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PATTERN AND CAUSES OF ANEMIA IN YEMENI PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT
Background and aims. The data on the patten and causes of anemia in patients with Diabetes Mellitus in developing countries including Yemen is scarce. The aim of this study was to determine the pattern and causes of anemia in a group of patients with type 2 DM evaluated in Sana'a, Yemen. **Methods:** The study was a prospective descriptive and analytical cross-sectional study which recruited all patients with type 2 DM evaluated between March 2016 and March 2o17 at Al-Jomhori Teaching Hospital in Sana'a, Yemen. **Results:** 324 (141 males and 183 females) patients were included in the study. The mean age ±SD was 53±12 years. 77 (23.8%) patients (32 males and 45 females) had anemia. Significantly higher number of anemic patients had renal insufficiency i.e. eGFR <60 ml/min/1.73 m² (32.5% vs. 14.6%) and uncontrolled DM i.e. FBS <7.0 mmol/L (37.7% vs. 28.7%). Anemic patients were significantly older (mean age 55.58±11.37 vs. 52.27±12.05 years) with longer duration of DM (mean duration 71.23±84.08 vs. 48.87±63.43 months) and with lower eGFR (69.07±24.72 vs. 82.51±22.99 ml/min/1.73m²). Hb level showed significantly negative correlation with age, duration of DM and creatinine and positive correlation with the eGFR. 24 patients had iron deficiency anemia, 27 patients had CKD, 21 patients had inflammatory disorders and five patients had megaloblastic anemia. Anemic patients with renal insufficiency (eGFR <60 ml/min/1.73 m²) were older, had higher HbA1c (uncontrolled DM) and also had lower MCV and MCH. **Conclusion:** Anemic patients were older, with longer duration of DM, and were more frequently to have uncontrolled DM and renal impairment. Nutritional anemia including iron deficiency and megaloblastic anemia were common causes of anemia.

KEYWORDS: Type 2 DM, Anemia, iron deficiency, megaloblastic anemia, causes, Yemen, Developing country.

INTRODUCTION
Diabetes Mellitus (DM) is a universal disease with great metabolic consequences. Chronic complications including nephropathy, neuropathy and macrovascular complications affect a significant proportion of patients with DM leading to tremendous morbidity and mortality. Anemia causing deficient supply of oxygen to tissues compounds the above effects when associated with DM causing earlier onset and more severe complications.[1-4] Anemic patients have more cardiovascular complications and earlier onset of nephropathy compared to patients without anemia. On the other hand, DM increases the severity of anemia in certain groups of patients with renal failure.[5] These effects underscore the importance of addressing the interaction between anemia and DM for their optimal management and care. The prevalence of anemia in patients with diabetes mellitus reaches up to 47% in some studies in developing countries.[6-9] Such findings indicate the need to screen patients with diabetes mellitus for anemia and importantly to evaluate the causes of anemia for the aim of proper management. Such an approach will help to decrease the morbidity and mortality of patients with diabetes mellitus.[10] The pathogenesis of anemia in patients with DM are multifactorial which include systemic inflammation, autonomic neuropathy causing efferent sympathetic denervation of the kidney with loss of appropriate erythropoietin production, damage to the renal interstitium, inhibition of erythropoietin release, erythropoietin resistance and even genetic predisposition.[11,12] Chronic renal insufficiency, which is a common complication of type 2 DM is a frequent cause of anemia.[13] Nutritional deficiencies of iron, folic acid and B12 are more likely to be important causes in developing countries in which poverty is prevalent. Metformin, the most recommended antidiabetic therapy

for patients with type 2 DM is also implicated to decrease the absorption of vitamin B12 causing megaloblastic anemia.[14] Although the pathogenesis of anemia in patients with type 2 DM has received particular interest in the literature, emphasis on the causes of anemia in these patients particularly in developing countries is lacking.

Yemen is a developing poor country which is currently further burdened by war and siege. It has a population of thirty millions and is located at the southern corner of Arabian Peninsula and its capital city Sana'a is one of the oldest cities since ancient times. The overall prevalence of type 2 DM in Yemen is approximately 4.6 % (7.4% in males and 2% in females.[15] Although anemia is a major health problem in the Yemeni population, the prevalence as well as the causes of anemia in Yemeni patients with DM are not known.[16] The aim of this study was to determine the pattern and causes of anemia in a group of patients with type 2 DM evaluated in Sana'a, Yemen.

