

Original article

Risk factors associated with anemia in diabetes mellitus type 2 with moderate renal impairment

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Abstract:

Background: Anemia is a common problem in type 2 diabetes mellitus (DM). The prevalence of anemia in moderate renal impairment (glomerular filtration rate; GFR 15-59 mL/min/1.73m²) was found predominantly among patients coexisting with type 2 DM compared with no type 2 DM. Related studies reported several factors such as diabetic retinopathy (DR), diabetic nephropathy (DN), erythropoietin resistance, obesity, metformin use and hyperglycemia associated with anemia. Identifying risk factors related to anemia may improve patient outcomes.

Objectives: The study aimed to determine the risk factors associated with anemia in type 2 DM coexisting with moderate renal impairment. **Material and Methods:** Medical records of diabetic patients visiting the HRH Princess Maha Chakri Sirindhorn Medical Center from 2009-2014 were gathered and reviewed as a retrospective cross-sectional study. Anemia was defined as hemoglobin (Hb) less than 13 g/dL among males and 12 g/dL among females. **Results:** From 699 medical records, 120 participants (17.7%) with moderate renal impairment were included in the study. Sixty-five (54.2%) of 120 patients had anemia. The median age of patients was 62.3 years (43-86). The complications of type 2 DM included DN 92 (76.7%) and DR 15 (12.5%). Mean Hb was 12.4 g/dL (8.9-17.6) and mean GFR was 48.5 mL/min/1.73m² (15.6-59.9). Females ($p = 0.001$), and DR ($p = 0.05$) were risk factors associated with anemia, after multivariate analysis. **Conclusion:** Anemia was found in 54.2% of diabetic cases. Females and DR were risk factors associated with anemia in type 2 DM with moderate renal function.

Keywords : ● Anemia in diabetes mellitus ● Anemia of chronic inflammation ● Diabetes mellitus type 2
● Renal impairment

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นิพนธ์ต้นฉบับ

ปัจจัยเสี่ยงต่อภาวะโลหิตจางในผู้ป่วยโรคเบาหวานชนิดที่ 2 ที่มีค่าการทำงานของไตเสื่อมปานกลาง

