

The telomere length of peripheral blood cells is associated with the risk of ischemic stroke in Han population of northern China

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Abstract

Background: Telomere length is closely related to the onset and prognosis of ischemic stroke. This study was to investigate the relationship between telomere length and the incidence of ischemic stroke in Han population of northern China.

Methods: In the present study, 152 patients with ischemic stroke were selected as the case group, and 152 healthy persons were used as the control group. Detection of telomere length was done by real-time polymerase chain reaction after extraction of genomic DNA from peripheral venous blood.

Results: Our results showed that the telomere length of the patients in the case group was significantly lower than that of the control group ($Z = -11.843$, $P < .0001$). Further analysis found that the telomere length of the control group was inversely correlated with age ($r = -0.234$, $P = .004$), and the telomere length and homocysteine (HCY) were inversely correlated in the case group ($r = -0.176$, $P = .03$), especially in women ($r = -0.357$, $P = .024$). Multivariate regression analysis showed that telomere length was a protective factor for ischemic stroke (odds ratio [OR] 95% confidence interval [95% CI] = 0.748 [0.681–0.823], $\beta = -0.29$, $P < .0001$). The receiver operating characteristic curve showed that telomere length was a good diagnostic biomarker of ischemic stroke (area under the curve: 0.894, sensitivity: 84.7%, specificity: 93.4%).

Conclusion: Our results indicate that shorter telomere length has some connection with the risk of ischemic stroke in the northern Chinese Han population. Telomere length might serve as a potential candidate biomarker for ischemic stroke. This requires a large sample to be further verified.

Abbreviations: CRP = C-reactive protein, DM = diabetes mellitus, GLU = fasting blood glucose, HCY = homocysteine, HDL = high-density lipoprotein, HTN = hypertension, LDL = low-density lipoprotein, non-DM = nondiabetes mellitus, non-HTN = non-hypertension, ROC = receiver operating characteristic, RT-PCR = real-time polymerase chain reaction, TC = total cholesterol, TG = triaurate glycerin.

Keywords: diagnosis, northern Chinese, stroke, telomere length

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1. Introduction

With characteristics of high morbidity, high mortality, and high disability rate, ischemic stroke has become the world's 2nd leading cause of death and the 3rd most disabling factor, thereby imposing great burden to patients and society.^[1,2] The increasingly more studies on the pathogenesis of ischemic stroke has made it necessary to find out the possible pathogenesis of ischemic stroke from the perspective of molecular biology.

Located at the end of the chromosomes of eukaryotic cells, telomeres have the function of stabilizing DNA structure,^[3] while the maintenance of telomere length is determined by genetic factors.^[4] Studies have shown that telomere length can affect mammalian lifespan, thereby serving as a hallmark of aging,^[5] while shortening of telomere length can lead to endothelial cell senescence, along with the prediction of the risk of atherosclerosis-related diseases.^[6,7] With the most common cause of ischemic stroke being atherosclerosis, we speculate that shortening of telomere length may also exist in ischemic stroke.

Although the shortening of telomere length increases the risk of cardiovascular disease,^[8] the association results of telomere length with ischemic stroke are not consistent. Studies have shown that the relative telomere length of female nurses and white males in the United States was not associated with the onset of ischemic stroke.^[9,10] However, being associated with ischemic

stroke, shorter telomere length levels can predict ischemic stroke mortality in Central China and East China.^[11,12] Nevertheless, a study in southern China has found that longer telomere length is associated with ischemic stroke.^[13]

With the possibility that racial differences and geographic differences might be responsible for the discrepancies mentioned earlier,^[14] the absolute telomere length of peripheral blood leukocytes in patients is compared with ischemic stroke and healthy people in northern Han Chinese population in this study.

