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Gender impact on baseline peritoneal transport properties in incident peritoneal dialysis patients

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Abstract

Objective To explore the impact of gender on baseline peritoneal transportation properties and ultrafiltration (UF) ability.

Method Patients who started peritoneal dialysis (PD) in our PD center were retrospectively analyzed. Peritoneal transportation characteristics and other UF-associated factors were compared between male and female patients. Stepwise linear regression analysis and propensity score (PS) analysis were used to identify the predictors of peritoneal equilibration test (PET) UF.

Results A total of 1052 patients (424 women and 628 men) were included. Compared with male PD patients, females were older (48.4 ± 15.3 vs 46.0 ± 14.6 years), with less total body water (27.6 ± 2.5 vs 36.9 ± 3.8 L), lower body mass index (21.2 ± 3.4 vs 22.0 ± 2.9 kg/m^2), lower albumin levels (37.4 ± 5.2 vs 38.0 ± 5.4 g/L), worse renal function [residual glomerular filtration rate: 2.6 (1.2, 3.9) vs 3.2 (1.8, 5.5) mL/min/1.73 m^2], lower dialysate-to-plasma ratio of creatinine at 4-h PET (D/PCr; 0.68 ± 0.11 vs 0.72 ± 0.12), higher 4-h effluent glucose level/0-h effluent glucose level of PET (D4/D0; 0.41 ± 0.08 vs 0.38 ± 0.08), better UF ability [PET UF: 320 (200, 400) vs 260 (150, 360) mL], and higher *Kt*/*V* (2.66 ± 0.60 vs 2.23 ± 0.66) (all $P < 0.05$) when starting PD. Stepwise linear regression analysis showed that D4/D0, residual urine output, D/PCr, and gender are independently associated with PET UF. By propensity score analysis, female patients still showed significantly more PET UF than male counterparts even with balanced D/PCr, D4/D0, and residual urine.

Conclusion Among the new PD patients, females showed much higher D4/D0, lower D/PCr, and more PET UF than males, with better PET UF being independent of slower solute transportation.

Keywords Peritoneal dialysis · Peritoneal equilibration test · Gender · Ultrafiltration

Introduction

The peritoneal equilibration test (PET) is a widely used assessment in common clinical practice to evaluate the capacity of the peritoneum to transport small solutes and its ability to generate ultrafiltration (UF). Although there are many modified PETs to evaluate the complex physiopathology of the peritoneal membrane, the standard PET of Twardowski et al. [1] is still the most popular because of its simplicity. Numerous studies about PET have shown that great variability exists among patients, which may be influenced by age, race, genetic factors, and clinical factors such as diabetic mellitus (DM), cardiovascular comorbidity and inflammation, and body area [2–4]. Although many years ago it was recognized that gender may have an impact on PET results [5], the widely variable time spent by patients on peritoneal dialysis (PD) makes it very difficult to recognize whether the difference of PD transport function is caused by gender or by other factors, such as glucose and inflammation, which may all damage the structure of the peritoneum as well as the peritoneal transportation status [6]. By focusing on the initial PET results of our PD patients, in this study we attempt to explore the impact of gender on the baseline peritoneal transportation properties and UF ability.

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Subjects and methods

Participants

All patients who started peritoneal dialysis from January 1, 2006 to April 30, 2012 at the PD center of The First Affiliated Hospital of Sun Yat-sen University were enrolled. The original patient inclusion criteria were: (1) undergoing continuous ambulatory PD (CAPD) ≥ 1 months; (2) age > 18 years; and (3) had signed an informed consent form. The patients who were not able to accomplish the PET for any reasons were excluded. Ultimately a total of 1052 CAPD patients completing the original study were recruited to the present study. The study protocols were approved by the Ethics Committee of The First Affiliated Hospital of Sun Yat-sen University (2016) no. 215. Informed consent was obtained from all participants.

Patient information

Patients were evaluated for clinical and biochemical data and PET during a routine clinical visit 1 month after the initiation of PD.

