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The Association between Glycated Hemoglobin and Estimated Glomerular Filtration Rate in Patients with Type 2 Diabetes based on the Hemoglobin Status

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Abstract

Background: The aim of the present study was to examine the association between hemoglobin A1C (HbA1C) and eGFR in patients with type 2 diabetes (T2D).

Methods: This cross-sectional study was conducted on 802 patients with T2D visiting Abu Reyhan Clinic of Shahid Mohammadi Hospital in Bandar Abbas, Iran. eGFR was determined using MDRD and EPI methods. The association between two levels of HbA1C (≤8% and >8%) and two levels of GFR (≤60% and >60%) were assessed in total population, and separately, in men and women using linear regression model.

Results: The mean ± SD age of the study population (27.7% male) was 53.5 ± 5.5 years. Based on the multivariable adjusted model, in subjects with HbA1C >8%, there was a negative association between HbA1C and eGFR-EPI (β = -0.14) and eGFR-MDRD (β = -0.12), (P<0.05). However, there was no significant relationship between HbA1C and eGFR in individuals with HbA1C ≤8%. Also, no significant association was found between HbA1C and eGFR as categorical variables (based on the two categories of eGFR ≤60 and >60). An inverse association was observed between GFR ≤60 and HbA1C >8% in women based on the Hb classification. This inverse association was found between HbA1C >8% and eGFR-EPI (β = -0.76) and eGFR-MDRD (β = -0.78) in women with Hb >12 mg/dl (P<0.01).

Conclusion: According to the results, the higher level of HbA1C can be associated with decreased eGFR level. This negative association was mostly observed between HbA1C >8% and GFR ≤60 in T2DM female patients without anemia (Hb>12). It seems that monitoring kidney function by eGFR estimation is a necessary action in patients with T2D with high HbA1C levels.

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Introduction

Type 2 diabetes (T2D) is one of the most important risk factors for the occurrence of chronic kidney disease (CKD). The glomerular filtration rate (GFR) is used as a tool for the assessment of renal function that declines in all types of renal failure. Creatinine is filtered freely at the glomerulus level and its concentration is inversely related to eGFR level. The serum creatinine level does not usually reach the abnormal range before eGFR reaches <60% of the normal limit (1). HbA1C indicates the average blood glucose level over the past three months and serves as an applicable index for the long-term management of diabetes. Recently, HbA1C has also been

adopted by the American Diabetes Association (ADA) as an index for the diagnosis of pre-diabetes and diabetes (2).

Albuminuria and decreased eGFR are components of the detection or staging of kidney dysfunction in T2D, and are considered as therapeutic indicators for the management or control of diabetic nephropathy (3,4). Also, GFR is a usual marker of renal function and albuminuria is a measure of renal damage (4). The role of eGFR and albuminuria in the clinical assessment of diabetic nephropathy can be complementary; however, they are not always closely coupled. Although increases in the albumin excretion rate precede a decrease in GFR, some subjects with diabetes showed a non-albuminuric pathway to diabetic nephropathy and renal failure (3). Some studies have reported that although micro-albuminuria is a main risk factor for diabetes and is obviously related to the increased risk of CVD and dyslipidemia (5,6). In epidemiological studies with a large population, the determination of eGFR is more common and applicable for the early diagnosis of diabetic nephropathy in patients with diabetes (7), because GFR is a more sensitive index for diagnosing glomerular dysfunction in its early stages. Also, it has been reported that micro-albuminuria does not necessarily imply progressive diabetic nephropathy, and can be returned to normo-albuminuria; in addition, early stage of CKD such as stage 3 can be observed in the absence of micro-albuminuria (7-10). Therefore, in epidemiological studies, GFR estimation can be performed to diagnose renal failure at its early stages (before the occurrence of micro-albuminuria), therefore, GFR estimation can be helpful in diagnosis and treatment of renal failure at its early stages in patients with T2D (11,12).

Several studies have been conducted on the association between HbA1C level and eGFR, and indicated a significant association between increased HbA1C and decreased eGFR (1,13-16). Also, a study by Lee et al. suggested that the variability of HbA1C is a main marker of long-term prediction of eGFR decline. This study reported that individuals with high HbA1C variation may experience eGFR decline even under control of HbA1C (<7) and before macroalbuminuira (17). Furthermore, a study by Subramanyam et al. on 60 Indian patients with CKD reported that higher levels of HbA1c were associated negatively with eGFR and positively with creatinine level (18).

