Prevalence of anemia and its associated factors among patients with type 2 diabetes mellitus in the north of Iran

Reyhane Hizomi Arani
Prevention of Metabolic Disorders Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

farima fakhri
- General Practitioner, Kerman University of Medical Science, Kerman 7616913555, Iran

Mohammad Naeimi Tabiee
Department of Internal Medicine, School of Medicine, Clinical Research Development Unit (CRDU), Sayad Shirazi Hospital, Golestan University of Medical Sciences, Gorgan, Iran

Fatemeh Talebi
Clinical Research Development Unit (CRDU), Sayad Shirazi Hospital, Golestan University of Medical Sciences, Gorgan, Iran

Zahra Talebi
Clinical Research Development Unit (CRDU), Sayad Shirazi Hospital, Golestan University of Medical Sciences, Gorgan, Iran

Negin Rashidi
Department of Medicine, Division of General Internal Medicine, Toronto General Hospital, Toronto, Canada.

Maryam Zahedi (drmaryam.zahedi@yahoo.com)
Department of Internal Medicine, School of Medicine, Clinical Research Development Unit (CRDU), Sayad Shirazi Hospital, Golestan University of Medical Sciences, Gorgan, Iran

---

Research Article

Keywords: Type 2 diabetes mellitus, Anemia, Prevalence, Iran

Posted Date: April 25th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1515543/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Endocrine Disorders on March 9th, 2023. See the published version at https://doi.org/10.1186/s12902-023-01306-5.

Abstract

Background:

Purpose: This study intended to investigate the prevalence of anemia and its associated factors among patients with type 2 diabetes mellitus (T2DM) in Gorgan, North of Iran.

Methods: This cross-sectional study was performed on 415 (109 men) patients with T2DM referred to Gorgan Diabetes Clinic in 2021. Demographic information was collected and some laboratory tests such as cell counts, serum blood glucose, creatinine, lipid and iron profiles, and urinary albumin were performed. Multivariable logistic regression analysis was applied to compute odds ratios (ORs) and 95% confidence intervals (CI) for potential associated factors, using SPSS version 21.

Results: The prevalence of anemia was about 21% among our participants. The adjusted model revealed that obesity (OR, 1.94 [95% CI, 1.17-1.23]), T2DM duration for more than five years (OR, 3.12 [CI, 1.78-5.47]), albuminuria (OR, 6.37 [CI, 3.13-10.91]), chronic kidney disease (OR, 4.30 [CI, 2.83-8.29]) and high triglycerides (OR, 1.72 [CI, 1.21-2.77]) were significantly associated with anemia among patients with T2DM. Moreover, using insulin both with (OR, 2.60 [CI, 1.42-6.42]) and without (OR, 1.87 [CI, 1.30-4.37]) oral glucose-lowering medications had a positive association with prevalence of anemia among our participants.

Conclusion: Anemia had a high prevalence among patients with T2DM in the north of Iran which is associated with obesity and hypertriglyceridemia, duration and severity of T2DM, and diabetic kidney disease.

Introduction

Anemia is a condition that the oxygen-carrying capacity of blood that could not meet the physiological needs of the body, due to decreased erythrocyte mass or hemoglobin concentration [1].

The prevalence of anemia is 27% worldwide, which is a public health problem with the greatest impact on developing countries (it accounts for more than 89 percent of the disease burden). Based on global burden disease (GBD) reports, Iran, as a developing country, has the age-standardized prevalence and years lived with disability (YLDs) of 23.0% and 677 (per 100,000), respectively [2], which was prominent among adult patients with type 2 diabetes mellitus (T2DM) (30.4%) [3].

Nearly 4.5 million cases of diabetes mellitus (DM) were detected among Iranian adults in 2011. According to estimations about 9.2 million will be affected by DM by the year 2030. This significant growth in the disease incidence reveals the high burden of DM in Iran, especially when taking into account the impact of its complications [4, 5]. It has been reported that patients with T2DM are twice more prone to anemia compared to patients without T2DM [6-8]. Anemia could increase the risk of end-stage renal and cardiovascular diseases, hospitalization, and premature death in patients with DM [9]. As

well as, it plays a role in the progression and development of macro vascular and microvascular complications of DM [10]. Hence, it could affect the patients' quality of life and their healthcare costs [11].

Many researchers have studied the potential associated factors with anemia such as diabetic nephropathy, glycemic control, gender, age, antihypertensive, and glucose-lowering drugs (GLDs) [12, 13]. Both T2DM and anemia have some similar symptoms like numbness or coldness in the extremities, pale skin, and shortness of breath. This may cause anemia to remain unrecognized in a considerable number of patients with T2DM [6]. Therefore, early diagnosis and management of anemia is an essential strategy to reduce its adverse effects on their health and quality of life.

Despite the high prevalence of anemia and its effect on T2DM complications, in Iran there are limited studies concerning its associated factors. Hence, this study aimed to estimate the prevalence of anemia and its correlations among patients with T2DM in Gorgan (southeast of Caspian Sea), north of Iran.