นิตา มะเคือลี ธนยา ศิริบุญญ์ รัชพร ทวีรุจนะ และ สุภาพ มะเคือลี

บทคัดย่อ

ความเป็นมา ภาวะโลหิตจางเป็นภาวะที่พบบ่อยในผู้ป่วยเบาหวานชนิดที่ 2 โดยพบว่าความชุกของภาวะโลหิตจางในผู้ป่วยที่มีค่าการทำงานของไตปานกลาง (GFR 15-59 mL/min/1.73m²) สูงถ้ามีโรคเบาหวานร่วมด้วย พบว่ามีหลายปัจจัยที่เป็นสาเหตุที่ทำให้เกิดภาวะโลหิตจาง ได้แก่ ภาวะแทรกซ้อนทางตาในเบาหวาน (Diabetic retinopathy) ภาวะแทรกซ้อนทางไตในเบาหวาน (Diabetic nephropathy) ภาวะดื้อต่ออีริโทรพอยอีติน (erythropoietin) ภาวะอ้วน และภาวะน้ำตาลที่สูงในผู้ป่วยที่เป็นโรคเบาหวาน ดังนั้นการศึกษาเพื่อหาปัจจัยที่มีผลต่อภาวะโลหิตจางในผู้ป่วยกลุ่มนี้ซึ่งจะทำให้ผลลัพธ์ของการรักษาดีขึ้น **วัตถุประสงค์** เพื่อศึกษาปัจจัยที่มีความเสี่ยงต่อภาวะโลหิตจางในผู้ป่วยเบาหวานชนิดที่ 2 ที่มีค่าการทำงานของไตปานกลาง **วิธีการ** เป็นการศึกษาย้อนหลังแบบภาคตัดขวาง โดยทบทวนจากเวชระเบียนของผู้ป่วยนอกที่ได้รับการวินิจฉัยเป็นโรคเบาหวานที่เข้ารับการรักษาในศูนย์การแพทย์สมเด็จพระรัตนราชสุตาฯ สยามบรมราชกุมารีระหว่างปี 2552 ถึงปี 2557 จะได้รับการวินิจฉัยว่ามีภาวะโลหิตจางเมื่อมีระดับฮีโมโกลบินน้อยกว่า 13 g/dL ในผู้ชาย และน้อยกว่า 12 g/dL ในผู้หญิง **ผลการศึกษา** จากการทบทวนเวชระเบียนของผู้ป่วยทั้งหมด 699 ราย พบผู้ป่วยที่เป็นเบาหวานที่มีค่าไตเสื่อมปานกลางทั้งหมด 120 คน คิดเป็นร้อยละ 17.7 โดยมีภาวะโลหิตจางอยู่ที่ร้อยละ 54.2 สำหรับค่ามัธยฐานของอายุผู้ป่วยคือ 62.3 ปี (ช่วง 43-86 ปี) มีผู้ป่วย 92 ราย คิดเป็นร้อยละ 76.7 มีภาวะแทรกซ้อนทางไตจากเบาหวาน และมีผู้ป่วย 15 ราย คิดเป็นร้อยละ 12.5 มีภาวะแทรกซ้อนทางตาจากเบาหวาน สำหรับค่าเฉลี่ยฮีโมโกลบินคือ 12.4 g/dL ค่าเฉลี่ยการกรองของไตอยู่ที่ 48.5 mL/min/1.73m² (ช่วง 15.6-59.9). จากการวิเคราะห์ทางพหุตัวแปรพบว่าปัจจัยเสี่ยงต่อภาวะโลหิตจางคือ เพศหญิงโดย (p = 0.001) และ ภาวะแทรกซ้อนเบาหวานทางตา (p = 0.05) **สรุป** ภาวะโลหิตจางในผู้ป่วยเบาหวานชนิดที่ 2 ที่มีค่าการทำงานของไตปานกลางอยู่ที่ร้อยละ 54.2 โดยพบว่าเพศหญิง และ ภาวะแทรกซ้อนทางตาเบาหวานเป็นปัจจัยเสี่ยงต่อภาวะโลหิตจาง

คำสำคัญ : ● ภาวะโลหิตจางในเบาหวาน ● ภาวะโลหิตจางจากการอักเสบเรื้อรัง ● เบาหวานชนิดที่ 2

● ภาวะการดำเนินงานของไตเสื่อม

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2565;32:139-48.

Introduction

Anemia is a common problem in chronic kidney disease (CKD) especially with declining glomerular filtration rate (GFR) less than 60 mL/min/1.73m². Patients with diabetes mellitus (DM) and moderate renal impairment experience anemia more than patients with CKD without comorbid DM. A related study reported the prevalence of anemia in moderate renal impairment between diabetic and nondiabetic cases reporting 22.2 vs. 7.9%, respectively¹. However, when GFR was less than 30 mL/min/1.73m², no difference was found regarding prevalence between DM and no DM (52.4 vs. 50%)¹. In the advanced stages of renal disease, anemia was associated with declining renal function, but could not explain absolutely that anemia involves moderate renal impairment.

Several studies have found many factors associated with anemia involving moderate renal impairment such as decreased GFR², microvascular complications including diabetic retinopathy (DR), diabetic neuropathy, and diabetic nephropathy (DN)³⁻⁸, decreased androgen hormone among males³, erythropoietin resistance³, albuminuria^{4,9-10}, hyperglycemia¹¹⁻¹², obesity and insulin resistance¹² causing chronic inflammation affecting erythropoietin production and resulting in anemia. The American Society of Diabetes Association and Thai guidelines do not recommend hemoglobin testing in newly diagnosed DM. Generally, patients with diabetes are tested for anemia when presenting anemic symptoms. Thus, early diagnosis and management can help prevent further complications.