The present study was conducted to investigate the association between HbA1C and eGFR in patients with T2D.

In this study, this association was assessed between two levels of HbA1C (≤8%and>8%) and two levels of GFR (≤60% and >60%) in total population and separately in men and women. Considering the possible role of anemia in HbA1C interpretation, the analysis was also performed based on three hemoglobin (Hb) categories (7-9, 9-13, >13 mg/dl in men and 7-9, 9-11, >12 mg/dl in women).

Materials and Methods

Study population

This cross-sectional study was conducted on 802 patients with diabetes visiting Abu Reyhan Clinic of Shahid Mohammadi Hospital in Bandar Abbas, Iran, from May 2016 to May 2018. The patients were selected based on the inclusion and exclusion criteria, their medical records were examined, and their clinical parameters and study variables were measured and recorded. The inclusion criteria were patients with a definitive diagnosis of type-2 diabetes by an endocrinologist or an internist based on the ADA criteria, age of 40-60 years, and those with diabetes duration of five years or more. Patients with a history of other known renal diseases (except for those related to diabetes) with acute renal failure and GFR <30 or ≥180 were excluded. Considering the role of confounding variables in the interpretation of HbA1C (19), patients with a history of transfusion or hemolytic anemia in the past three months, those who had developed anemia after acute blood loss in the past three months, those with a history of hypertriglyceridemia >1750 mg/dl, those with a history of taking ribavirin and

interferon-alpha in the past three months, those with a history of severe hyperbilirubinemia (bilirubin level >20 mg/dl), those with GFR <30, pregnant women, and those with Hb <7 were also excluded from the study.

Ethical statement

The protocol of this study was approved by the Ethics Committee of Hormozgan University of Medical Sciences, Bandar Abbas, Iran (Ethical code: IR.HUMS.REC.1398.049) Written informed consent was obtained from all participants included in the study.

Measurements

The blood samples were collected after 12-14 h of overnight fasting based on the standard protocol and centrifuged within 30-45 min of collection. The blood samples were analyzed using the Selectra 2 auto-analyzer (Vital Scientific, Spankeren, and Netherlands) at laboratory of the Abu Reyhan Clinic of Shahid Mohammadi Hospital on the day of blood collection. The enzymatic colorimetric method with glucose oxidase was used to measure fasting blood sugar (FBS). The enzymatic colorimetric method was used to measure total cholesterol with cholesterol esterase and cholesterol oxidase. High density lipoprotein cholesterol (HDL-C) was measured with phosphotungstic acid after precipitation of apolipoprotein β. Low density lipoprotein cholesterol (LDL-C) was also calculated by measuring the serum and TC, TG, and HDL-C concentrations and reported it in mg/dl using the Friedewald formula. These analyses were performed using commercial kits (Pars Azmoon, Tehran, Iran). Also, an amount of collected blood samples were poured into a tube containing EDTA, and HbA1c concentration was measured by the enzymatic colorimetric method using commercial kits (Pars Azmoon Co., Tehran, Iran) on an automatic biochemistry analyzer (Mindray BS-800). Serum creatinine was assessed by standard colorimetric Jaffe_Kinetic reaction method. Both intra- and inter-assay coefficients of variation were <3.1%. The mean creatinine was measured at least twice over six months, and if there was not a ±10% difference, eGFR was calculated based on the mean creatinine level using the modification of diet in renal disease (MDRD) and the epidemiology collaboration equation (EPI) (20).

MDRD:

eGFR = 175 (or 186) × serum creatinine - 1.154 × Age - 0,203 × (0.742 if female) × (1.212 if black

EPI:

eGFR = 141 × min (Scr/κ,1)α × max(Scr/κ, 1) - 1.209 × (0.993× Age) × 1.018 [if female] × 1.159 [if black]

Then, the association between HbA1C and eGFR was examined overall and the association between HbA1C and different eGFRs (GFR <60 and eGFR ≥60) was also separately evaluated.

