 ± 1.8% compared to the control subjects 5.5% ± 0.8. Thus, IDA could cause problems in the diagnosis of uncontrolled diabetes mellitus so iron status must be considered during the interpretation of the HbA1c concentrations in DM [43].

In 2015, Sucu *et al* in Turkey reported that higher hemoglobin A1C levels in IDA were found 5,53 %(±0,2) when compared to the control groups 5,36 %(±0,2) thus in interpreting hemoglobin A1C levels in diabetes mellitus diagnosis and follow-up, presence of iron deficiency anemia and the other sources of error should be considered [44].

In Turkey, in 1999 Tarim *et al* carried out a study to investigate the effect of iron deficiency on HbA1c in diabetic patients. The finding revealed that iron deficiency anemia is associated with higher concentrations of HbA1c among type 1 DM patients and after iron treatment the concentration decreased [45].

In India, a cross sectional study conducted in 2014 by Sudhakar *et al* to look the correlation between serum ferritin, free iron concentrations with glycaemic control. There were significant positive correlation between serum ferritin and free iron concentration with HbA1c [46].

Similarly, a recent study in 2015 in India, Chhabra *et al* assess to determine the effect of IDA on the HbA1c levels in non-diabetic patients. IDA patients were selected by based on their hemoglobin levels (Hgb<11 g/dl), and on their peripheral blood smears (microcytic hypochromic). The result showed the mean HbA1c (8.9 ± 1.8%) level in the patients with IDA was higher than that in the control group (5.4 ± 0.8%) which were significantly increased among the IDA [47].

A cross sectional study was carried out by Sasekala *et al* in India in 2014 to evaluate the HbA1c levels among non-GDM mothers with IDA. Like majority of the studies, the finding revealed an increased HbA1c levels among mothers with IDA compared to the mothers without anemia. Thus the study proposed that in iron deficient pregnant women the deficient iron status plays a role in elevating the HbA1c level so the elevated levels may alter the interpretation of the glycemic status in individual‟s particularly pregnant women [48].

Another investigation was conducted in India in 2014 by Kalasker *et al* to study the effects of iron deficiency anemia on HbA1c levels in non-diabetic adults. In contrast to most studies, HbA1c concentration tended to be lower in the presence of iron deficiency anemia. Thus the study suggests that iron deficiency anemia is unlikely to be a major concern in diagnosing diabetes using concentration of HbA1c [49].

In 2010, Hashimoto *et.al* did longitudinal study in Japan to assess if HbA1c increase in pregnant women with diabetes. It revealed an increase in A1C levels occur in late pregnancy with diabetes as well as in non-diabetic because of iron deficiency [50].

A cross-sectional study conducted by Kim *et al* in Korea in 2011 to assess whether glycated albumin (GA) or HbA1c is the best diagnostic tools for DM with anemia. HbA1c was misjudging in diabetic patients with anemia than in those without anemia, which indicate GA may be a possible alternative to HbA1c in diabetic patients with anemia however, HbA1c is for long-term diabetic control [8].

In 2015, a study done in China suggests that HbA1c should only be used for glycemic assessment in the absence of ID and/or anemia. So while interpreting glycaemia status in patients with ID and/or anemia parallel measurement of iron, hemoglobin and HbA1c is critical [51].

3. Objectives

3.1 General objective

- To determine the effect of IDA on HbA1c in diabetic patients at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia.

3.2 Specific objectives

- To assess IDA in diabetic patients
- To evaluate the association of IDA and HbA1C in diabetic patients

4. Hypothesis

- There is no statistically significant mean difference of hematological parameters and HbA1c between case and control.

5. Material and method

5.1 Study area

Tikur anbessa specialized hospital (TASH) or Black lion hospital is Ethiopia"s largest specialized and referral public hospital. In 1998, the hospital was given to Addis Ababa University by the ministry of health for the faculty as a main teaching hospital. The TASH has 200 doctors, 130 specialists, 50 non-teaching doctors, 379 nurses and 115 other health professionals dedicated to providing health care service. The hospital offers diagnosis and treatment for approximately 370,000-400,000 patients a year. As Black lion hospital aspires to become a center of excellence in diagnosis, treatment and care of patients with cancer. The hospital receives patients who are referred from across the country as well as from Addis Ababa [52].

There are different units and clinics that provide specialized service for patients. Among the clinics is the diabetes center which was inaugurated by Prof Dr. Giuseppe "pino" Grimaldi, president of the international association of lions club, on Saturday 12th November 1994. The endocrine and metabolism unit sees around 1300-1500 patients per month and this unit compromise the DM clinic, endocrine clinic, foot clinic and GDM clinic. Around 70-90 diabetic patients get service on the DM clinic day which is every Monday and Wednesday. The DM clinic screen for retinopathy, foot ulcer and also provide laser therapy for those with diabetic complications. In approximate the clinic sees around 690 diabetic patients per month and out of this 517 are type 2 diabetic and 173 are type 1 diabetic patients.

The reason for selecting TASH is that there is diabetes center only for diabetic patients, so the study participant are available at one place thus it will minimize time and cost. Secondly, the hospital has different types of automated hematology analyzers both with 3- and 5-part differential counts.

5.2 Study period

The study was conducted from October 2015 to October 2016, the data collection was from April 2016 to July 2016 at TASH.

5.3 Study design

A case-control study was conducted to assess the effect of iron deficiency anemia on HbA1c in diabetic patients at TASH, Addis Ababa, Ethiopia.

5.4. Population

5.4.1 Source population

The source population was all diabetic patients who come to TASH from April to July, 2016.

5.4.2 Study population

All diabetic patients with IDA with a baseline laboratory data attaining at TASH diabetic clinic from April to July 2016 were the study population.

5.4.3 Control

Diabetic patients without iron deficiency anemia with baseline laboratory data were taken as a control.

5.5 Inclusion and exclusion criteria

5.5.1 Inclusion criteria

- Adults aged above 18 with type 1 and type 2 DM were included
- Patients who are iron deficient and iron sufficient with DM with a baseline laboratory data were included
- Those who are voluntary to participate were included.

5.5.2 Exclusion criteria

- Pregnant women with DM are excluded
- Any history of renal failure, blood loss, who donates blood in the previous 3 months, recurrent malaria.
- Diabetic patients on iron treatment and diagnose with other form of anemia
- Febrile patients and those who are on antibiotic medication in the previous one week.

