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Anemia in Patients with Type 2 Diabetes in Absence of Renal Impairment

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ABSTRACT

Objectives: *this study aims to determine the prevalence of anemia in patients with type 2 diabetes in absence of renal impairment and its association with microalbuminuria and estimated glomerular filtration rate (eGFR).*

Materials and Methods: *this study included 100 patients with type 2 diabetes without renal impairment (eGFR ≥ 90ml/min) and 35 healthy subjects as control group. For all studied subjects, full history, physical examination and the following laboratory investigations were done: complete blood count, fasting and postprandial serum glucose, hemoglobin A1c, serum creatinine, eGFR, spot urine analysis for microalbuminuria.*

Results: *the study showed that anemia was present in 34% of patients with type 2DM in absence of renal impairment. The anemic diabetic group had a significantly longer duration of diabetes, significantly lower eGFR and significantly higher urinary albumin excretion rate than the non-anemic diabetic group with P-value=0.000 for each. In the diabetic patients, the study showed that the hemoglobin level had highly significant negative correlation with the duration of diabetes (r=-0.370, P=0.000) and the urinary albumin excretion rate (r=-0. 508, P=0. 000), while it had highly significant positive correlation with eGFR (r=0. 359, P=0. 000).*

Conclusions: *we concluded that the prevalence of anemia in patients with type 2 diabetes in absence of renal impairment was 34%. The diagnosis of anemia in diabetic patients may be good indicator for early diagnosis of kidney injury and diabetic nephropathy, therefore hemoglobin level can be used as a simple tool for diabetic patients' follow up.*

Keywords: *anemia, type 2 diabetes, normal renal function.*

INTRODUCTION

According to WHO criteria [1], the lower limit of normal hemoglobin (Hb) in adults is 13 g/dl in men and 12 g/dl in women. Anemia is the most common blood disorder in patients with diabetes and a significant risk factor for diabetic complications [2]. A reduced Hb concentration, even within the normal range, identifies patients with diabetes at increased risk for hospitalization and premature death [3]. Previous studies have shown that the incidence of anemia in diabetic patients is mostly associated with the presence of renal insufficiency. Thus, patients with diabetes have a greater degree of anemia for their level of renal impairment than non-diabetic patients presenting with other causes of renal failure [4]. Factors suggested as the reason for the earlier onset of anemia in patients with diabetes include severe symptomatic autonomic neuropathy causing efferent sympathetic denervation of the kidney and loss of appropriate erythropoietin; damage to the renal interstitium; systemic inflammation; and inhibition of erythropoietin release [5].

There are several previous studies regarding the presence of anemia in diabetic patients with renal impairment, but few studies exist on the presence of anemia in diabetic patients with normal renal function. This may explain why most diabetic patients with normal renal function are rarely tested for anemia. The need for more studies on presence of anemia in diabetic patients with normal renal function has therefore become highly important for better understanding of anemia among the diabetic patients. Therefore, this study aims to determine the prevalence of anemia in patients with type 2 diabetes in absence of renal impairment and its association with microalbuminuria and estimated glomerular filtration rate (eGFR).

PATIENTS AND METHODS

This cross-sectional study was conducted on one hundred patients with type 2 diabetes mellitus without renal impairment (eGFR≥90 ml/min) attending the diabetes clinic of Internal Medicine Department at Assiut University Hospitals, Assiut, Egypt, in the period between January 2012 and January 2014. Also, the study included thirty five nondiabetic healthy subjects as control group. Informed consent was taken from all subjects of the study. This study was approved by ethical committee of college of medicine, Assiut University.Any diabetic patient with reduced kidney function was excluded from the study. All patients and controls were subjected to the following; full history taking including: age, sex, the duration of diabetes and thorough clinical examination including: measurement of height and weight, calculation of body mass index (BMI) with the following formula:BMI=weight (kg)/height^2 (m^2), measurement of systolic and diastolic blood pressure.