Considering the possible role of anemia in the interpretation of HbA1C, both male and female patients were divided into two groups including T2D patients with anemia (men: 7 <Hb < 9, 9 < Hb < 13; women: 7 < Hb < 9, 9 < Hb < 12) and T2D patients without anemia (men: Hb ≥13; women: Hb ≥12). Then, the association between HbA1C and GFR was assessed overall and the association between HbA1C and different eGFRs (eGFR <60 and eGFR ≥60) was also separately examined.

In addition, the patients with diabetes who had a history of cardiovascular diseases (those with MI or angioplasty or those with angina pectoris as diagnosed by a cardiologist based on the clinical, laboratory or electrocardiographic criteria were also selected and the noted association was evaluated in these patients as well.

Statistical analysis

Data were analyzed using SPSS (version 23.0; SPSS Inc., Chicago, IL, USA). Statistical significant level was considered at P<0.05. The Kolmogorov-Smirnov test and histogram chart

were used to assess the normality of the data. The characteristics of subjects were expressed as mean ± SD for continuous variables and numbers (percentages) for categorical variables. Chi-square and Mann-Whitney U test were used for the comparison of qualitative and quantitative variables in participants. β was estimated for the eGFR status (estimated by EPI and MDRD methods), according to the HbA1C status and Hb categories in men and women by linear regression model. Linear regression test was performed in two models including crude model and multivariable adjusted model. In the linear regression model, HbA1C and eGFR were considered as independent and dependent variables, respectively. Multivariable adjusted model was adjusted for potential confounding factors including age, sex, body mass index (BMI), cardiovascular diseases, and Hb level. Due to the possible confounding effect of some variables such as gender, different levels of hemoglobin (anemia status) on the relationship between HbA1C and eGFR, subgroup analyses (stratified analysis) were performed based on the sex categories and hemoglobin levels . Finally, the linear relationship between FBS and eGFR was assessed by linear regression test in total population and in men and women, separately.

Results

In this study, conducted on 802 patients with T2D, the mean ± SD age of the study population (27.7% male) was 53.5 ± 5.5 years. The mean ± SD of HbA1C in individuals with eGFR >60 (HbA1C =9.9 ± 2.6) was significantly higher than those with eGFR ≤60 (HbA1C =8.8 ± 2.0) (P=0.004).

Baseline characteristics of participants are shown in Table 1. Findings revealed that 74.5% of the participants with HbA1C <8% and 71% of those with HbA1C >8% were women. Also, 14.6% of the participants in the HbA1C ≤8% group and 17% of those in the HbA1C >8% group had cardiovascular diseases. Compared with subjects in group with HbA1C ≤8%, participants with HbA1C >8% had significantly lower eGFR-EPI and eGFR-MDRD and higher total cholesterol, LDL-C, FBS, and creatinine (P<0.05).

Table 1. Baseline characteristics of the study population based on the HbA1C levels categories

Variable	Total	A1C≤8%	A1C>8%	P-value
Age (year)	53.55±5.56	53.75±5.49	53.43±5.60	0.427
Male n (%)	222(27.7%)	77(25.5%)	145(29.0%)	0.283
Cardiovascular diseases n (%)	129(16.1%)	44(14.6%)	85(17.0%)	0.210
Height (cm)	162.68±8.68	162.57±8.59	162.75±8.75	0.781
Weight (kg)	69.53±28.17	68.26±13.11	70.29±34.18	0.323
BMI (kg/m^2)	25.82±4.72	25.68±4.62	34.18±4.78	0.516
Hemoglobin (g/dl)	12.59±1.63	12.48±1.65	12.66±1.61	0.120
Fasting blood glucose (mg/dl)	196.80±82.14	145.88±48.92	227± 82.80	<0.001
Cholesterol (mg/dl)	171.98±43.98	165.57±42.33	175.86±44.55	0.001
Triglyceride (mg/dl)	159.87±93.09	153.92±92.87	163.46±93.13	0.160
LDL-C (mg/dl)	95.30±36.13	90.32±33.80	98.32±37.18	0.002
HDL-C (mg/dl)	42.79±11.69	42.36±11.10	43.06±12.03	0.412
Creatinine (mg/dl)	0.8471±0.22	0.8123±0.18	0.8681±0.24	0.001
GFR_EPI	86.30±17.48	88.45±15.96	84.99±18.23	0.007
GFR_MDRD	86.79±23.29	89.12±22.02	85.38±23.94	0.027

*Data are presented as mean ± standard deviation for continuous variables and as percentage for categorical variables.
BMI: Body mass index; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol.

