



## COMPARISON OF ESTIMATED GLOMERULAR FILTRATION RATE USING DIFFERENT FORMULAS IN TURKISH POPULATION

### TAHMİNİ GLOMERÜLER FİLTASYON HIZININ FARKLI FORMÜLLERLE TÜRK POPÜLASYONUNDA KIYASLANMASI

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#### ABSTRACT

**Objective:** Creatinine-based equations are generally used in clinical practice to estimate glomerular filtration rates (GFR), but values are not usually consistent. This study aimed to evaluate the difference between estimated GFR values using different equations.

**Material and Method:** Adult Turkish patients with serum creatinine measurements between January to December 2021 and complete demographic data were included. GFR values were calculated using 5 different formulas. GFR calculated with Cockcroft-Gault were normalized to body surface area and added to the comparison. Difference between GFR values and KDIGO stages were evaluated. Albumin/creatinine ratio (ACR) of patients was also assessed.

**Result and Discussion:** A total of 305 patients with average age of 52.92 years were included. Six different GFR calculations were recorded with median values between 51.70 to 71.77 ml/min/1.73m<sup>2</sup>. Formula of The Modification of Diet in Renal Disease with the race factor for Turkish population resulted in the lowest eGFR values. The ACR values of only 42 patients were available and it was negatively correlated to all GFR values and positively correlated to all KDIGO stages ( $p < 0.05$ ). There were noteworthy variations in GFR values, based on patient demographics and/or equations. The need for novel practical methods for estimating GFR in general and specific patient populations are necessary.

**Keywords:** Creatinine clearance, eGFR variations, GFR calculation, glomerular filtration rate, kidney function

#### ÖZ

**Amaç:** Glomerüler filtrasyon hızlarını (GFR) tahmin etmek için klinik uygulamada genellikle kreatinin bazlı formüller kullanılır, ancak değerler genellikle tutarlı değildir. Bu çalışmanın amacı farklı formüller kullanarak eGFR değerleri arasındaki farkı değerlendirmektir.

**Gereç ve Yöntem:** Ocak-Aralık 2021 tarihleri arasında serum kreatinin ölçümü yapılan ve demografik verileri eksiksiz olan yetişkin Türk hastalar çalışmaya dahil edildi. GFR değerleri 5 farklı formül kullanılarak hesaplandı. Cockcroft-Gault ile hesaplanan GFR, vücut yüzey alanına göre normalize edilerek karşılaştırmaya eklendi. GFR değerleri ile KDIGO evreleri arasındaki fark değerlendirildi. Hastaların albumin/kreatinin oranı (ACR) da değerlendirmeye alındı.

**Sonuç ve Tartışma:** Ortalama yaşı 52.92 yıl olan toplam 305 hasta çalışmaya dahil olmuştur. Ortanca değerleri 51.70 ila 71.77 ml/dak/1.73m<sup>2</sup> arasında değişen altı farklı GFR hesaplaması kaydedilmiştir. Türk popülasyonu için ırk kastsayısı içeren The Modification of Diet in Renal Disease formülü en düşük eGFR değerleri ile sonuçlanmıştır. Sadece 42 hastanın ACR verisi

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*bulunmuştur ve bu değerleri tüm GFR değerleri ile negatif, tüm KDIGO evreleri ile pozitif korelasyon göstermiştir ( $p < 0.05$ ). Hasta demografisine ve/veya denklemlere bağlı olarak GFR değerlerinde kayda değer farklılıklar görülmüştür. Genel ve spesifik hasta popülasyonlarında GFR'yi tahmin etmek için yeni pratik yöntemlere ihtiyaç vardır.*

**Anahtar Kelimeler:** Böbrek fonksiyonu, eGFR varyasyonları, GFR hesaplaması, glomerüler filtrasyon hızı, kreatinin klerensi

## INTRODUCTION

Glomerular filtration rate (GFR) is the indirect measurement of functional nephrons. It is considered the best overall measure of kidney function. A decline in GFR represents a malfunction of the excretion capacity which may be directly or indirectly related to the kidneys. But it is not correlated with kidney mass loss. The normal GFR value ranges between 90 to 120 ml/min/1.73 m<sup>2</sup> but it is considerably variable among individuals as it depends on age, sex, and body size. Measured GFR (mGFR) is the most accurate method of assessment [1]. But it is less convenient and may involve the administration of exogenous filtration markers excreted exclusively by the kidneys. In clinical setting, estimated GFR (eGFR), calculated from endogenous biomarkers excreted mostly by the kidneys, is preferred as a more convenient way of determining baseline kidney function, diagnosing kidney disease, evaluating kidney disease progression and dosing medications [2,3].