Laboratory investigations were done for all subjects including: fasting and postprandial serum glucose:done by Enzymatic Colorimetric Assay (Roche/Hitachi912/Modular,analyzers:ACN512,Cobas)[6]. Glycated hemoglobin (HbA1c) was done

by Turbidimetric Inhibition Immunoassay[7]. Complete blood count (CBC) was done by automated blood coulter(Cell-Dyn3700). Anemia was defined according to WHO criteria [1].Serum creatinine(sCr) was measured by using the modified kinetic Jaffe method (Roche/Hitachi 912 analyzer:ACN439Blank(twin mode),Cobas) [8]. Estimated glomerular filtration rate (eGFR) was calculated by using Cockcroft and Gault equation: eGFR (ml/min.)= ((140-age) x weight in Kg.)/ (serum creatinine in mg/dl x 72) (x 0.85 for females) [9]. Urine analysis for microalbuminuria: urinary albumin excretion rate was measured in a spot urine sample by using Immunoturbidimetric assay(Roche/HitachiModular,Analyzer/Cobas)[1]. Classification of microalbuminuria was shown in table 1 according to American Diabetes Association [11]. Abdominal ultrasound was also done for all subjects.

Statistical Analysis

Statistical analysis was performed using the SPSS 21.0 statistical software Package. Continuous variables were expressed as mean ± SD. Categorical variable were expressed as numbers and percentages. Paired-samples and independent-samples student t tests were used to compare variables between patients and controls or between patients` groups. Bivariate-pearson correlation was used to investigate for potential relations between variables. P-value < 0.05 was considered statistically significant.

RESULTS

Characteristics of Patients and Controls

The study included 100 patients with type 2 diabetes without renal impairment, 65 cases (65%) were males and 35 cases (35%) were females. Their ages ranged from 40 to 73 years with mean ± SD was 55.50 ± 8.04 years. The duration of diabetes ranged from 1year to 22year with mean ±SD was 8.13 ± 4.18 years. The study also included 35 nondiabetic healthy controls, 22 of them (62.9%) were males, while the remaining 13 (37.1%) were females. Their ages ranged from 40 to 69 years with mean ± SD was 54.60 ± 8.99 years. Other clinical and laboratory characteristics of the patients and controls were shown in table 2.

Prevalence of Anemia in Type 2 Diabetes without Renal Impairment

The study showed that anemia was present in 34 patients (34%) from total 100 diabetic patients,; 20 patients (58.8%) from anemic patients had normocytic anemia and 14 patients (41.2%) had microcytic anemia (figure1). Also the study showed that anemia was present in 8 subjects (22.9%) from the total 35 healthy controls,; 5 subjects (62.5%) had microcytic anemia and 3 subjects (37.5%) had normocytic anemia.

Comparison between Patients and Controls

The study revealed that the diabetic group had insignificantly lower mean Hb level than the control group (12.64 ± 1.80 gm/dl vs 13.14 ± 1.56, p-value=0.145). The diabetic group had a significantly higher systolic blood pressure, fasting serum glucose, postprandial serum glucose and glycated hemoglobin (HbA1c) levels than

control group (135.55 ± 12.97 vs 127.86 ± 10.73 mmHg,P=0.002) (171.39 ± 24.57vs 96.06 ± 9.59mg/dl,P-value=0.000) (261.23 ± 63.22 vs 127.20 ± 6.0 mg/dl,P=0.000) (6.80 ± 0.80 vs 4.90 ± 0.49%, P=0.000) respectively. The study showed that the diabetic group had a significantly higher serum creatinine level, higher urinary albumin excretion rate, and a highly significant lower eGFR than the control group (1.04 ± 0.13 vs 0.90 ± 0.12mg/dl P=0.000)(69.91 ± 77.22 vs 6.97 ± 3.10µg/mg with P-value=0.000) (97.52 ±7.91 vs 108.20 ±8.69 ml/min with P-value=0.000) respectively (table 2). Also, the study showed that the males diabetic group had a significantly higher number of anemic patients than the males control group (21/65 vs 2/22 with P-value=0.033) **(table3).**

Comparison between Anemic and Non-anemic Groups in Diabetic Patients

In the diabetic patients , the study showed that the anemic group had a significantly longer duration of diabetes (10.21 ± 4.75yrs vs 7.06 ± 3.42 yrs with P=0. 000) and a significantly lower number of red blood cells than the non-anemic group (3.89 ± 0.76 vs 4.40 ± 0.74 million/l, P-value=0.002). The anemic group had insignificantly higher glycated hemoglobin than the non-anemic group (6.94 ± 0.94% vs 6.73 ± 0.71%, P-value=0.208). Also, the anemic group had a highly significant lower eGFR (92.99 ± 2.87 vs 99.86 ± 8.65 ml/min, P-value=0.000) and significantly higher urinary albumin excretion rate than the non-anemic group (138.62 ± 84.29 vs 26.94 ± 34.20 µg/mg , P-value=0.000) (figures 2, 3 and table 4).