Patients and Methods

The study was conducted at the Department of Medicine and Hematology Unit of Al-Jomhori Teaching Hospital in the capital city, Sana'a, Yemen. The study was a prospective descriptive and analytical cross-sectional study which recruited all patients with type 2 DM who attended the internal medicine-hematology clinic between March 2016 and March 2o17. Exclusions were patients with type 1 DM, pregnant patients with DM, drug induced DM and patients with MODY. A total number of 324 patients (141 males and 183 females) with type 2 DM were evaluated during this period. The data regarding age, sex, duration of diabetes, weight, height, metformin use and hypertension were obtained during the initial visit. BMI for each patient was calculated according to the formula, weight (kg)/ height $(m)^2$, and patients were categorized into underweight when BMI < 18.5 kg/m^2, healthy weight when BMI 18.5-25, overweight when BMI ≥25-30, obese when BMI ≥ 30-40 and extreme obesity when BMI ≥40 kg/m^2. During the initial visit at the specialized internal medicine and hematology clinic, every patient underwent screening for anemia in addition to other biochemical tests including FBS, HbA1c, creatinine and lipid profile. Blood samples (EDTA and clotted) were obtained from each patient. A complete blood count (CBC) was performed on fresh blood using the Automated Hematology Analayzer KX-21N (Symex Corporation, Kobe, Japan). Quantitive assessment of HbA1c was performed by High Performance Liquid Chromatography (HLPC). Serum creatinine, FBS, lipid profile and other related laboratory data were measured using standard laboratory methods.[17] The glomerular filtration rate (eGFR) was Calculated using the CKD-EPI formula.[18,19] Whenever a patient was found to have anemia, he or she was subjected to intensive evaluation to diagnose the cause of anemia including when indicated evaluation of serum ferritin, vitamin B12 and Folic acid by ElISA and bone marrow examination. When suspected, other specialized tests were performed to evaluate systemic inflammatory disorders including C-RP, ESR, RF and ANA. Screening for infectious diseases including HIV testing were performed based on the clinical context. Further specialized tests were performed to specifically diagnose the cause including imaging studies and histopathology to diagnose malignant disorders. The study was approved by the Ethical Committee of the Faculty of Medicine and Health Sciences of Sana'a University.

Definitions

Anemia was defined according to WHO criteria i.e. a female patient was considered anemic if the Hb level was < 12 g/dl and a male patient was considered anemic if the Hb level < 13 g/dl.[20] Anemia was further subclassified into three categories, severe anemia when the Hb level was < 8.0 g/dl, moderate anemia when the Hb was between 8.0 and 11 g/dl and mild anemia when Hb ≥ 11 g/dl. The anemia was further categorized into microcytic when the MCV was < 80 fl, normocytic when the MCV was between 80 and 95 fl and macrocytic when the MCV was ≥ 95 fl.[21] The causes of anemia were categorized into four categories, iron deficiency anemia, anemia due to chronic kidney disease (CKD), anemia due to systemic inflammation and megaloblastic anemia. A patient was considered to have iron deficiency anemia when he or she had microcytic anemia associated with low serum ferritin (<30 ng/ml in males and postmenopausal females or <6 ng/ml in premenopausal females) and does not fit to the other categories. A patient was considered to have megaloblastic anemia if he or she had anemia associated with low serum folic acid (< 2.0 ng/ml) and or low serum vitamin B12 (<191 pg/ml). A patient was considered to have anemia due to CKD if the anemia was associated with eGFR < 60 ml/min/1.73 m^2. If in addition the patient had low serum ferritin, low serum folic acid or low serum B12 he or she was considered to have CKD associated with iron deficiency anemia or megaloblastic anemia according to the deficient vitamin. The anemia due to systemic inflammation included patients with collagenous diseases, autoimmune diseases, inflammatory bowel disease, infectious diseases and anemia due to malignant diseases. Based on the CKD staging from The National Kidney Foundation Kidney Disease Improving Global Outcomes (KDIGO) 2012 guidelines, eGFR values were categorised into five categories, I, II, III, VI and V when the eGFR ≥ 90, 60-90, 30-60, 15-30 and <15 ml/min/1.73 m^2 respectively.[22] The patient was considered to have controlled DM when the FBS < 126 mg/dl and or HbA1c < 7.0%. The patient was considered to have dyslipidemia when the low density lipoprotein (LDL) was > 130 mg/dl and or Triglyceride level > 170 mg/dl.