5.6 Study variable

5.6.1 Dependent variable

- Concentration of HbA1c

5.6.2 Independent variable

- Sex
- Age
- Hematological parameters(RBC, Hgb, MCV, MCH,MCHC,RDW)

5.7 Measurement and data collection

5.7.1 Sampling method

Non probable convenient sampling method was used to enroll study participants. All diabetic patients with and without IDA with a base line laboratory data that came to Tikur Anbessa Specialized Hospital during the study period and fulfill the inclusion criteria and volunteered to participate were included.

5.7.2 Sample size determination

For determination of sample size case-control sample size calculation were used

$$\text{sample size} = \frac{r+1\ (p*)(1-p*)\left(Z\beta+\frac{Z\alpha}{2}\right)2}{r\qquad\qquad(P1-P2)2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 53$$

r= ratio of control to cases, 1 for equal number of case and control

P*= average proportion exposed=proportion of exposed cases + proportion of control exposed/2

Z_β= standard normal variate for power 80% power= 0.08

$Z_{\alpha/2}$= standard normal variate at 5% type 1 error ($p<0.05$) is 1.96

P_1-P_2= different in proportion expected based on previous studies. P_1 is proportion in cases and P_2 is proportion in control.

Therefore, r=1

Cases (P_1) = DM with anemia=19%=0.19…….32

Control (P_2) =prevalence of DM=5.1%=0.051……..54

P*= $\underline{0.19 + 0.051}$= 0.12
2

$(P1-P2)^2= (0.19-0.052)^2= 0.019$

Sample size= $2/1\ \underline{(0.12)\ (1-0.12)\ (0.84+1.96)^2}$
0.019

=$0.262\ \underline{(0.87)\ (7.84)}$ = 87.14
0.019

= ~87

Thus, a total of 174 participants where 87 for case and 87 for control were enrolled.

5.7.3 Data collection procedure

For laboratory analysis, five (5) ml venous blood was collected by sterile syringe in ethylene diamine tetra acetic acid (EDTA) anti-coagulant for complete blood count (CBC) and HbA1c then mixed properly and in serum separator tube for serum ferritin determination. CBC was performed using Cell dyn 1800 hematology analyzer and HbA1c by COBAS C 111 analyzer. The whole blood in serum separator tube allowed to clot 15-30 minutes then centrifuge at 3000 revolution per minute (rpm) then serum were extracted. Serum ferritin was determined using COBAS INTEGRA 400 plus COBAS INTEGRA 800 Chemistry analyzer.

Physical examination, CBC and serum ferritin was done for all the iron deficient patients to differentiate from the control group and on the basis of hemoglobin levels, IDA patients were categorized as having mild, moderate, or severe anemia: mild anemia (male, 12-12.9 g/dL and female, 11- 11.9 g/dL), moderate anemia (male, 9-11.9 g/dL and female, 8-10.9); and severe anemia (male, less than 9 g/dL and female, less than 8 g/dL) [49]. The serum ferritin was taken for the iron deficient male < 30 ng/ml and for the female <13 ng/ml. Then the collected blood was transported from TASH to Ethiopian public health institute (EPHI) clinical chemistry laboratory according to the standard operating procedure (SOP). The socio demographic information, the patient‟s history and the result of CBC, HbA1c and serum ferritin was recorded on the checklist.

5.7.4 Hematological analysis

The collected sample was examined for CBC, serum ferritin and HbA1c by using different hematological and chemistry analyzers.

5.7.4.1 Principle of CBC by CELL-DYN 1800

The CELL-DYN 1800 uses two independent measurement methods: the electrical impedance method for determining WBC, RBC and platelet (PLT) data and modified methemoglobin method for determining HGB. Electrical impedance is based on the measurement of changes in electrical resistance produced by a particle suspended in conductive diluents as it passes through the aperture.

The CELL-DYN 1800 uses electronic sizing to determine three distinct WBC subpopulations. Cells correlating to lymphocyte are included in the small cell subpopulation and cells correlating to granulocytes (neutrophils) are included in the large cell subpopulation. The remaining cells

correlating to monocytes, basophils, eosinophil, blasts and other precursor white cell are generally included in the mid-size population.

After the WBC have been counted and sized, the remainder of the lysed dilution is transferred to the HGB flow cell assembly. The electrical impedance is used to count RBC and PLT as well as they pass through the aperture.

Mean cell volume (MCV) and red cell distribution width (RDW) is determined from the RBC size distribution data and hematocrit (HCT) results are calculated from the RBC count and MCV value. Mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) values are calculated automatically whenever appropriately parameters are measured like RBC, HCT and HGB.

5.7.4.2 Principle of Ferritin

The ferritin assay is based on the immunological agglutination principle with enhancement of the reaction by latex. Human ferritin agglutinates with latex particles coated with anti-ferritin antibodies. The precipitate is determined turbidimetrically at 552 nm on the COBAS INTEGRA 4OO plus/800 analyzers with COBAS INTEGRA. The result is expressed as µg/L or ng/ml.

5.7.4.3 Principle of HbA1c

The anti-coagulated whole blood specimen is hemolyzed automatically on the COBAS C 111 Hemolyzing Reagent Gen 2. This method uses tetra decyl trimethyl ammonium bromide (TTAB) as a detergent in the hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes). Sample pretreatment to remove labile HbA1c is not necessary. All hemoglobin variants which are glycated at the β‑chain N‑terminus and which have antibody-recognizable regions identical to that of HbA1c are measured by this assay.

The HbA1c determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood. HbA1c in the sample reacts with anti- HbA1c antibody to form soluble antigen-antibody complexes. Since the specific HbA1c antibody site is present only once on the HbA1c molecule, complex formation does not take place. The polyhaptens react with excess anti- HbA1c antibodies to form an insoluble antibody-polyhapten complex which can be determined turbidmetrically. Liberated hemoglobin in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum which is measured bichromatically during

the pre-incubation phase (sample + anti- HbA1c antibody) of the above immunological reaction. A separate Hgb reagent is consequently not necessary. The final result is expressed as mmol/mol HbA1c (International Federation of clinical Chemistry IFCC) or % HbA1c (Diabetes Control and Complication Trial DCCT).