Materials And Methods

Study population

This clinical-based, cross-sectional study included out-patients with T2DM referred to Gorgan Diabetes Clinic in 2021. The sample size was calculated based on this formula: $N = (pq/e^2) * z_{1-/\alpha}{}^2$. Here, p was the anticipation of anemia prevalence of 19.6% in the diabetic population, according to a study by Bonakdaran et al [14]; q = 1 - p; e was an allowable error (5%); and $Z_{1-\alpha/2}$= 1.96. So, 242 participants were an acceptable sample size for this study. Patients were selected by a systematic random sampling technique among volunteer patients with T2DM.

Exclusion criteria compromise the : (1) age ≤18 or ≥75 years, (2) T2DM duration less than 1 year (3) known hematologic diseases (thalassemia, lymphoma, and leukemia) or other systemic disorders (such as infectious diseases) that could result in anemia, (4) presence of an acute condition (such as acute bleeding) or hospitalization within the last two weeks before sampling, (5) blood transfusions in the three months before sampling, (6) pregnancy, (7) type 1 DM, (8) smoking, (9) missing clinical and demographic data. Finally, 415 (109 men) eligible participants remained for analysis.

The Ethics Committee of Golestan University of Medical Sciences approved this study (ethics Code: IR.GOUMS.REC.1398.170). Verbal informed consent was obtained from all subjects, and they were also assured that their personal information would remain confidential. All methods of this study were performed following the relevant guidelines and regulations.

Measurements

A trained interviewer recorded patients' information, including demographic characteristics, duration of T2DM, and medical history. The height and weight of patients without shoes and with light clothes to the

nearest 100 grams were measured using the same device (Seca weighing scale, made in Germany), and body mass index (BMI) was calculated by dividing weight (kg) into height squared (meters).

After 8 to 12 hours of overnight fasting, a blood sample was taken from all participants to measure cell counts, iron profiles including total iron-binding capacity (TIBC), hemoglobin concentration, serum ferritin, and iron levels, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), fasting plasma glucose (FPG), glycated hemoglobin (Hb A1c). Also, urine sample was taken from the participants. All of them were measured in the same lab with the same kit, device, and methods.

FPG was measured by the colorimetric glucose oxidase method (Human, Heidelberg Germany). TC, HDL-C, and triglyceride were measured by enzymatic method using the available kit (Lipid, Pars Azmoon Co., Karaj, Iran). Also, if triglycerides were <400 mg/dL, the Friedewald formula (LDL= total cholesterol - (HDL + TG/5) was applied for calculating LDL-C, and if triglycerides were ≥400 mg/dL, LDL-C was measured by direct assay. Hb A1C was assessed by column chromatography. TIBC was measured by the chemical precipitation method. Also, ferritin was measured by the immunoassay method using a gamma counter. Urinary albumin excretion was assessed by the immunoturbidometry method of a fresh early morning sample. Serum creatinine levels were measured using kinetic colorimetric Jaffe with the sensitivity of 0.2 mg/dL (range, 0.2–15 mg/dL). Based on the recommendation of manufacturer, reference intervals were (0.9–1.3 mg/dL) in men and (0.6– 1.1 mg/dL) in women[15]. Modification of Diet in Renal Disease (MDRD) equation was employed for calculating the estimated glomerular filtration rate (e-GFR) of the participants [16].

Definition of Outcomes and Variables

Anemia was considered as hemoglobin less than 12 g/dl in women and less than 13 g/dl in men according to the World Health Organization (WHO) criteria [1]. DM was defined as FPG ≥ 126 mg/dL and/or Hb A1c≥ 6.5 or taking any GLDs (glucose lowering drugs). Obesity was categorized in two groups: BMI <30 kg/m^2 (normal/overweight) and ≥30 kg/m^2 (obese). Diabetic kidney disease (DKD) was considered as e-GFR lower than 60 mL/ min/1.73m^2 according to the kidney disease outcomes quality initiative (KDQOI) guidelines [17]. Albuminuria was considered as urinary albumin creatinine ratio (ACR) of 30 mg or more in 24 hours' urine collection.

Data analysis

Statistical analyses were performed using SPSS version 21 for Windows (Chicago, Illinois, USA). To report the quantitative variables, mean (standard deviation) and mean (inter-quarter range) were used for variables with normal and abnormal distribution, respectively. The distribution of quantitative data was measured using the Kolmogorov-Smirnov test. For comparison between the two groups, t-test for quantitative data with normal distribution and Mann-Whitney test for quantitative data with abnormal

distribution were used (**Table 1**). The prevalence [95% confidence interval (CI)] of each group of categorical variables were estimated and compared by Chi-square test (**Table2**).