Statistical analysis: The data were collected, tabulated and compiled in a computer database. SPSS version 21 was used to analyze data. Frequencies and percentages were used to describe categorical data. Unpaired Independent Samples T test was used to evaluate the comparison between anemic and non-anemic patients

regarding the means of Hb, creatinine, eGFR, weight, height, BMI, FBS, HbA1c and duration of Diabetes mellitus. It was also used to compare the mean values of clinical and laboratory parameters of anemic patients with DM according to gender and according to kidney function. Chi squared test was used to compare patients with anemia and without anemia regarding sex, hypertension, obesity, dyslipidemia, metformin use and categories of eGFR, BMI, FBS, HbA1c and duration of DM. It was also used to test the distribution of the causes of anemia in patients with DM in relation to the categories of sex, age, Hb categories (anemia severity), MCV and eGFR categories. Spearman's correlation test was used to correlate Hb level with age, weight, BMI, creatinine, eGFR, FBS, HbA1c and duration of DM. The significance level was considered at $p < 0.05$.

RESULTS

A total number of 324 (141 males and 183 females) patients were included in the study. The mean age ±SD was 53±12 years. 77 patients (32 males and 45 females) out of the 324 patients had anemia i.e 23.8%. Table 1 shows the characteristics of anemic and non-anemic patients with type 2 DM. There were significant differences regarding FBS, eGFR, dyslipidemia and duration of DM (Higher number of anemic patients had longer duration of DM, significant renal insufficiency and uncontrolled DM but lower dyslipidemia). Table 2 shows the comparison regarding the mean values of cliniclal and laboratory data. There were significant differences regarding the mean vlaues of age, duration of DM, eGFR and creatinine (anemic patients were older with longer duration of DM and had higher serum creatinine and lower eGFR values). Table 3 shows the correlation between Hb level and the parameters of age, weight, BMI, FBS, creatinine, HBA1c, duration of DM and eGFR. There was significant negative correlation with age, duration of DM and creatinine i.e. the lower the Hb the older the patient, the longer the duration of DM and the higher the serum creatinine. There was positive correlation with the eGFR i.e. the lower the Hb the lower the eGFR. There was no significant correlation with FBS and HbA1c. Out of the 77 patients who had anemia, 32 patients (41.6%) were males and 45 patients (58.4%) were females. Table 4 shows gender differences of the mean values of clinical and laboratory parameters of Yemeni anemic patients with type 2 DM. Female patients with anemia were significantly younger and shorter, had significantly higher HbA1c, significantly higher BMI and higher Hb than male anemic patients. On the other hand, there were no significant differences regarding the mean values of weight, FBS, duration of DM, creatinine and eGFR. Table 5 shows distribution of the causes of anemia. 24 patients had iron deficiency anemia, 27 patients had CKD, 21 patients had inflammatory disorders (two patients with rheumatoid arthritis, two patients with inflammatory bowel disease, three patients with thyroiditis, one patient with HIV associated with HCV inection, two with HBV associated chronic liver disease, one patient with pneumonia, one patient with myelofibrosis, one patient with myelodysplastic syndrome, one patient with hepatocellular carcinoma and seven patients with chronic lymphocytic leukemia (CLL), one of them associated with pneumonia and one associated with renal insuffficiency) and five patients had megaloblastic anemia (three patients due to folic acid deficiency and two patients due to vitamin B12 deficiency). Two of these patients had non-macrocytic red blood cells due to associated low serum ferritin and were categorized with megaloblastic anemia. The two patients who had anemia due to vitamin B 12 deficiency were using metformin. Two additional patients with CLL had no anemia. Iron deficiency anemia was more common in females particularly young females and showed dominant microcytic anemia and mild renal insufficiency i.e. eGFR 60-90 ml/min/1.73 m^2. One of these patients had low serum ferritin and normocytic rather than microcytic red blood cells but did not fit to any of the other categories and was categorized with iron deficiency anemia. Six patients with CKD and microcytic anemia had low serum ferritin and were considered to have CKD associated iron deficiency anemia. Three patients with CKD had macrocytic anemia, which was confirmed to be due to associated folic acid deficiency. In addition, microcytic anemia was also frequent in patients with inflammatory diseases who had also normal to high serum ferritin (a picture of anemia of chronic disease). Table 6 shows the mean clinical and laboratory values of anemic patients according to kidney function. Anemic patients with renal insufficiency (eGFR <60 ml/min/1.73 m^2) were older, had higher HbA1c (uncontrolled DM) and had lower MCV and MCH.

Table 1: Characteristics of Yemeni Patients with type 2 Diabetes Mellitus and the differences between anemic and non-anemic patients.