The study aimed to identify the factors associated with anemia in type 2 DM coexisting moderate renal impairment.

Materials and Methods

Study Population

A retrospective chart review was conducted from 2009 to 2014 as a cross-sectional study. Patients with type 2 DM and moderate renal impairment (GFR 15-59

mL/min/1.73m²), not receiving erythropoietin and normal MCV (80-99 fL) were included in our study. Patients coexisting with both acquired and inherited hemolytic anemia, severe cardiovascular disease, severe peripheral vascular disease, blood donation or blood loss within three months, active cancer or cancer receiving treatment, surgery within one month, chronic inflammatory disease such as rheumatoid arthritis, systemic lupus erythematosus, history of gastrointestinal bleeding within six months, megaloblastic anemia or bone marrow disease were excluded from the study. The study was approved by the ethics committee of Srinakharinwirot University (EC 175/58E).

Definition

According to World Health Organization (WHO), anemia is defined as hemoglobin (Hb) less than 13.0 g/dL among males and less than 12.0 g/dL among females.

Baseline Data Collection

Baseline characteristics including sex, age, comorbidities, complete blood count, a microvascular complication of DM, creatinine and GFR were collected.

Statistical analysis

Descriptive analysis was used to test baseline characteristics. To identify differences between groups, categorical factors were analyzed using the Chi-square test. The student's t-test was used to analyze continuous factors. Logistic regression was used to analyze the risk factor for anemia and *p*-value less than 0.05 was considered statistically significant.

Results

Baseline Characteristics

Six hundred and ninety-nine participants with a diagnosis of type 2 DM in the diabetic clinic from 2009 to 2014 were reviewed. Of 120 (17.7%) patients with diabetes and moderate renal impairment were included in our study. Altogether, 55 (45.8%) and 36 (30%) patients resided in Nakhon Nayok and Pathumthani, respectively (Table 1). Eighty-six (71.7%), and 26 (71.7%) participants had reimbursement and universal coverage insurance, respectively (Table 1).

Table 1 Baseline characteristics of patients with anemia in diabetic mellitus type 2 with moderate renal impairment. (n = 120)

Characteristic	Number of patients	Rate (%)
Sex		
Male	57	47.5
Female	63	52.5
Primary reimbursement scheme		
Universal coverage (UC)	26	21.7
Civil Servant Medical Benefit Scheme (CSMBS)	86	71.7
Social Security Scheme	5	4.2
Self-payment	3	2.5
Province		
Nakhon Nayok	55	45.8
Pathumthani	36	30.0
Prachinburi	15	12.5
Chachoengsao	4	3.3
Bangkok	2	1.7
Ayutthaya	2	1.7
Others	6	5.0
Hypertension		
Yes	115	95.8
No	5	4.2
Dyslipidemia		
Yes	112	93.3
No	8	6.7
Heart disease		
Yes	19	15.8
No	101	84.2
Gout		
Yes	5	4.2
No	115	95.8
Cancer		
Yes	0	0
No	120	100

The median age of patients was 61.91 years (range, 43.12 to 86.21). Fifty-seven (47.5%) patients and 63 (53.5%) were male and female, respectively. Patients with coexisting hypertension, and dyslipidemia totaled 115 (95.8%) and 112 (93.3%), respectively, (Table 1). Metformin, glipizide and insulin were used to treat 68 (56.7%), 52 (43.3%) and 37 (30.8%) patients, respectively, (Table 2).

In all, 89, 75, and 81 patients received lipid-lowering drugs (statins), aspirin and angiotensin-converting enzyme inhibitor/angiotensin receptor blocker (ACEI/ARB), respectively (Table 2). The common antihypertensive drugs were losartan (66.7%), enalapril (30.9%), and telmisartan (2.5%).