Comparison between Anemic Groups in both Patients and Controls

The study showed that the anemic diabetic group had a significantly older age (58.82 ± 7.47 vs 47.75 ± 8.19 years, P-value=0.001), a significantly higher systolic blood pressure and higher diastolic blood pressure than the anemic control group (138.82 ± 11.22 vs 120.63 ± 11.48mmHg, P=0.000) (86.62 ± 5.47vs 81.25 ± 7.44mmHg, P=0.025) respectively.The anemic diabetic group had significantly higher fasting serum glucose , higher postprandial serum glucose, and higher glycated hemoglobin (HbA1c) than the anemic control group (176.15 ± 26.12 vs 99.87 ± 16.82 mg/dl, P=0.000) (271.00 ± 68.85 vs139.88 ± 14.40mg/dl, P=0.000) (6.94 ± 0.94 vs 5.01 ± 0.76% P=0.000) respectively. The anemic diabetic group had significantly higher serum creatinine , highly significant lower eGFR and significantly higher urinary albumin excretion rate than the anemic control group(1.07 ± 0.15 vs 0.89 ± 0.14 mg/dl, P=0.003)(92.99 ± 2.87vs 95.50 ± 3.55 ml/min, P value=0. 0.039) (138.62 ± 84.29 vs 7.50 ± 2.14 µg/mg, P value=0.000) respectively **(table 5).**

Correlations between Hemoglobin Levels and Clinical & Laboratory Data in Diabetic Patients

In diabetic patients, there was a highly significant negative correlation between hemoglobin level and duration of DM (r=-0.370, p<0.000). Also, there was a highly significant negative correlation between hemoglobin level and urinary albumin excretion rate (r=-0.508, P=0.000). Furthermore,

there was a highly significant positive correlation between hemoglobin level and eGFR (r-value=0.359, P-value=0.000) (table 6 and figures 4,5,6).

Table 1 American Diabetes Association Classification of Microalbuminuria.

Urinary albumin excretion rate	Category
Less than 30 µg/mg	Normal
30-300 µg/mg	Microalbuminuria
More than 300 µg/mg	Clinical albuminuria

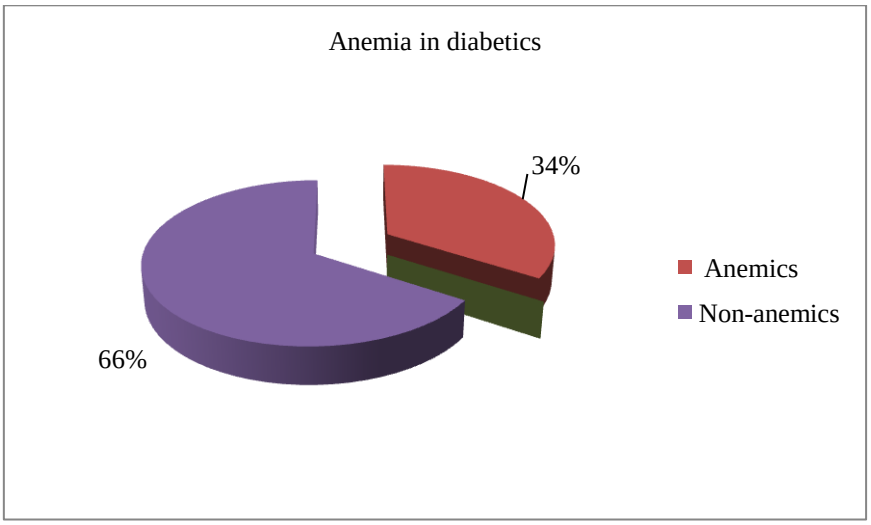

Figure 1 Frequency of anemia in diabetic patients.

Table 2 The comparison between patients and controls.