2. Materials and methods

2.1. Sample selection

A total of 152 hospitalized patients with ischemic stroke were obtained from the 148th Hospital of PLA in Zibo City, Shandong Province of China. Ischemic stroke was diagnosed by typical neurologic positive signs and imaging evidence. We also recruited 152 age-matched healthy controls from medical examination center of 148th Hospital of PLA. Both the case and control groups excluded malignant tumors, blood diseases, coronary heart disease, tuberculosis, and Parkinson disease, Alzheimer disease, and other neurologic diseases. The control group also excluded hypertension, hyperlipidemia, diabetes, and family history of stroke disease. Informed consent forms were obtained from both the groups of subjects at the time of sample collection and were approved by The Medical Ethics Committee of the Second Affiliated Hospital of Zhengzhou University and The Medical Ethics Committee of the 148th Hospital of PLA. After screening, the eligible subjects were selected and the fasting peripheral venous blood was taken before treatment. The cells were thoroughly mixed in the EDTA anticoagulation tube and stored in a refrigerator at -80°C .

2.2. DNA extraction and detection of telomere length

The purified DNA of each specimen was extracted by a DNA kit (Omega Bio-Tek, Norcross, GA) and transferred to a centrifuge tube, the specific steps were carried out according to the kit instructions, and then placed in a -20°C refrigerator for use. The reaction system in a 384-well plate consisted of $5\mu\text{L}$ $2\times$ MasterMix, $0.5\mu\text{L}$ of each primer, $1.5\mu\text{L}$ template DNA, $3\mu\text{L}$ H_2O . Real-time polymerase chain reaction (RT-PCR) was performed using a Roche 480 fluorescence qPCR instrument (Roche, Basel, Switzerland). The PCR program consisted of 95°C for 10 minutes, 45 cycles of 95°C for 15 seconds and 60°C for 1 minutes. The dissolution curve was collected at 95°C for 15 seconds, 60°C for 1 minutes, 95°C for 10 seconds, and a temperature drop of 40°C for 10 seconds to collect fluorescent signals. The telomere repeat number (T) of the peripheral blood leukocytes and the copy number (S) of the internal reference single copy gene (36B4) were detected, and the standard sample diluted with the fold ratio and the sample to be tested were subjected to a PCR. A standard curve was used to analyze the gene copy number of the sample. The detection of telomere length was based on a previous study.^[15]

2.3. Statistical analysis

Statistical analysis was performed with SPSS 22.0 (SPSS Inc, Chicago, IL). Kolmogorov–Smirnov normal distribution test was used to test the distribution of variables. The normal

Table 1

Comparison of general clinical data in 2 groups.

Basic clinical data	Case N=152	Control N=152	T or Z	P
Age, yr	60.37 ± 12.02	60.45 ± 12.23	0.061	.951
Sex (male/female)	112/40	112/40	—	1
HCY, $\mu\text{mol/L}$	1.18 ± 0.23	1.14 ± 0.16	1.835	.047*
GLU, mmol/L	5.70 (5.05–7.36)	5.36 (5.01–6.29)	−2.251	.024*
TG, mmol/L	1.31 (0.98–1.83)	1.35 (0.92–1.86)	−0.624	.533
TC, mmol/L	4.54 ± 1.13	5.04 ± 1.02	−4.106	<.0001*
HDL, mmol/L	1.16 ± 0.31	1.68 ± 0.43	−12.089	<.0001*
LDL, mmol/L	2.46 ± 0.78	2.61 ± 0.66	−2.027	.071
CRP, mg/L	6.60 (1.70–14.20)	4.80 (4.10–6.20)	−1.709	.087

CRP = C-reactive protein, GLU = fasting blood-glucose, HCY = homocysteine, HDL = high-density lipoprotein, LDL = low-density lipoprotein, TC = cholesterol total, TG = glycerin triaurate.

* P -value $\leq .05$ is in bold.

distribution of data was compared by T test and expressed by mean ± standard deviation, and the data of non-normal distribution was compared by nonparametric test and expressed by median (quartile spacing). The risk factors of ischemic stroke were analyzed by logistic regression and receiver operating characteristic (ROC) analysis was employed to evaluate the possible diagnostic value of telomere length for stroke. Spearman rank correlation test was used to analyze the associations between telomere length and clinical indexes. A 2-sided $P < .05$ was considered statistically significant.