Dialysate-to-plasma ratio of creatinine at 4 h of PET (D/PCr) and 4-h effluent glucose level/0-h effluent glucose level of PET (D4/D0) were determined based on the results of the standard 4-h PET. Prior to the PET, a standard CAPD treatment was conducted with 8–12 h dwell overnight with 2 L of 1.5% dextrose (1.36% glucose solution). The effluent was drained for 20 min, then 2 L of 2.5% dextrose (2.27% glucose solution) was infused into the peritoneal cavity and dwelt for 4 h. Dialysate samples were collected at 0, 2, and 4 h and a blood sample was collected at 2 h. Net UF volumes were recorded. In addition, patients provided a 24-h collection of urine and effluent, completed in the morning of the study visit date, to evaluate *Kt/V* (a number used to quantify dialysis treatment adequacy) and residual renal function. Adequest 2.0 software (Baxter Healthcare, Deerfield, IL, USA) was applied to calculate the D/PCr, D4/D0, *Kt/V* [total (peritoneal + residual) weekly urea *Kt/V*] [7], and residual glomerular filtration rate (rGFR) (Table 1).

Diabetes was defined as use of insulin or oral hypoglycemic agents, a fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL), and/or a 2-h postprandial plasma glucose ≥ 11.1 mmol/L (200 mg/dL).

Blood was taken from patients to measure biochemical markers. Serum creatinine (SCr), serum albumin, and glucose concentrations were determined for all dialysate and blood samples using an automatic analyzer (Aeroset; Abbott Laboratories, Chicago, IL, USA). Creatinine was measured by enzymic method and serum albumin by the bromocresol green method. Fasting blood glucose was measured by enzymatic method (glucose oxidase method). At the same time, data regarding patient demographics were collected.

All 1052 patients were using dialysis systems from Baxter Healthcare. The program uses a variety of dextrose glucose solutions of 2 L volume. Icodextrin was not used in this program because it was not commercially available in China. All patients performed CAPD therapy (no one used APD).

Statistics

The patients' characteristics are presented as mean ± SD or median and interquartile range for continuous variables, and percentages and frequencies for categorical variables. We performed normality tests for all continuous variables in each group using the Kolmogorov–Smirnov test in SPSS software before addressing any statistical descriptions and inferences. A variance inflation factor test was also conducted whereby all the variables selected, except D/PCr and D4/D0, were not correlated. An independent-sample *t* test was used for a comparison of normally distributed continuous variables. A comparison of non-normally distributed continuous variables was performed using the Mann–Whitney *U* test. For categorical variables, a Chi-squared test was used. Correlation analysis of PET UF with clinical index was performed to obtain the potential correlated factors, then

Table 1 Formulas for calculations

Variables	Formula
D/P Cr	PET 4-h dialysate-corrected Cr level/PET 2-h blood-corrected Cr level
D4/D0	PET 4-h effluent glucose level/PET 0-h effluent glucose level
PET UF volume	Volume infusion of PET − volume drainage of PET
BMI	Weight (in kilograms)/height squared (in square meters)
Total body water (L) [21]	Male: 2.447 − 0.09516 × age (years) + 0.1074 × height (cm) + 0.3362 × weight (kg)
rGFR	Female: − 2.097 + 0.1069 × height (cm) + 0.2466 × weight (kg) [(urine urea nitrogen concentration/serum urea nitrogen concentration) × 24 h urine volume (mL)/1000 × 7 + (urine creatinine concentration/serum creatinine concentration) × 24 h urine volume (mL)/1000 × 7]/2

Corrected creatinine (mg/dL) = creatinine (mg/dL) − glucose (mg/dL) × 0.000531415

stepwise linear regression analysis was performed by SPSS for Windows, version 16.0 (SPSS, Chicago, IL, USA).

To balance the differences in D/PCr, D4/D0, and residual urine volume between males and females, we used propensity score (PS) analysis to obtain a cohort of 414 male and 414 female patients, creating a database of 828 patients. Formula = gender ~ age + UrinVolOut + D4D0 + DP; method = "nearest"; distance = "logit". The PS was estimated using a logistic regression model including covariates (D/P Cr, D4/D0, and residual urine volume), which have been proven to have effects on the PET UF.