Creatinine and Cystatin-c are efficiently used endogenous filtration markers with some limitations attached to their use. In clinical practice, creatinine is more commonly used but variations in production and secretion mainly affected by individual patient characteristics, extrarenal excretion, and measurement issues are the core limitations to the use of creatinine. Cystatin C levels are also variable and its higher levels are associated with male sex, greater height and weight, higher lean body mass, higher fat mass, diabetes mellitus, higher levels of inflammatory markers, hyper- and hypothyroidism, and glucocorticoid use [3]. The equations that contain both markers give the most accurate GFR estimates [3,4]. But this is not always feasible in most clinical settings and creatinine-based estimates remain the primary approach.

Some creatinine-based estimating equations include the 2009 chronic kidney disease epidemiology (CKD- EPI) equation, the Modification of Diet in Renal Disease (MDRD) study equation, the Cockcroft-Gault (CG) equation and the recently published CKD-EPI 2021. All equations incorporate serum creatinine concentrations with different patient variables like age, weight, sex, and race. The inclusion of different variables in the different equations leads to variations in GFR values. As such the accuracy of all the equations is not universal in all patient populations. Certain formulas provide more accurate results in certain patient groups or kidney function range.

The CG equation was developed to determine creatinine clearance. Drug dosing is based on the CG as it was used in pharmacokinetic studies to establish drug dosing in kidney dysfunction [5]. But it was developed before the standardization of creatinine assays and has not been revised to suit updated versions. This leads to overestimation when used with creatinine values measured by most laboratories. Furthermore, the accuracy of eGFR values is affected by variations in body weight and body mass index [6,7]. When using the CG equation, the use of actual body weight in underweight patients, ideal body weight in patients with normal weight and adjusted body weight (0.4 correction) for overweight and obese patients give more accurate and less biased GFR values [7].

The MDRD was developed from nondiabetic patients' data, and it excludes patient weight in estimations. The equation has been re-evaluated to be used with standardized creatinine measurements and was considered to be more accurate than CG [8,9]. The chronic kidney disease epidemiology (CKD-EPI) equation was first published in 2009 and recently modified in 2021 to exclude the race parameter [10,11]. The estimation of GFR in the Turkish population was evaluated in a previous study and MDRD was established as the most suitable equation. A race factor of 0.804 was also suggested to get more accurate GFR values in this population [12]. But there is no evidence of the integration of this equation in clinical practice.

Recommendations are now put in place for the use of the most accurate method in establishing GFR for individual patients. The American Society of Nephrology (ASN) and the National Kidney

Foundation (NKF) recommend the use of the CKD-EPI 2021 equation in most clinical settings. It is considered accurate and acceptable among different populations. Though it is slightly less accurate than the CKD-EPI 2009 as it underestimates mGFR in blacks and overestimates mGFR in other individuals [3].

In this study, we aimed to investigate the difference between eGFR levels calculated using CG, MDRD, CKD-EPI equations in addition to the newly recommended CKD-EPI 2021 equation and MDRD Turkish version with the race factor of 0.804.

## MATERIAL AND METHOD

In this retrospective study, adult Turkish patients with serum creatinine measurements between January and December 2021 were searched from the online record system of the Medipol Mega University Hospital in Istanbul. Patients with required demographic (age, weight, height) and health-related data were included in the study. Five serum creatinine-based equations were used to calculate the glomerular filtration rates of all patients. These equations include CG, MDRD, MDRD-TR, CKD-EPI 2009 and CKD-EPI 2021 (Table 1). In the CG equation, the adjusted body weight (0.4 correction) was used for obese patients [2,7]. GFR calculated with CG were normalized to body surface area for all patients to compare with GFR values calculated with other equations [3] and reported separately as CG-BSA. The urine albumin/creatinine ratio (ACR) was also assessed. The Kidney Disease: Improving Global Outcomes (KDIGO) kidney function classification was used to classify patients' GFR values, and ACR [13]. The GFR values and stages were compared and variations between equations were analysed.