	Diabetics (n= 100) Mean ± SD	Controls (n= 35) Mean ± SD	P-value
Age: (years)	56.99 ± 8.08	54.60 ± 8.99	0.108
Sex:			
Males	65 (65.0%)	22 (62.9%)	0.820
Females	35 (35.0%)	13 (37.1%)	
BMI (kg/m2)	27.77 ± 2.93	27.94 ± 2.65	0.760
Duration of DM (years)	8.13 ± 4.18	--	--
Systolic BP (mmHg)	135.55 ± 12.97	127.86 ± 10.73	0.002*
Diastolic BP (mmHg)	84.95 ± 7.80	82.71 ± 6.68	0.133
Fasting serum glucose (mg/dl)	171.39 ± 24.57	96.63 ± 11.21	0.000*
Postprandial serum glucose(mg/dl)	261.23 ± 63.22	134.83 ± 10.97	0.000*
HbA1c(%):	6.80 ± 0.80	4.90 ± 0.49	0.000*
< 7	53 (53.0%)	35 (100.0%)	0.000*
≥ 7	47 (47.0%)	0 (0.0%)	
RBC (million/l)	4.22 ± 0.78	4.27 ± 0.57	0.744
Hb (g/dl):	12.64 ± 1.80	13.14 ± 1.56	0.145
Anemic	34 (34.0%)	8 (22.9%)	0.220

Non-anemic	66 (66.0%)	27 (77.1%)	
MCV(fl):	82.31 ± 6.54	82.94 ± 4.88	0.599
Microcytic anemia	14 (41.2%)	5 (62.5%)	0.487
Normocytic anemia	20 (58.8%)	3 (37.5%)	
MCHC (g/dl)	31.50 ± 2.54	30.29 ± 2.91	0.000*
MCH (pg)	28.29 ± 3.10	30.09 ± 1.63	0.001*
Serum creatinine (mg/dl)	1.04 ± 0.13	0.90 ± 0.12	0.000*
eGFR (ml/min)	97.52 ± 7.91	108.20 ±8.69	0.000*
Microalbuminuria	38 (38.0%)	0 (0.0%)	0.000*
UA excretion rate (µg/mg)	69.91 ± 77.22	6.97 ± 3.10	0.000*

Table 3 Comparison of anemia in diabetic and control groups according to sex.

Sex	Diabetics (n=100)		Controls (n= 35)		P-value
	No.	%	No.	%	
Males (n=65)/(n=22)	21	32.3	2	9.1	0.033*
Females (n=35)/(n=13)	13	37.1	6	46.2	0.571

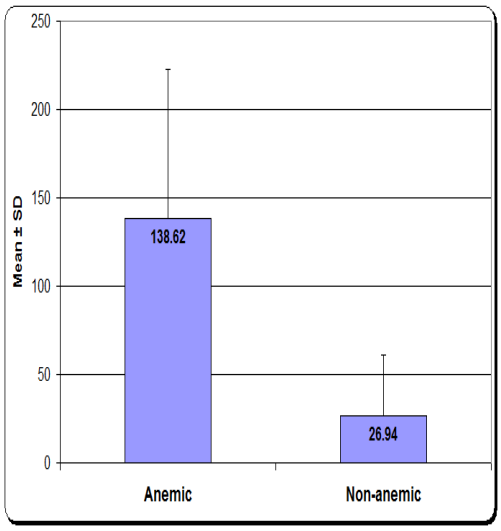

Figure 2 Comparison between anemic and non anemic groups in diabetic patients according to urinary albumin excretion rate

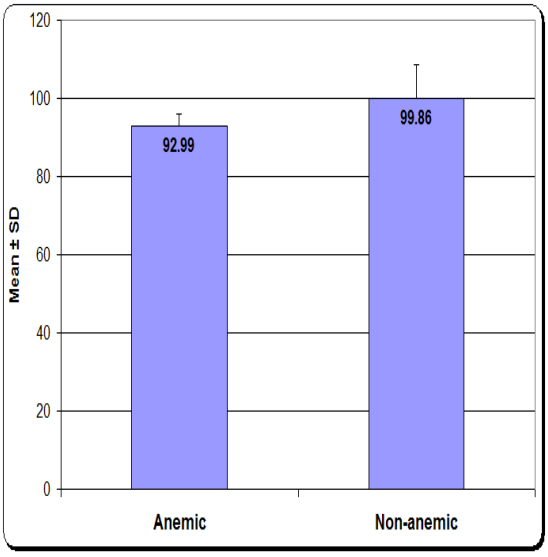

Figure 3 Comparison between anemic and non anemic groups in diabetic patients according to eGFR.