Multivariable logistic regression analysis was performed for categorical variables to evaluate the independent association between variables and outcome in 2 levels: (1) without adjustment (crude odds ratios (ORs) and 95% CI); (2) full adjustment, which is adjusted for obesity (non-obese as reference), Hb A1c (Hb A1c≤7% as reference), T2DM duration (less than five years as reference), GLDs usage (oral as reference), DKD (e-GFR 60 mL/ min/1.73m^2, albuminuria (< 30 mg/24 hr as reference), triglyceride (> 150 mg/dL as reference) and TC (> 200 mg/dL as reference). Covariates with p-values < 0.20 in table 2 were then selected to enter the multivariable analysis.

Results

The study population consisted of 415 participants (73.7% women) with a mean (SD) age of 57.5 (8.6). The prevalence of anemia was 21.9% (19.3-23.1) and 20.2% (17.9-24.2) among women and men, respectively. The clinical and demographic data of participants were shown in **Table 1**. Generally, in comparison with T2DM patients without anemia, T2DM patients with anemia had a longer T2DM duration, higher FPG, HDL-C, and triglycerides levels. Iron indices (iron, ferritin, TIBC) were similar among patients with and without anemia since we defined anemia based on hemoglobin, and both groups may have negative iron balances. **Table 2** showed the prevalence of anemia in different subgroups. Anemia was more prevalent among obese individuals compared to non-obese ones. In addition, the prevalence of anemia was higher in participants with more than five years of T2DM duration than in ones with less than five years. In addition, participants with Hb A1C more than 7% had a higher prevalence of anemia than ones with Hb A1C less than 7. Also, patients with albuminuria and DKD were more likely to have anemia. Furthermore, participants who used insulin and oral GLDs at the same time were more likely to have anemia compared to those who were treated with one of them only. Finally, participants with high triglycerides (>150 mg/dL) had a higher prevalence of anemia than those with low triglyceride levels.

Multivariable logistic regression analysis (**Table 3**) showed that the presence of obesity (OR, 1.94 [95%CI, 1.17-1.23]), T2DM duration more than 5 years (OR, 3.12 [CI, 1.78-5.47]), albuminuria (OR, 6.37 [CI, 3.13-10.91]), high triglycerides (OR, 1.72 [CI, 1.21-2.77]) as well as DKD (OR, 4.30 [CI, 2.83-8.29]) had the independent positive associations with anemia. Furthermore, using insulin and oral GLDs at the same time and insulin alone were significantly associated with prevalence of anemia with the ORs of 2.60 [CI, 1.42-6.42] and (OR, 1.87 [CI, 1.30-4.37])), respectively.

Discussion

In this clinic-based study conducted in 2021, about one-fifth of the Gorgan diabetic women and men were found to have anemia. Obesity, T2DM duration more than five years, albuminuria, D DKD, high triglyceride, as well as using insulin both with and without oral GLDs were independently associated with prevalence of anemia among our participants.

Several studies have reported the prevalence of anemia among patients with T2DM to be common, especially among developing countries. Our result were similar to the previous study from Iran(Mashhad city) in 2011(19.6%)[14] and lower than another one(Tehran city) in 2014 (30.4%) [3]. A recent meta-analysis estimated the prevalence of 35% among patients with T2DM in Africa[18]. Other studies conducted in Kuwait[6], Malaysia[19], Brazil [20], California (DISTANCE study) [21], Greece (patients with DKD stages 2-4) [22], Saudi[23], England [24] and Pakistan [25], revealed the higher prevalence of 29.7% (5,655 /19,059), 31.7% (256/808), 34.2% (50/146), 34.7% (22,812/65), 47.8% (88/184), 55.5% (126/227), 59% (68/115) and 63% (126/200) among patients with T2DM. However, a study from India [10] and a community-based cohort from Australia [8] showed a lower prevalence of 12.13% (174 /1414) and 11.5% (178/1551), respectively. These differences may be due to the quality of health care services such as accessibility of patients to visit specialist and laboratory testing besides, selection bias could be the underlie the difference; for instance, in countries with high quality of health care services, the participants are in close follow up in specialized centers therefore were not representative of the whole population diabetic patients.

The differences observed could be the result of variations in studies' methodology and participant characteristics such as lifestyle, feeding habits, type of GLD usage, duration of T2DM, ethnicity, and mean age [1, 21].

The current study showed that T2DM duration for more than five years (regardless of glycemic indices and nephropathy) had a strong independent association with anemia, in line with some other studies [8, 26]. It seems that chronic hyperglycemia could decrease erythropoiesis and increase red blood cells (RBCs) destruction due to more exposure to inflammation and oxidative stress, and bone marrow impairment [27, 28].

In this study, although the levels of FPG and HbA1c were higher among diabetics with anemia, but statistically they were not significantly associated with anemia. However, some studies showed lower HbA1c among patients with anemia [29, 30]. They explained that deceased hemoglobin concentration and the enhanced RBC turnover in anemia of chronic disease could reduce the glycation process and consequently lead to falsely reported lower HbA1c levels [31].