3. Results

3.1. Comparison of general clinical data in 2 groups

The general clinical medical records of the 2 groups of subjects were analyzed (Table 1). Our results showed that there was no significant difference between the 2 groups in age ($P = .951$) and gender ($P = 1$). We found that HCY and GLU were significantly higher in the case group than in the control group, which was consistent with previous literatures.^[16,17] Our study also indicated that TC and high-density lipoprotein (HDL) in the case group were significantly lower than those in the control group ($P < .05$). This might be related to the use of lipid-lowering drugs in patients with ischemic stroke.

3.2. Comparison of telomere length in case and control groups

We compared the telomere lengths of the case and control subjects (Fig. 1A) and found that the telomere length of the patients in the case group was significantly lower than that of the healthy control group (0.0447 [0.0432, 0.0463] vs 0.0499 [0.0491, 0.0517], $Z = -11.843$, $P < 0.0001$). We further performed a gender-based subgroup comparison (Fig. 1B). In both men and women, the telomere length was significantly lower in the case group than in the control group ($P < .0001$). In addition, no differences were found between the male and female subgroups in the same group ($P > .05$). We further subgroup analysis of patients in the case group according to whether they had hypertension or diabetes, and whether or not they had smoking history (Fig. 1C). No significant difference was found in the telomere length between the subgroups ($P > .05$).