Table 4 Comparison between anemic and non-anemic groups in diabetic patients.

	Anemic (n= 34) Mean ± SD	Non-anemic (n= 66) Mean ± SD	P-value
Age: (years)	58.82 ± 7.47	56.05 ± 8.28	0.104
Sex:			
Males	21 (61.8%)	44 (66.7%)	0.626
Females	13 (38.2%)	22 (33.3%)	
BMI(kg/m2)	27.84 ± 2.88	27.74 ± 2.97	0.870
Duration of DM(year)	10.21 ± 4.75	7.06 ± 3.42	0.000*
Systolic BP(mmHg)	138.82 ± 11.22	133.86 ± 13.55	0.070
Diastolic BP(mmHg)	86.62 ± 5.47	84.09 ± 8.68	0.126
Fasting serum glucose(mg/dl)	176.15 ± 26.12	168.94 ± 23.56	0.166
Postprandial serum glucose(mg/dl)	271.00 ± 68.85	256.20 ± 60.04	0.269
HBA1c %:	6.94 ± 0.94	6.73 ± 0.71	0.208
< 7	17 (50.0%)	36 (54.5%)	0.666
≥ 7	17 (50.0%)	30 (45.5%)	
RBC(million/l)	3.89 ± 0.76	4.40 ± 0.74	0.002*
MCV(fl):	80.10 ± 7.29	83.44 ± 5.85	0.015*
Microcytic anemia	14 (41.2%)	16 (24.2%)	0.080
Normocytic anemia	20 (58.8%)	50 (75.8%)	
MCHC(g/dl)	31.27 ± 2.92	31.62 ± 2.34	0.527
MCH(pg)	27.32 ± 3.39	28.79 ± 2.85	0.024*
Serum creatinine (mg/dl)	1.07 ± 0.15	1.02 ± 0.12	0.090
eGFR (ml/min)	92.99 ± 2.87	99.86 ± 8.65	0.000*
UA excretion rate (µg/mg)	138.62 ± 84.29	26.94 ± 34.20	0.000*

Table 5 Comparison between anemic diabetic group and anemic control group.

	Anemic Diabetic (n= 34) Mean ± SD	Anemic Control (n= 8) Mean ± SD	P-value
Age: (years)	58.82 ± 7.47	47.75 ± 8.19	0.001*
Sex:			
Males	21 (61.8%)	2 (25.0%)	0.138
Females	13 (38.2%)	6 (75.0%)	
BMI (kg/m2)	27.84 ± 2.88	27.13 ± 2.47	0.522
Duration of DM(years)	10.21 ± 4.75	--	--
Systolic BP (mmHg)	138.82 ± 11.22	120.63 ± 11.48	0.000*
Diastolic BP (mmHg)	86.62 ± 5.47	81.25 ± 7.44	0.025*
Fasting serum glucose (mg/dl)	176.15 ± 26.12	99.87 ± 16.82	0.000*
Postprandial serum glucose(mg/dl)	271.00 ± 68.85	139.88 ± 14.40	0.000*
HBA1c (%):	6.94 ± 0.94	5.01 ± 0.76	0.000*
< 7	17 (50.0%)	8 (100.0%)	0.028*
≥ 7	17 (50.0%)	0 (0.0%)	
RBC(million/l)	3.89 ± 0.76	3.94 ± 0.56	0.865
MCV(fl):	80.10 ± 7.29	77.50 ± 2.56	0.330
Microcytic anemia	14 (41.2%)	5 (62.5%)	0.487
Normocytic anemia	20 (58.8%)	3 (37.5%)	
MCHC (g/dl)	31.27 ± 2.92	26.38 ± 2.62	0.000*
MCH (pg)	27.32 ± 3.39	29.38 ± 2.26	0.112
Serum creatinine (mg/dl)	1.07 ± 0.15	0.89 ± 0.14	0.003*
eGFR (ml/min)	92.99 ± 2.87	95.50 ± 3.55	0.039*
UA excretion rate (µg/mg)	138.62 ± 84.29	7.50 ± 2.14	0.000*

Table 6 Correlation between haemoglobin levels and clinical & lab. data in diabetic patients.