The findings on the association between two categories of eGFR (estimated by EPI and MDRD methods) and HbA1C level categories are reported in Table 2. Based on the multivariable adjusted model, eGFR-EPI (β=-0.14, P=0.001) and eGFR-MDRD (β=-0.12, P=0.006) were negatively associated with HbA1C >8%. However, there was no significant association between eGFR (measured by EPI and MDRD) and HbA1C ≤8% in the population study. Also, in the present study, based on the eGFR status, the participants were divided into two groups including subjects with eGFR ≤60 and those with eGFR >60 (Table 2). Then, the association between HbA1C (≤8% and >8%) and eGFR (≤60 and >60) was measured by the EPI and MDRD. The results indicated that there was no significant association between any of the two eGFR classes and HbA1C groups (≤8% and >8%).

Table 2. The association between two categories of eGFR (estimated by EPI and MDRD methods) and HbA1C level categories

GFR		A1C≤8					A1C>8				
		Mean ± SD*	Model 1		Model 2		Mean ± SD*	Model 1		Model 2	
			Beta	P-value	Beta	P-value		Beta	P-value	Beta	P-value
GFR	EPI	88.45±15.96	-0.02	0.700	-0.04	0.410	84.99±18.23	-0.12	0.006	-0.14	0.001
	MDRD	89.12±22.02	-0.03	0.607	-0.04	0.491	85.36±23.94	-0.10	0.019	-0.12	0.006
GFR≤60	EPI	52.54±6.07	0.43	0.079	0.33	0.253	49.91±8.31	-0.02	0.897	-0.04	0.753
	MDRD	51.54±5.57	0.48	0.048	0.26	0.358	49.32±8.05	-0.02	0.987	-0.03	0.816
GFR>60	EPI	90.60±13.65	-0.03	0.539	-0.06	0.293	88.48±14.99	-0.04	0.389	-0.05	0.295
	MDRD	91.37±20.55	-0.04	0.430	-0.05	0.368	88.95±21.96	-0.03	0.503	-0.03	0.453

*Data are presented as mean ± standard deviation.
P-value was determined using linear regression model.
Model 1: Crude model; Model 2: Adjusted for age, sex, body mass index, cardiovascular diseases, and hemoglobin level.

In Table 3, the association between eGFR status and HbA1C level was investigated separately in women and men based on the hemoglobin levels classification (7-9, 9-13, >13 mg/dl in men and 7-9, 9-12, >12 mg/dl in women). It should be noted that, due to the small sample size, it was not possible to determine the relationship between HbA1C and eGFR in subjects with Hb= 7 to 9 mg/dl. Also, due to the small sample size, it was not possible to determine the relationship between eGFR and HbA1C levels (≤8% and >8%) in men with eGFR ≤60, based on the hemoglobin levels classification. β was estimated for the eGFR status (estimated by EPI and MDRD methods), according to the HbA1C status and Hb categories in men and women by linear regression model. Based on the results obtained from linear regression analysis, there was an inverse association between HbA1C and eGFR-EPI (β= -0.76, P=0.007) and eGFR-MDRD (β= -0.78, P=0.004) in women with Hb >12 mg/dl. However, there was no significant association between eGFR and HbA1C ≤8% based on other hemoglobin levels categories in women. Also, no significant association was observed between eGFR (including two classes) and HbA1C levels (≤8% and >8%) in men based on the hemoglobin levels classification (Table 3).