Table 2 Medication used by patients with diabetes mellitus type 2 (n = 120)

Medication	Number of patients	Rate (%)
Metformin		
Yes	68	56.7
No	52	43.3
Glipizide		
Yes	52	43.3
No	68	56.7
Acarbose		
Yes	2	1.7
No	118	98.3
TZD*		
Yes	1	0.8
No	119	99.2
Insulin		
Yes	37	30.8
No	83	69.2
Aspirin		
Yes	75	62.5
No	45	37.5
Statin		
Yes	89	74.2
No	31	25.8
Fibrate		
Yes	9	7.5
No	111	92.5
ARB/ACEI*		
Yes	81	67.5
No	39	32.5

*TZD; thiazolidinediones, ARB/ACEI; angiotensin receptor blocker/angiotensin-converting-enzyme inhibitor

Of 120 participants included in the study, DN and DR were found among 92 (76.7%) and 15 (12.5%) patients (Table 3). For vascular complications, coronary artery disease, cardiovascular accident and peripheral vascular disease were found in 12, 5, and 2 participants (Table 3). Microalbuminuria and macroalbuminuria were found in 63 (68.5%), and 29 (31.5%) patients, respectively (Table 4).

Of a total of 120 participants, anemia was found in 54.2% (Table 5). Median Hb was 12.35 (range 8.9 to 17.6) and median GFR was 48.5 (range 15.6 to 59.9). Approximately 90% of patients (n = 108) had GFR 30 to 60 mL/min/1.73m². Twelve patients (10%) had GFR 15 to 29 mL/min/1.73m². Median HbA1C at 12 to 15 months before evaluation of CBC was 6.9 (range 4.7 to 12.53) (Table 5).

We found factors such as sex ($p = 0.004$) and DR ($p = 0.007$) were associated with anemia after univariate analysis as shown in Table 6. Multivariate analyses found being male ($p = 0.001$, OR = 0.24, 95%CI: 0.10 to 0.56), a protective factor, and DR ($p = 0.005$, OR 10.37, 95%CI: 2.01 to 53.65) were risk factors to anemia, after adjusting for age, gout, acarbose, glipizide, metformin, statin, DN, HbA1C, and GFR, data as shown in Table 7.

Discussion

Anemia was identified in 54.2% of patients with type 2 DM and coexisting moderate renal impairment, greater than the related study¹. A related study from JP and et al. reported the frequency of anemia in stage 3 of diabetic chronic kidney disease up to 18%¹³, but a limitation was encountered in this study due to the smaller population.

In our study, female and diabetic retinopathy were risk factors affecting anemia in type 2 DM with moderate renal impairment similar to the study findings of Feteih, et al¹⁴. However, the mechanism of diabetic retinopathy and anemia remain unclear.

Traveset, et al¹⁵ reported the association of anemia and severe diabetic eye complications. Anemia is a long term complication of DM that may contribute to retinal ischemia, consistent with the study results by He, et al.¹⁶ and the Early Treatment Diabetic Retinopathy Study¹⁷

Moreover, female was a risk factor for anemia. This may be caused by iron deficiency anemia in women of childbearing age¹⁸. However, this could not be explained in our study because the median age was 63.2 years.

Table 3 Complications of diabetes mellitus type 2 (n = 120)

Complication	Number of patients	Rate (%)
Diabetic retinopathy		
Yes	15	12.5
No	105	87.5
Diabetic nephropathy		
Yes	92	76.7
No	28	23.3
Cardiovascular accident		
Yes	5	4.2
No	115	95.8
Coronary artery disease		
Yes	12	10
No	108	90
Peripheral arterial disease		
Yes	2	1.7
No	118	98.3

Table 4 Types of diabetic nephropathy (n=92)

Type of diabetic nephropathy	Number of patients	Rate (%)
Microalbuminuria	63	68.5
Macroalbuminuria	29	31.5

Table 5 Laboratory results of patients (n=120)