	Hemoglobin (HB)	
	r-value	P-value
Duration of DM	-0.370	0.000*
eGFR	0.359	0.000*
Urinary albumin excretion rate	-0.690	0.000*

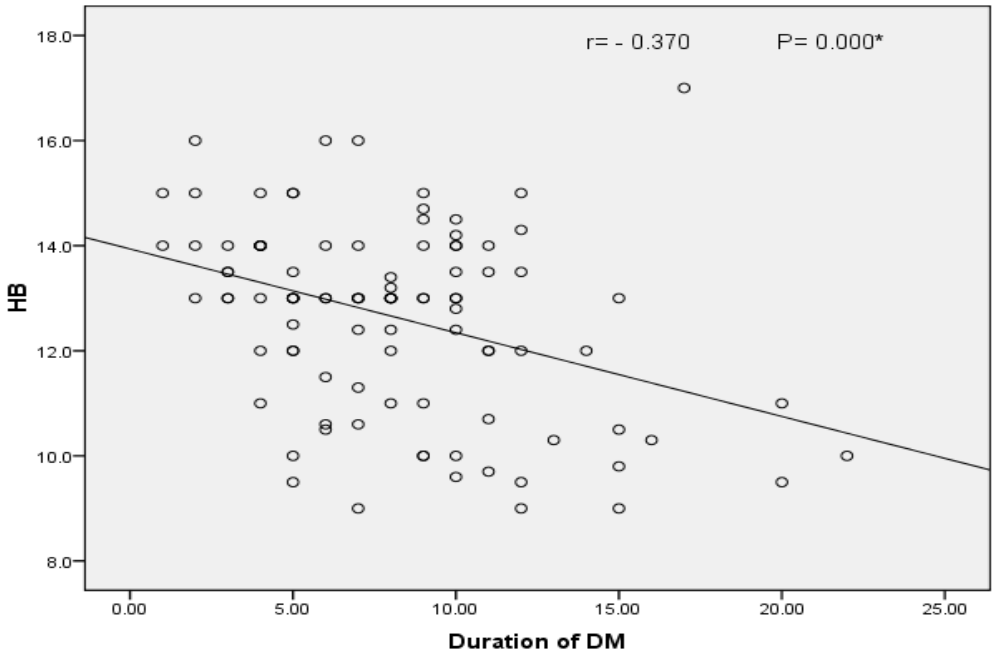

Figure 4 The negative correlation between hemoglobin level and duration of diabetes in diabetic patients.