Variable	All patients No=324(%)	Anemic patients No=77(%)	Non-anemic patients No=247(%)	*P* value
Sex category Male Female	 141 (43.5%) 183 (56.5%)	 32 (41.6%) 45 (58.4%)	 109 (44.1%) 138 (55.9%)	0.691
Age category (years) <60 >60	 247 (76.2%) 77 (23.8%)	 55 (71.4%) 22 (28.6%)	 192 (77.7%) 55 (22.3%)	0.256
eGFR category (ml/min) I II III VI V	 114 (35.2%) 151 (46.6%) 49 (15.1%) 7 (2.2%) 3 (0.9%)	 15 (19.5%) 38 (49.3%) 19 (24.7%) 4 (5.2%) 1 (1.3%)	 99 (40.1%) 113 (45.8%) 30 (12.1%) 3 (1.2%) 2 (0.8%)	0.002
eGFR category (ml/min) <60 >60	 59 (18.2%) 265 (81.8%)	 24 (32.5%) 53 (67.5%)	 35 (14.6%) 212 (85.4%)	0.001
Hb A1c category (%) <7 >7	 93 (28.7%) 231 (71.3%)	 22(28.6%)9 55(71.4%)	 71(28.7%)32 176(71.3%)79	0.982
Hypertension Yes no	 158 (48.8%) 166 (51.2%)	 37 (48%) 40 (52%)	 121 (49%) 126 (51%)	0.858
Obesity (BMI categories) (kg/m^2) BMI <18.5 BMI 18.5-25 BMI 25-30 BMI >30 BMI>40	 12 102 108 96 6	 5 (6.5%) 23 (29.9%) 25 (32.5%) 21 (27.2%) 3 (3.9%)	 7 (2.8%) 79 (32%) 83 (33.6%) 75 (30.4%) 3 (1.2%)	0.075
Metformin use Yes no	 123 (38%) 201 (62%)	 35 (45.5%) 42 (54.5%)	 88 (35.6%) 159 (64.4%)	0.229
Dyslipidemia Yes no	 183 141	 34(44.2%) 43 (55.8%)	 149 (60.3%) 98 (39.7%)	<0.001
Duration (months) <60 60-120 >120	 228 51 45	 49 (63.6%) 11 (14.3%) 17 (22.1%)	 179 (72.5%) 40 (16.2%) 28 (11.3%)	0.020
FBS category (mg/dl) <126 >126	 104 220	 29 (37.7%) 48 (62.3%)	 71 (28.7%) 176 (71.3%)	<0.001

Table 2: Comparison of the mean clinical and laboratory values between Yemeni anemic and non-anemic patients with type 2 Diabetes Mellitus.

Variable	All patients No=324	Anemic patients No=77	Non-anemic patients No=247	*P* value
Age (years)	53±12	55.58±11.37	52.27±12.05	0.033
Weight (kg)	68.4±14.9	67.77±15.86	68.62±14.62	0.666
Height (cm)	157.2±8.9	157.08±8.46	157.21±9.08	0.918
BMI (kg/m²)	27.7±5.6	27.54±6.32	27.73±5.43	0.804
Duration (months)	53.5±68.9	71.23±84.08	48.87±63.43	0.036
FBS (mg/dl)	183.4±78.5	186.80±92.43	187.13±97.61	0.983
Hb A1c (%)	9.0±2.6	9.18±2.62	8.94±2.63	0.649
Creatinine (mg/dl)	1.0±0.58	1.22±0.94	.96±0.38	0.001
Hb (g/dl)	13.4±2.5	9.97±1.79	15.02±9.0	<0.001
eGFR (ml/min)	79.1±24.0	69.07±24.72	82.51±22.99	<0.001

Table 3: Correlation of hemoglobin level with clinical and laboratory parameters in Yemeni patients with type 2 Diabetes mellitus.

variable	*p*	R
Age	0.030	-.120
Weight	0.072	0.101
BMI	0.754	0.018
Duration	0.011	-0.160
FBS	0.264	0.068
Creatinine	0.002	-0.229
Hb A1c	0.221	0.103
eGFR	<0.001	0.292

Table 4: Gender differences of the mean values of clinical and laboratory parameters of Yemeni anemic patients with type 2 Diabetes Mellitus.