**Table 1.** GFR estimation equations

Formulae	Equation
<b>CG</b> (Creatinine clearance measurement, ml/min)	$(140 \times \text{age}) \times \text{weight (kg)} / 72 \times \text{Scr} \times (0.85 \text{ if female})$
<b>MDRD</b> (GFR measurement, ml/min per 1.73 m <sup>2</sup> )	$175 \times (\text{Scr})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American})$
<b>MDRD-TR</b> (GFR measurement, ml/min per 1.73 m <sup>2</sup> )	$175 \times (\text{Scr})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times 0.804$
<b>CKD-EPI Creatinine Equation (2009)</b> (GFR measurement, ml/min per 1.73 m <sup>2</sup> )	$A \times (\text{Scr}/B)^C \times 0.993^{\text{age}} \times (1.159 \text{ if black})$ Where: A and B are the following Female: for $\text{Scr} \leq 0.7$ , A = 144, B = 0.7, C = -0.329; and $\text{Scr} > 0.7$ , A = 144, B = 0.7, C = -1.209 Male: for $\text{Scr} \leq 0.9$ , A = 141, B = 0.9, C = -0.411; and $\text{Scr} > 0.9$ , A = 141, B = 0.9, C = -1.209
<b>CKD-EPI Creatinine Equation (2021)</b> (GFR measurement, ml/min per 1.73 m <sup>2</sup> )	$142 \times (\text{Scr}/A)^B \times 0.9938^{\text{age}} \times (1.012 \text{ if female})$ Where: A and B are the following Female: for $\text{Scr} \leq 0.7$ , A = 0.7, B = -0.241 and $\text{Scr} > 0.7$ , A = 0.7, B = -1.2 Male: for $\text{Scr} > 0.9$ , A = 0.9, B = -1.2 and $\text{Scr} > 0.9$ , A = 0.9, B = -1.2

CG: Cockcroft-Gault; CKD- EPI: Chronic Kidney Disease Epidemiology Equation; MDRD: Modification of Diet in Renal Disease; MDRD-TR: Modification of Diet in Renal Disease Turkish version Scr: serum creatinine

SPSS Version 25.0 was used for statistical analysis. The Kolmogorov-Smirnov test was done to determine the distribution pattern. Normal distributed continuous variables were expressed as mean  $\pm$  standard deviation while not normally distributed variables were presented as median and interquartile range and ordinal and nominal data were expressed as n (%). Spearman's correlation analysis was used to analyze the relationship between continuous variables. Friedmann and Wilcoxon's tests were used to assess the difference between GFR estimates. The Mann-Whitney U test was used to analyze the

difference between eGFR median values between different groups. A  $p$ -value  $< 0.05$  within a confidence interval of 95% was considered significant.

## RESULT AND DISCUSSION

A total of 305 patients with complete demographic data were included in the study. The average age of patients was 52.92 years. Most (59%) of the patients were male. The average weight, BMI and serum creatinine level were 80.45 kg, 29.20 kg/m<sup>2</sup> and 1.68 mg/dl respectively. The adjusted body weight was calculated for 119 obese patients (BMI  $> 30$ kg/m<sup>2</sup>). The diagnosis of acute kidney injury (AKI) and chronic kidney disease (CKD) was present in 18 and 42 patients respectively. Four patients with CKD had an AKI diagnosis. Patient demographic and health details are given in Table 2.

**Table 1.** Patients' demographic and health related data

Characteristics	Median (Quartile1-Quartile3)
Age (years)	53 (41-67)
Weight (kg)	80 (67.5-91)
Height (cm)	167 (160-173)
Body mass index (kg/m <sup>2</sup> )	28.4 (24.7-32.7)
Body surface area (m <sup>2</sup> )	1.92 (1.75-2.07)
Serum creatinine	1.09 (0.78-1.82)
	<b>n (%)</b>
	<b>305 (100)</b>
Gender	Male
	Female
Age group	18-29
	30-59
	>60
Patients with kidney disease diagnosis	Acute kidney diseases
	Chronic kidney disease
	Both
	None
Weight status	Under-weight
	Normal
	Over-weight
	Obese

The median values of eGFR calculated using CG-ADJ, CG-BSA, MDRD, MDRD-TR, CKD-EPI 2009 and CKD-EPI 2021 equations were 70.83 ml/min, 65.82, 64.30, 51.70, 71.14 and 71.77 ml/min/1.73m<sup>2</sup> respectively. Similar eGFR values were obtained from CKD-EPI 2009 and CKD-EPI 2021. The lowest eGFR values were obtained from the MDRD-TR equation.