5.8 Data quality control

All the hematological analysis was conducted in TASH emergency laboratory and HbA1c was done in Ethiopian public health institute clinical chemistry laboratory.

Pre-analytical

The exclusion criteria were checked to assure the right study participant is included and then the checklist was filled for those who are willing to participate. Blood was collected from those who are willing to participate and labeled by a code and the drawn sample was transported from TASH to EPHI clinical chemistry laboratory, where the test was run, following the SOP.

Analytical

As internal quality control, before running the samples daily start up procedure, daily calibration was performed. And also commercial controls were used according to the manufacture‟s recommendations. All reagents lot‟s number and expire date was checked. The samples were collected, labeled and transported properly and when they were analyzed according to SOP. All the tests were performed under the supervision of senior laboratory personnel.

Post-analytical

The checklist was completed after all the results are combined and the results of the patients were recorded in caution to avoid patient misidentification.

5.9 Data analysis and interpretation

All collected data checked manually and entered into Excel spread sheet and then exported to Statistical Package for Social Sciences (SPSS) version 21 software systems for analysis. Frequency and summary statistics like mean, standard deviation and percentage were used to describe the distribution of age, sex among the IDA and the control group. Pearson‟s correlation was used to determine the association between hematological parameters and HbA1c and chi-square test was used for categorical data. Independent t-test was calculated for comparison of the

hematological parameters and HbA1c mean between the IDA and control groups. The result were presented in the form of tables, figures and texts and the results were presented as mean ± SD. P-value of <0.05 was taken as statistical significant.

5.10 Operational definition

Iron deficiency anemia: a decreased red cell production due to low iron stores in the body

Good control: is when the HbA1c is above < 6.5%

Bad control: is when the HbA1c is above ≥ 6.5%

5.11 Ethical consideration

After the study proposal reviewed by department of research and ethical review committee (DRERC), Addis Ababa university, department of medical laboratory science, a letter asking approval of this research project sent to department of internal medicine endocrinology and metabolism unit (diabetic center), School of Medicine at Tikur Anbessa Specialized Hospital College of Health Sciences, Addis Ababa, Ethiopia. Then support letter was sent to EPHI clinical chemistry laboratory for permission to perform the tests. Informed consent was obtained from the study population after explaining the objective and benefit of the study and those who refuse to participate were not forced. The collected 5 ml blood was used only for CBC, serum ferritin and HbA1c and the result of each participant was confidential by using codes and the used for this study only.

6. Result

6.1 Socio demographic status of study participant

A total of 174 diabetic patients (87 cases and 87 controls) participated in the study to determine the effect of IDA and HbA1c in diabetic patients. From the total number of 174 diabetic patient, 89(51.1%) were male and 85 (48.9%) were female and the mean age was 47.5 ± 15.83. A total of 87 iron deficient diabetic patients involved in the study where 53 (60.9%) were male and 34 (39.1%) were female. For the control group a total of 87 non-iron deficient diabetic patients involved where 51 (58.6%) were female and 36 (41.4%) were male. The mean age group was 46.06±16.2 and 49.01±15.3 for the case and control respectively (table 1).

Table 1: Distributions of Sex, Age group among IDA and control group at TASH from April to July 2016.

Variable		**IDA(n=87) No (%)**	**Control(n=87) No (%)**	**Total**
Sex	M	53 (60.9)	36(41.4)	89
	F	34(39.1)	51(58.6)	85
Age group	18-27	12(13.8)	9(10.3)	21
	28-37	21(24.2)	11(12.7)	32
	38-47	13(14.9)	17(19.5)	30
	48-57	11(12.7)	21(24.2)	32
	58-67	20(22.9)	19(21.8)	39
	≥68	10(11.5)	10(11.5)	20
Total		87	87	174

6.2 Comparison of HbA1c and hematological parameters among IDA and control group

All hematological parameters, serum ferritin and HbA1c were done for both IDA and the control groups. Mean ± SD was calculated for both group and Independent t-test was used to compare the mean of RBC, Hgb, HCT, MCV, MCH, MCHC, HbA1c among IDA and the control group.

The mean RBC, Hgb, HCT, MCV, MCH, MCHC, HbA1c were significantly lower in IDA group compared to the control group (P<0.05) (table 2).

Table 2: Independent t test for various parameters among the IDA and control group at TASH from April to July, 2016

Parameters	IDA(N=87)	Control(N=87)	t-test for equality of Means		
			P value	95% Confidence Interval of the Difference	
				Lower	Upper
RBC, 10^6/ µl	3.45 ± 0.80	4.91 ± 0.39	.000	-1.65032	-1.26945
Hgb, g/dl	9.97 ± 2.04	15.17 ± 1.21	.000	-5.7010	-4.6944
HCT, %	30.43 ± 6.37	45.29 ± 3.53	.000	-16.4000	-13.3126
MCV, fl	88.5 ±8.56	92.12 ± 3.76	.001	-5.5368	-1.5759
MCH, pg	29.89 ± 4.04	30.93 ± 1.62	.027	-1.9672	-.1224
MCHC, g/dl	32.97 ± 2.19	33.57± 0.99	.022	-1.1073	-.0881
RDW, %	19.08 ± 4.24	14.42 ± 1.66	.000	3.7013	5.6297
HbA1c,%	6.18 ±1.57	7.74 ±1.81	.000	-2.05946	-1.04169

6.3 Association between hematological parameters and HbA1c

The mean RBC, MCV, MCH, MCHC, RDW, Hgb were calculated and 3.45±0.8, 88.57±8.56, 29.89±4.04, 32.97±2.19, 19.08±4 and 24, 9.97±2.04 respectively. Pearson correlation test was used to determine the association between hematological parameters and HbA1c of the IDA patients. HbA1c has no significant association with RBC, MCV, MCH, MCHC, RDW, Hgb (r= -0.10; r= -0.28; r= -0.08; r= -0.73; r= -0.10; r=-0.18; p> 0.05 respectively).

6.4 Severity of anemia in IDA diabetic patients

Frequency was calculated to determine the degree of anemia among the 87 IDA diabetic patients. Thus, mild anemia were seen in 25 (28.7%) patients, moderate anemia in 40 (46%) patients and severe anemia was seen in 22 (25.3%) of the patient (fig 1).