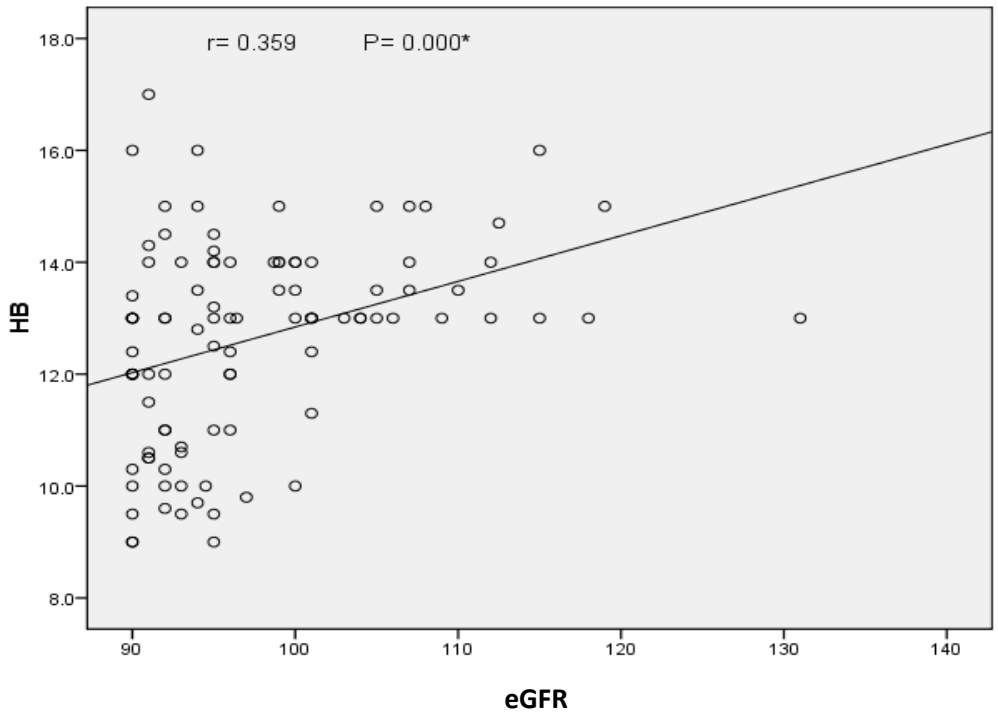

Figure 5 The positive correlation between hemoglobin level and eGFR in diabetic patients.

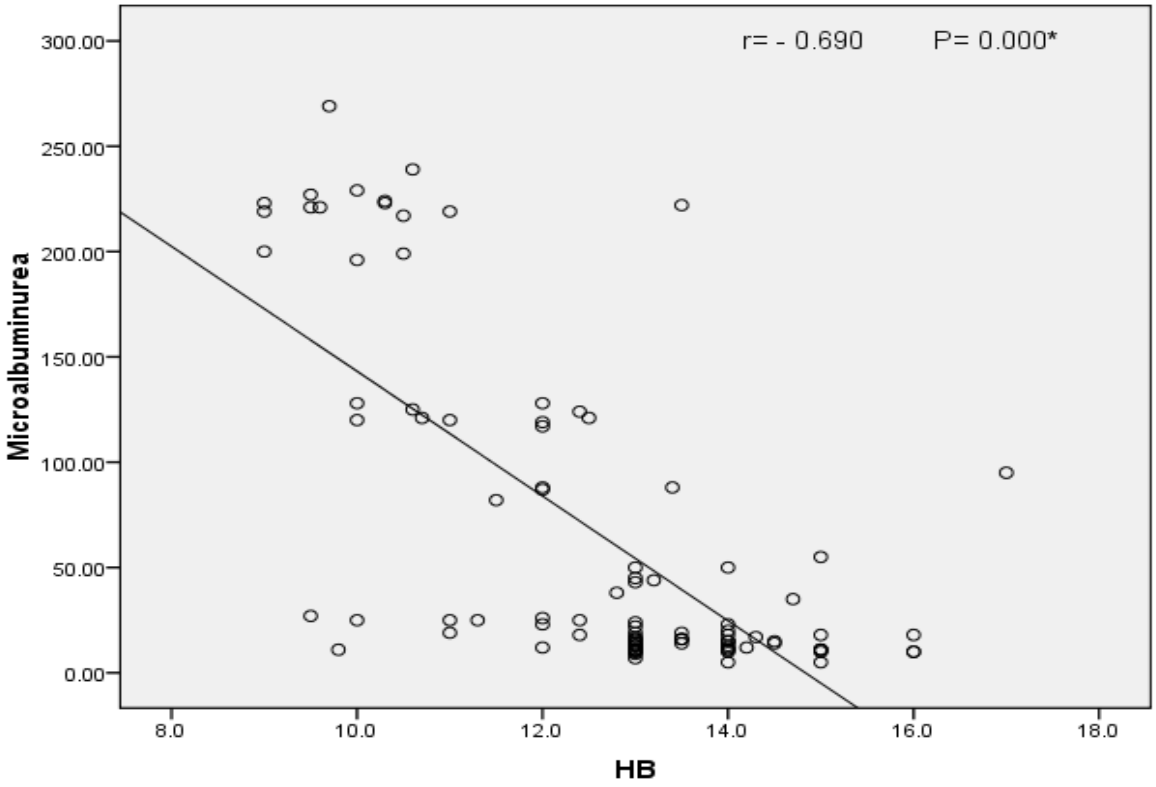

Figure 6 The negative correlation between hemoglobin level and urinary albumin excretion rate in diabetic patients.

