



Markers of Iron Metabolism and Stroke Risk: Cross-Sectional and Longitudinal Findings from the China Health and Nutrition Survey (CHNS)

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Abstract

Background: The association of iron metabolism or status with the stroke risk remains unclear. We aimed to examine the associations between markers of iron metabolism or status and stroke risk using data from the China Health and Nutrition Survey (CHNS).

Methods: Overall, 8589 in the CHNS in 2009, and 7290 participants between 2009 and 2015 were included in the cross-sectional and longitudinal analyses, respectively. Markers included hemoglobin, ferritin (FET), transferrin (TRF), soluble transferrin receptor (sTRF-R), and ratio of sTRF-R/log FET (sTfR-F index). Multivariable logistic regression and Cox proportional hazards models were used to analyze the associations between those markers and risk of stroke. Age, gender, high-sensitivity CRP (hsCRP), body mass index (BMI), current smoking, drinking status, diabetes and hypertension were included as potential confounding factors.

Results: We observed longitudinal associations of hemoglobin (HR: 1.54, 95% CI: 1.15 – 2.06, $P = 0.004$), and sTfR-F index (HR: 0.68, 95% CI: 0.46 – 0.99, $P = 0.044$) with stroke risk among the participants whose BMI ≤ 23 kg/m². In addition, FET levels were significantly associated with stroke risk among female (HR: 1.45, 95% CI: 1.00 – 2.09, $P = 0.049$) after a median of 6.1 years follow-up. Hemoglobin, FET, TRF, sTRF-R, and sTfR-F index were not associated with the risk of stroke in overall analyses.

Conclusion: FET among female, hemoglobin and sTfR-F index among those BMI ≤ 23 kg/m² may be contributing factors for stroke.

Keywords: Iron metabolism; Ferritin; Soluble transferrin receptor; Stroke

Introduction

Iron metabolism or status markers such as hemoglobin (a well-known iron-containing oxygen-

transport protein), ferritin (FET, a protein serving as body iron reservoir, stores excess iron) and



transferrin (TRF, the main protein that accepts ferric ions and transports them to other cells) have been reported as risk factors of stroke (1-5). Furthermore, soluble transferrin receptor (sTRF-R) expressed in cells that requires iron has been introduced as a promising diagnostic tool to distinguish between iron-deficiency anemia and anemia of chronic disease (6). In addition, sTRF-R/log FET ratio (sTfR-F index), serves as a more sensitive parameter for the identification of iron homeostasis compared to the sole use of sTRF-R (7). Nowadays, the low- and middle-income countries are experiencing a growing crisis of stroke (8). Particularly, stroke was the leading cause of death and disability-adjusted life-years in adults from China (9). The hypothesis of iron accumulation related to cardiovascular disease has been proposed by Sullivan more than 30 years ago (10). However, the evidences in systemic iron status and stroke were still insufficient, and the associations between specific markers of iron status and stroke risk remain inconclusive (2, 11-17). Furthermore, most of the previous studies used a single or very limited number of iron indicators. There is a lack of comprehensive studies to investigate the risk associations between systemic iron status and stroke. Notably, to our best knowledge, there was still no report about sTRF-R and sTfR-F index related to the stroke risk.

Therefore, we aimed to examine the associations of five markers of iron metabolism or status (hemoglobin, FET, TRF, sTRF-R and sTfR-F index) with the risk of stroke using the cross-sectional and longitudinal data from the China Health and Nutrition Survey (CHNS).

Methods

Study subjects

The CHNS is an ongoing large-scale survey performed by the Chinese Center for Disease Control and Prevention and the University of North Carolina Population Center (18). Approximately

20000 individuals from 4500 families between 1989 and 2015 were included in CHNS and the detail has been described in the previous report (19).