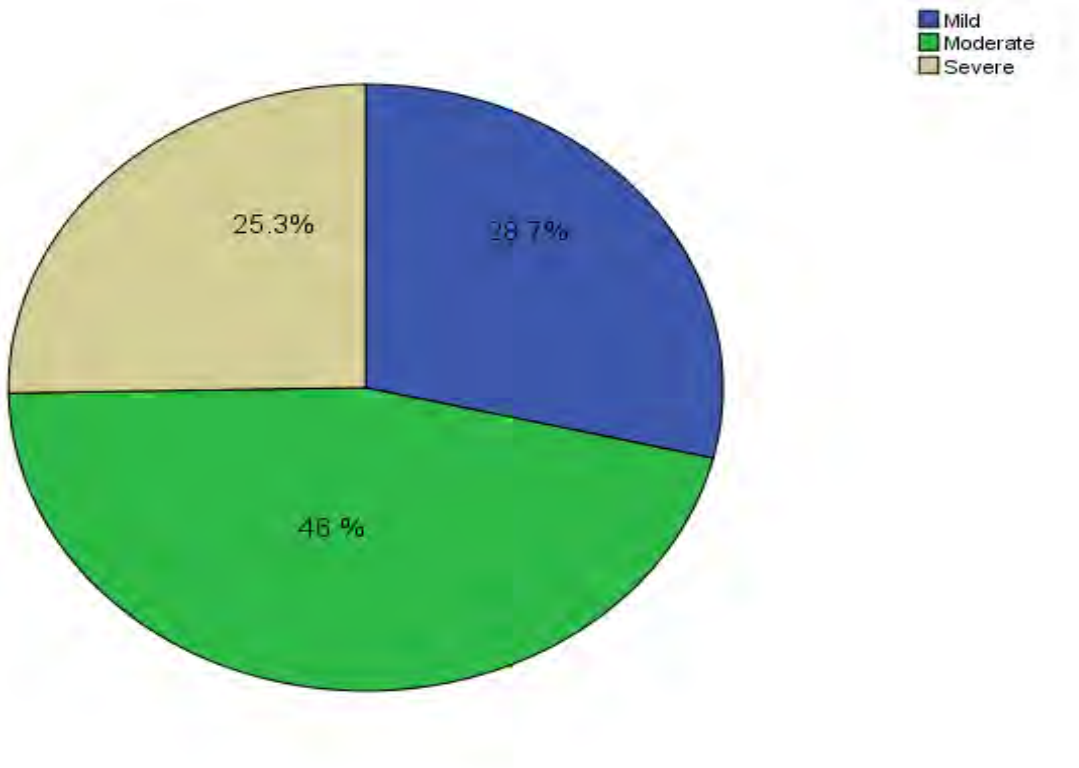

Fig 1: Pie chart of frequency of severity of anemia among the IDA diabetic patient at TASH from April to July 2016.

6.5 Association of elevated HbA1c with sex in the IDA group

Based on their HbA1c diabetic patients were divided into two groups, good control was HbA1c < 6.5 and poor control was HbA1c ≥ 6.5 [15]. Frequency was done to determine the distribution of HbA1c among sex and from 34 IDA female participants 23 (39%) had HbA1c < 6.5 and 36 (61%) from the male IDA participant. Odds ratio was calculated to describe the odds of having HbA1c ≥ 6.5 in IDA diabetic patients. Thus, the odds ratio of HbA1c ≥ 6.5 in IDA male and female participant were not significant [1.005 (95% CI 0.70-1.44], [0.99 (95%CI 0.56-1.73] respectively.

Table 3: Odds ratio of elevated HbA1c among IDA group at TASH from April to July, 2016

HbA1c	Female		Male	
	Odds ratio	95% CI	Odds ratio	95% CI
≥ 6.5	**0.99**	**0.56-1.73**	**1.005**	**0.70-1.44**

6.6 Association of HbA1c with sex and age in IDA and control groups

As shown in table 4, in IDA group the mean HbA1c were 6.18 ± 1.55 for female and 6.19 ± 1.60 for male. The student t-test shows there is no mean difference between sex and HbA1c as well as between age and HbA1c. The association of sex and HbA1c were not found to be statistically significant (p=0.97) similarly the association of age and HbA1c was not statistically significant (p=0.8).

Table 4: Association of HbA1c with sex and age in IDA group at TASH from April to July, 2016

Parameters		HbA1c	
Age		46.06±16.2	
Sex	Female	6.18 ± 1.55	
	Male	6.19 ± 1.60	
	t-test for equality of Means		
	P value	95% Confidence Interval of the Difference	
		Lower	Upper
Age	0.8	-6.240	-8.07
Sex	0.97	-0.684	0.702

7. Discussion

HbA1c is on its way to celebrate 50 years of existence and is being considered as one of the best achievements in the history of DM. HbA1c has emerged as a marker of glycemic control, glycemic risk and predictor of diabetic complication and as screening tool for diagnosis of DM [16]. Anemia may either increase or reduce the A1C values due to changes in the half-life of RBC [37]. Numerous studies have been conducted on the effect of iron deficiency anemia on HbA1c in diabetic patient or non-diabetic patients and different results have been generated, but there is no clear explanation on mechanism how iron-deficiency affects HbA1c. Likewise, HbA1c is affected by ethnicity/race [33]. This study was conducted to assess the effect of iron deficiency anemia and HbA1c in diabetic patient at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

The present study revealed HbA1c (%) were significantly lower in IDA group (6.18 ± 1.57) compared to the control group (7.74 ± 1.81) ($p< 0.05$). This is supported by studies done by Sinha *et al* in 2012 [7], Kalasker *et al* in 2014 [49] and Cavagnolli *et al* in 2015 [37] stated HbA1c concentration tends to be lower in the presence of iron deficiency anemia. According to Sinha *et al* suggestion, the reason for lower HbA1c was due to the severity of anemia in the study participants. This could be, in iron deficiency red cell production decreases thus microcytic hypochromic cell will be release to the circulation [23]. This microcytic hypochromic cell have a short life span compared to the normal cell in the circulation so, glycated hemoglobin will decrease in iron deficient patients.