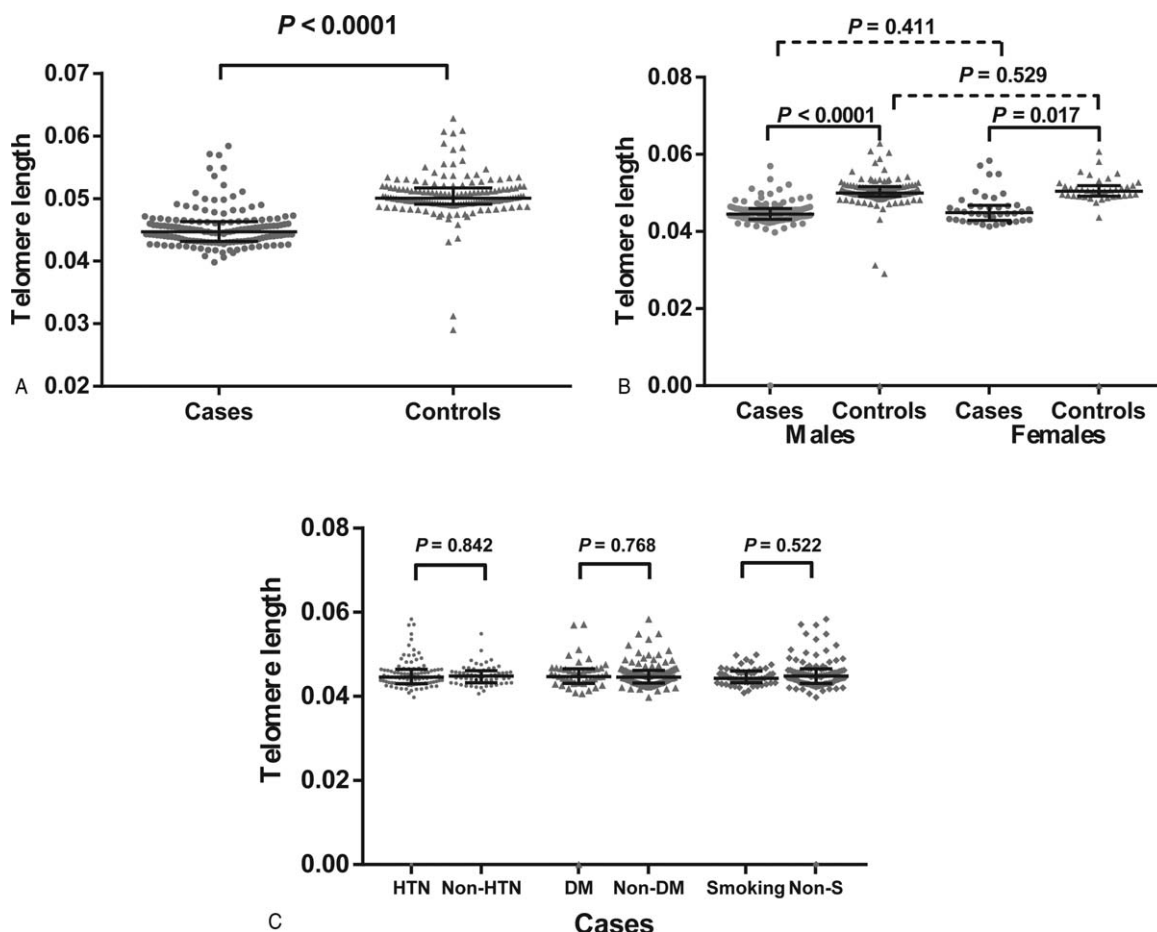


Figure 1. (A) Comparison of telomere length in case group and control group. (B) Comparison of telomere length in the gender-based subgroup. (C) Comparison of telomere length in stroke risk factors subgroup of cases. DM = diabetes mellitus, Females = female subgroup, HTN = hypertension, Males = male subgroup, non-HTN = nonhypertension, non-DM = nondiabetes mellitus, Non-S = no smoking history, Smoking = smoking history.

3.3. Correlation between telomere length and clinical index

We correlated the telomere length with the age of the subjects (Fig. 2A). Our results showed that there was no significant correlation between telomere length and age in the case group ($r = -0.114, P = .162$). The telomere length of the control group was inversely correlated with age ($r = -0.234, P = .004$).

Subsequently, we analyzed the correlation between the telomere length and clinical biochemical indicators. Our results indicated that the telomere length and HCY were inversely correlated in the case group ($r = -0.176, P = .03$), especially in women ($r = -0.357, P = .024, \text{Fig. 2B}$). And the remaining indicators were not found to be related to telomere length. In addition, the telomere length in the control group was not found to correlate with biochemical indicators.

3.4. Multiple regression analysis of risk factors for ischemic stroke

We performed multiple regression analysis of ischemic stroke risk factors and telomere length in the 2 groups (Table 2), showing that telomere length was a protective factor for ischemic stroke (odds ratio [OR] 95% confidence interval [95% CI]=0.748

[0.681–0.823], $\beta = -0.29, P < .0001$), and HDL was also a protective factor for ischemic stroke (OR [95% CI]=0.748 [0.681–0.823], $\beta = -3.519, P < .0001$). No correlation was found between telomere length and HDL in the 2 groups in this study.

3.5. ROC curve analysis for the diagnostic value of telomere length in ischemic stroke

We analyzed the diagnostic capacity of telomere length for ischemic stroke (Fig. 3). Our results found: area under the curve of 0.894 (95% CI=0.051–0.937), a sensitivity of 84.7%, and a specificity of 93.4% (cut-off=0.0473 and $P < .0001$). These results suggest that telomere length may be a potential biomarker for early diagnosis of ischemic stroke.

4. Discussion

The shortening of telomere length has been recognized as a biomarker of human aging,^[18] and atherosclerosis is formed with aging of vascular endothelial cells and other factors.^[19] Telomere length is related to genetic factors, and the relative length of telomeres at the time of birth will lead to differences in cardiovascular risk in individuals.^[20] However, the results of