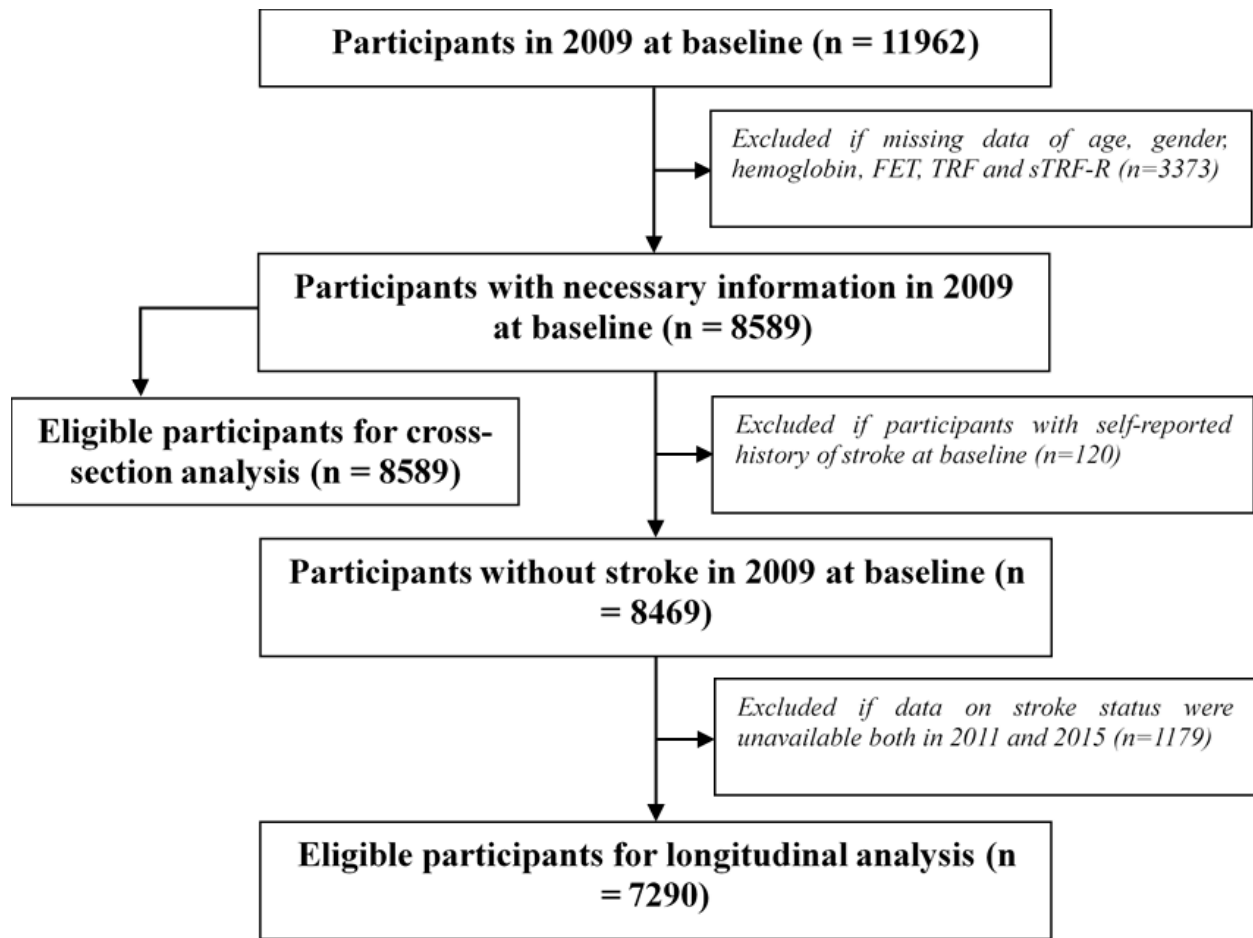
At the 2009 wave of the survey, 11962 participants who finished both the questionnaires and physical examination were included in this study. We further excluded those with missing data of age, gender, hemoglobin, FET, TRF, sTRF-R levels ($n = 3373$). As a result, 8589 eligible participants were included as the cross-sectional analysis. Of these participants, 8469 free of stroke in 2009 were followed up until the subsequent waves of survey in 2011 and 2015. Finally, 7290 participants who had outcome information were included in the longitudinal study (median follow-up time: 6.1 years) (Fig. 1).

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional review committees of the University of North Carolina, the Chinese Center for Disease Control and Prevention, and the 1964 Helsinki declaration as well as its later amendments or comparable ethical standards.

