Original Research Article

Estimating Glomerular Filtration Rate Using Full Age Spectrum Equation to Assess Kidney Function in Hypertensive Patients: A Cross-sectional Study

ABSTRACT

Background: Estimated glomerular filtration rate (eGFR) is accepted as the best indicator of kidney function and commonly assessed from serum creatinine (Cr) and cystatin C (Cys-C) based equations. The present cross-sectional, observational study aimed to assess eGFR using a new and validated Full Age Spectrum (FAS) equation and compared with eGFR assessed using old and established equations in hypertensive patients.

Materials and Methods: Overall, 60 subjects were recruited for the study, including 30 hypertensive patients and 30 age and sex matched healthy subjects. Serum creatinine and cystatin C were measured using commercial biochemical kits. These levels were used to derive and compare eGFR using our different equations, namely, Cockcroft and Gault (CG), Modification of Diet in Renal Disease (MDRD), Chronic Kidney Disease-epidemiology collaboration (CKD-EPI), and FAS equation. Student t-test was used for comparison between two groups and One-way ANOVA test was used to find multiple comparison with-in the hypertensive and control group. Pearson's Univariate correlation followed by multiple linear regression analysis was applied to find independent predictors of eGFR. All data were analyzed using Sigma-Stat.

Results: There was significant difference found in the eGFR levels using different equations in hypertensive subjects as compared to healthy subjects (P<0.01). With–in hypertensive subjects and with-in heathy subjects, a significant difference was also reported (both P<0.01). For FAS-based GFR, age was found as independent predictor of eGFR by all FAS equations. eGFR estimated using Cr based equations resulted in significant difference in categorizing number of subjects into CKD v/s non-CKD depending on their eGFR levels. But there was no difference found for the above in serum cystatin C based equations (P=0.26).

Conclusion: Present data showed that eGFR derived using all set of equations resulted in variable eGFR levels. But, use of Cr based equations instead of Cys-C or combine Cr-Cys based equations resulted in wide variation *i.e.* change in GFR due to change in marker.

Keywords: Full age spectrum; glomerular filtration rate; hypertensive patients; equations; creatinine; cystatin C.

1. INTRODUCTION

Hypertension is risk factor for cardiovascular disease (CVD), chronic kidney disease (CKD) and end stage renal disease (ESRD) [1]. Accurate measurement of glomerular filtration rate (GFR) is utmost important for proper diagnosis of renal impairment in these patients, but is often difficult and frequently imprecise [2]. As per the guidelines and recommendations of the National Kidney Foundation, exogenous GFR markers like Inulin clearance and clearances of radioisotope labeled or non-labeled trace quantities of Chromium 51-EDTA (Cr-EDTA), technetium 99-diethylenetriamine pentacetic acid (^{99}Tr-DTPA), iothalamate or iohexol have been considered the reference standards for GFR (rGFR) measurement. However, these techniques are expensive, labor and time intensive, thus not ideal for clinical practice [3].

Endogenous filtration markers are easy to measure, less complex and provide more rapid result. The most commonly used endogenous filtration markers in clinical practice is serum creatinine (Cr). But Cr based measured GFR is

erratic due to its dependence on age, sex, changing muscle mass, bilirubin, fluid variations, and its tubular secretion and re-absorption. Recently, serum cystatin C (Cys-C), an alternative marker of kidney function, has been described as a better predictor of GFR than Cr [4]. Cys-C is a cysteine protease inhibitor found in virtually all human tissues and body fluids, which, in contrast to Cr, is independent of muscle mass, bilirubin, age, sex, race, weight, or diet [2]. Many equations and rules for calculating eGFR has been used from last decades by using Cr and Cys-C as biomarkers for finding the kidney function, but these equations are still imprecise. Mainly, the very first Cockcroft and Gault (CG) equation [5] followed by Modification of Diet in Renal Disease (MDRD) equation [6], Hoek's formula [7], then Chronic Kidney Disease-Epidemiological collaboration (CKD-EPI) equation [8,9] and very recent Full Age Spectrum (FAS) equations [10,11] have been developed for estimating GFR (eGFR). eGFR based on FAS equation has been evaluated in Korean adults [12], black Africans [13], Europeans [14] and Chinese subjects [15,16].