Despite the lack of significant association between glucose indices and prevalence of anemia, using insulin had a significant association in the present study. Insulin users had potentially worse baseline characteristics rather than non-insulin users; also, using insulin could be a representative of poor control DM (allocation bias) [32, 33]. So this result is in agreement with some other studies that mentioned poor control DM has an association with anemia [12, 34]. Since neuropathy is common in patients with poor glycemic control, one of the reasons for the increased risk of anemia is the impairment of production and release of erythropoietin stimulated by the autonomic nervous system [35]. DM could negatively affect interstitial and peritubular structures (where erythropoietin is produced), and anemia could be the result of Decreased erythropoietin production by the failing kidney. Moreover, the exposure of erythroblasts or the mature erythrocytes to oxidative stress (due to glucose toxicity) could cause erythrocyte

dysfunction [28]. Furthermore, metformin is the first-line choice for T2DM management unless in the patients with e-GFR <45 ml/min/1.73 m^2, according to ADA 2021 guidelines. It has been reported that metformin interferes with cyanocobalamin absorption and is associated with vitamin B12 deficiency, resulting in an increased risk of anemia among patients with T2DM [36]. We still doubt whether the long duration of poor-control T2DM could increase the risk of anemia or not.

We found that obesity and high triglyceride were independently associated with prevalence of anemia. There are controversial results in this area as some studies reported that obesity is positively associated with anemia. They believe that obesity could lead to insulin resistance which could result in a hyperglycemic state. The adipose tissue is the source of different cytokines. The increase in inflammatory activity of adipose tissue in obese patients could cause an increase in hepcidin levels, causing a reduction in serum iron and limiting the availability of iron [37]. In this line, studies reported an obesity paradox in anemia and observed that normal/overweight T2DM patients were more anemic than obese patients [29]. However, some studies could not show any independent association between anemia and BMI among T2DM patients [38, 39]. Over nutrition may be associated with increased consumption of protein, iron, and other micronutrients with a protective effect against iron and/ or B12 deficiency in.diabetic population.[40]

We found that albuminuria> 30 mg/24hr and e-GFR 0 ml/min/1.73 m^2 as two important parameters of Diabetic kidney disease (DKD) had a significant independent association with anemia among patients with T2DM. Our findings were in accordance with other studies showing a higher frequency of anemia in patients with T2DM and nephropathy [41-43]. A large multicenter US study of the kidney early evaluation program (KEEP) demonstrated that development and aggravation of anemia in patients with DKD had a statistical relationship with severity of albuminuria and e-GFR [44]. DM could damage tubulointerstitial tissue (associated with the degree of albuminuria) in the early stage, even before any reduction in e-GFR. It could cause decreased erythropoietin production and iron metabolism impairment leading to reduced production of RBCs [45]. Blood urea may increase due to renal dysfunction and could negatively affect RBC's lifespan [22].

We found no significant difference in the prevalence of anemia among T2DM regarding gender in contrast to other studies [12, 46]. However, Bekele et al. showed a higher prevalence of anemia among men [26]. As the mean age of our participants was 57.8 years, men and women were similar due to decreased occurrence of anemia in women due to menopause. In the Iranian diabetic society, men and women have identical diets, education, and health awareness [47, 48].

AlDallal and Idris et al. [6, 19] showed a positive association of age and prevalence of anemia, which is in contrast with our results that could be due to bone marrow disorders occurring in older age, deficiencies in vitamins such as cyanocobalamin and folate, and a higher number of comorbidities [49].

The strengths of the research were: first, we assess various baseline characteristics such as various laboratory and clinical data in the study. We considered different potential associated factors in our

model in a sufficient sample size. Thus, we could discuss some important independent associated factors in detail. Second, we used a random sampling method that could mitigate selection bias and enhance the ability to generalize our results to the Iranian population (due to different ethnic groups living in Gorgan, Indigenous inhabitants, Turkman, Sistani, and Baloch). Third, to the best of our knowledge, there were no studies concerning the prevalence of anemia among diabetic population in the north of Iran. This research had several limitations. First, as it was a cross-sectional study, we could not show the casualty, so a longitudinal study is needed to assess the relationship over time. Second, we did not consider some drug usage including iron supplements, erythropoietin (EPO) in case of DKD. Third, we did not record dietary patterns, particularly iron intake was not considered in our study, although all patients are routinely educated about diet and health care in the Gorgan diabetes clinic. Fourth, we did not measure erythropoietin, B12, and folate levels in our participants, so lack of definition of anemia etiology was another limitation.

Conclusion

We demonstrated a high prevalence of anemia among patients with T2DM in North of Iran associated with obesity and hypertriglyceridemia, duration, and severity of diabetes, and renal dysfunction. It is necessary to evaluate and treat anemia in the diabetic population. They must be warned against the potential risk and complications of anemia and the importance of regular screening, especially among those with stated associated factors.

The prevalence of Anemia with the same study method is suggested to be done to have a valid and trustable results.