Biochemical measurements

The levels of hemoglobin were measured using the Beckman Coulter LH750 (Beckman Coulter, USA). Serum FET levels were measured using the method of radioimmunity (North Institute of Bio-Tech, China). Serum TRF and sTRF-R levels were measured on Siemens BNP machine (Siemens, Germany). The levels of hemoglobin A1c in whole fresh blood were measured using HLC/HLC/HPLC method (Tosoh, Japan/Bio-Rad, USA/Primus, USA). Serum high-sensitivity CRP (hsCRP) levels were measured on a Hitachi 7600 analyzer (Denka Seiken, Japan).



FET, ferritin; TRF, transferrin; sTRF-R, soluble transferrin receptor.

Fig. 1: Participants flow diagram

Outcome definitions

The assessment of stroke events was based on self-report. Stroke was defined if the respondents answered “yes” to the question “Has the doctor ever given you the diagnosis of apoplexy?”. In longitudinal analysis, only the first-ever stroke over the period of follow-up were recorded.

Covariates

Demographic and lifestyle information was obtained from questionnaires, including age, gender, current smoking and drinking status. Current smoker was defined as participants who currently smoke cigarettes. Drinking status was defined as yes if participants who drank beer or any other alcohol beverages within one year before the survey.

Body weight and height were measured by a physician, nurse or other health professionals. BMI (kg/m^2) was calculated as body weight (kg) divided by the square of height (m^2). Hypertension was defined if participants answered “yes” to the question “Do you have high blood pressure?”, had anti-hypertensive medication, or had a mean blood pressure $\geq 140/90$ mmHg in the physical examination. Diabetes was defined if participants answered “yes” to the question “Has a doctor ever told you that you suffer from diabetes?”, had a fasting blood glucose of ≥ 7.0 mmol/L, or a hemoglobin A1c of $\geq 6.5\%$.

Statistical analysis

Categorical variables were presented as number (N) and percentage (%), while continuous variables were shown as median and interquartile ranges. *Wilcoxon rank-sum* analysis for continuous variables and Chi-square analysis for categorical variables was used to test the differences between groups. Natural logarithmic transformation was applied to hemoglobin, FET, TRF, and sTRF-R, sTfR-F index [calculated as sTRF-R (mg/L) divided by the log FET (ng/mL)] to normalize their distributions. Logistic regression and Cox proportional hazards models with person-year as the time scale were used to estimate the independent stroke risk associated with the five-iron metabolism factors. Odds ratios (ORs) and hazard ratios (HRs) with 95% confidence intervals (CIs) were reported accordingly. The follow-up of the study cohort began at the baseline survey in 2009, and ended at the date of stroke diagnosis or the survey in 2015, whichever came first.

Unadjusted, age & gender-adjusted, and multivariable adjusted (further adjusted for BMI, smoking status, drinking status, diabetes, hypertension, and hsCRP) analyses were applied to the logistic and Cox models. Additionally, we performed stratified analysis by gender (male/female), age (by median), BMI (by median), and hypertension (yes/no). The R 4.0.2 (www.r-project.org) was used for data analyses, and a two-sided *P* value of < 0.05 was considered as statistically significant.

Results

Demographic characteristics

During a median of 6.1 years of follow-up, a total of 128-incident stroke events were identified (Table 1). The participants who developed stroke during the follow-up period were significantly older than those who did not, and more likely to be male, and had a higher BMI. Moreover, the participants who developed stroke were more likely to have hypertension and diabetes. However, no significant difference in smoking and drinking status was found between the two groups. Furthermore,

levels of sTRF-R, sTfR-F index and hemoglobin were not different between the two groups, but TRF were significantly lower in the stroke group than their counterparts were. While FET, hsCRP levels were significantly higher in the stroke group compared to their counterparts. In addition, demographic and biochemical characteristics of the 8589 participants by stroke status from cross-sectional data were also analyzed. Most of characteristics shared the similar significance to longitudinal data, beside BMI (*P* = 0.050) and sTfR-F index (*P* = 0.004).