Variable	All anemic patients No=77	Male patients No=32	Female patients No=45	*P* value
Age (years)	55.23±11.7	60.6±11.2	51.7±10.9	0.001
Weight (kg)	67.8±15.9	66.2±14.6	68.7±16.8	0.521
Height (cm)	157.1±8.5	164.4±7.9	152.4±4.8	<0.001
BMI (kg/m²)	27.5±6.3	24.4±4.8	29.5±6.5	0.001
Duration (months)	71.3±84.1	89.9±80.8	59.6±85.2	0.210
FBS (mg/dl)	186.8±92.4	187.4±100.5	186.5±89.1	0.973
Hb A1c (%)	9.2±2.6	7.9±2.3	9.8±2.6	0.039
Creatinine (mg/dl)	1.22±0.94	1.24±0.46	1.2±1.16	0.850
eGFR (ml/min)	69.07±24.72	69.2±24.8	68.7±24.9	0.929
Hb (g/dl)	9.0±	9.4±2.0	10.3±1.6	0.033

Table 5: Distribution of the causes of anemia in Yemeni patients with type 2 Diabetes Mellitus

Variable	Iron deficiency anemia No=24(%)	CKD No=27(%)	Inflammation No=21(%)	Megaloblastic anemia No=5(%)	*P value*
Sex category Male Female	 7(29.2%) 17 (70.8%)	 10 (37%) 17 (63%)	 11 (52.4%) 10 (47.6%)	 4 (80%) 1 (20%)	0.072
Age category (years) <60 >60	 21 (87.5%) 3 (12.5%)	 16 (59.3%) 11 (40.7%)	 16 (76.2%) 5 (23.8%)	 2 (40%) 3 (60%)	0.054
Hb category (g/dl) <8 8-11 >11	 1 (4.2% 14 (58.3%) 9 (37.5%)	 4 (14.8%) 14 (51.9%) 9 (33.3%)	 5 (23.8%) 12 (57.1%) 4 (19.1%)	 0 3 (60%) 2 (40%)	0.465
MCV category (fl) Microcytic Normocytic Macrocytic	 23 (95.8%) 1 (4.2%) 0	 14 (51.9%) 10 (37%) 3 (11.1%)	 11 (52.4%) 7 (33.3%) 3 (14.3%)	 1 (20%)[a] 1 (20%)[a] 3 (40%)	0.014
eGFR category (ml/min) I II III VI V	 6 (25%) 18 (75%) 0 0 0	 0 (19.5%) 3 (11.1%) 19 (70.4%) 4 (14.8%) 1 (3.7%	 6 (28.6%) 15 (71.4%) 0 0 0	 3 (60%) 2 (40%) 0 0 0	<0.001

[a]Have associated low serum ferritin

Table 6: Mean values of clinical and laboratory parameters of Yemeni anemic patients with type 2 Diabetes Mellitus according to kidney function.

Variable	All patients No=77	eGFR more than 60 (ml/min) No=24	eGFR less than 60 (ml/min) No=53	*P* value
Age (years)	55.58±11.4	53.34±11.7	59.15±11.69	0.043
Weight (kg)	67.8±15.9	68.51±16.9	65.8±12.8	0.478
Height (cm)	157.1±8.5	157.88±8.3	155.8±8.2	0.295
BMI (kg/m²)	27.5±6.3	27.5±6.5	27.3±5.6	0.926
Duration (months)	71.2±84.1	64.1±70.4	85.1±66.5	0.213
FBS (mg/dl)	186.8±92.4	178.8±63.9	202.3±89.5	0.191
Hb A1c (%)	9.2±2.6	8.9±1.4	9.7±2.0	0.031
Creatinine (mg/dl)	1.22±0.94	0.92±0.18	1.79±1.44	<0.001
Hb (g/dl)	9.97±1.79	9.93±1.82	9.95±1.83	0.910
MCV (fl)	78.2±10.7	76.5±9.1	81.4±9.0	0.024
MCH (pg)	24.5±4.3	23.8.1±3.6	25.8±3.6	0.016
MCHC g/l	217.3±131.2	223.7±123.6	201.3±113.3	0.345
eGFR (ml/min)	69.07±24.72	82.2±17.5	43.3±14.2	<0.001

DISCUSSION

Patients with DM are burdened by chronic complications including diabetic nephropathy and cardiovascular morbidity and mortality.[23] Anemia is associated with left ventricular dysfunction and heart failure and causes symptoms related to decreased delivery of oxygen to tissues leading to further impairment of the quality of life of these patients. Anemia has been found to be two to three times more prevalent in patients with diabetes compared with the general population at all levels of GFR.[4,6,13] In developing countries type 2 diabetes mellitus is increasing in prevalence and anemia is a frequent cause of morbidity and mortality.[24,25] The association of anemia with type 2 DM and their interaction deserves evaluation to optimize the care of patients with DM. Our study showed a high frequency of anemia among patients with DM (23.8%) which is intermediate between the frequency seen in African countries (41%)[9] and the frequency seen in a Caucasian population (12%)[26] and similar to that seen in a Chinese population.[7] Our anemic patients had significantly longer duration of DM and also the Hb level was significantly negatively correlated with the duration of DM which indicates that Hb levels become lower with