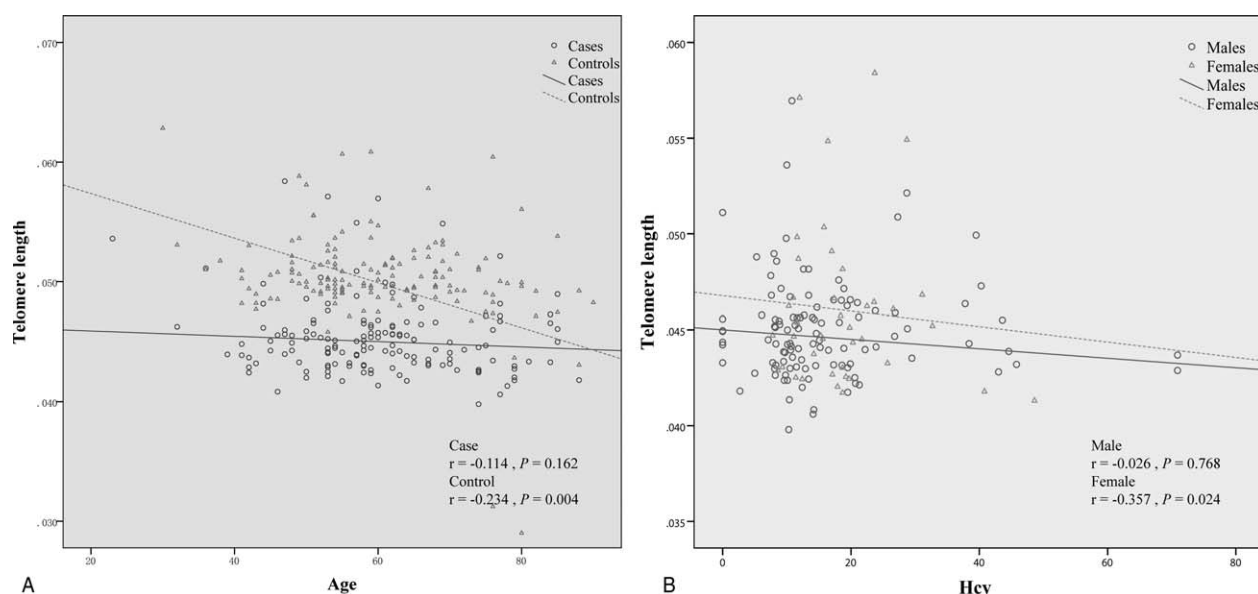


Figure 2. Correlation between telomere length and clinical index. (A) Comparison between telomere length and age in case group and control group. (B) Comparison of telomere length and homocysteine (HCY) between men and women in the case group.

ischemic stroke and telomere length are inconsistent. The shortening of telomere length may be affected by factors such as hypertension, diabetes, and smoking history. In this study, no significant difference in telomere length was found in subgroup tests by factors of hypertension, diabetes, and smoking history, therefore our study concluded that shortening telomere length was an independent risk factor for ischemic stroke.

In this study, the telomere length of the subjects was significantly inversely correlated with age in the control group, which is consistent with the previous study.^[18] Studies have found that taking statins and delaying the reduction of telomere length,^[21,22] considering that most patients with ischemic stroke take long-term statins. This might explain why there was a lack of association of telomere length with age in the cases.

Increased HCY is considered a risk factor in cardiovascular and cerebrovascular diseases, and elevated HCY also plays a role in accelerating biologic aging.^[23] Our results showed that telomere length was inversely correlated with HCY in patients with ischemic stroke, especially in women. These results were consistent with previous findings.^[24] Individual with shorter telomere length is more likely to have a high level of HCY,

which might subsequently increase the risk of cardiovascular and cerebrovascular diseases. It was found that shorter telomere length only affects women’s immune cell function and is associated with female obesity.^[25] Our findings provided a novel hint on the effect of telomere length in females.

In addition, we found that blood HDL and telomere length are a protective factor for the onset of ischemic stroke, the longer telomere length of the individual has a significantly lower risk of ischemic stroke. And our ROC curve analysis showed that telomere length had some potential diagnostic value for ischemic stroke. However, there are still some limitations. Our study was not involved with different types of ischemic stroke, and the samples were all from the same area. A large number of studies were needed to repeat the experiment and prospective experiments to observe the role of telomere length in the prediction of ischemic stroke mortality.

In summary, this study suggests that the shortening telomere length has some connection with the risk of ischemic stroke in the northern Chinese Han population. In addition, we also find that telomere length is associated with blood HCY levels in female patients. The above findings provide some new theoretical ideas

Table 2

Multiple regression analysis of risk factors for cerebral infarction.

	β	SE	P	OR	95% CI
Telomere length	-0.29	0.048	<.0001*	0.748	0.681–0.823
HCY	0.001	0.022	.953	1.001	0.959–1.046
GLU	0.121	0.079	.125	1.129	0.967–1.318
TC	-0.017	0.193	.928	0.983	0.673–1.435
HDL	3.519	0.6	<.0001*	0.03	0.009–0.096

GLU = fasting blood glucose, HCY = homocysteine, HDL = high-density lipoprotein, TC = total cholesterol.

* P-value $\leq .05$ is in bold.