Cross-sectional and longitudinal associations

According to cross-sectional data in Table 2, TRF and sTfR-F index were inversely associated with stroke risk, whereas FET was positively associated with stroke risk in unadjusted model. However, not all of iron indicators were significantly associated with stroke risk both in age and gender adjusted, and further multivariable adjusted models. In longitudinal analyses, FET was positively, whereas TRF and sTfR-F index were inversely associated with risk of stroke in unadjusted models. However, these associations vanished in age and gender adjusted model (except hemoglobin), and multivariable adjusted model (Table 2). Similar to the results of Cox regression using the markers as continuous variables, there were no significant risk association hemoglobin, FET, TRF, sTRF-R, and sTfR-F index in quartiles with stroke.

Stratified analyses of longitudinal data

As shown in Table 3, significant associations of FET among female, hemoglobin and sTfR-F index were observed among those participants with BMI ≤ 23 kg/m². There were no significant associations between TRF, sTRF-R and stroke risk after stratifications by gender, age, BMI or hypertension, however, both TRF and sTRF-R tended to be significantly associated with the stroke risk among those participants with hypertension and BMI ≤ 23 kg/m², respectively.

Table 1: Demographic and biochemical characteristics of the participants from longitudinal data

Variable	Total	Non-Stroke	Stroke	P value
N, %	7290 (100)	7162 (98.2)	128 (1.76)	
Age, years	51 (41, 61)	51 (41, 61)	65 (57, 71)	< 0.001
Gender, %				0.005
Male	3371 (46.2)	3296 (46.0)	75 (58.6)	
Female	3919 (53.8)	3866 (54.0)	53 (41.4)	
BMI, kg/m ²	23.2 (21.0, 25.6)	23.1 (21.0, 25.6)	24.3 (22.0, 26.3)	< 0.001
Hypertension, %				< 0.001
No	5223 (71.7)	5186 (72.4)	37 (28.9)	
Yes	2067 (28.3)	1976 (27.6)	91 (71.1)	
Diabetes, %				0.013
No	6521 (89.5)	6416 (89.6)	105 (82.0)	
Yes	769 (10.5)	746 (10.4)	23 (18.0)	
Current smoker, %				0.617
No	5289 (72.6)	5193 (72.5)	96 (75.0)	
Yes	2001 (27.4)	1969 (27.5)	32 (25.0)	
Drinking status, %				0.939
No	4913 (67.4)	4825 (67.4)	88 (68.8)	
Yes	2376 (32.6)	2336 (32.6)	40 (31.2)	
FET, ng/mL	80.3 (40.1, 147)	79.8 (39.4, 147)	99.2 (61.9, 171)	< 0.001
TRF, mg/dL	283 (251, 319)	283 (252, 319)	268 (238, 303)	0.002
sTRF-R, mg/L	1.34 (1.09, 1.66)	1.34 (1.09, 1.67)	1.35 (1.10, 1.61)	0.859
sTfR-F index	0.31 (0.24, 0.42)	0.31 (0.23, 0.42)	0.29 (0.24, 0.37)	0.050
Hemoglobin, g/dL	14.0 (12.8, 15.4)	14.0 (12.8, 15.3)	14.3 (13.1, 15.7)	0.122
HsCRP, mg/L	1.00 (1.00, 2.00)	1.00 (< LOD, 2.00)	1.00 (1.00, 3.00)	< 0.001

The continuous variables are expressed as median (interquartile ranges), and categorical variables as number (n) and percentage (%); P values were calculated by *Wilcoxon* test for continuous variables and Chi-square test for categorical variables.

FET, ferritin; TRF, transferrin; sTRF-R, soluble transferrin receptor; sTfR-F index, sTRF-R/log FET; HsCRP, high-sensitivity CRP; LOD, limit of detection

Table 2: Associations of hemoglobin, FET, TRF, sTRF-R, and sTfR-F levels with stroke risk from cross-sectional and longitudinal data