Table 3. The results of linear regression models assessing the association between two levels of eGFR and HbA1C levels based on hemoglobin classification in the female male groups†

eGFR	Female	Hb (mg/dl)	A1C≤8			A1C>8		
			Mean ± SD*	Beta	P-value**	Mean ± SD*	Beta	P-value**
Female								
eGFR ≤60	eGFR-EPI	9-12	51.78±5.76	0.51	0.197	49.02±8.99	0.20	0.399
		>12	56.13±1.20	-0.37	0.757	51.55±5.93	-0.78	0.004
	eGFR-MDRD	9-12	50.28±5.05	0.54	0.166	47.90±8.49	0.20	0.411
		>12	54.76±0.66	-0.70	0.502	50.59±5.85	-0.76	0.007
eGFR >60	eGFR-EPI	9-12	89.42±13.44	-0.04	0.674	89.89±14.89	-0.02	0.797
		>12	89.82±13.33	-0.08	0.367	88.14±15.70	-0.05	0.429
	eGFR-MDRD	9-12	87.00±17.55	-0.01	0.931	90.63±22.69	-0.04	0.637
		>12	90.24±21.34	-0.09	0.327	88.34±24.21	-0.03	0.670
Male								
GFR >60	GFR	9-13	93.65±14.80	0.26	0.203	83.76±13.99	0.05	0.764
	EPI	>13	92.98±13.57	-0.11	0.433	88.78±13.89	-0.07	0.482
	GFR	9-13	99.52±24.61	0.28	0.166	85.36±18.89	0.01	0.932
	MDRD	>13	96.28±20.60	-0.16	0.286	89.31±17.62	-0.12	0.243

†Due to the low sample size, it was not possible to determine the relationship between eGFR and HbA1C levels (≤8% and >8%) in the male patients with eGFR ≤60, based on the hemoglobin level classification.
*Data are presented as mean ± standard deviation.
**P-value was determined using linear regression model.

The association between eGFR and FBS level was assessed by the linear regression test, and the results are shown in Table 4. The findings indicated that there was a negative association between FBS and eGFR-EPI in total population (β= -0.189, P=0.016), men (β= -0.197, P<0.001), and women (β= -0.181, P≤0.001). Also, a negative relationship was observed between FBS and eGFR-EPI in all individuals (β= -0.156, P<0.001), men (β= -0.221, P=0.001), and women (β= -0.129, P=0.002).

Table 4. The linear relationship between two categories of eGFR (estimated by EPI and MDRD methods) and fasting blood glucose in the total population and based on the gender classification

GFR		Total population				Male				Female			
		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
		Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
GFR	EPI	-0.136	<0.001	-0.189	<0.001	-0.162	0.016	-0.197	<0.001	-0.126	0.002	-0.181	<0.001
	MDRD	-0.123	<0.001	-0.156	<0.001	-0.193	0.004	-0.221	0.001	-0.096	0.021	-0.129	0.002
GFR>60	EPI	-0.049	0.706	-0.090	0.487	0.122	0.619	0.150	0.505	-0.124	0.428	-0.164	0.343
	MDRD	0.064	0.620	0.016	0.908	0.457	0.075	0.279	0.306	-0.040	0.791	-0.102	0.550
GFR≤60	EPI	-0.104	0.005	-0.156	<0.001	-0.111	0.114	-0.155	0.025	-0.100	0.020	-0.156	<0.001
	MDRD	-0.078	0.033	-0.104	0.006	-0.136	0.052	-0.169	0.017	-0.055	0.204	-0.080	0.071

P-value was determined using linear regression model.
Model 1: Crude model; Model 2: Adjusted for age, sex, body mass index, cardiovascular diseases, and hemoglobin level.

Another stratified analysis indicated that there was also no significant association between different eGFR groups (≤60 and >60) and HbA1C levels (≤ 8% and >8%) in those with cardiovascular diseases (Supplementary Table 1). The differences of HbA1C and fating blood glucose level in male and female patients based on the hemoglobin categories were assessed (Supplementary Table 2). The findings indicated that there was no significant difference in HbA1C and fating blood glucose level based on the hemoglobin categories in male and female patients.