In contrary, Ford *et al* study in 2011 [38], Shekhae *et al* in 2014 [43], Silva *et al* study in 2015 [39], Chhabra *et al* study in 2015 [47] all reveled HbA1c level in the patients with IDA was higher than the control groups. Moreover, the earliest study of Brooks *et al* stated that HbA1c concentration increase in IDA patients and they suggest in IDA, the quaternary structure of the Hgb molecule was altered due to the absence of iron and thus the glycation occurred more readily in the globin chain [40]. In Turkey, a study by Tarim *et al* [45] on type 1 DM patients as well as Christy *et al* [55] study on IDA diabetic patients finding reveled there is a positive correlation between IDA and increased HbA1c which contradict with the result of the present study. According to Sluiter *et al* explanation if iron deficiency has been worsening in previous months, the red cell production rate will have fallen, leading to anemia and also higher than normal average age of circulating erythrocytes consequently the HbA1c increase. Therefore, as

long as the average age of the red cells is not constant, HbA1c concentration will be affected [41].

Among the hematological parameters RBC, Hgb, MCV, MCH showed statistically significant mean difference between IDA subject and the control group. This finding is similar with the Indian study in 2016 [56**]**, RBC and Hgb shows significant difference between the IDA and the control group. Korean study finding showed [57] unlike MCH and Hgb which show significant difference, MCV showed no significant difference in IDA group which contradict this study.

Association between RBC, red cell indices and HbA1c were determined in IDA group and the result was not statistically significant. Similarly Indian study in 2014 [55] showed no significant correlation between MCV and HbA1c but a borderline significant association were found between MCH and HbA1c in IDA diabetic patient (P=0.05). In contradiction with Koga *et al* study [17] where RBC count was positively associated with HbA1c in pre menopause women which are relatively iron deficient. Correspondingly, Hardikar *et al* [58] study reported there is significant association between MCV, MCH, MCHC and HbA1c in IDA pre diabetic and diabetic patients.

The present study reported there was no significant correlation between Hgb and HbA1c in IDA group. Likewise, the other studies revealed there is no significant correlation between these parameters [55]. Sinha *et al* study [7] and Adeoye study in 2014 [59] observed significant correlation of Hgb and HbA1c in IDA patients this finding contradicts with the present study.

On the basis of hemoglobin, iron deficient groups were categorized into three groups, mild anemia, moderate anemia and severe anemia. From our finding, 25 (28.7%) of patients were mild, 40 (46%) were moderate anemia and 22 (25.3%) of the patient were severe anemia. Severe anemia was seen in 38 (76%) patients, and moderate anemia, in 12 (24%) patients and no cases of mild anemia were seen in Indian study [39]. Christy *et al* found the association of elevated A1C with severity of iron deficiency anemia remains unexplained [55].

According to the American diabetic federation, HbA1c level ≥6.5% is sufficiently sensitive and specific to identify individuals who are at risk for developing retinopathy and who should be diagnosed as diabetic [15]. Odds ratio was calculated to describe the odds of having ≥6.5 HbA1c in IDA group thus both in male and female HbA1c were not statistical significance. Christy *et al*

[55] revealed similar finding with the present study which they reported odds ratios for HbA1c above 6.5 in females were statistically non-significant [0.690 (95% CI 0.368-1.294). Moreover, Kim *et al* study [60] reported the odds of having HbA1c ≥6.5 in iron-deficient women was not statistically significant but the male odds ratio were not calculated because of the small number of participant having HbA1c ≥6.5%.

The present study revealed no significant association between Sex, Age and HbA1c in IDA diabetic patients. Similarly, Hardikar study revealed there was no association between age, sex and HbA1c in IDA diabetic patients [58]. Indian study in 2014 also stated age did not show any significant correlation with HbA1c [55]

Even though, most of the studies contradict with our finding, this study depicts patients with IDA have significantly lower HbA1c compared to non-IDA diabetic patients. Therefore monitoring these patients using HbA1c could be misleading because their actual HbA1c level could be lower than the actual value hence physician and health care providers should take this into account before making any therapeutic decision. However, this is the first study done here in Ethiopia future studies must be conducted to explain exact mechanism on how hemoglobin is glycated in IDA patients as well as in diabetic patients. As a base, the present study performed various hematological parameters and HbA1c for the diabetic patients but there are different factors like ethnicity/race, type of the diabetic and the treatment which was not considered could affect the result, thus future study is needed.

8. Strength and limitation of the study

8.1 Strength

- Different tests CBC and HbA1c have been performed for each of the study participant
- It is the first to provide the scientific finding on the effect of IDA and HbA1c in Ethiopia and provide as a base or starting point for future research to be conducted

8.2 Limitation

- Peripheral morphology, plasma blood glucose were not incorporated
- Follow up and iron supplement were not given for the iron deficient diabetic patients.

9. Conclusion

- The mean RBC, Hgb, HCT, MCV, MCH, MCHC, HbA1c were significantly lower in IDA group compared to the control group.
- HbA1c were significantly lower in IDA group compared to the control group. This could be in iron deficiency red cell production decreases thus microcytic hypochromic cell will be released into the circulation. This cells microcytic hypochromic cell have a short life span compared to the normal cell in the circulation so that the glycation time with glucose will decrease therefore, glycated hemoglobin will decrease in iron deficient patients.
- There was no statistically significant association between HbA1c and RBC, red cell indices in the IDA group. Similarly, there was no significant association found between Hgb and HbA1c in IDA group.
- Odds ratio of having ≥6.5 HbA1c in IDA both male and female were not statistical significance.

10. Recommendation

- A cohort study must be undertaken with a large number of study participants to clarify the relationship between IDA and HbA1c.
- Since this is the first study done here in Ethiopia and future studies must be conducted to explain exact mechanism on how hemoglobin is glycated in IDA patients as well as in diabetic patients.
- Physician and health care provider should consider treating the iron deficiency anemia before diagnosing the diabetic using HbA1c.

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12. Annexes

Annex I: Procedure and reagents of cell dyn 1800

Procedure of cell dyn 1800

Standard precautions should be followed when handling specimens and performing all laboratory testing.

Manual Mode

1. 30 uL of whole blood aspirated from open collection tube.
2. Enter the specimen number using the keyboard or the handheld barcode wand.
3. Click [enter].
4. Mix the patient sample. Uncap the tube.
5. Place sample under the sample aspiration probe and transfers the sample to the pre-mixing cup.
6. After sample aspiration 7.5 ml volume of diluents is added to the pre-mixing cup to achieve a dilution ratio of 1:251.
7. The diluted sample is divided into two samples
8. 100 uL of the 1:251 sample dilutions are aspirated and mixed with an additional 5 ml of diluents in the RBC/PLT mixing chamber to create a dilution ratio of 1:12801. A specimen of the 1:12801 dilutions is analyzed to generate result for the RBC and PLT parameters.
9. The remainder of the 1:251 sample dilution is mixed with 1.0 ml of lyse reagent in the WBC mixing chamber. This dilution is used to measure the number and modified size of the WBC and amount of HGB released.