Abbreviations

GBD, global burden disease; YLDs, years lived with disability; T2DM, type 2 diabetes mellitus; DM, diabetes mellitus; GLDs, glucose-lowering drugs; BMI, body mass index; TIBC, total iron-binding capacity; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC ,total cholesterol ; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin;. MDRD, Modification of Diet in Renal Disease; e-GFR, estimated glomerular filtration rate; WHO, World Health Organization; DKD, Diabetic kidney disease; KDQOI, kidney disease outcomes quality initiative; ACR, albumin creatinine ratio; CI, confidence interval; ORs, odds ratios; RBCs , increase red blood cells; KEEP ,kidney early evaluation program; EPO, erythropoietin.

Declarations

Ethics approval and Consent to participate

The Ethics Committee of Golestan University of Medical Sciences approved this study (ethics Code: IR.GOUMS.REC.1398.170). This research complied with the principles of the declaration of Helsinki. All

methods were carried out in accordance with relevant guidelines and regulations. Informed consent was obtained from all subjects and/or their legal guardian(s) participating in this study. A copy of the written consent is available for review by the Series Editor of this journal.The authors declare that they have no competing interests

Consent for publication.

Not applicable.

Availability of data and materials.

The data that support the findings of this study **are available from** Golestan University of Medical Sciences **but restrictions apply** to the availability of these data, **which were used under license** for the current study, and so are not publicly available. Data are **however available from the authors upon**reasonable request **and with permission of** Golestan University of Medical Sciences.

Conflict of Interest Statement

The authors declare that they have no competing interests.

Funding Sources.

This work was funded by a Golestan university of Medical Sciences, Gorgan, Iran. The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript. There was no additional external funding received for this study.

Author Contributions.

M.Z. Conceptualized and designed the study, analyzed and interpreted the data, prepared the manuscript, and approved the final manuscript as submitted. M.Z, RH, F.F, FT, ZT, MTN and NR designed the study and drafted the initial manuscript, and approved the final manuscript as submitted. F.F and NR interpreted the data and critically revised the manuscript and approved the final manuscript as submitted.

Acknowledgement: We would like to acknowledge Dr. Seyyed Saeed Moazzeni for his valuable comments

References

1. Organization WH: **Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity**. In.: World Health Organization; 2011.
2. Kassebaum NJ, Collaborators GA: **The global burden of anemia**. Hematology/oncology clinics of North America 2016, **30**(2):247–308.
3. Hosseini MS, Rostami Z, Saadat A, Saadatmand SM, Naeimi E: **Anemia and microvascular complications in patients with type 2 diabetes mellitus**. Nephro-urology monthly 2014, **6**(4).

4. Esteghamati A, Larijani B, Aghajani MH, Ghaemi F, Kermanchi J, Shahrami A, Saadat M, Esfahani EN, Ganji M, Noshad S: **Diabetes in Iran**: **prospective analysis from first nationwide diabetes report of National Program for Prevention and Control of Diabetes** (**NPPCD**-2016). Scientific reports 2017, **7**(1):1–10.
5. Derakhshan A, Sardarinia M, Khalili D, Momenan AA, Azizi F, Hadaegh F: **Sex specific incidence rates of type 2 diabetes and its risk factors over 9 years of follow-up: Tehran Lipid and Glucose Study**. PLoS One 2014, **9**(7):e102563.
6. AlDallal SM, Jena N: **Prevalence of anemia in type 2 diabetic patients**. Journal of hematology 2018, **7**(2):57.
7. Deray G, Heurtier A, Grimaldi A, Vacher VL, Bagnis CI: **Anemia and diabetes**. American journal of nephrology 2004, **24**(5):522–526.
8. Gauci R, Hunter M, Bruce DG, Davis WA, Davis TM: **Anemia complicating type 2 diabetes: Prevalence, risk factors and prognosis**. Journal of diabetes and its complications 2017, **31**(7):1169–1174.
9. Vlagopoulos PT, Tighiouart H, Weiner DE, Griffith J, Pettitt D, Salem DN, Levey AS, Sarnak MJ: **Anemia as a risk factor for cardiovascular disease and all-cause mortality in diabetes: the impact of chronic kidney disease**. Journal of the American Society of Nephrology 2005, **16**(11):3403–3410.
10. Ranil PK, Raman R, Rachepalli SR, Pal SS, Kulothungan V, Lakshmipathy P, Satagopan U, Kumaramanickavel G, Sharma T: **Anemia and diabetic retinopathy in type 2 diabetes mellitus**. J Assoc Physicians India 2010, **58**:91–94.
11. Azmi S, Goh A, Muhammad NA, Tohid H, Rashid MRA: **The cost and quality of life of Malaysian type 2 diabetes mellitus patients with chronic kidney disease and anemia**. Value in health regional issues 2018, **15**:42–49.
12. Antwi-Bafour S, Hammond S, Adjei JK, Kyeremeh R, Martin-Odoom A, Ekem I: **A case–control study of prevalence of anemia among patients with type 2 diabetes**. Journal of medical case reports 2016, **10**(1):1–8.
13. Sahay M, Kalra S, Badani R, Bantwal G, Bhoraskar A, Das A, Dhorepatil B, Ghosh S, Jeloka T, Khandelwal D: **Diabetes and Anemia: International Diabetes Federation (IDF)–Southeast Asian Region (SEAR) position statement**. Diabetes & Metabolic Syndrome: Clinical Research & Reviews 2017, **11**:S685-S695.
14. Bonakdaran S, Gharebaghi M, Vahedian M: **Prevalence of anemia in type 2 diabetes and role of renal involvement**. Saudi Journal of Kidney Diseases and Transplantation 2011, **22**(2):286.
15. Moazzeni SS, Arani RH, Hasheminia M, Tohidi M, Azizi F, Hadaegh F: **High Incidence of Chronic Kidney Disease among Iranian Diabetic Adults: Using CKD-EPI and MDRD Equations for Estimated Glomerular Filtration Rate**. Korean Diabetes Journal 2021.
16. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, Van Lente F, Collaboration CKDE: **Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values**. Clinical chemistry 2007, **53**(4):766–772.