ongoing time and points to the need to screen patients with DM regularly for anemia. Such progressive decrease in Hb with time has been observed in another study in which patients were followed prospectively for a median of seven years and showed an ongoing, small but significant decrease in Hb since presentation.[11] Our anemic patients had significantly more frequent patients with poorly controlled DM as determined by one marker of control (FBS). This observation has been reported in other studies which showed a significantly increased FBS in the cases than in the controls.[4,27] However, in general most of our patients (both anemia and non-anemic) had uncontrolled DM (71%). This poor glycemic control among the majority of Yemeni patients with DM was also reported by others.[25,28] These findings indicate that the quality of diabetic care in Yemen is not proper and calls for establishment of measures to improve the control of blood sugar of these patients. There was no significant difference regarding HBA1c, which is consistent with that seen by others.[29] Anemic patients in this study had significantly lower eGFR with significantly more patients with moderate to severe kidney insufficiency. This finding is plausible as CKD is a common cause of anemia due to decreased erythropoietin production. This finding also has been reported by others showing higher incidence of anemia in patients with diabetes and renal insufficiency.[4,27,29,4] However, a significant number of our patients with CKD had also iron deficiency anemia and folic acid deficiency which points to associated nutritional deficiencies, which is, expected considering that our country is a poor developing country. Even in patients with acceptable eGFR, iron deficiency anemia was common (24/77), compared to other causes of anemia, coming second to CKD. This is in contrast to that seen in developed countries in which iron indices were found to be elevated in patients with DM.[30] Nutritional factors need to be addressed as causes of anemia in patients with type 2 DM in developing countries whether associated or not associated with CKD. 123 (38%) patients in our study were using metformin, which is a low proportion considering that it is highly recommended as initial therapy and as part of combination regimen even in patients with declining renal function.[31] Although metformin has been implicated to cause vitamin B12 deficiency, there was no difference between our anemic and non-anemic patients regarding metformin use.[32] Only two of the 35 our anemic patients (5.7%) who used metformin developed vitamin B12 deficiency, which is lower than the estimated frequency of 10-30%.[33] Such low association also has been reported in a Malay population where there was no significant difference between anemic and non-anemic patients regarding metformin use and the frequency of macrocytic anemia was only 3.1%.[29] The severity of anemia had no relation to the cause of anemia which means that even patients with nutritional anemia (iron deficiency or folic acid deficiency) may present with moderate to severe anemia. Interestingly that seven of our anemic patients had CLL in addition to two other patients who had DM with CLL without anemia. This finding may be partly explained by the fact that the study was conducted in a specialist internal medicine and hematology clinic. However, such a prevalence of CLL in patients with DM in our center where the total prevalence of CLL patients were reported to be 75 patients over ten years is considered to be high.[34] The prevalence of CLL in patients with type 2 DM was reported in the blood journal to be 16% (47 out of 291 patients) compared to only 0.7% in patients with type 1 DM.[35] Further clarification of the relation between type 2 DM and CLL warrants future investigation. Our patients with moderate to severe kidney insufficiency (eGFR < 60) were older and had significantly higher HbA1c compared to patients with normal or mild renal insufficiency (eGFR >60). These findings are similar to that seen in other studies.[9] The mean age of our anemic patients was significantly higher than non-anemic patients and 22 (28.6%) of our anemic patients were elderly which is consistent with other studies showing higher prevalence in the elderly.[2,9] In addition to being elderly, having DM and having renal insufficiency, anemia will further pose a great burden on cardiovascular reserve leading to marked fatigue, exercise intolerance and may precipitate myocardial ischemic events. Anemia has been reported to be associated with increased morbidity and mortality and can lead to cardiovascular and neurological events in old age.[36-38] Particular emphasis to screen and evaluate anemia in elderly diabetic patients should be considered to decrease their morbidity and mortality. Our study showed a few gender differences including significantly higher HbA1c and higher BMI among females compared to males which is similar to the finding seen in a sub-Saharan African hospital and is consistent with the reported finding that Yemeni females with type 2 DM are generally more obese than males.[9,28]

In conclusion, this study on anemic patients with type 2 DM compared to non-anemic patients with type 2 DM showed that significantly higher number of anemic patients had significant renal insufficiency and uncontrolled DM and that the lower the Hb the older the patient, the longer the duration of DM, the higher the serum creatinine and the lower the eGFR. It showed also that in addition to chronic CKD, nutritional anemia is an important and frequent cause of anemia whether associated or not associated with CKD. It showed also that inflammatory disorders are also frequent causes of anemia, which need to be evaluated for, and that a relation between type 2 DM and CLL warrants investigation.