Results

Comparison of baseline data in male and female patients

A total of 1052 patients, 424 (40.3%) women and 628 (59.7%) men, were studied. As shown in Table 2, compared with the male patients, females were older (48.37 ± 15.33 vs 46.02 ± 14.60 years, $P = 0.012$) and had significantly less total body water (TBW) (27.64 ± 2.50 vs 36.88 ± 3.79 L, $P < 0.001$), lower body surface area (BSA) (1.51 ± 0.12 vs 1.70 ± 0.14 m^2, $P < 0.001$), body mass index (BMI) (21.19 ± 3.43 vs 21.95 ± 2.85 kg/m^2, $P < 0.001$), lower serum creatinine level (673.48 ± 220.52 vs 820.66 ± 321.87 μmol/L, $P < 0.001$), poorer residual renal function [2.55 (1.24, 3.91) vs 3.23 (1.76, 5.48) mL/min/1.73 m^2, $P < 0.001$], and less residual urine volume [500 (300, 800) vs 700 (400, 1050) mL, $P < 0.001$]. Compared with the male patients, females showed a significantly slower peritoneal transport property (D/PCr: 0.68 ± 0.11 vs 0.72 ± 0.12, $P < 0.001$; D4/D0: 0.41 ± 0.08 vs 0.38 ± 0.08, $P < 0.001$) and much better UF ability [PET UF: 320 (200, 400) vs 260 (150, 360) mL, $P < 0.001$], and had a significantly higher total *Kt/V* (2.66 ± 0.60 vs 2.23 ± 0.66, $P < 0.001$). However, there was no significant difference in the percentage of DM (female vs male: 20.8% vs 18.8%, $P > 0.05$) between patients of different gender.

Correlation of PET UF and clinical index

In brief, univariate analysis showed that PET UF was positively related to gender ($r = -0.173$, $P < 0.001$), BSA ($r = -0.119$, $P < 0.001$), total body water ($r = -0.143$, $P < 0.001$), serum glucose ($r = -0.056$, $P = 0.071$), residual urine volume ($r = -0.204$, $P = <0.001$), rGFR ($r = -0.096$, $P = 0.002$), and D/PCr ($r = -0.336$, $P < 0.001$), and negatively related to serum albumin ($r = 0.093$, $P = 0.003$) and D4/D0 ($r = 0.350$, $P < 0.001$) (Table 3).

Independent associated factors of PET UF

As shown in Table 4, PET UF was independently associated with D4/D0 (higher D4/D0 correlates with more PET UF; $R^2 = 0.124$, adjusted $R^2 = 0.123$, $B = 424.94$, $P < 0.0001$), residual urine volume (more residual urine volume is related

Table 2 Comparison of baseline data of 1052 peritoneal dialysis patients

	Total ($n = 1052$)	Female ($n = 424$)	Male ($n = 628$)	$\chi^2/t/Z$	*P* value
Age (years)	46.96 ± 14.94	48.37 ± 15.33	46.02 ± 14.60	2.507	0.012*
Height (cm)	163.5 ± 7.49	157.22 ± 4.97	167.75 ± 5.72	− 31.712	< 0.001**
Weight (kg)	58.07 ± 10.38	52.44 ± 9.13	61.87 ± 9.40	− 16.149	< 0.001**
BSA (m^2)	1.62 ± 0.16	1.51 ± 0.12	1.70 ± 0.14	− 22.790	< 0.001**
BMI (kg/m^2)	21.64 ± 3.12	21.19 ± 3.43	21.95 ± 2.85	− 3.742	< 0.001**
DM [no. (%)]	206 (19.6)	88 (20.8)	118 (18.8)	0.621	0.431
TBW (L)	33.16 ± 5.63	27.64 ± 2.50	36.88 ± 3.79	− 47.61	< 0.001**
Residual urine (mL/day)	600 (325, 1000)	500 (300, 800)	700 (400, 1050)	− 5.431	< 0.001**
rGRF (mL/min/1.73 m^2)	2.92 (1.51, 4.80)	2.55 (1.24, 3.91)	3.23 (1.76, 5.48)	− 5.445	< 0.001**
Glucose (mmol/L)	6.50 ± 3.16	6.70 ± 3.44	6.37 ± 2.95	1.663	0.097
Albumin (g/L)	37.77 ± 5.34	37.37 ± 5.24	38.04 ± 5.39	− 1.992	0.047*
SCr (μmol/L)	761.64 ± 294.44	673.48 ± 220.52	820.66 ± 321.87	− 8.729	< 0.001**
Total weekly *Kt/V*	2.40 ± 0.67	2.66 ± 0.60	2.23 ± 0.66	10.802	< 0.001**
D4/D0	0.39 ± 0.08	0.41 ± 0.08	0.38 ± 0.08	7.086	< 0.001**
D/PCr	0.71 ± 0.11	0.68 ± 0.11	0.72 ± 0.12	− 5.130	< 0.001**
PET UF (mL)	300 (180, 400)	320 (200, 400)	260 (150, 360)	− 5.601	< 0.001**