Laboratory result	Mean±SD	Median	Range
Hb (g/dL)	12.38±1.90	12.35	8.9-17.6
Hct (%)	37.87±6.56	37.65	25.2-60
MCV (fL)	85.22±4.45	84.65	80-96.2
WBC (cell/ μ L)	8,221.92±2829.94	7,650	3,900-20,600
Platelet (cell/ μ L)	270,258.83±74,993.39	273,000	144,000-547,000
GFR (mL/min/1.73m ²)	46.17±10.76	48.5	15.6-59.9
Average HbA1C	7.29±1.5	6.9	4.7-12.53

GFR; glomerular filtration rate (mL/min/1.73m²)

Table 6 Factors associated with anemia in diabetes mellitus type 2 with moderate renal impairment (n = 120)

Characteristic	No. of patients (%)	Hemoglobin (Hb)		
		Anemia (n = 65)	No anemia (n = 55)	p-value
Age				
< 60 year	48 (40)	23 (35.4)	25 (45.5)	0.26
≥60 year	72 (60)	42 (64.6)	30 (54.5)	
Sex				
Male	57 (47.5)	23 (35.4)	34 (61.8)	0.004
Female	63 (52.5)	42 (64.6)	21 (38.2)	
Hypertension				
Yes	115 (95.8)	61 (93.8)	54 (98.2)	0.37
No	5 (4.2)	4 (6.2)	1 (1.8)	
Dyslipidemia				
Yes	112 (93.3)	62 (95.4)	50 (90.9)	0.47
No	8 (6.7)	3 (4.6)	5 (9.1)	
Heart disease				
Yes	19 (15.8)	12 (18.5)	7 (12.7)	0.39
No	101 (84.2)	53 (81.5)	48 (87.3)	
Gout				
Yes	5 (4.2)	1 (1.5)	4 (7.3)	0.135
No	115 (95.8)	64 (98.5)	51 (92.7)	
Metformin				
Yes	68 (56.7)	39 (60.0)	29 (52.7)	0.423
No	52 (43.3)	26 (40.0)	26 (47.3)	
Glipizide				
Yes	52 (43.3)	32 (49.2)	20 (36.4)	0.156
No	68 (56.7)	33 (50.8)	35 (63.6)	
Acarbose				
Yes	2 (1.7)	0 (0)	2 (3.6)	0.208
No	118 (98.3)	65 (100)	53 (96.4)	
TZD				
Yes	1 (0.8)	1 (1.5)	0 (0)	1.0
No	119 (99.2)	64 (98.5)	55 (100)	
Insulin				
Yes	37 (30.8)	21 (32.3)	16 (29.1)	0.704
No	83 (69.2)	44 (67.7)	39 (70.9)	
Aspirin				
Yes	75 (62.5)	39 (60.0)	36 (65.5)	0.539
No	45 (37.5)	26 (40.0)	19 (34.5)	
Statin				
Yes	89 (74.2)	45 (69.2)	44 (80.0)	0.179
No	31 (25.8)	20 (30.8)	11 (20.0)	
Fibrate				
Yes	9 (7.5)	5 (7.7)	4 (7.3)	1.00
No	111 (92.5)	60 (92.3)	51 (92.7)	

Table 6 Factors associated with anemia in diabetes mellitus type 2 with moderate renal impairment (n = 120) (continue)

Characteristic	No. of patients (%)	Hemoglobin (Hb)		
		Anemia (n = 65)	No anemia (n = 55)	p-value
ARB/ACEI				
Yes	81 (67.5)	44 (67.7)	37 (67.3)	0.961
No	39 (32.5)	21 (32.3)	18 (32.7)	
Diabetic nephropathy				
Yes	92 (76.7)	52 (80.0)	40 (72.7)	0.348
No	28 (23.3)	13 (20.0)	15 (27.3)	
Diabetic retinopathy				
Yes	15 (12.5)	13 (20.0)	2 (3.6)	0.007
No	105 (87.5)	52 (80.0)	53 (96.4)	
Hb A1C				
> 6.5	76 (63.9)	37 (56.9)	39 (72.2)	0.084
≤6.5	43 (36.1)	28 (43.1)	15 (27.8)	
GFR				
<30	11 (9.2)	8 (12.3)	3 (5.6)	0.34
30-60	108 (90.8)	57 (87.7)	51 (94.4)	