In hypertensive patients, Cr based GFR estimates often give imprecise results that may lead to the over diagnosis of CKD in these patients. SBP, DBP and PP are well known to predict CV risk, but their respective association with eGFR (determined using advanced new equations) *i.e.* Cys-C based eGFR, had not been widely explored as compared to Cr based eGFR in hypertensive patients. In current clinical practice, CKD is defined by GFR estimated from Cr, not from Cys-C. Even knowing Cys-C as a better marker, practically it is underutilized for estimating GFR. To the best of our knowledge, eGFR estimation has not yet reported by any study using FAS, 2017 equation in hypertensive patients in India. Thus, the study was aimed to determine eGFR using FAS, 2017 equation and comparing it with eGFR determined using other standard equations, so that clinical utilization of new equations for determining eGFR could be assessed during routine renal function evaluation in hypertensive patients.

2. MATERIALS AND METHODS

2.1 Study Site and Design

A cross-sectional, observational clinical study was conducted at Department of Medicine, Govt. Medical College and Rajindra Hospital, Patiala and Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala. All the patients were assessed for their eligibility of inclusion and exclusion criteria. Clinical assessment of eligible patients was carried out on hospital visit after inclusion in the study. The study protocol was approved by the Institutional Ethics Committee (approval no. IEC-2019/123), Punjabi University, Patiala. The study was conducted in accordance with the principles of the declaration of Helsinki, the code of Good Clinical Practice, and in accordance with "Ethical guidelines for biomedical research on human participants" issued by Indian Council of Medical Research (ICMR). All the patients provided a written informed consent to participate after a full explanation of the study.

2.2 Inclusion/Exclusion Criteria

The Inclusion criteria were: hypertension defined as SBP >140mmHg and DBP> 90mmHg [17]; patients of both sexes and age≥ 18 years; with/without- alcoholism and smoking; able and willing to give informed consent. The Exclusion criteria were: Patients having all other types of hypertension; type-1 and type-2 diabetes; pregnant or lactating women; history of congestive heart failure; any chronic inflammatory rheumatic disorder specially affecting renal function; thyroid disorders, malignancies and liver disorders; patients with any other concurrent acute and chronic medical conditions; patients on drugs affecting renal function.

2.3 Sampling and Assessment

Overall, 60 subjects were recruited for the study, including 30 hypertensive patients and, 30 age- and sex- matched healthy subjects. Subjects were assigned into two major groups, healthy control Group I (GP I) and hypertensive patients Group II (GP II). A sample of 3ml blood was collected from each subject by trained lab technicians. Samples were then centrifuged immediately on a laboratory centrifuge to separate out the serum. Separated serum samples were stored at -40^{o}C till analysis.

Overall, following anthropometric, clinical and biochemical parameters were assessed:

1. Body mass index: Height and weight of all the subjects were measured and body mass index (BMI) was calculated

according to the formulae, weight in kilogram/ square meter height.

2. Blood pressure: Blood pressure was measured using manual Sphygmomanometer.
3. Renal Function Tests (Serum creatinine and cystatin C): Serum Cr was measured using Erba Liquixx Creatinine kit (Transasia Bio-Medicals Ltd.) and Cystatin-C by using quantitative turbidimetric immunoassay kit (Accurex Bio-Medicals Ltd.) according to manufacturer's recommended protocol. The concentration was measured using semi autoanalyzer (ChEM-4 plus v-2 auto analyzer).
4. Estimated Glomerular filtration rate (eGFR): eGFR was estimated using four different equations. The equations are: a) Cockcroft and Gault (CG) equation [5]; b) Modification of Diet in Renal Disease (MDRD) study-based equation [MDRD equation] [6]; c) Chronic kidney Disease-Epidemiology Collaboration (CKD-EPI) equation comprising three different serum markers *i.e.* Cr [8], Cys C, and Cr-Cys (both Cr and Cys C) [9]; d) Full Age Spectrum (FAS) Equation by Pottel *et al*., 2017 for assessing GFR, based on European healthy subjects with a novel modeling approach [10].

Both CG and MDRD equations derive eGFR based on serum creatinine levels, whereas, Both CKD-EPI and FAS equations further consists eGFR estimation based on serum creatinine (eGFR Cr), serum cystatin C (eGFR Cys) and combination of both creatinine and cystatin C (eGFR Cr-Cys) (Table 1).