17. Initiative KDOQ: **Clinical practice guidelines for chronic kidney disease: evaluation, classification and stratification**. 2002.
18. Olum R, Bongomin F, Kaggwa MM, Andia-Biraro I, Baluku JB: **Anemia in diabetes mellitus in Africa: a systematic review and meta-analysis**. Diabetes & Metabolic Syndrome: Clinical Research & Reviews 2021:102260.
19. Idris I, Tohid H, Muhammad NA, Rashid MRA, Ahad AM, Ali N, Sharifuddin N, Aris JH: **Anaemia among primary care patients with type 2 diabetes mellitus (T2DM) and chronic kidney disease (CKD): a multicentred cross-sectional study**. BMJ open 2018, **8**(12):e025125.
20. Barbieri J, Fontela PC, Winkelmann ER, Zimmermann CEP, Sandri YP, Mallet EKV, Frizzo MN: **Anemia in patients with type 2 diabetes mellitus**. Anemia 2015, **2015**.
21. Ahmed AT, Go AS, Warton EM, Parker MM, Karter AJ: **Ethnic differences in anemia among patients with diabetes mellitus: the Diabetes Study of Northern California (DISTANCE)**. American journal of hematology 2010, **85**(1):57–61.
22. Loutradis C, Skodra A, Georgianos P, Tolika P, Alexandrou D, Avdelidou A, Sarafidis PA: **Diabetes mellitus increases the prevalence of anemia in patients with chronic kidney disease: A nested case-control study**. World journal of nephrology 2016, **5**(4):358.
23. Al-Salman M: **Anemia in patients with diabetes mellitus: prevalence and progression**. General Medicine: Open Access 2015:1–4.
24. Trevest K, Treadway H, Hawkins-van der Cingel G, Bailey C, Abdelhafiz AH: **Prevalence and determinants of anemia in older people with diabetes attending an outpatient clinic: a cross-sectional audit**. Clinical diabetes: a publication of the American Diabetes Association 2014, **32**(4):158.
25. Sharif A, Younus S, Baig K, Ali NH: **Prevalence and risk of anemia in type-2 diabetic patients**. Health 2014, **2014**.
26. Bekele A, Teji Roba K, Egata G, Gebremichael B: **Anemia and associated factors among type-2 diabetes mellitus patients attending public hospitals in Harari Region, Eastern Ethiopia**. PloS one 2019, **14**(12):e0225725.
27. Pappa M, Dounousi E, Duni A, Katopodis K: **Less known pathophysiological mechanisms of anemia in patients with diabetic nephropathy**. International urology and nephrology 2015, **47**(8):1365–1372.
28. Singh DK, Winocour P, Farrington K: **Erythropoietic stress and anemia in diabetes mellitus**. Nature Reviews Endocrinology 2009, **5**(4):204–210.
29. Awofisoye OI, Adeleye JO, Olaniyi JA, Esan A: **Prevalence and correlates of anemia in type 2 diabetes mellitus: a study of a Nigerian outpatient diabetic population**. Sahel Medical Journal 2019, **22**(2):55.
30. Anaemia **and type 2 diabetes: implications from a retrospectively studied primary care case series**. Hong Kong Med J 2013, **19**(3):214–221.
31. English E, Idris I, Smith G, Dhatariya K, Kilpatrick ES, John WG: **The effect of anaemia and abnormalities of erythrocyte indices on HbA 1c analysis: a systematic review**. Diabetologia 2015, **58**(7):1409–1421.