Supplementary Table 1. The association between two levels of eGFR and HbA1C levels in patients with cardiovascular disease

		A1C≤8			A1C>8		
		Mean ± SD*	Beta	P-value**	Mean ± SD*	Beta	P-value**
GFR	EPI	82.27±18.67	-0.065	0.690	79.32±19.75	-0.219	0.061
	MDRD	83.06±23.53	0.038	0.821	82.18±23.48	-0.197	0.093
GFR<60	EPI	51.51±8.06	NE†	NE	50.16±8.52	0.092	0.788
	MDRD	51.10±7.43	NE	NE	49.60±8.17	-0.059	0.869
GFR>60	EPI	87.12±14.79	0.101	0.589	86.08±14.81	0.137	0.296
	MDRD	88.11±21.08	0.096	0.596	87.27±19.89	0.076	0.557

*Data are presented as mean ± standard deviation.
**P-value was determined using linear regression model.
†Not estimated because of low sample size.

Supplementary Table 2. The HbA1C and fating blood glucose level in male and female patients based on the hemoglobin categories

	Male			P-value	Female			P-value
Hemoglobin Categories	**<9**	**9-13**	**>13**		**<9**	**9-13**	**>13**	
Fasting blood Glucose (mg/dl)	209.0±95.3	193.01±83.8	192.7±84.4	0.834	170.8±98.8	195.4±81.5	207.0±80.1	0.193
HbA1C	11.8±3.5	8.9±2.1	9.2±2.2	0.149	8.3±1.8	8.8±2.0	9.1±2.1	0.219

Data are presented as mean ± standard deviation.
†Not estimated because of low sample size.

Discussion

This study was conducted on 802 patients with T2D (72.3% female), and it was found a negative association between HbA1C and eGFR-EPI and eGFR-MDRD in total population, manifested by a significant drop in eGFR with an increase in HbA1C. Also, there was an inverse association between GFR ≤60 and HbA1C >8% in women based on the Hb classification. However, no significant association was found between eGFR and HbA1C ≤8% based on other hemoglobin levels categories in women. Finally, the association between eGFR and HbA1C based on the hemoglobin levels categories in men was not significant. Furthermore, the present study showed that higher levels of FBS are associated with decreased eGFR in patients with T2D.

Several studies have been conducted on the association between HbA1C and eGFR and showed a significant association between increased HbA1C and decreased eGFR (1,13-16). Consistent with the results of the present study, the study of Prabhu et al. showed an inverse association between HbA1C and GFR in the control group, DM group, and DM and CVD groups, but not in the CVD group (1). In comparison with the results of the present study, the study of Prabhu et al. investigated eGFR level only using MDRD method. In their study, no classification or analysis was made based on the anemia level (as a confounding variable), however, in the present study, the association between HbA1c and eGFR value was assessed based on various categories of Hb in men and women (1). In a study by Rigalleau et al. conducted on 193 patients with diabetes, a significant association was observed between eGFR and HbA1C levels (15). Contrary to the present study, Rigalleau et al. isotopically measured eGFR (51Cr-EDTA). Also, the association between different levels of glycemic control and eGFR was significant only in HbA1C >8% and eGFR-MDRD; however, in the present study, HbA1C >8% was significantly related to the levels of both eGFR-MDRD and eGFR-EPI. An additional step in the present study was performing an analysis based on different anemia classifications to determine the role of anemia as a confounding variable in the interpretation of HbA1C (15).

The results of the present study was also consistent with the findings of previous studies conducted on various populations in the USA (16), Korea (13), and Taiwan (14). A population-based cohort study on 15,792 adult population from four USA communities reported that higher levels of fasting blood glucose and HbA1C were strongly associated with increased risk of chronic kidney disease and end-stage renal disease after adjustment of potential confounding factors (16). The KNHANES study revealed that eGFR in individuals without diabetes was inversely related to the HbA1c level, in other words, a decline in eGFR occurred in patients with higher levels

of HbA1C (13). Furthermore, a longitudinal survey on Taiwan population indicated that higher baseline levels of HbA1C resulted in a higher decline in eGFR (14).