DISCUSSION

Anemia is the most common blood disorder and a common finding in patients with diabetes [2]. Also, patients with diabetes have a greater degree of anemia for their level of renal impairment than non-diabetic patients presenting with other causes of renal failure [4]. Several studies have shown the presence of anemia in diabetic patients with renal impairment while few studies exist on the presence of anemia in diabetics with normal renal function. The need for more studies on presence of anemia in diabetic patients with normal renal function has become highly important, therefore this study aimed to determine the prevalence of anemia in patients with type 2 diabetes in absence of renal impairment for more understanding of anemia among the diabetic patients.

The study showed that anemia was present in 34% of patients with type 2 DM in absence of renal impairment.This result was consistent with the study of Goldhaber et al. [12] who reported that the prevalence of anemia in unselected patients with diabetes and normal serum creatinine levels was 32%. Also the study showed that, in the diabetic group, 32.3% of the diabetic males were anemic and 37.1% of the diabetic females were anemic and 50 % of overall anemic patients had poorly controlled DM. These results were concordant to the results reported by Adejumo et al. [13], who studied 72

patients with type 2 DM in absence of renal impairment, and stated that anemia is common among the diabetic patients, the prevalence of anemia was 15. 3%, 20.6% of the diabetic men were anemic, and 10.5% of the diabetic women were anemic , 25% of the anemic patients had poorly controlled diabetes and 3.1% of the anemic patients had controlled diabetes. Also, Grossman et al.[14] reported that anemia is more common in diabetic patients even when eGFR > 60 ml/min and anemia was observed in 10.8% of the diabetic group and in only 2.7% in non-diabetic group. Also a previous study by Bonakdaran et al.[15] reported that 7.2% of diabetics with normal renal function had anemia. In the present study, 58.2% of anemic diabetic patients had normocytic anemia and 41.8% had microcytic anemia. This result was in concordance to the results reported by Chen et al.[16] who found that normocytic anemia was present in 61% of patients , whilst fewer patients had microcytic (27%) or macrocytic (12%) anemia. Factors suggested as the reasons for the earlier onset of anemia in patients with diabetes include severe symptomatic autonomic neuropathy causing efferent sympathetic denervation of the kidney and loss of appropriate erythropoietin; damage to the renal interstitium; systemic inflammation; and inhibition of erythropoietin release [5].

The study showed that the diabetic group had a significantly higher serum creatinine level, a significantly higher urinary albumin excretion rate, and highly significant lower eGFR than the control group with P-value=0.000 for each. These results were also similar to the results reported by Kundu et al.[17]. Also in this study, the anemic diabetic group had a significantly longer duration of diabetes, highly significant lower eGFR and significantly higher urinary albumin excretion rate than the non-anemic diabetic group with P- value=0.000 for each. These results were in agreement with the results reported by Ezenwaka et al.[18]. In addition, the study showed that, in the diabetic patients, the hemoglobin level had highly significant negative correlation with the duration of diabetes (r-value=-0.370, P-value=0.000) and the urinary albumin excretion rate (r-value=-0. 508, P-value=0. 000) and also the hemoglobin level had highly significant positive correlation with eGFR (r-value=0. 359, P-value=0. 000). These results were in concordance to the results reported by Chen et al.[16] and Abate et al.[19].

Diabetes mellitus is the leading cause of end-stage renal disease in several countries. It is also the cause of chronic hemodialysis and renal transplantation. Cardiovascular disease is the most common cause of death in diabetic patients with chronic kidney disease (CKD) and anemia appears to be a risk factor for mortality among these patients. Patients with diabetes may be more vulnerable to the effects of anemia, since many patients have significant cardiovascular disease and hypoxia-induced organ damage [20]. Observational studies indicate that low Hb levels in diabetic patients may increase risk for progression of kidney disease and cardiovascular morbidity and mortality [21]. Anemia is often

more severe and occurs at an earlier stage of CKD in individuals with diabetes compared with those with other causes of CKD.The diagnosis of anemia in diabetic patients may be an early indicator for the diagnosis of kidney injury and diabetic nephropathy and hemoglobin level can be used as a simple indicator for early diagnosis of diabetic nephropathy.

CONCLUSIONS

We concluded that the prevalence of anemia in patients with type 2 diabetes in absence of renal impairment was 34%. The diagnosis of anemia in diabetic patients may be good indicator for early diagnosis of kidney injury and diabetic nephropathy, therefore hemoglobin level can be used as a simple tool for diabetic patients' follow up.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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