BSA body surface area, *BMI* body mass index, *TBW* total body water, *rGRF* residual glomerular filtration rate, *SCr* serum creatinine, *D4/D0* 4-h effluent glucose level/0-h effluent glucose level of PET, *D/PCr* dialysate-to-plasma ratio of creatinine at 4 h of PET, *PET UF* peritoneal equilibration test ultrafiltration

*$P < 0.05$; **$P < 0.01$

Table 3 Correlation analysis of PET UF with clinical indices

Clinical index	*r* value	*P* value
Age	−0.008	0.803
Gender	−0.173	<0.001**
Height	−0.144	<0.001**
Weight	−0.090	0.004**
BSA	−0.119	<0.001**
BMI	−0.023	0.454
DM	−0.49	0.111
TBW	−0.143	<0.001**
Residual urine (mL/24 h)	−0.204	<0.001**
rGRF	−0.096	0.002**
Glucose	−0.056	0.071
Albumin	0.093	0.003**
SCr	0.049	0.114
Total weekly *Kt/V*	0.002	0.954
D4/D0	0.350	<0.001**
D/PCr	−0.336	<0.001**

BSA body surface area, *BMI* body mass index, *TBW* total body water, *rGRF* residual glomerular filtration rate, *SCr* serum creatinine, *D4/D0* 4-h effluent glucose level/0-h effluent glucose level of PET, *D/PCr* dialysate-to-plasma ratio of creatinine at 4 h of PET, *PET UF* peritoneal equilibration test ultrafiltration

*$P<0.05$; **$P<0.01$

to less PET UF; $R^2=0.146$, adjusted $R^2=0.144$, $B=-0.05$, $P<0.0001$), D/PCr (higher D/PCr is associated with less PET UF; $R^2=0.154$, adjusted $R^2=0.151$, $B=-229.20$, $P=0.002$), and gender (male = 0, female = 1: being female suggests more PET UF; $R^2=0.160$, adjusted $R^2=0.157$, $B=30.56$, $P=0.005$). All of these variables were normally distributed and not correlated.

Propensity score analysis

We performed a PS analysis to balance the varied distribution of D/PCr, D4/D0, and residual urine output between the male and female patients. A total of 828 patients, including 414 men and 414 women, were included in the PS-matched population. At the outset the included male and female patients had similar D/PCr, D4/D0, and residual urine output. PET UF was then compared between the two groups. Compared with the male PD patients, the females still had more PET UF (320 (200, 400) vs 300 (188, 380) mL, $P=0.006$), as shown in Table 5. In the PS-matched population we repeated the stepwise multiple regression analysis for PET UF, and obtained results (Table 6; $R^2=0.111$, adjusted $R^2=0.107$) similar to those from the unmatched data (Table 4). Our results showed that the female patients had significantly more PET UF than the males even when they had similar D/PCr, D4/D0, and residual urine.

Table 4 Linear regression of PET UF ($n=1052$)

	B	Std. error	*β*	*t* value	*P* value
(Constant)	323.877	87.027		3.722	<0.0001**
D4/D0	424.941	102.734	0.195	4.136	<0.0001**
Urine volume (mL/24 h)	−0.047	0.01	−0.134	−4.585	<0.0001**
D/PCr	−229.202	72.186	−0.148	−3.175	0.002**
Gender (female)	30.56	10.746	0.084	2.844	0.005**

Male = 0, female = 1, male group as reference

PET UF peritoneal equilibration test ultrafiltration, *D4/D0* 4-h effluent glucose level/0-h effluent glucose level of PET, *D/PCr* dialysate-to-plasma ratio of creatinine at 4 h of PET

*$P<0.05$; **$P<0.01$

Table 5 Comparison of initial PET results in propensity score-matched population ($n=828$)