32. Khattab M, Khader YS, Al-Khawaldeh A, Ajlouni K: **Factors associated with poor glycemic control among patients with type 2 diabetes**. Journal of Diabetes and its Complications 2010, **24**(2):84–89.
33. Nunan D, Heneghan C, Spencer EA: **Catalogue of bias: allocation bias**. BMJ evidence-based medicine 2018, **23**(1):20.
34. Taderegew MM, Gebremariam T, Tareke AA, Woldeamanuel GG: **Anemia and its associated factors among type 2 diabetes mellitus patients attending debre berhan referral hospital, north-east Ethiopia: a cross-sectional study**. Journal of blood medicine 2020, **11**:47.
35. Töyry JP, Niskanen LK, Mäntysaari MJ, Länsimies EA, Uusitupa MI: **Occurrence, predictors, and clinical significance of autonomic neuropathy in NIDDM: ten-year follow-up from the diagnosis**. Diabetes 1996, **45**(3):308–315.
36. Katroth A, Narayanam A, Chauhan S, Singh A: **Diabetes mellitus and metformin: fatal vitamin B12 deficiency associated with anemia and sepsis in a young adult**. Journal of Applied Pharmaceutical Science 2018, **8**(3):165–167.
37. Weiss G: **Anemia of chronic disorders**: **new diagnostic tools and new treatment strategies**. In: Seminars in hematology: 2015: Elsevier; 2015: 313–320.
38. Goldhaber A, Ness-Abramof R, Ellis MH: **Prevalence of anemia among unselected adults with diabetes mellitus and normal serum creatinine levels**. Endocrine Practice 2009, **15**(7):714–719.
39. Adetunji O, Mani H, Olujohungbe A, Abraham K, Gill G: **'Microalbuminuric anaemia'—The relationship between haemoglobin levels and albuminuria in diabetes**. Diabetes research and clinical practice 2009, **85**(2):179–182.
40. Jéssica Barbieri, Paula Caitano Fontela, Eliane Roseli Winkelmann, Carine Eloise Prestes Zimmermann, Yana Picinin Sandri, Emanelle Kerber Viera Mallet, Matias Nunes Frizzo, "Anemia in Patients with Type 2 Diabetes Mellitus", Anemia, vol. 2015, Article ID 354737, 7 pages, 2015. https://doi.org/10.1155/2015/354737
41. Martynov SA, Shestakova MV, Shilov EM, Shamkhalova MS, Vikulova OK, Sukhareva OY, Trubitsyna NP, Egorova DN, Bondarenko ON, Dedov II: **Prevalence of anemia in patients with type 1 and type 2 diabetes mellitus with chronic renal disease**. Diabetes mellitus 2017, **20**(5):318–328.
42. El-Achkar TM, Ohmit SE, Mccullough PA, Crook ED, Brown WW, Grimm R, Bakris GL, Keane WF, Flack JM: **Higher prevalence of anemia with diabetes mellitus in moderate kidney insufficiency: The Kidney Early Evaluation Program**. Kidney international 2005, **67**(4):1483–1488.
43. Abate A, Birhan W, Alemu A: **Association of anemia and renal function test among diabetes mellitus patients attending Fenote Selam Hospital, West Gojam, Northwest Ethiopia: a cross sectional study**. BMC Blood Disorders 2013, **13**(1):1–7.
44. Thomas MC, Tsalamandris C, MacIsaac RJ, Jerums G: **The epidemiology of hemoglobin levels in patients with type 2 diabetes**. American journal of kidney diseases 2006, **48**(4):537–545.
45. Fishbane S, Spinowitz B: **Update on anemia in ESRD and earlier stages of CKD: core curriculum 2018**. American Journal of Kidney Diseases 2018, **71**(3):423–435.

46. Alsayegh F, Waheedi M, Bayoud T, Al Hubail A, Al-Refaei F, Sharma P: **Anemia in diabetes: experience of a single treatment center in Kuwait**. Primary care diabetes 2017, **11**(4):383–388.
47. Tol A, Mohebbi B, Sadeghi R: **Evaluation of dietary habits and related factors among type 2 diabetic patients: An innovative study in Iran**. Journal of education and health promotion 2014, **3**.
48. Azimi-Nezhad M, Ghayour-Mobarhan M, Parizadeh M, Safarian M, Esmaeili H, Parizadeh S, Khodaee G, Hosseini J, Abasalti Z, Hassankhani B: **Prevalence of type 2 diabetes mellitus in Iran and its relationship with gender, urbanisation, education, marital status and occupation**. Singapore medical journal 2008, **49**(7):571.
49. Gaskell H, Derry S, Moore RA, McQuay HJ: **Prevalence of anaemia in older persons: systematic review**. BMC geriatrics 2008, **8**(1):1–8.

Tables

Table1. Baseline characteristics of 415 participants with T2DM

	Total (N=415)	**Without Anemia (N=89)**	**With Anemia (N=326)**	**P-value**
Age (year)	57.8 (9.0)	57.4 (8.5)	57.9 (9.1)	0.742
BMI (kg/m^2)	28.4 (4.0)	28.2 (4.2)	28.3 (4.0)	0.939
Diabetes duration (month)	10.7 (6.2)	7.2 (4.8)	11.1 (6.6)	**<0.001**
FPG (mg/dL)	180.1 (141-244)	162.0 (129-198)	185.0 (145-257)	**<0.001**
Hb A1c (%)	8.1 (8.9)	8.5 (1.8)	8.9 (1.7)	0.176
TC (mg/dL)	175.7 (3.2)	165.4 (0.7)	198.5 (3.9)	0.073
LDL-C(mg/dL)	76.8 (59-103)	83.5 (67-103)	75.0 (58-103)	0.108
HDL-C(mg/dL)	48.4 (43-64)	46.0 (38-55)	49.0 (45-67)	**0.009**
Triglyceride(mg/dL)	158.9 (106-225)	140 (101-182)	164.0 (108-237)	**0.014**
e-GFR (ml/min/1.73 m^2)	82.1 (3.5)	86.3 (2.5)	80.9 (3.8)	0.202
Hemoglobin(g/dL)	12.0 (1.4)	13.2 (1.6)	11.7 (1.4)	**<0.001**
Ferritin (µg/L)	37.1 (21-72)	34.0 (27-65)	37.9 (20-74)	0.740
Iron (µg/dL)	82.1 (59-98)	79.0 (65-98)	83.0 (57-98)	0.254
TIBC (µg/dL)	351.7 (36.2)	347.1 (33.0)	352.9 (37.1)	0.292