3. RESULTS

The distribution of demographic, biochemical and hemodynamic characteristics of subjects have shown in Table 2. Serum Cr and Cys-C levels were found high in hypertensive subjects as compared to healthy subjects.

Table 3 shows the eGFR estimation by various equations. There was significant difference found in the eGFR levels using different equations in hypertensive subjects as compared to healthy subjects ($p<0.01$). With–in hypertensive subjects and with-in heathy subjects, a significant difference was also reported (both $p<0.01$) that signified the change in GFR level with the change in equation used for calculating eGFR.

Pearson's univariate correlation analysis was used in hypertensive patients to find the correlation/association of different traditional risk factors with eGFR calculated by four different equations. We found that eGFR based on different equations were significantly correlated (P=0.05) with many variables: a) CG equation-Age (r= -0.64), BMI (r=0.44) and SBP (r= -0.40); MDRD equation: Age (r= -0.39) and SBP (r= -0.32); CKD EPIcr equation:

Table 1. Equation for calculating eGFR

1. Cockcroft and Gault Equation

$$\text{CLcr (ml/min)} = \frac{(140 - \text{age}) \times \text{lean body weight (kg)} \times (0.85 \text{ if female})}{\text{Serum creatinine (mg/dl)} \times 72}$$

2. MDRD Equation

$$\text{MDRD eGFR} = 170 \times \text{Cr}^{-1.154} \times \text{age}^{-0.203} \times [0.742 \text{ if female}] \times [1.212 \text{ if black}]$$

3. CKD-EPI - Cr Equations

Basis of Equation and Sex	Serum Creatinine mg/dl	Serum Cystatin C mg/dl	Equations for estimating GFR
Female	≤0.7		$144\times(\text{Cr}/0.7)^{-0.329}\times0.993^{\text{age}}$ [×1.159 if black]
Female	≥0.7		$144\times(\text{Cr}/0.7)^{-1.209}\times0.993^{\text{age}}$ [×1.159 if black]
Male	≤0.9		$141\times(\text{Cr}/0.9)^{-0.411}\times0.993^{\text{age}}$ [×1.159 if black]
Male	≥0.9		$141\times(\text{Cr}/0.9)^{-1.209}\times0.993^{\text{age}}$ [×1.159 if black]

CKD-EPI - Cys C equation

Female or Male		≤0.8	$133 \times (Cys/0.8)^{-0.499} \times 0.996^{age}$ [×0.932 if female]
Female or Male		≥0.8	$133 \times (Cys/0.8)^{-1.328} \times 0.996^{age}$ [×0.932 if female]
CKD-EPI Cr-Cys C equation			
Female	≤0.7	≤0.8	$130 \times (Cr/0.7)^{-0.248} \times (Cys/0.8)^{-0.375} \times 0.995^{age}$ [×1.08 if black]
		≥0.8	$130 \times (Cr/0.7)^{-0.248} \times (Cys/0.8)^{-0.711} \times 0.995^{age}$ [×1.08 if black]
Female	≥0.7	≤0.8	$130 \times (Cr/0.7)^{-0.601} \times (Cys/0.8)^{-0.375} \times 0.995^{age}$ [×1.08 if black]
		≥0.8	$130 \times (Cr/0.7)^{-0.601} \times (Cys/0.8)^{-0.711} \times 0.995^{age}$ [×1.08 if black]
Male	≤0.9	≤0.8	$130 \times (Cr/0.7)^{-0.207} \times (Cys/0.8)^{-0.375} \times 0.995^{age}$ [×1.08 if black]
		≥0.8	$130 \times (Cr/0.7)^{-0.207} \times (Cys/0.8)^{-0.711} \times 0.995^{age}$ [×1.08 if black]
Male	≥0.9	≤0.8	$130 \times (Cr/0.7)^{-0.601} \times (Cys/0.8)^{-0.375} \times 0.995^{age}$ [×1.08 if black]
		≥0.8	$130 \times (Cr/0.7)^{-0.601} \times (Cys/0.8)^{-0.711} \times 0.995^{age}$ [×1.08 if black]

4. FAS Equations

$FAS_{Cr} = 107.3/(Cr/Qcr) \times [\ 0.988^{(Age-40)}$ when age > 40 years]

$FAS_{Cys} = 107.3/(Cys/Q_{Cys}) \times [0.988^{(Age-40)}$, age > 40years]