	Female ($n=414$)	Male ($n=414$)	*t/V*	*P*
D4/D0	0.41 ± 0.08	0.40 ± 0.07	−1.442	0.150
Urine volume (mL)	600.30 ± 445.90	636.72 ± 453.31	37,145	0.150
D/PCr	0.68 ± 0.11	0.69 ± 0.11	0.965	0.335
PET UF (mL)	320 (200, 400)	300 (188, 380)	−2.74	0.006**

Paired-sample *t* test and Wilcoxon signed-rank test with continuity correction

D4/D0 4-h effluent glucose level/0-h effluent glucose level of PET, *D/PCr* dialysate-to-plasma ratio of creatinine at 4 h of PET, *PET UF* peritoneal equilibration test ultrafiltration

*$P<0.05$; **$P<0.01$

Table 6 Linear regression of PET UF after propensity score analysis ($n=828$)

	B	Std. error	*β*	*t* value	*P* value
(Constant)	329.209	94.858		3.471	0.002**
D4/D0	332.062	114.329	0.149	2.904	0.001**
Urine volume/24 h (mL)	−0.055	0.013	−0.144	−4.351	<0.001**
D/PCr	−212.321	78.577	−0.139	−2.702	0.040*
Gender (female)	26.222	11.222	0.077	2.337	0.018*

D4/D0 4-h effluent glucose level/0-h effluent glucose level of PET, *D/PCr* dialysate-to-plasma ratio of creatinine at 4 h of PET

*$P<0.05$; **$P<0.01$

Discussion

Our study showed that female PD patients in our PD center were older, smaller, and slimmer, with less total body water, slightly lower albumin levels, worse renal function, higher total weekly *Kt*/*V*, and lower peritoneal transport rate of small solutes, but higher PET UF when they initiated PD. Residual urine output, D/PCr, D4/D0, and gender are all independent predictors of PET UF. Compared with male patients, female counterparts have more PET UF that is independent of the slower decrease in osmotic pressure induced by slower small solute transportation.

Although enough proof has been provided that time on PD can increase the transport rate of peritoneal membrane on small solutes, which may be related to the increased vascular surface area and progressive fibrosis [3, 8], most research on PET has included a wide diversity of patients on PD. One of the few baseline PET reports is from the ANZA-DATA study [9], which showed a much lower level of the D/PCr (0.69) than our result (0.71). This disparity is likely caused by the demographic difference, since the PD patient cohort in that study was much older (59.4 ± 14.8 vs 46.96 ± 14.94 years) and with a majority of white patients (70%). Considering the significant diversity of peritoneal transport characteristics in different ethnic populations, we compared our results with its Asian subgroup and found the mean D/PCr of Asian patients in that study to be still much lower than in our study (0.68 ± 0.11 vs 0.71 ± 0.11) [9]. Given the smaller percentage male PD patients with faster transport (47.8% vs 59.7%) in the Asian subgroup of that study, this difference can be thereby explained, while the data also suggest that gender may have an impact on the initial PET results.

Ates et al. [5] first noticed that gender also had an impact on the ratios of D/PCr and D4/D0, which was independent from the BSA index (effective surface transport area). Due to the small sample of his study (26 males and 15 females), as well as the variable time on PD (range 3–56 months), not much attention was raised by this study. Now for the first time we have explored a fairly sizeable sample size of a single race—Chinese—based on the baseline PET results, making it possible for us to delineate the gap between male and female ability to transport small solutes peritoneally. Compared with males, our study found that female PD patients had much lower D/PCr (0.68 ± 0.11 vs 0.72 ± 0.12, $P<0.001$) and higher D4/D0 (0.41 ± 0.08 vs 0.38 ± 0.08, $P<0.001$), which suggested slower peritoneal transport. It is therefore preferable to classify patients according to the results of the initial PETs in different genders using the mean and SD of the patients to obtain the classification of peritoneal transport properties. Of course, it is best to consider the values of peritoneal permeability (expressed by D/PCr) as a continuous entity and not as categories.