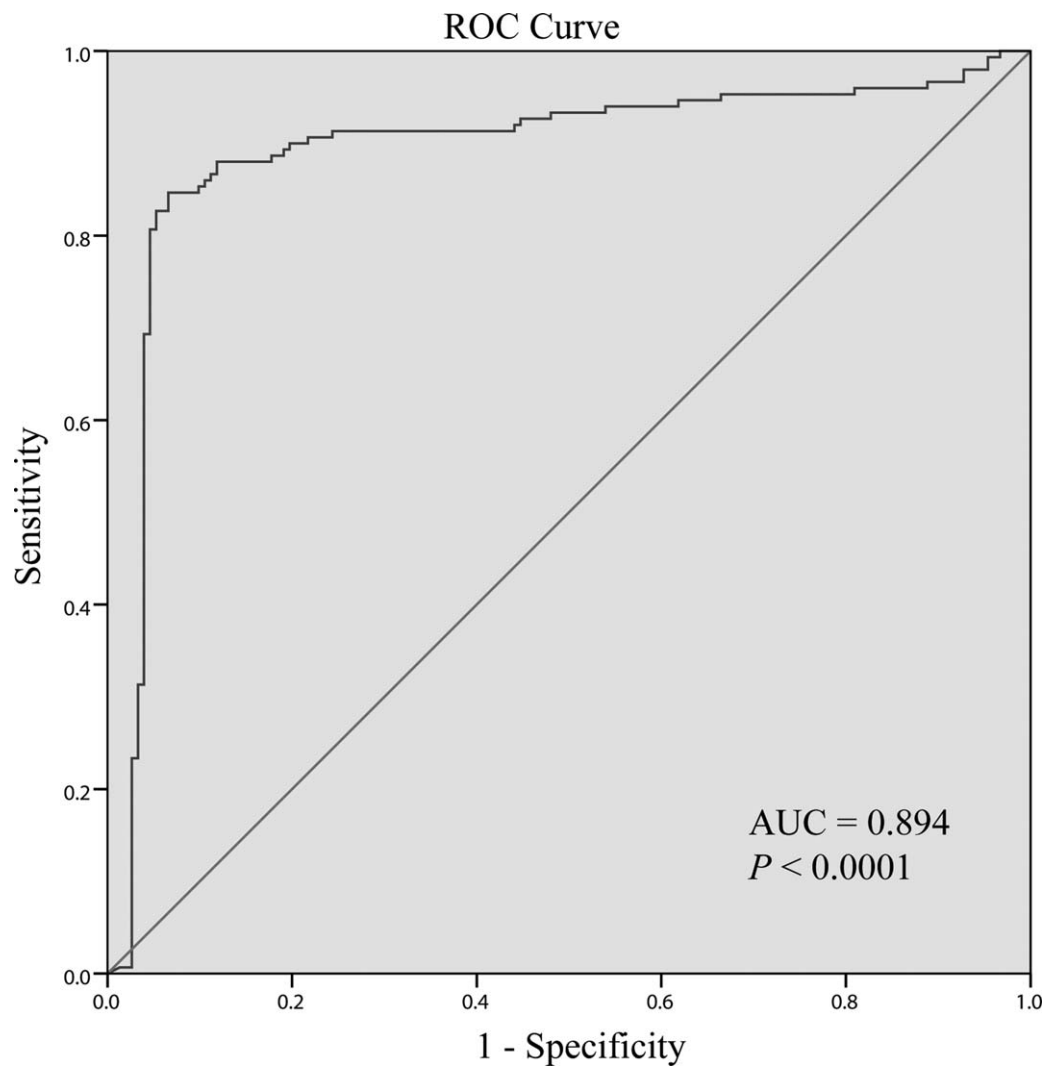


Figure 3. Receiver operating characteristic (ROC) curve analysis for the diagnostic value of telomere length in ischemic stroke. The ROC curve showed that telomere length was a potential diagnostic biomarker of ischemic stroke (area under the curve [AUC]: 0.894, sensitivity: 84.7%, and specificity: 93.4%, $P < .0001$).

for early screening and diagnosis of ischemic stroke and prognosis of ischemic stroke.

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