Markers	Unadjusted		Age and gender adjusted		Multivariable adjusted ^a	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
Cross-sectional data (n = 8589)						
Hemoglobin	1.14 (0.94, 1.37)	0.182	1.14 (0.93, 1.40)	0.209	1.08 (0.88, 1.34)	0.450
FET	1.38 (1.14, 1.66)	0.001	1.08 (0.86, 1.35)	0.523	1.02 (0.80, 1.29)	0.900
TRF	0.82 (0.73, 0.92)	0.001	0.92 (0.77, 1.09)	0.331	0.90 (0.76, 1.07)	0.217
sTRF-R	0.88 (0.73, 1.05)	0.153	0.88 (0.73, 1.07)	0.208	0.83 (0.68, 1.01)	0.061
sTfR-F index	0.74 (0.60, 0.90)	0.004	0.85 (0.67, 1.08)	0.182	0.82 (0.64, 1.05)	0.115
Longitudinal data (n = 7290)						
	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
Hemoglobin	1.19 (1.00, 1.43)	0.053	1.27 (1.05, 1.55)	0.015	1.14 (0.93, 1.39)	0.209
FET	1.42 (1.19, 1.71)	< 0.001	1.20 (0.97, 1.49)	0.098	1.11 (0.89, 1.39)	0.363
TRF	0.85 (0.76, 0.95)	0.003	0.96 (0.80, 1.14)	0.617	0.92 (0.79, 1.08)	0.300
sTRF-R	0.97 (0.82, 1.16)	0.732	0.99 (0.83, 1.20)	0.948	0.99 (0.82, 1.19)	0.891
sTfR-F index	0.78 (0.64, 0.95)	0.014	0.89 (0.71, 1.12)	0.318	0.92 (0.73, 1.16)	0.495

FET, ferritin; TRF, transferrin; sTRF-R, soluble transferrin receptor; sTfR-F index, sTRF-R/log FET; HsCRP, high-sensitivity CRP.

^a Models were adjusted for age, gender, BMI, smoking status, drinking status, diabetes, hypertension, and hsCRP

Table 3: Hazard ratios (HR) of stratified analysis by gender, age, BMI and hypertension for stroke risk from longitudinal data (n = 7290)

Variables	Case/N	Hemoglobin		FET		TRF		sTRF-R		sTfR-F index		
		HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	
Gender	Male	75/3371	1.18 (0.90, 1.54)	0.234	0.93 (0.69, 1.26)	0.652	1.01 (0.78, 1.30)	0.950	1.09 (0.84, 1.40)	0.530	1.11 (0.82, 1.50)	0.518
	Female	53/3919	1.08 (0.80, 1.46)	0.606	1.45 (1.00, 2.09)	0.049	0.88 (0.74, 1.04)	0.125	0.89 (0.67, 1.17)	0.388	0.74 (0.52, 1.05)	0.093
Age, years	≤ 51	13/3683	0.75 (0.45, 1.25)	0.270	1.00 (0.53, 1.86)	0.989	0.81 (0.65, 1.02)	0.069	0.81 (0.49, 1.34)	0.414	0.79 (0.42, 1.48)	0.461
	> 51	115/3607	1.11 (0.90, 1.37)	0.328	1.07 (0.84, 1.37)	0.592	0.92 (0.77, 1.10)	0.349	1.02 (0.83, 1.25)	0.850	0.98 (0.76, 1.26)	0.847
BMI, kg/m ²	≤ 23	47/3595	1.54 (1.15, 2.06)	0.004	1.21 (0.84, 1.75)	0.312	0.93 (0.70, 1.24)	0.639	0.75 (0.56, 1.01)	0.057	0.68 (0.46, 0.99)	0.044
	> 23	81/3695	0.95 (0.75, 1.22)	0.697	1.07 (0.80, 1.42)	0.654	0.92 (0.76, 1.12)	0.405	1.12 (0.88, 1.43)	0.346	1.07 (0.80, 1.44)	0.645
Hyper- tension	Yes	91/2067	1.10 (0.87, 1.40)	0.416	1.01 (0.77, 1.33)	0.946	0.87 (0.75, 1.00)	0.054	0.90 (0.72, 1.12)	0.348	0.88 (0.66, 1.17)	0.379
	No	37/5223	1.22 (0.83, 1.79)	0.315	1.32 (0.88, 1.99)	0.176	1.23 (0.84, 1.81)	0.297	1.23 (0.89, 1.71)	0.206	1.04 (0.71, 1.52)	0.856

BMI, body mass index; FET, ferritin; TRF, transferrin; sTRF-R, soluble transferrin receptor; sTfR-F index, sTRF-R/log FET; HsCRP, high-sensitivity CRP. All of models were adjusted for age, gender, BMI, smoking status, drinking status, diabetes, hypertension, and hsCRP

Discussion

In the present study, we observed that the levels of hemoglobin, FET, TRF, sTRF-R, and sTfR-F index were not associated with the risk of stroke in both cross-sectional and longitudinal analyses. Nonetheless, there were significant associations of FET among female, hemoglobin and sTfR-F index with stroke risk among those participants with BMI ≤ 23 kg/m² after a median of 6.1 years follow-up.