$FAS_{Cr\text{-}Cys} = 107.3/[\alpha \times (Cr/Q_{cr}) + (1-\alpha) \times (Cys/Q_{Cys})] \times [0.988^{(Age-40)}$, age >40 years]

Qcr normalized serum creatinine (female: Qcr = 0.70 mg/dl; male: Qcr = 0.90 mg/dl); QCys: normalized Cystatin C (age<70 years old: QCys=0.82 mg/l; age ≥70 years old: QCys=0.95 mg/l); α=0.5 (Yong et al., 2019)

Table 2. Demographic, Biochemical and hemodynamic characteristics of subjects

Parameter	**Hypertensive patients (Group I)**	**Healthy subjects (Group II)**	***P value***
Age (year)	55.36 ± 14.53	25.26 ± 8.0	<0.01
Sex (M/F)	8/22	19/11	
BMI (kg/m^2)	25.43 ± 4.21	23.04 ± 3.32	0.01
Disease duration (years)	3.01 ± 1.93	--	--
SBP (mmHg)	161.20 ± 15.84	117.93 ± 3.50	0.01
DBP (mmHg)	93.66 ± 7.74	79.66 ± 1.15	0.01
PP (mmHg)	67.53 ± 13.40	38.26 ± 3.76	0.01
Serum Cr (mg/dL)	1.36 ± 0.38	1.05 ± 0.17	0.01
Serum Cys C (mg/dL)	1.19 ± 0.35	0.92 ± 0.03	0.01

Data is presented as mean ± standard deviation; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; PP: Pulse pressure; Cr: Creatinine; Cys C: Cystatin C

Table 3. eGFR levels in hypertensive and control subjects

S. N.	Equations	Marker used	eGFR (ml/min/1.73 m^2)		*P value*
			Hypertensive	**Control**	
1	CG	Cr	55.60± 20.57	93.81± 21.58	0.01
2	MDRD	Cr	47.35± 14.48	79.35±17.33	0.01
3	CKD EPI	Cr[a]	50.83± 17.04	89.38± 17.32	0.01
		Cys[b]	66.24± 23.19	96.74± 5.48	0.01
		Cr-Cys[c]	56.84± 19.51	90.09± 16.62	0.01
4	FAS	Cr[a]	51.77± 16.26	85.00± 16.01	0.01
		Cys[b]	66.47± 19.93	94.539±5.04	0.01
		Cr-Cys[c]	50.87± 20.73	83.67± 10.36	0.01
P value			2 v/s 4[a], *P*=0.04 2 v/s 4[b], *P*=0.01 3a v/s 4[a], *P*=0.01	2 v/s 3[b], P=0.01 2 v/s 4[b], *P*=0.01 3[b] v/s 4[c], *P*=0.01 3[b] v/s 4[a], *P*=0.01 4[b] v/s 4[c], *P*=0.03 4[a] v/s 4[b], *P*=0.01	

Table 4. Multiple linear regression analysis in hypertensive subjects

S. No.	Equations	Markers	Regression Equation	*t* and *P* value
1	CG	Cr	59.33 - (0.81 x Age) + (1.63 x BMI)	(t=2.72, *P*= 0.01)
2	MDRD	Cr	68.95 - (0.39 x Age)	(t= 6.95, *P*=0.01)
3	CKD EPI	Cr	-	-
		Cys	-	-
		Cr-Cys	-	-
4	FAS	Cr	89.259 - (0.67 x Age)	(t= 9.27, *P*=0.01)
		Cys	102.49 - (0.65 x Age)	(t=7.85, *P*=0.01)
		Cr-Cys	81.75 - (0.55 x Age)	(t=5.76, P=0.01)

Table 5. CKD and Non-CKD subjects

			Hypertensive subjects			**Healthy subjects**		
S. No	**Equations**	**Marker**	**CKD patients**	**Non-CKD patients**	***P***	**CKD patients**	**Non-CKD patients**	***P***
1	CG	Cr	18 (60%)	12 (40%)	0.01	1(3.3%)	29 (96.66%)	0.28
	MDRD		27 (90%)	3 (10%)		2 (%)	28 (93.33%)	
	CKD EPI		23 (76.66%)	7 (23.33%)		0	30 (100%)	
	FAS		13 (43.33%)	17 (56.66%)		0	30 (100%)	
2	CKD EPI	Cys	18 (60%)	12 (40%)	0.26	0	30 (100%)	1
	FAS		23 (76.66%)	7 (23.33%)		0	30 (100%)	
3	CKD EPI	Cr-Cys	10 (33.33%)	20 (66.66%)	0.01	0	30 (100%)	1
	FAS		21 (70%)	9 (30%)		0	30 (100%)	