Reagents of cell dyn 1800

Diluent

- Maintain the cell volume of each RBC and PLT during the count and sizing portion of the measurement cycle.
- Provide a conductive medium for impedance counting and sizing of the cell and platelets
- And also the sample probe and flow system

CN-FREE Diff Lyse (cyanide free)

- Rapidly lyse the RBC and minimize the resultant cell stroma
- Alter the WBC membrane to allow the cytoplasm to slowly diffuse and shrink the membrane around the nucleus and any granules that may be present.
- Convert HGB to the modified hemoglobin complex that is measurable at 540 nm.

Detergent

- Provide an optically clear solution that is used to obtain the zero reference during the HGB measurement cycle.

Enzymatic cleaner

- Is used to remove protein buildup and provide detergent cleaning within the instrument.

Annex II: Procedure of serum ferritin

1. Bring the sample at room temperature
2. Mix all brand new (non‑punctured) cobas c packs for 1 minute on a cassette mixer before loading on the analyzer.
3. After cobas c packs puncture, the analyzer automatically mixes the reagent for 1 minute.
4. Bar-coded cobas c reagents pack is properly load on the analyzer. Glycine buffer (R1) and Latex particles coated with anti‑human ferritin (rabbit) (SR) in position B and in position C respectively.

- Working Reagent is ready to use

5. The requested test will be fed into the computer which is connected to the machine.
6. Bar coded serum sample will be place on the machine rack and it reads the bar code then run the test accordingly.
7. The serum ferritin result will be expressed in µmol/L

Annex III: Procedure of HbA1c

1. Bring EDTA sample at room temperature and mix.
2. Bar-coded cobas c reagents pack is properly load on the analyzer. Antibody reagent (R1) and polyhapten(SR) in position B and position C respectively.

- Working Reagent is ready to use

3. The requested test will be fed into the computer which is connected to the analyzer.
4. Bar coded EDTA sample will be place on the machine rack and it reads the bar code then run the test accordingly.
5. The EDTA sample and the antibody form a antigen-antibody reaction then the polyhapten will be added and the reaction will be started.
6. The final result will be expressed as mmol/mol HbA1c (IFCC) or % HbA1c (DCCT).

- Protocol 2 (mmol/mol HbA1c acc. to IFCC):
  HbA1c (mmol/mol) = (HbA1c/Hgb) * 1000
- Protocol 2 (% HbA1c acc. to DCCT/NGSP):
  HbA1c (%) = (HbA1c/Hgb) * 91.5 + 2.15

Annex IV: Patient information sheet (English)

Title: Effect of Iron Deficiency Anemia and HbA1c in Diabetic Patients at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia: A case control study from October 2015 to June 2016

Name of principal investigator: Absra Solomon (BSc.)

Purpose: This study is conducted by MSC student at Addis Ababa University, College of health sciences, School of Allied Health Science, Department of Medical Laboratory Sciences and you are cordially invited to participate in this study. It is aimed at determining the effect of IDA and HbA1c in diabetic patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

The purpose of this study is to determine the effect of IDA on HbA1c in diabetic patients in Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia and the final result will help the patient and also clinician or other health care provider to consider IDA before making any diagnostic or therapeutic decision. Therefore, please read the following description about the study and ask any unclear points before you agree to participate.

Duration: you will only take 10-15 minute to understand the objective of the study and sign the consent and to give the sample for the study.

Procedure to be carried out: first you will be asked few questions about previous medical records and then you will be requested to give 5 ml venous blood. After the unique code is labeled the sample will be transported to EPHI laboratory.

Risk: there will be a slight pain or discomfort while the blood is collected at the puncture site but not any other problem because well trained laboratory personnel will be responsible for the collection the sample.

Benefits: Even though there is no payment for participating in this study but the information you are giving will help you and other diabetic patients from taking or changing medications based on the HBA1C result without considering the IDA.

Confidentiality: The unique code will be given for the information you gave and the sample you provide so that your result cannot be identified. Only the principal investigated and selected

health professionals have access to your result of the sample and the result will be used for this study only.

Voluntary Participation and Withdrawal from the Study: The participation is completely voluntary and you have the right not to participate in this study. You can stop participating in the study at any time after giving your consent. If you want to participate you can give your consent by signing on the consent form. This decision will not affect in any way yours current or future medical care in the health facility.

Contact information: If you have any questions about this study you can contact Absra Solomon, Department of medical laboratory science School of allied health Sciences College of health sciences Addis Ababa University and the ethical committee for further information.

Absra Solomon: 0960277684

School of medical laboratory sciences Department (office): 0112755170

Annex V: Patient information sheet (Amharic)

ርእስ-በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል በስካር ታካሚዎች ላይ በአይረን እጥረት ምክንያት የሚመጣ የደም ማነስ ያለው ተዕእኖ በሄሞግሎብሊን ኤዋን ሲ የሚደረግ ጥናታዊ ምርምር ነው፡፡

የተመራማሪዋ ስም-አብስራ ሰለሞን

አላማ-የአዲስ አበባ ዩንቨርሲቲ የጤና ሣይንስና ኮሌጅ የአላይድ ጤና ሣይንስ ት/ቤት የህክምና ላብራቶሪ ሣይንስ ለማጥናት ታስቦ ለተሳታፎዎች የተዘጋጀ መረጃ ሲሆን እርሶም በአዲስ አበባ ዩንቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ የማስተርስ ድግሪ ተማሪ መመረቂያ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል፡፡ የጥናቱ አላማ በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል በስካር ታካሚዎች ላይ በአይረን እጥረት ምክንያት የሚመጣ የደም ማነስ በሄሞግሎብሊን ኤ ዋን ሲ ያለው ተዕእኖ ለማወቅ፡፡