A Korean Heart Study reported a U-shaped relationship between hemoglobin level and stroke among overall population and male, while a positive trend among female (14). Inversely, a US study exhibited the U-shaped association among

female, not male (15). However, hemoglobin was not associated with stroke risk in the present study both in the overall and gender stratified analyses. Social environments, confounding factors, and different population characteristics including genetic backgrounds may explain such inconsistency. Moreover, our study observed that there were no significant association of markers of FET and TRF with stroke risk in both cross-sectional and longitudinal analyses of overall. Similarly, the Jackson Heart Study reported no longitudinal association between the two markers and stroke risk (11). Moreover, a 17-year prospective follow-up study in Busselton also suggested that FET was not a risk factor for cardiovascular disease (CVD) and stroke (17). In contrast, two European studies

reported that FET was positively associated with stroke, and TRF was inversely associated with stroke (13, 16). It is worth mentioning that the European studies used cross-sectional design (13, 16), while the Jackson Heart study (11), the study in Busselton (17) used longitudinal design. The cross-sectional study design is unable to investigate the causal relationship between iron status and stroke (17). Our study reported the consistent results using cross-sectional and longitudinal design, which may provide stronger evidence than the previous studies.

Although there were no associations between indicators of hemoglobin, FET, sTfR-F index and stroke risk in overall analysis, the association of FET with the stroke risk existed between female, and the association of hemoglobin and sTfR-F index with the risk among those $BMI \leq 23 \text{ kg/m}^2$ by stratified analyses. Previously, Van Der et al. reported that high FET was significantly associated with stroke risk among postmenopausal female, longitudinally (20). The markedly accumulation of FET and stored iron after menopause may promote incident stroke with inflammation by the production of highly reactive free radicals that cause lipid peroxidation contributing to the expansion of foam cells and atherosclerosis, and may result in cell death (21). Thus, the FET and iron-accumulated effects after menopause (22), and the role of estrogen in release of free iron from FET (23, 24) may together contribute to the significant association between FET and stroke risk in female, but not in male in the present study. In addition, the participants who developed stroke had a significant increased BMI at baseline than others. While in those participants with lower BMI ($\leq 23 \text{ kg/m}^2$), hemoglobin and sTfR-F index were significantly associated with the stroke risk, but not in those with higher BMI. These results could be explained that those with lower BMI usually exhibit a more insulin-sensitive environment than obese individuals (25). Otherwise, insulin can directly promote erythropoiesis then elevate hemoglobin level (26), further, the action and secretion of insulin could closely interact with the iron homeostasis (27). Moreover, higher hemoglobin may affect the cardiovascular system, due to its ability

to consume nitric oxide, produce free oxygen radicals and alter blood viscosity (28, 29). Furthermore, sTfR-F index as a more sensitive parameter than sTRF-R, is closely related to cellular iron demands, and notably exerts a marker of erythropoiesis (7, 27).

The main strengths of our study include the use of both cross-sectional and prospective design, a relatively large sample size, and blood measurements in a central lab with standard protocol. However, the present study also has some limitations. First, recall and mis-classification bias could exist because the definition of stroke was not based on medical examinations, such as MRI and radiography. Second, we were not able to look into different stroke types for analysis due to unavailability of such data. Third, other parameters related to iron metabolism including hepcidin and transferrin saturation were not measured in the CHNS, which limits more extensive evaluations of iron metabolism in association with the stroke risk.

Conclusion

There were no significant associations of iron metabolism markers including hemoglobin, FET, TRF, sTRF-R, and sTfR-F index with stroke risk in both cross-sectional and longitudinal analyses. Nevertheless, longitudinal analyses associated the risk with FET among female, and with hemoglobin and sTfR-F index among those participants with $BMI \leq 23 \text{ kg/m}^2$ and stroke risk, which warrants further studies.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interests.

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