Age (r= -0.47) and SBP (r=-0.32); CKD EPIcr-cys equation: Age (r= -0.38), SBP (r= -0.36) and DBP (r= -0.37); FAScr and FAScr-cys equations: with age only (r= -0.60) and (r= -0.39), respectively. eGFR by FAScys was not correlated with any variable.

The variables that showed significantly correlation with eGFR levels were further subjected to regression analysis to reveal the dependency of one variable on the other variable (Table 4). As eGFR using CG equation was significantly correlated with age, BMI, and SBP, these variables were entered in to multiple linear regression analysis to find the independent predictors of GFR. For CG based GFR: age and BMI were found as independent predictors; MDRD and FAS based GFR: age was only independent predictor of eGFR. There was found no independent predictor of CKD EPI based GFR.

Subjects were categorized on the basis of eGFR level into two categories- chronic kidney disease subjects (CKD: eGFR <60ml/min/1.73m^2) and no chronic kidney disease subjects (Non-CKD: eGFR >60ml/min/1.73m^2). In hypertensive subjects: eGFR estimated using four Cr based equations (CG, MDRD, CKD-EPI and FAS, P<0.001) and two combined Cr-Cys based equations (CKD-EPI and FAS, P=0.01) resulted in significant difference in categorizing number of subjects into CKD v/s non CKD depending on their eGFR levels. But there was no difference found for the above in serum cystatin C based equations (p=0.26). On the other hand, in control subjects eGFR based on different equations also did not show any significant difference in categorizing number of subjects into CKD v/s Non- CKD depending on their eGFR levels (all P>0.05) (Table 5).

4. DISCUSSION

Cystatin C has emerged as reliable biomarker for determination of kidney function due to its negligible interference with dietary intake, muscle mass, body height, weight, and renal tubular re-absorptive mechanisms compared to conventional biomarker creatinine [18]. mGFR using urinary or plasma clearance of exogenous filtration markers is considered the gold standard for evaluation of kidney function but is not routinely available because of the complexity of measurement protocols. Due to this limitation, eGFR based on creatinine is now widely reported by clinical laboratories and is available in most clinical encounters as a “first line” test of kidney function [19]. In clinical practice, most common creatinine and cystatin C based equations used for the assessment of kidney functioning are CG, MDRD and CKD-EPI [20]. FAS equation has been recently introduced for estimating eGFR and very scarce data is available for FAS-eGFR in CKD patients and to best of our knowledge there is no yet published evidence for FAS-eGFR in hypertensive patients. Thus, in present study we estimated eGFR by FAS equation and compared with the GFR estimated from other three standard equations (CG, MDRD, and CKD-EPI).

GFR estimation using CG equation is a common practice. It is the oldest, still widely acceptable and most reliable formula and has been reported in various studies for estimating GFR in hypertensives with kidney dysfunction or CKD patients [21,22,23]. Similarly, MDRD equation is widely used for assessment of eGFR in patient with renal impairment [22,23,24].

In the present study, we found that eGFR levels were low in hypertensive patients as compared to healthy subjects. Present finding of low GFR calculated using CG equation [21,22,23], MDRD and CKD EPI equation [23,24] in hypertensive as compared to healthy are in accordance with the previous published data. At present we have not yet any published evidence available for comparing GFR derived using FAS equation. As per our study, eGFR determined using all FAS equations was observed lower in hypertensive patients than healthy subjects.

With-in hypertensive group, we found different mean level of GFR when estimated using different equations, especially of the case (MDRD v/s FAS_{Cr}, P=0.04), (CKD EPI_{Cr} v/s FAS_{Cr}; p<0.01), (MDRD v/s FAS_{Cys}; P<0.01). From this, it was interpreted that eGFR result vary by varying the equation based on serum marker.