According to the KDIGO classification, 108 and 109 of the patients were in G1 stage based on the CKD-EPI 2009 and CKD-EPI 2021, while only 32 patients were in this category based on MDRD-TR. The number of patients in stage G3a was similar based on all equations. Based on MDRD-TR equation, 43 patients were in G5 stage while only 17 and 22 patients were in this category based on CG and CG-BSA respectively. The distribution of patients' KDIGO stages is given in Table 3.

**Table 2.** Distribution of patients based on their Kidney function stages and the presence of kidney disease

Formular	GFR Median (Quartile1- Quartile3)	KDIGO stages	Number of Patients (n)	Presence of kidney disease diagnosis				BMI			
				None (241)	AKI (18)	CKD (42)	Both (4)	<18.5 (10)	18.5-25 (74)	25-30 (102)	>30 (119)
CG	70.83 (40-111) ml/min	G1	115	113	2	0	0	1	25	42	47
		G2	71	68	1	2	0	4	15	28	24
		G3a	33	27	2	4	0	1	14	5	13
		G3b	31	19	6	5	1	1	5	11	14
		G4	38	12	5	20	1	1	8	12	17
		G5	17	2	2	11	2	2	7	4	4
CG-BSA	65.82 (36-98) ml/min/1.73 m <sup>2</sup>	G1	95	92	2	1	0	3	25	35	32
		G2	76	76	0	0	0	2	16	29	29
		G3a	43	35	3	5	0	2	12	11	18
		G3b	27	20	4	3	0	0	7	8	12
		G4	42	16	6	18	2	2	7	14	19
		G5	22	2	3	15	2	1	7	5	9
MDRD	64.30 (37-92) ml/min/1.73 m <sup>2</sup>	G1	86	83	2	1	0	3	22	28	33
		G2	79	79	0	0	0	3	17	28	31
		G3a	44	41	1	2	0	1	12	16	15
		G3b	34	21	6	7	0	0	8	11	15
		G4	33	11	5	15	2	1	8	8	16
		G5	29	6	4	17	2	2	7	11	9
MDRD-TR	51.70 (29-74) ml/min/1.73 m <sup>2</sup>	G1	32	30	2	0	0	1	10	9	12
		G2	98	97	0	1	0	4	19	36	39
		G3a	45	43	1	1	0	2	16	13	14
		G3b	53	44	4	5	0	0	13	20	20
		G4	34	19	5	9	1	1	4	11	18
		G5	43	8	6	26	3	2	12	13	16
CKD-EPI 2009	71.14 (36-100) ml/min/1.73 m <sup>2</sup>	G1	108	105	2	1	0	4	26	35	43
		G2	65	63	1	1	0	3	17	22	23
		G3a	40	37	0	3	0	0	11	15	14
		G3b	29	18	6	5	0	0	6	9	14
		G4	31	11	5	13	2	1	5	9	16
		G5	32	7	4	19	2	2	9	12	9
CKD-EPI 2021	71.77 (37-101) ml/min/1.73 m <sup>2</sup>	G1	109	106	2	1	0	4	26	36	43
		G2	67	65	1	1	0	3	18	23	23
		G3a	39	36	0	3	0	0	10	14	15
		G3b	27	16	6	5	0	0	6	8	13
		G4	31	11	5	13	2	1	5	9	16
		G5	32	7	4	19	2	2	9	12	9

AKI: Acute kidney injury; BMI: Body mass index; CG: Cockcroft-Gault; CG-BSA: Cockcroft-Gault normalized to body surface area; CKD: Chronic kidney disease; CKD-EPI: Chronic Kidney Disease Epidemiology Equation; GFR: glomerular filtration rate; MDRD: Modification of Diet in Renal Disease; MDRD-TR: Modification of Diet in Renal Disease Turkish version

The Mann-Whitney U was used to analyze the difference between eGFR median values and patients' BMI and age groups. There was a significant difference between the >60 age group and the other two age groups ( $p < 0.001$ ). There was no difference among BMI subgroups. The distribution of

patients based on KDIGO stages, their BMI and the presence of kidney disease is given in Table 3. There were two patients in G1 stage with AKI diagnosis based on all equations and one patient with CKD diagnosis based on CG-BSA, MDRD and both CKD-EPI equations. The distribution of patients with kidney disease diagnosis was similar in the G5 stage among all the equations but the highest number ( $n=35$ ) of patients was based on the MDRD-TR equation.