In the present study, the determination of glycemic control using HbA1C as a main glycemic marker may be underestimated in the case of anemia. Borg et al. reported that the relationship between FBS and HbA1c is affected in severe anemia, so that blood glucose levels may be underestimated by HbA1c in anemia (21). For this reason, the measurement of HbA1C variation could be a useful marker to assess glycemic glucose in patients with T2D. Also, the variability of HbA1C may be an independent risk factor for the development of albuminuria and eGFR decline in patients with T2D (17,22). Lee et al. reported that the variability of HbA1C is a main marker of long-term prediction of eGFR decline. They suggested that subjects with high HbA1C variation may have a decline in eGFR even in normal level of HbA1C (<7) and before macro-albuminuria (17). Also, Ceriello et al. have reported a significant association between variability in HbA1c and higher risk of developing albuminuria (22). Variability in HbA1c in the present study could act as a confounding factor in assessing the association between HbA1C and eGFR level. However, since there was no data on HbA1c variability to assess its potential impact on the results of the present study, so, in addition to HbA1C, the relationship between FBS and eGFR was assessed and it was revealed that the uncontrolled FBS can be associated with reduction of eGFR level and higher risk of kidney dysfunction. Finally, as previous studies (23,24) suggested that iron-deficiency anemia can affect HbA1c levels in patients with T2D, in the present study, the association between HbA1C and eGFR was assessed based on the hemoglobin categories and it was found a negative association between HbA1C >8% and eGFR ≤60 (estimated by both MDRD and EPI) in T2D female patients without anemia (Hb>12), but not in male patients.

Although, there was no significant association between eGFR as a categorical variable (eGFR <60 and eGFR >60) and HbA1C, this association between HbA1C and eGFR was significant in women. Considering the role of anemia as a confounding variable in the interpretation of HbA1C (although people with Hb <7 was excluded at the onset), the results were assessed based on different categories of Hb levels in men and women, separately. The male patients were therefore divided into two groups with (7 < Hb < 9, 9 < Hb < 13) and without anemia (Hb ≥13) and the female patients were similarly divided into groups with (7 < Hb < 9, 9 < Hb < 12) and without anemia (Hb ≥12), and the association between HbA1C and eGFR ≥60 as well as the association between HbA1C and eGFR <60 were assessed in these patients, separately. The findings revealed a significant association between HbA1C >8 and eGFR <60 in the female patients with Hb >12. In other words, in the female patients without anemia, a significant reduction in eGFR was observed as HbA1C increased at levels of >8, which could be due to the role of anemia as a confounding variable in the interpretation of HbA1C. No such association was observed in men, probably because women constituted the majority of the statistical population in this study (74.5%), therefore, a significant association could be observed in men in the case of having a larger and more adequate sample size for men.

Strengths and limitations

The present study has several strengths. First, eGFR was measured using both EPI and MDRD for both levels of eGFR. Second, due to the confounding effect of anemia in the

interpretation of HbA1C, the participants (men and women) were classified based on the anemia status and a stratified analysis was performed. However, the present study has also some limitations. First, the low sample size of men compared to women led to obtaining no significant finding in this group; the association between eGFR and HbA1C could be significant for men with a larger sample size. Second, the cross-sectional nature of the study limited authors from establishing a cause-and-effect relationship between HbA1C and eGFR level. Third, there was no data on albuminuria to assess the status of diabetic nephropathy in T2D patients with low GFR. Furthermore, some comorbidities such as hypertension were not determined in the participants to assess its effect on kidney function. However, a stratified analysis was performed based on the cardiovascular diseases status to assess the association between eGFR and HbA1C levels. Finally, there were no comprehensive data on the medication use related to the control of hypertension or dyslipidemia in T2D, some of which may have a mild nephrotoxic effect.

Conclusion

According to the results, in diabetic patients with HbA1C >8%, higher HbA1C is associated with decreased level of eGFR-EPI and eGFR-MDRD. Also, it was revealed that there was a negative association between HbA1C >8% and eGFR ≤60 (estimated by both MDRD and EPI) in T2D women without anemia (Hb>12), but not in men. No significant association was observed between HbA1C >8% and eGFR ≤60 in T2D patients with Hb <12, which could confirm the role of anemia as a confounding variable in the interpretation of HbA1C. A significant decrease in eGFR with an increase in HbA1C indicate that the management of HbA1C level in T2D is an essential goal in the prevention of decreased renal function.

Authors' contributions

HR. S and M. KJ contributed to the project conception, design, and statistical analysis. M.T and M.KJ contributed to the data collection and manuscript drafting. M.KJ supervised the study. All authors approved the final version of the manuscript.

Conflict of interests

The authors declared that they have no conflict of interests.

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