Data were presented as mean (SD); FPG, LDL-C, HDL-C, Triglyceride, Ferritin and Iron were presented as median (interquartile range [IQR]); For comparison between the two groups, t-test for data with normal distribution and Mann-Whitney test ones with abnormal distribution; BMI: body mass index; FPG: fasting plasma glucose; Hb A1c: Hemoglobin A1c; TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; e-GFR: estimated glomerular filtration rate e-GFR: estimated glomerular filtration rate; TIBC: total iron binding capacity; T2DM: Type 2 diabetes mellitus

Prevalence of anemia among patients with T2DM in different subgroup Table2.

	Case/Total	Crude prevalence % (95% CI)	P-value
Obesity			0.012
Non-obese	53/292	18.2 (16.4-20.1)	
obese	36/132	29.3 (26.6-31.1)	
Gender			0.708
Women	67/306	21.9 (19.3-23.1)	
Men	22/109	20.2 (17.9-24.2)	
Diabetes Duration			**<0.001**
<5 years	18/162	11.1 (10.2-12.4)	
≥5 years	71/253	28.1 (26.5-31.0)	
Hb A1c			**0.001**
7	11/106	10.4 (8.8-12.6)	
>7	78/309	25.2 (23.4-27.8)	
Albuminuria			**<001/0**
No	64/375	17.1 (15.8-20.2)	
Yes	25/40	62.5 (60.0-65.1)	
GLD			**<0.001**
Oral	52/300	17.3 (16.2-18.4)	
Insulin	24/77	31.2 (29.3-32.8)	
Oral + Insulin	13/38	34.2 (31.7-36.5)	
DKD			**<0.001**
No	62/362	17.1 (15.1-18.9)	
Yes	27/53	50.9 (49.0-52.1)	
Triglyceride			**0.023**
150	39/226	17.3 (15.7-19.3)	
>150	50/189	26.5 (23.7-28.9)	
TC			0.066
≤200	67/340	19.7 (17.6-21.4)	
>200	22/75	29.3 (28.1-31.3)	

LDL-C			0.688
≤100	65/296	22.0 (20.5-24.7)	
>100	24/119	20.2 (18.9-22.1)	
HDL-C			0.240
≥40	63/272	32.2 (28.8-36.2)	
<40	26/143	18.2(17.0-20.1)	

Each group of categorical variables compared by Chi-square test; GLD: glucose lowering drug; DKD: chronic kidney disease; TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; T2DM: Type 2 diabetes mellitus

Table3. Crude and adjusted odds ratios of associated factors with prevalent anemia among patients with T2DM

	Crude OR (95% CI)	Adjusted OR (95% CI)	P-value
Obesity			
Non-obese	1	1	
obese	2.28 (1.72-3.19)	1.94 (1.17-3.23)	0.010
Hb A1c			
7		1	
7	1.61 (1.31-2.12)	1.15 (0.60-3.10)	0.610
Diabetes Duration			
<5 years		1	
5 years	4.23 (2.16-6.01)	3.12 (1.78-5.47)	**<0.001**
Albuminuria			
No		1	
Yes	7.31 (4.61-12.54)	6.37 (3.13-10.91)	**<0.001**
GLD			
Oral		1	
Insulin	2.42 (1.89-5.17)	1.87 (1.30-4.37)	**0.039**
Oral + Insulin	3.19 (2.03-6.73)	2.60 (1.42-5.42)	**0.046**
DKD			
No		1	
Yes	5.87 (3.51-8.15)	4.30 (2.83-7.29)	**<0.001**
Triglyceride			
≤150		1	
>150	2.31 (2.79-3.98)	1.72 (1.21-2.77)	**0.024**
TC			
200		1	
>200	2.09 (0.67-5.45)	1.58 (0.84-4.88)	0.124

Multivariable logistic regression analysis was performed in 2 levels: (1) without adjustment (crude odds ratios (ORs) and 95% CI); (2) full adjustment, which is adjusted for obesity, Hb A1c, diabetes duration, GLDs usage, DKD, albuminuria, triglyceride and TC. GLD: glucose lowering drug; DKD: chronic kidney

disease; TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; T2DM: Type 2 diabetes mellitus.