Poor UF is always associated with increased mortality and morbidity [10, 11]. Theoretically, the patients who have higher transport function in the peritoneum will have less UF because of the rapid dissipation of the peritoneal osmotic gradient. Therefore, it is indisputable that male PD patients had less PET UF than female counterparts because they had higher D/P Cr and lower D4/D0 as shown in our results. The correlation analysis showed that, besides the peritoneal transport characteristics (D/PCr and D4/D0), gender, body size (height, weight, and BSA), residual urine output, rGFR, glucose, albumin, and TBW were all associated with PET UF. Assuming that the difference in body size and TBW could be because of the gender difference (males are usually taller and have more muscle than females), and that stepwise linear regression analysis further showed that only D4/D0, residual urine output, D/PCr, and gender were independent predictors of PET UF, we further performed a PS analysis to balance the effects of higher D/PCr, lower D4/D0, and more residual urine output in male patients, and obtained a balanced sample of 828 patients (male/female 414:414). In the new sample, male and female PD patients had similar D/PCr, D4/D0, and residual urine output, but there was still a significant difference in PET UF between men and women after PS analysis, suggesting that the different PET UF may not be the result of different peritoneal transport ability of small solutes or different residual urine output. We repeated the stepwise linear regression and arrived at exactly the same result, which suggested that female PD patients tend to have more UF not because of slower peritoneal transport or less

residual urine output, but simply because they are female. Women tend to be smaller and have less muscle and TBW, which may partly explain why they have higher PET UF levels. Given the importance of volume control in PD patients, our finding can explain why more females remain on PD for more than 10 years [12].

UF during the PET depends on the balance between the different forces acting across the peritoneal capillaries and interstitial tissues. On one hand, capillary hydrostatic pressure moves fluid, passing through aquaporin water channels and small pores outward from the capillaries because the intraperitoneal hydrostatic pressure is typically much lower. On the other hand, capillary oncotic and osmotic pressures counteract the hydrostatic forces. In PD, hypertonic glucose dialysate raises the osmolality in excess of the capillary, which creates the main initial driving force of UF. In this study we found a significant difference between male and female baseline UF. Since the PET parameters are related to small solute transport and not directly to water transport, they impact on water transport through osmotic pressure changes. Moreover, the difference in PET UF may not have been totally the result of the differing ability to transport small solutes (small pores), but rather because of the initial difference in BSA and TBW between different genders or the difference in aquaporin water channel (ultrasmall pore) transport. The transcapillary movement of free water via aquaporin-1 accounts for 40–50% of total UF across the peritoneal membrane [13, 14]. Further investigation of the expression and function of aquaporins in the peritoneum of different genders is needed. Previous studies in rats have shown that the peritoneal expression of aquaporin-1 can be upregulated by high-dose corticosteroids [15, 16]. Therefore, the reason for the different UF ability in patients of different gender may also lie in the differences in underlying disease and the treatments of patients of different gender.

Although it has been reported that hyperglycemia in diabetic patients could lead to reduced PET UF volumes [4, 17], we found that DM and blood glucose were not predictors of PET UF, in line with other reports [18, 19]. The main factor causing the disparity might be that our research was focused on the initial peritoneal function when the patient's peritoneum was almost free of fibrosis. For diabetic PD patients, high glucose dialysate induces the formation of advanced glycation end-products and then induces the progression of peritoneal fibrosis and microvascular sclerosis, which in turn results in impaired UF capacity of the peritoneal perineum [20]. The different residual renal functions of the patients included may also be involved in this discrepancy.

There are some limitations to this study, the first of which is the estimation of TBW, which was calculated according to a formula rather than bioimpedance analysis, and thus may not provide information on actual fluid status. Second, since it is an observational study, there may have been other confounding factors even after PS matching was performed to reduce selection bias. Third, because it is an observational study it cannot prove causality, and further research to explore the underlying mechanism is needed.

In conclusion, our findings suggest that males and females have different initial peritoneal transport properties and UF ability, and gender is a predictor of PET UF independent of peritoneal transport. However, the underlying mechanism of why gender has an impact on fluid transport is lacking. The different underlying diseases and treatments, as well as expression of aquaporin in PD patients of different gender, need to be investigated in future studies.

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Compliance with ethical standards

Conflict of interest Xin Wang declares that she has no conflict of interest. Qunying Guo declares that she has no conflict of interest. Qian Zhou declares that she has no conflict of interest. Chunyan Yi declares that she has no conflict of interest. Jianxiong Lin declares that she has no conflict of interest. Haiping Mao declares that she has no conflict of interest. Xiao Yang declares that she has no conflict of interest. Xin Wang declares that she has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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