GFR; glomerular filtration rate (ml/min/1.73m²)

Table 7 Multivariate analysis of factor associated with anemia in diabetes mellitus type 2 with moderate renal impairment (n = 120)

Characteristic	Anemia		
	AOR	95%CI	p-value
Diabetic retinopathy	10.37	2.01-53.65	0.005
Male	0.24	0.10-0.56	0.001

AOR; adjusted odds ratio. After adjusted with age, gout, acarbose, glipizide, metformin, statin, diabetic nephropathy, HbA1C and GFR

Another reason may be because the number of patients with anemia included significantly more women than men. This could be a confounding factor, making the results unreliable.

A related study found poor glycemic control was unrelated to anemia in type 2 DM with moderate renal dysfunction. Other factors contributing to uncontrolled anemia in this study were receiving an ACEI¹⁹ that constituted one limitation of our study.

Several studies reported the prevalence of vitamin B12 deficiency among patients receiving metformin, ranging from 5.8% to 33%²⁰⁻²². Rising age, dose and

duration of metformin were associated risks to vitamin B12 deficiency²³⁻²⁴. A case control study in a Chinese population reported that every 1 g/day of metformin could increase OR 2.9 (95%CI: 2.15-3.87). Moreover, patients taking metformin over or equal to three years increased OR 2.4 (95%CI: 1.46-3.91) compared with metformin less than three years²⁴. The absorption of vitamin B12 decreases after receiving metformin for four months⁽²⁵⁾. Several mechanisms of vitamin B 12 deficiency include alteration of small bowel motility inducing bacterial overgrowth and resulting in vitamin B 12 deficiency, inhibition or interruption of the absorption of vitamin

B12, changing level of intrinsic factor (IF) and interaction with cubulin endocytic receptor²⁶. However, no data of vitamin B 12 level were included in our study.

Limitations of our study included a small population, uncontrolled variable in receiving ACE, no data of erythropoietin level and vitamin B 12 level, and no information of dose and duration of metformin among patients.

Conclusion

Female and diabetic retinopathy were factors associated with anemia resulting in type 2 DM with moderate renal impairment.

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Potential conflicts of interest

The authors declare that they have no conflicts of interest.