We recommend that patients with type 2 DM need to be screened for anemia at presentation and regularly thereafter for example annually. When anemia is found, search for the cause, whether or not CKD is present, is essential including screening for serum ferritin, folic acid and vitamin B 12 particularly in developing countries. We also recommend further investigation of the role of

the type of diet as a predisposing factor for anemia in patients with type 2 DM.

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REFERENCES

1. Bosman DR, Winkler AS, Marsden JT, Macdougall IC, Watkins PJ. Anemia with erythropoietin deficiency occurs early in diabetic nephropathy. Diabetes Care, 2001; 24(3): 495–499.
2. Thomas MC, MacIsaac RJ, Tsalamandris C, Power D, Jerums G. Unrecognized anemia in patients with diabetes: a crosssectional survey. Diabetes Care, 2003; 26(4): 1164– 1169.
3. Rossing K, Christensen PK, Hovind P, Tarnow L, Rossing P, Parving HH: Progression of nephropathy in type 2 diabetic patients. Kidney Int, 2004; 66(4): 1596–1605.
4. Thomas MC, MacIsaac RJ, Tsalamandris C, Molyneaux L, Goubina I, Fulcher G, Yue D, Jerums G. The burden of anaemia in type 2 diabetes and the role of nephropathy: A cross-sectional audit. Nephrol Dial Transplant, 2004; 19(7): 1792-1797.
5. Ishimura E, Nishizawa Y, Okuno S, Matsumoto N, Emoto M, Inaba M, Kawagishi T, Kim CW, Morii H. Diabetes mellitus increases the severity of anaemia in non-dialyzed patients with renal failure. J Nephrol, 1998; 11(2): 83-86.
6. Angelousi A, Larger E. Anaemia, a common but often unrecognized risk in diabetic patients: a review. Diabetes Metab, 2014; 41(1): 18–27.
7. Chen CX, Li YC, Chan SL, Chan KH. Anaemia and type 2 diabetes: implications from a retrospectively studied primary care case series. Hong Kong Med J, 2013; 19(3): 214–21.
8. 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 Hematol, 2013; 13(1): 6.
9. Feteh VF, Choukem SP, Kengne AP, Nebongo DN, Ngowe-Ngowe M. Anemia in type 2 diabetic patients and correlation with kidney function in a tertiary care sub-Saharan African hospital: a cross-sectional study. BMC Nephrol, 2016; 17: 29.
10. Gouva C, Nikolopoulos P, Ioannidis JP, Siamopoulos KC: Treating anemia early in renal failure patients slows the decline of renal function: a randomized controlled trial. *Kidney Int, 2004;* 66: 753–760.
11. Craig KJ, Williams JD, Riley SG, Smith H, Owens DR, Worthing D, Cavill I, Phillips AO. Anemia and Diabetes in the Absence of Nephropathy. *Diabetes Care, 2005;* 28(5): 1118–1123.
12. Chiou TT, Lee JJ, Wang MC, Chung MS, Pan LL, Hsieh CJ, Huang ST, Chang HW, Yang KD, Lee CT, Liu RT. Genetic disposition and modifiable factors independently associated with anemia in patients with type 2 diabetes mellitus. Diabetes Res Clin Pract, 2015; 108(1): 164-169.
13. El-Achkar TM, Ohmit SE, McCullough PA, Crook ED, Brown WW, Grimm R, Bakris GL, Keane WF, Flack JM; Kidney Early Evaluation Program.. Higher prevalence of anaemia with diabetes mellitus in moderate kidney insufficiency: The Kidney Early Evaluation Program. Kidney Int, 2005; 67(4): 1483-1488.
14. Mazokopakis EE, Starakis IK. Recommendations for diagnosis and management of metformin-induced vitamin B12 (Cbl) deficiency. Diabetes Res Clin Pract, 2012; 97(3): 359-367.
15. Al-Habori M, Al-Mamari M, Al-Meeri A. Type II Diabetes Mellitus and impaired glucose tolerance in Yemen: prevalence, associated metabolic changes and risk factors. Diabetes Res Clin Prac, 2004; 65(3): 275–281.
16. Al-Ghazaly J., Al-Selwi AH., Abdullah M., Al-Jahafi AK, Al-Dubai W., Al-Hashdi A. Pattern of haematological diseases diagnosed by bone marrow examination in Yemen: a developing country experience. Clin Lab Haematol, 2006; 28(6): 376-81.
17. Bishop ML, Duben-Enelkrik JL and Fody EP (eds). Clinical Chemistry: Principles, Procedures, Correlations. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2000.
18. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A New Equation to Estimate Glomerular Filtration Rate. *Ann Intern Med*, 2009; 150(9): 604–612.
19. Calculate eGFR using the CKD-EPI. Calculate by QxMD formula. Available at: eGFR%20using%20CKD-EPI%20_%20Calculate%20by%20QxMD2.html (last accessed: 3o October 2017).
20. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011 (WHO/NMH/NHD/ MNM/11.1) http://www.who.int/vmnis/indicators/haemoglobin.pdf. Accessed 02/01/2018.
21. Hoffbrand V, Moss P. Essential Haematology (6th ed). Wiley-Blackwell. 2011. Page 27.
22. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guidelines for the Evaluation and Management of Chronic Kidney Disease. *Kidney International Suppl*, 2013; 3(1): 1-1630.
23. Kengne AP, Czernichow S, Hamer M, Batty GD, Stamatakis E. Anaemia, haemoglobin level and