A significant difference ( $p<0.001$ ) was found between all equations when the KDIGO stages were compared. Wilcoxon test was done to clarify further the disparity between the equations. There was significant difference between all equations except between both CKD-EPI equations, and between these two equations and CG-BSA (Table 4). But when the test was repeated based on patients' BMI, a significant difference was also recorded between both CKD-EPI and CG-BSA in obese patients ( $p<0.008$ ). There was no difference between CG and CG-BSA in patients with BMI<30. On the contrary, no difference was recorded between CG-BSA and MDRD ( $p=0.162$ ) in obese patients.

**Table 3.** Variation in patients' eGFR stages calculated with different equations

KDIGO Stages	CG	CG-BSA	MDRD	MDRD-TR	CKD-EPI 2009	CKD-EPI 2021	Friedmann test p value
CG		<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	$p<0.001$
CG-BSA	<0.001*		0.003*	<0.001*	0.515	0.159	
MDRD	<0.001*	0.003*		<0.001*	<0.001*	<0.001*	
MDRD-TR	<0.001*	<0.001*	<0.001*		<0.001*	<0.001*	
CKD-EPI 2009	<0.001*	0.515	<0.001*	<0.001*		0.014	
CKD-EPI 2021	<0.001*	0.159	<0.001*	<0.001*	0.014		

\* $p<0.008$  was considered significant (Bonferroni correction  $0.05/6=0.008$ ) CG: Cockcroft-Gault; CG-BSA: Cockcroft-Gault normalized to body surface area; CKD-EPI: Chronic Kidney Disease Epidemiology Equation; KDIGO: Kidney Disease: Improving Global Outcomes; MDRD: Modification of Diet in Renal Disease; MDRD-TR: Modification of Diet in Renal Disease Turkish version

The ACR was recorded for only 42 patients. The ACR of 17 patients (40.5%) was below 30 and that of 11 patients was above 300. Four of these patients had CKD and one had both AKI and CKD diagnosis (Table 5). Spearman's correlation showed a significant negative correlation between ACR and eGFR values ( $p<0.05$ ) calculated using all equations with CG having the largest coefficient (-0.380) and CKD-EPI 2021 having the smallest (-0.450). Likewise, there was a positive correlation between ACR stages and all eGFR stages ( $p<0.05$ ) with coefficients of 0.357, 0.392, 0.425, 0.418, 0.466, and 0.434 for CG, CG-BSA, MDRD, MDRD-TR, CKD-EPI 2009 and CKD-EPI 2021 respectively.

GFR is an essential information in the assessment of kidney function and a main determinant of drug dosage in patients with kidney dysfunction. The accurate estimation of the GFR is critical and has been a matter of debate as different equations yield variable results in diverse patient populations. The performance of equations is based on patient-related factors which have variable extent of influence on eGFR [1]. Therefore, the selection of the most suitable equation for a particular patient subset is important. In this study, we evaluated the discrepancy in GFR values calculated using different equations in Turkish patients.

MDRD is said to have an accuracy close to that of CKD-EPI [6] and is reported to be less dispersed than CG [14]. It was reported to provide the best estimate in patients with different health issues [15] and different races including the Turkish populace [12]. Altıparmak reported that the addition of a race factor of 0.804 to the 4-variable MDRD resulted in a more accurate estimation of GFR in the Turkish population and, also produced a better classification performance over various GFR equations [12]. We evaluated this formula in addition to CG, MDRD, CKD-EPI 2009 and the newly recommended CKD-EPI 2021. The eGFR values calculated using the revised MDRD-TR equation were significantly lower compared to those calculated using other equations and most patients with kidney disease diagnosis were placed in lower KDIGO stages based on this equation. We recorded a significant

difference between the MDRD and MDRD-TR. Although our study population were relatively similar to that of Altıparmak, in terms of demographic data, the average serum creatinine concentration of our patients was lower. This may have affected our results as they reported that MDRD overestimated in patients with GFR values below 30 ml/min/1.73m<sup>2</sup> and underestimated in others.