A correlation matrix was used to find the correlation/association of different traditional risk factors with eGFR. Salgado et al., 2013 [25] had reported a significant correlation of Cr and Cys C levels with age (r=0.22, p=0.01 and r=0.22, p<0.01) and SBP (r=0.19, p<0.01 and r=0.28, p<0.01). Monteiro et al., 2012 [26] has also observed a significant correlation of Cys C level with age (r=0.40, p<0.01) but, not with BMI and SBP. In present study both Cr and Cys C levels were not found correlated with neither age nor SBP and BMI. These observations are not in support of previous finding [25,26]. It may be due to insufficient sample size in our study. Cr and Cys C level were found highly correlated with each other (r=0.87, p=0.03). Cys C levels were also found negatively correlated with eGFR assessed by CG, MDRD, CKD EPI and FAS equation. The same pattern has been reported by Monteiro et al., 2012 revealing decrease CG-eGFR (r=-0.52, p<0.01) and MDRD-eGFR (r=-0.52, p<0.01) by rising serum Cys-C levels.

We found CG-eGFR was significantly correlated with three traditional risk factors of renal impairment (age, BMI and SBP). Similarly, eGFR by MDRD and CKD-EPI $_{Cr\text{-}Cys}$ were found correlated with age and SBP. Multiple linear regression analysis also revealed only age as independent predictor for eGFR derived using CG, MDRD and FAS equation. In present study,

age was the only independent risk factor for declined eGFR derived using FAS equation in hypertensive subjects. This signifies the age associated decline in GFR [27,28,29]. Duan et al., 2019 [30] has reported BMI level as independent and negative predictor of CKD-EPI eGFR in elderly subjects with GFR <60 ml/min m^2. Wang *et al*., 2018 [1] has found that 24 hr SBP variability was associated with decline in eGFR. In present study, BP and BMI were not the variables that could affect the eGFR levels derived by FAS equation in hypertensive patients.

In healthy subjects, as they were assumed to have no CKD, based on their eGFR levels they were categorized into non-CKD (n=100%) by all the equations, except CG equation, in which only one patient was categorized into CKD category.

In hypertensive subjects, Cr based equations (CG, MDRD, CKD-EPI and FAS) and combined Cr and Cys C based equation (CKD-EPI and FAS) resulted in significantly different number of patients in to CKD v/s non-CKD (all $p<0.001$) based on their GFR levels. Only Cys C based equations ($CKD\text{-}EPI_{cys}$ and FAS_{cys}) resulted in no difference in categorizing patients into CKD v/s non-CKD based on their eGFR levels. Thus, eGFR based on Cr and combined Cr-Cys based equations, but not Cys-C based equations, produced different results i.e. either over estimate or underestimate the GFR that vary from equation to equation.

5. CONCLUSION

In present study, the new equation evaluated for eGFR estimation was FAS equation that further constituted three set of equations (FASCr, FASCys and FASCr-Cys) and compared with eGFR estimation with other standard equations. Present data showed that the eGFR derived using all set of equations [four Cr based equations (CG, MDRD, CKD-EPI and FAS), two Cys-C based equations (CKD-EPI and FAS) and, two combined Cr-Cys C based equations (CKD-EPI and FAS)] resulted in different eGFR levels when compared with-in hypertensive patients. But, using of Cr based equations instead of Cys-C or combined Cr-CysC based equations resulted in wide variation *i.e.* change in GFR due to change in marker. Lowest mean eGFR levels were reported using $MDRD_{Cr}$ equation (47.35±14.48) and highest levels were reported with FAS_{Cys} equation (66.47±19.93). In present study, age was the only independent risk factor for declined eGFR derived using FAS equation in both healthy and hypertensive subjects. It was found that eGFR levels by FASCys were significantly higher than by FASCr and FASCr-Cys. Moreover, it resulted in under estimation of eGFR as compared to eGFR (FASCr) and (FASCr-Cys) when patients were categorized in to CKD (eGFR < 60ml/min/1.73m^2) and Non-CKD (eGFR > 60ml/min/ 1.73m^2) based on their eGFR levels. Thus, there is need to find a robust equation that results in least variability in eGFR levels.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved (approval number- IEC-2019/123) by human institutional ethics committee (IEC) of Punjabi university, Patiala and was performed in accordance with the declaration of Helsinki and the code of Good Clinical Practice. All patients provided written informed consent to participate after a full explanation of the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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