የጥናቱ የመጨረሻ ውጤት የምርመራም ሆነ የህክምና አገልግሎት ከመሰጠቱ በፊት ለታካሚውም ሆነ ለጤና ባለሙያው በእጥረት ምክንያት የሚመጣ የደም ማነስ ከግምት ውስጥ እዲከቱት ከፍተኛ እርዳታ ያደርጋል፡፡ ስለሆንም እባክዎ በዚህ ጥናት ለመሳተፍ ከመስመማትዎ በፊት ከዚህ ቀጥሎ የሚገኘውን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነውን/ትን ማንኛውም ሃሳብ ይጠይቁ፡፡

የሚፈጀውጊዜ-ከ10-15 ደቂቃ በሚሆን ጊዜ ውስጥ ስለጥናቱ አላማ ተረድተው በስምምነት ማረጋገጫ ቅጽ ላይ ይፈርማሉ በመቀጠልም ለጥናቱ ናሙና በመስጠት ይጨርሳሉ፡፡

የሚከናወኑትተግባራትቅደምተከተል-መጀመሪያ ስለበፊት የህክምና መረጃዎት የተወሰነ ጥያቄ ይጠየቃሉ፣ ቀጥሎ አምስት ሚሊ ሊትር የሚሆን የደም ናሙና ከደም ስርዎት ያሰጣሉ፡፡ በመጨረሻም የሰጡት የደም ናሙና ልዩ ቁጥር ተሰቶት ወደ ላብራቶሪ ይላካል፡፡

አደጋ-ከደም ስርዎት የደም ናሙና በሚወሰድበት ጊዜ ያለመመቸት ስሜት ሊሰማዎት ይችላል፡፡ ከዚህ ሌላ ችግር ወይም አደጋ አያጋጥሞትም ምክንያቱም የላብራቶሪ ባለሙያ በሙያው የሰለጠነ፣በቂ እውቀት ያለው ስለሆነ ለስራው ሙሉ ሃላፊነት ይወስዳል፡፡

ጥቅም-በዚህ ጥናት ላይ በመሳተፎ ምንም አይነት የገንዘብ ክፈያ ባያገኙም እርሶ የሚሰጡት መረጃ ለርሶም ሆነ ለሌሎችም የስካር ታካሚዎች ከፍተኛ ጥቅም አለው ምክንያቱም

በሄሞግሎብሊን ኤዋንሲ ውጤት መሰረት በአይረን እጥረት ምክንያት የሚመጣ የደም ማነስ ከግምት ውስጥ ሳይገባ መድሃኒት ከመውሰድም ሆነ ከመለወጥ ይረዳል፡፡

ሚስጥር ስለመጠበቅ- እርስዎ የሚሰጡት መረጃና የደም ናሙና ልዩ የሆነ ቁጥር ይሰጠዋል ስለዚህ የእርስዎን ከሌሎች የጥናቱ ተሳታፊዎች መለየት አይቻልም፡፡ ውጤትዎን የማየት ፈቃድ ያላቸው ጥናቱን አድራጊና የተወሰኑ የጤና ባለሙያዎች ብቻ ናቸው፤ጥቅም ላይም ሚውልው ለዚህ ጥናታዊ ምርምር ብቻ ነው፡፡

ስለ በጎ ፈቃድ ተሳትፎ እና ራስን ከጥናቱ ስለማግለል-ይሄ ጥናታዊ ምርምር በርስዎ ሙሉ በሙሉ የበጎ ፈቃደኝነት ተሳትፎ ላይ የተመሰረተ ነው፡፡በጥናቱ ላይ ለመሳተፍ ከተስማሙ መስማማወዎትን ለማሳየት በስምምነት ማረጋገጫ ቅጽ ላይ ይፈርማሉ፡፡ በማንኛውም ጊዜ ተሳትፎዎን ማቆም ሆነ ከጥናቱ ራሳዎን ማግለል ይችላሉ፡፡ እዚህ ጥናት ላይ በመሳተፎ አሁን የሚያገኙትን ሆነ የወደፊት የጤና አገልግሎት ፤ ክትትል በምንም አይነት መልኩ ችግር አይፈጥርብዎትም፡፡

አድራሻ-ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ለሚያጋጥሙን ችግሮች ወይም ጥያቄ ካሎት በሚከተለው አድራሻ ይጠቀሙ፡፡

አብስራ ሰለሞን
የህክምና ላባራቶሪ ሳይንስ ት/ቤት
የጤና ሳይንስ ኮሌጅ
አዲስ አበባ ዩንቨርሲቲ
ሞባይል፡-251 960 27 26 84

Annex VI: Consent form (English)

Greeting! My name is Absra Solomon and I am MSC student at Addis Ababa University, College of health sciences, School of Allied Health Science, Department of Medical Laboratory Sciences. I am doing my thesis on the Effect of Iron Deficiency Anemia on HbA1c in Diabetic Patients at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia: A case control study from October 2015 to June 2016 and you are cordially invited to participate in this study. The purpose of this study is to determine the effect of IDA on HbA1c in diabetic patients in Tikur Anbessa specialized hospital and the final result will help the patient and also clinician or other health care provider to consider IDA before making any diagnostic or therapeutic decision.

You will be requested to give 5 ml venom blood and asked few questions about your previous medical records. There will be a slight pain or discomfort while the blood is collected at the puncture site but not any other problem. Unique code will be given for the information you gave and the sample you provide so that your result cannot be identified and the participation is completely voluntary and you have the right not to participate in this study.

I ……do interestingly give consent to Absra Solomon to include me in the proposed research after a clear understanding of the objectives and conditions of the study & with recognition of my right to withdraw from the study if I change my mind. The proposal has been explained to me in the language I understand.

Code of the participant: _____

Participant‟s signature: _____

Name and Signature of data collector: _____

Date: _____

I thank you for your cooperation

Please direct any questions or problems you may encounter during this study to:

Absra Solomon, Department of Medical Laboratory Sciences Addis Ababa University.