**Table 4.** Distribution of patients based on their ACR

	Frequency (n)	A1 17	A2 14	A3 11
<b>Sex</b>				
<b>Male</b>	28	13	7	8
<b>Female</b>	14	4	7	3
<b>Age group</b>				
<b>18-29</b>	3	1	2	0
<b>30-59</b>	19	7	5	7
<b>&gt;60</b>	20	9	7	4
<b>BMI</b>				
<b>&lt;18.5</b>	1	1	0	0
<b>≥18.5 - &lt;25</b>	8	4	3	1
<b>≥25- &lt;30</b>	15	5	4	6
<b>≥30</b>	18	7	7	4
<b>Presence of kidney disease</b>				
<b>None</b>	34	16	13	5
<b>AKI</b>	2	0	1	1
<b>CKD</b>	5	1	0	4
<b>Both</b>	1	0	0	1

ACR: Albumin creatinine ratio, AKI: Acute kidney injury; BMI: Body mass index; CKD: Chronic kidney disease

The CKD-EPI has been considered the best estimation equation in different populations [6]. In a study involving Turkish participants, 6-variable MDRD and CKD-EPI were found to demonstrate the best performance in estimating GFR in reference to 24-hour creatinine clearance [16]. In our study, the eGFR values from both CKD-EPI equations were statistically similar to CG-BSA with comparable median values. The median GFR from MDRD and MDRD-TR were significantly lower. We used the 4-variable MDRD in place of the 6-variable, this may have affected our results. This further highlights the importance of variability among the equations. Another study reported better performance of CG with lean body weight over MDRD and CKD-EPI in patients with metabolic diseases [17]. But the inaccuracy of all formulas to reveal a decline in GFR in patients has been emphasized [18].

GFR estimating formulas have failed to be fully accurate in overweight and obese patients and the choice of equation has been a controversial issue. Some researchers have considered the use of CG with lean body weight to be more appropriate [19,20] while others consider MDRD and CKD-EPI in patients with stable kidney function, especially for dose adjustments [2]. To compare GFR values calculated using CG with that from other formulas, adjustment to BSA is recommended [14], but this was reported to lead to underestimation of GFR in this patient population, which may have serious health consequences [21]. In our study, there was a noticeable shift in the distribution of patients based on the KDIGO stages when CG GFR values were adjusted to BSA as the GFR values dropped after adjustment. A significant proportion of our study population was overweight or obese. In the obese patients, the adjusted CG GFR values became similar to only that calculated using MDRD. After adjustment, while the difference in non-obese patients between CG and CG-BSA was neutralized, it persisted in obese patients. Routine use of gold-standard measurement methods may be indicated particularly in this patient population.

The ACR measured in spot urine is used to measure albuminuria and used to assess kidney function. In our study, this ratio was checked in only a few patients, and it correlated to patients' eGFR values and KDIGO stages. The association of lower eGFR and higher ACR in determining the risk of kidney injury in patients with or without chronic diseases is consistent [22,23]. The use of both eGFR

and ACR to assess kidney function in all general populations may provide better insight into the general kidney status although the ACR is not translated to GFR and cannot be directly used in drug dose modifications.

Our study has limitations in generalizing the results to the Turkish population, such as being conducted retrospectively with limited data and at a single center. We were not able to determine the most accurate estimation equation for the Turkish population as patients' measured GFR values were not available to be used as a standard reference. The previously recommended MDRD revised for the Turkish population resulted in the lowest GFR estimates; therefore, further research is needed.

The most accurate GFR measurement methods are not practical in clinical practice and estimation methods using endogenous biomarkers are more readily used. Our results show valuable variation in estimations based on patient demographics and/or equations. The need for novel practical methods for estimating glomerular filtration rates in general and specific patient populations are urgently needed.

## AUTHOR CONTRIBUTIONS

Concept: R.M.U.; Design: B.N.Ç., R.M.U.; Control: R.M.U. Sources: - ; Materials: - ; Data Collection and/or Processing: B.N.Ç.; Analysis and/or Interpretation: B.N.Ç., R.M.U.; Literature Review: B.N.Ç., R.M.U.; Manuscript Writing B.N.Ç., R.M.U.; Critical Review: B.N.Ç., R.M.U.; Other: -

## CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

## ETHICS COMMITTEE APPROVAL

The institutional review board approved this study (No:2022-389) and waived consent.

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