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References

1. El-ACHkar TM, Ohmit SE, McCullough PA, Crook ED, Brown WW, GrimmR, et al. Higher prevalence of anemia with diabetes mellitus in moderate kidney insufficiency. *The Kidney Evaluation Program. Kidney Inter* 2005;67:1483-88.
2. 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: 6.
3. Hosseini MS, Rostami Z, Saadat A, Saadatmand SM, Naeimi E. Anemia and microvascular complications in patients with type 2 diabetes mellitus. *Nephro Urol Mon*. 2014;6(4) e19976.
4. Thomas M, Tsalamandris C, Maclsaac R, Jerums G. Anemia in diabetes: an emerging complication of microvascular disease. *Curr Diabetes Rev* 2005;1:107-26.
5. Anand I, McMurray JJ, Whitmore J, Warren M, Pham A, McCamish MA, et al. Anemia and its relationship to clinical outcome in heart failure. *Circulation* 2004;110:149-54.
6. Vlagopoulos PT, Tighiouart H, Weiner DE, Griffith J, Pettitt D, Salem ND, et al. Anemia as a risk factor for cardiovascular disease and all-cause mortality in diabetes: the impact of chronic kidney disease. *J Am Soc Nephrol*. 2005;16:3403-10.
7. Joss N, Patel R, Paterson K, Simpson K, Perry C, Stirling C. Anaemia is common and predicts mortality in diabetic nephropathy. *QJM*. 2007;100:641-7.
8. Thomas MC, Cooper ME, Rossing K, Parving HH. Anaemia in diabetes: Is there a rationale to TREAT? *Diabetologia*. 2006; 49:1151-7.
9. Bonakdaran SH, Gharebagi M, Vahedian M. Prevalence of anemia in type 2 diabetic patients and the role of nephropathy. *Im J Endocrinol Metab*. 2009;11:127-33.
10. Thomas MC, Maclsaac RJ, Tsalamandris C, Molyneaux L, Goubina I, Fulcher G, et al. The burden of anaemia in type 2 diabetes and the role of nephropathy: a cross-sectional audit. *Nephrol Dial transplant*. 2004;19:1792-7.
11. Adejumo BI, Dimkpa U, Ewenighi CO, Onifade AA, Mokogwu AT, Erhabor TA, et al. Incidence and risk of anemia in type-2 diabetic patients in the absence of renal impairment. *Health*. 2012;304-8.
12. King DE, Mainous A3, Buchanan TA, Pearson WS. C-reactive protein and glycemic control in adults with diabetes. *Diabetes Care* 2003;26:1535-9.
13. Shaheen FA, Souqiyeh MZ, Al-Attar BA, Karkar A, Al Jazairi AM, Badawi LS, et al. Prevalence of Anemia in Predialysis Chronic Kidney Disease Patients. *Saudi J Kidney Dis Transpl*. 2011;22:456-63
14. Fetei VF, Choukem S-P, Kengne A-P, 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.
15. Traveset A, Rubinat E, Ortega E, Alcuibierre N, Vazquez B, Hernández M, et al. Lower Hemoglobin Concentration Is Associated with Retinal Ischemia and the Severity of Diabetic Retinopathy in Type 2 Diabetes. *J Diabetes Res*. 2016;2016:3674946.
16. He B Bin, Xu M, Wei L, Gu YJ, Han JF, Liu YX, et al. Relationship between Anemia and Chronic Complications in Chinese Patients with Type 2 Diabetes Mellitus. *Arch Iran Med*. 2015;18:277-83.
17. Early Treatment Diabetic Retinopathy Study design and baseline patient characteristics. ETDRS report number 7. *Ophthalmology*. 1991;98(5 Suppl):741-56.
18. Bedwell RM, Spielvogel H, Bellido D, Vitzthum VJ. Factors Influencing the Use of Biomedical Health Care by Rural Bolivian Anemic Women: Structural Barriers, Reproductive Status, Gender Roles, and Concepts of Anemia. *PLoS One* 2017;12:e0170475.
19. Ajmal A, Gessert CE, Johnson BP, Renier CM, Palcher JA. Effect of angiotensin converting enzyme inhibitors and angiotensin receptor blockers on hemoglobin levels. *BMC Res Notes*. 2013;6:443.
20. Pfiipsen M, Oh R, Saguil A, Seehusen D, Topolski R. The Prevalence of Vitamin B12 Deficiency in Patients with Type 2 Diabetes: a Cross-Sectional Study. *J Am Board Fam Med*. 2009;22:528-34.

21. Reinstatler L, Qi Y, Williamson R, Garn J, Oakley-Jr G. Association of Biochemical B12 Deficiency With Metformin Therapy and Vitamin B12 Supplements. *The National Health and Nutrition Examination Survey, 1999-2006. Diabetes Care.* 2012;35:327-33.
22. Qureshi S, Ainsworth A, Winocour P. Metformin therapy and assessment for vitamin B12 deficiency: is it necessary? *Practical Diabetes.* 2011;28:302-4.
23. De-Jager J, Kooy A, Lehert P. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. *BMJ.* 2010;340:c2181.
24. Ting R, Szeto C, Chan M, Ma K, Chow K. Risk Factors of Vitamin B12 Deficiency in Patients Receiving Metformin. *Arch Intern Med.* 2006;166:1975-9.
25. Wulffele M, Kooy A, Lehert P. Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial. *J Intern Med.* 2003;254:455-63.
26. Andre's E, Noel E, Goichot B. Metformin-associated vitamin B12 deficiency. *Arch Intern Med.* 2002;162:2251-2.