cause-specific mortality in people with and without diabetes. PLoS One, 2012; 7(8): e41875.
24. WHO Library Cataloguing-in-Publication Data Global report on diabetes. Available at: http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf. Last accessed: December 15, 2017.
25. Gunaid AA, El Khally FM, Hassan NA, Mukhtar el D. Demographic and clinical features of diabetes mellitus in 1095 Yemeni patients. Ann Saudi Med, 1997; 17(4): 402–9.
26. Cawood TJ, Buckley U, Murray A, Corbett M, Dillon D, Goodwin B, Sreenan S. Prevalence of anaemia in patients with diabetes mellitus. Ir J Med Sci, 2006; 175(2): 25–7.
27. 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. J Med Case Rep, 2016; 10(1): 110.
28. Al-Sharafi BA, Gunaid AA. Prevalence of Obesity in Patients With Type 2 Diabetes Mellitus in Yemen. Int J Endocrinol Metab, 2014; 12(2): e13633.
29. Subashini CT, Intan NS, Elizabeth G, Lydiar KR, Nur SS, Zanariah H, Nurain MN, Masni M. Anaemia in Type 2 Diabetes Mellitus (T2DM) Patients in Hospital Putrajaya. Malaysian J Med Health Sci, 2015; 11(1): 49-61.
30. Thomas MC, MacIsaac RJ, Tsalamandris C, Jerums G. Elevated iron indices in patients with diabetes. Diabet Med, 2004; 21(7): 798–802, 2004.
31. Chamberlain JJ, Rhinehart AS, Shaefer CF Jr, Neuman A. Diagnosis and Management of Diabetes: Synopsis of the 2016 American Diabetes Association Standards of Medical Care in Diabetes. *Ann Intern Med*, 2016; 164(8): 542-552.
32. Elias E. Mazokopakis a, Ioannis K. Starakis. Recommendations for diagnosis and management of metformin-induced vitamin B12 (Cbl) deficiency. Diabetes Res Clin Prac, 2012; 79(3): 359-367.
33. de Groot-Kamphuis DM, van Dijk PR, Groenier KH, Houweling ST, Bilo HJ, Kleefstra N. Vitamin B12 deficiency and the lack of its consequences in type 2 diabetes patients using metformin. Neth J M, 2013; 71(7): 386-390.
34. Al-Ghazaly J, Al-Dubai W, Abdullah M, Al-Mahagri A, Al-Gharasi L. A Ten Year Descriptive Study of Adult Leukaemia at Al-Jomhori Teaching Hospital in Sana'a, Yemen. Yemeni J Med Sci, 2014; (8).
35. Seymour EK, Saiya-Cork K, Parkin B, Shedden K, Griggs J, Malek SN. The Impact of Diabetes on Clinical Outcomes in Chronic Lymphocytic Leulemia. Blood, 2015; 126(23): 2095.
36. Izaks GJ, Westendorp RG, Knook DL. The definition of anemia in older persons. *JAMA*, 1999; 281(18): 1714–1717.
37. Penninx BW, Pahor M, Woodman RC, Guralnik JM. Anemia in old age is associated with increased mortality and hospitalization. *J Gerontol A Biol Sci Med Sci*, 2006; 61(5): 474–479.
38. Culleton BF, Manns BJ, Zhang J, Tonelli M, Klarenbach S, Hemmelgarn BR. Impact of anemia on hospitalization and mortality in older adults. *Blood*, 2006; 107(10): 3841–3856.