Mobile 0960277684

Annex VII: Consent form (Amharic)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

ሰላም! እኔ ስሜ አብስራ ሰለሞን በአዲስ አበባ ዩንቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ የማስተርስ ድግሪ ተማሪ ስሆን መመረቂያ ጥናት በመስራት ላይ እገኛለሁ፡፡ የጥናቱ አላማ በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል በስካር ታካሚዎች ላይ በአይረን እጥረት ምክንያት የሚመጣ የደም ማነስ በሄሞግሎብሊን ኤ ዋን ሲ ያለው ተዕእኖ ለማወቅ ሲሆን እርሶ በዚህ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል፡፡ የጥናቱ የመጨረሻ ውጤት የምርመራም ሆነ የህክምና አገልግሎት ከመሰጠቱ በፊት ለታካሚውም ሆነ ለጤና ባለሙያው በእጥረት ምክንያት የሚመጣ የደም ማነስ ከግምት ውስጥ እዲከቱት ከፍተኛ እርዳታ ያደርጋል፡፡

መጀመሪያ ስለበፊት የህክምና መረጃዎት የተወሰነ ጥያቄ ይጠየቃሉ፣ ቀጥሎ አምስት ሚሊሊትር የሚሆን የደም ናሙና ከደም ስርዎት ያሰጣሉ፡፡ ከደም ስርዎት የደም ናሙና በሚወሰድበት ጊዜ ያለመመቸት ስሜት ሊሰማዎት ይችላል፡፡ እርስዎ የሚሰጡት መረጃና የደም ናሙና ልዩ የሆነ ቁጥር ይሰጠዋል ስለዚህ የእርስዎን ከሌሎች የጥናቱ ተሳታፊዎች መለየት አይቻልም፡፡ይሄ ጥናታዊ ምርምር በርስዎ ሙሉ በሙሉ የበጎ ፈቃደኝነት ተሳትፎ ላይ የተመሰረተ ነው፡፡

እኔ አብስራ ሰለሞን እየሰራችው ባለው ጥናት ላይ ለመሳተፍ ፈቃዴን የሰጠሁት በፍላጎቱ ሲሆን ስለጥናቱ ግልፅ የሆነ እውቀትና ግንዛቤ ኖሮኝ ነው፡፡ ነገር ግን ራሴን ከጥናቱ ለማግለል የሃሳብ ለውጥ ካለኝ ጥናቱን የማቋአረጥ መብት አለኝ፡፡ ስለጥናቱ ግልፅ፣ ቀላል እና በሚገባኝ ቋንቋ ገለፃ ተደርጎልኛ ነው፡፡

የተሳታፊው ሚስጥራዊ ቁጥር……

የተሳታፊው ፊርማ……..

የመረጃ ሰብሳቢ ስምና ፊርማ ……

ቀን……..

አመሰግናለሁ ስለተባበሩን

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ለሚያጋጥሙን ችግሮች ወይም ጥያቄ ካሎት በሚከተለው አድራሻ ይጠቀሙ፡፡

አብስራ ሰለሞን ሞባይል፡-251 960 27 26 84

የህክምና ላባራቶሪ ሳይንስ ት/ቤት፤የህክምና ላባራቶሪ ሳይንስ ት/ቤት፤አዲስ አበባ ዩንቨርሲቲ

Annex VIII: Check list

Patient code	Sex	Age	Medical records							Result								
			Recurrent Malaria	Renal failure	Blood loss	Donated blood	Iron treatment	Pregnant	Febrile	Serum ferritin	HbA1c	Hgb	MCV	MCH	MCHC	HCT	RDW	RBC count

Annex IX: SOP for venous blood collection

1. Assemble the necessary materials and equipment.

Remove the syringe from its protective wrapper and the needle from the cap and assemble them allowing the cap to remain covering the needle until use. Attach the needle so that the bevel faces in the same direction as the graduation mark on the syringe.

Check to make sure the needle is sharp, the syringe moves smoothly and there is no air left in the barrel. The gauge and the length of the needle used depend on the size and depth of the vein to be punctured. The gauge number varies inversely with the diameter of the needle. The needle should not be too fine or too long; those of 19 or 21G are suitable for most adults, and 23G for children, the latter especially with a short shaft (about 15mm).

2. Identify the right patient and allow him/her to sit comfortably preferably in an armchair stretching his/her arm.

Allow it to dry in the air or use a dry pad or cotton. The area should not be touched once cleaned.

3. Apply a tourniquet at a point about 6-8cm above the bend of the elbow making a loop in such a way that a gentle tug on the protruding ends will release it.

It should be just tight enough to reduce venous blood flow in the area and enlarge the veins and make them prominent and palpable. The patient should also be instructed to grasp and open his/her fist to aid in the buildup of pressure in the area of the puncture. Alternatively, the veins can be visualized by gently tapping the antecubital fossa.

4. Prepare the arm by swabbing the ante cubital fossa with a gauze pad or cotton moistened with 70% alcohol.
5. Grasp the back of the patient‟s arm at the elbow and anchor the selected vein by drawing the skin slightly taut over the vein
6. Using the assembled syringe and needle, enter the skin first and then the vein

The index finger is placed alongside of the hub of the needle with the bevel facing up and the needle should be pointing in the same direction as the vein.

7. The plunger is drown back to create suction pressure to draw the blood
8. The tourniquet should be released the moment blood starts entering the syringe

Otherwise, some hemoconcentration will develop after one minute of venous stasis.

9. After drawing the required blood sample, apply a ball of cotton to the puncture site and gently withdraw the needle. Instruct the patient to press on the cotton
10. Transfer the blood to the appropriate test tube, either by directly puncturing the top of the EDTA tube in the center (rubber black area) or remove the tube top and gently inject the blood into the empty tube prior to replacing the cap.
    Invert 8-10 times for EDTA tube
11. Label the tubes with patient‟s name, hospital number and other information required by the hospital (before the patient leaves the collection area)
12. Re-inspect the vein puncture site to ascertain that the bleeding has stopped. Do not let the patient go until the bleeding stops.

Annex X: SOP for blood sample transportation

1. Make sure the specimen is tightly closed and the cap is not leaking.
2. Place the blood sample in a plastic container or box and then cotton or packing material around the sample to prevent moving in the container or box.
3. Pack the blood sample along with the check list or request form but separate enough to avoid contamination of paper.
4. Label the outer container „Biological specimens – Infectious substance".
5. Deliver samples as soon as possible from the time they were collected to the laboratory.
6. After the arrival of the sample to the EPHI clinical chemistry laboratory, the sample and the check list will be cross checked and the test will be run accordingly.

Annex XI: Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the PI: Absra Solomon (BSc)

Signature_____

Date of submission_____/_____/_____

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Date of submission_____/_____/_____

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