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Investigation of telomere length in young age ischemic stroke patients

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Abstract

Background & Objective: Telomere length is frequently accepted as a marker of biological aging. Various studies examine the relationship between telomere length, accepted as a marker of biological aging, and stroke, but this relationship has not been clearly demonstrated. The objective of this study is to determine whether short DNA telomere length is an independent risk factor for stroke. *Methods:* Our study examined telomere length in young patients with ischemic stroke. The study included 60 patients with a history of ischemic stroke at a young age within the last 3 years and a control group consisting of 40 volunteers with similar age, gender, and disease history defined as risk factors for stroke. *Results:* In our study, the mean telomere length of the patient group was significantly higher than the mean telomere length of the control group. However, the telomere length of the patients in the study did not show a significant difference between socio-demographic and risk factors defined for ischemic stroke.
Conclusion: Our study is very important in being the first to show such a relationship between telomere length and young age ischemic stroke. The low sample size may partially explain this result compared to other studies, as well as the study's design, the study population's age, ethnicity, and methods of studying telomere length. More studies with a larger sample size with younger patients are needed to better understand the relationship between telomere length and stroke in the future.

Keywords:Telomere length, ischemic stroke, young age

INTRODUCTION

Age, gender, and race have been identified as non-modifiable risk factors for ischemic stroke, whereas hypertension (HT), diabetes mellitus (DM), smoking, alcohol use, diet, physical inactivity, cardiac causes, obesity, hypercholesterolemia, and psychosocial causes are modifiable risk factors.[1] It is well known that people with such traditional neurovascular risk factors have a higher risk of developing atherosclerosis and neurovascular aging. However, the sensitivity of these identified risk factors to neurovascular disease is approximately 50-60%.[1] Therefore, further consideration of other risk factors for stroke and stroke-related disability is needed, possibly leading to the discovery of new mechanisms. As stroke is an age-related disease, early biological (chronological) aging may contribute to this risk, and therefore, telomere length, a molecular marker of aging, may represent an important cause of stroke risk factors.

Telomeres play an important role in maintaining the integrity and stability of genetic material by preventing fusion between chromosomes (end-to-end fusion), especially during cell replication. The length of telomeres decreases in each cell cycle. In addition to genetic factors, there are also environmental factors that affect telomere length. The most important of these factors is oxidative stress and inflammation.[2-4] The telomere length in white blood cells may be a marker of the cumulative burden of circulating oxidative stress and inflammation throughout an individual's lifetime.

Whether the telomere length is an independent marker of vascular injury or is shortened by the identified vascular risk factors remains unsolved. Understanding more about the basic functions, regulation, and clinical implications of telomeres may lead to developing future therapeutic targets.

Our aim in this study was to determine whether short DNA telomere length is an independent risk

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factor for stroke. The main reason for choosing the young adult patient population is that traditional stroke risk factors are relatively lower in this age group.

METHODS

Study population

Ischemic stroke patients admitted to our center between July 2017 and July 2020 were prospectively included in the study. We included 60 patients aged 18-45 years who presented within seven days of the onset of stroke symptoms and had no contraindications for magnetic resonance imaging (MRI). Patients with acute diffusion restriction on MRI consistent with acute ischemic stroke were included. We wanted to examine the relationship between telomere length and ischemic stroke based on vascular damage. Therefore, we preferred a young patient population and excluded patients with high-risk factors for cardioembolism (acute myocardial infarction, congenital heart disease, atrial fibrillation, infective endocarditis, mechanical prosthetic valve, EF <28). Patients with a history of cancer, liver, or renal failure were excluded. The control group was selected from patients presenting to the neurology outpatient clinic with infrequent episodic tension-type headaches. Volunteers with similar age, gender, and similar risk factors for ischemic stroke were included in the study. Among the volunteers, only those with normal brain MRI and neurologic examinations were included in the study. As in the patient group, volunteers with a history of cancer, liver, or kidney failure in the control group were not included in the study. A scientific research project at our university supported our study. Therefore, patient and control groups were formed before starting the study. 60 patients and 40 controls who met the inclusion and exclusion criteria were included in the study. DNA telomere length was prospectively investigated from peripheral blood leukocytes in all individuals included in the study.

Age, gender, concomitant risk factors (HT, DM, congestive heart failure, hyperlipidemia, smoking, and alcohol use history, hemoglobin, hematocrit values, and body mass index), transthoracic and/or transesophageal echocardiogram, bilateral carotid vertebral arterial Doppler ultrasonography and cranial MRI of 100 individuals included in the study were examined. The study was approved by the 01/08/2018 dated and 03 numbered decision of the ethics committee of our university clinical research ethics committee. Each patient who participated in the study was informed about the study, and informed consent was obtained.

A blood pressure above 140 mmHg systolic and 90 mmHg diastolic was defined as hypertension; a blood pressure between 120-140 mmHg systolic and 80-90 mmHg diastolic was defined as prehypertension. Blood pressure assessment of the patients participating in the study was performed at two different times at rest at least two weeks after stroke. Diabetes mellitus was defined as a preprandial blood glucose level above 125 mg/dl, a glucose level above 200 mg/dl at the 2nd hour of the oral glucose tolerance test, or an HbA1c level above 6.5%; prediabetes was defined as a preprandial blood glucose level between 110-125 mg/dl, a glucose level between 140-199 mg/dl at the 2nd hour of the oral glucose tolerance test or an HbA1c level between 5.7-6.4%. A body mass index of <18 kg/m2 was considered underweight, 18-25 as normal, 25-30 as overweight, and >30 as obese.

Telomere measurement

DNA isolation was performed with PureLink® Genomic DNA Mini Kit. The heating block was set to 55 C during the protocol steps. 200 μl peripheral blood was added to a sterile microcentrifuge tube. 20 μl proteinase K was added. 0 μl RNase was added, vortexed, and kept at room temperature for 2 minutes. 200 μl PureLink® Genomic Lysis/ Binding Buffer was added and vortexed until homogenized. Incubated at 55 C for 10 minutes for protein degradation. 200 μl of 100% ethanol was added and vortexed for 5 seconds until homogenized. The lysate was transferred to PureLink® Spin Column. Centrifuged at 10,000 × g for 1 min. The bottom tube was discarded, and a new PureLink® Collection Tube was used. 500 μl of Wash Buffer I was added to the column. The column was centrifuged at 10,000 × g for 1 min. 500 μl of Wash Buffer II was added to the column. Centrifuged for 3 minutes at maximum speed. The bottom tube was discarded, and the column was transferred to a new 1.5 ml centrifuge tube. 60 μl PureLink® Genomic Elution Buffer was added and allowed to stand at room temperature for 1 minute. Then, centrifugation was performed at maximum speed for 1 minute. The resulting DNA was placed at -20C for use.

The amounts of the DNAs obtained were quantified with the Qubit® 3.0 Fluorometer and Qubit® dsDNA HS Assay Kits. The telomere length of a human cell population was measured

directly as an average with ScienCell's Absolute Human Telomere Length Quantification qPCR Assay Kit. Two qPCR reactions were prepared for the reference genomic DNA sample, one with telomere primer stock solution and one with SCR primer stock solution. Two qPCR reactions were prepared for each genomic DNA sample, one with telomere primer stock solution and one with SCR primer stock solution.

Statistical analysis

The normality test (Shapiro Wilks), descriptive statistics (frequency analysis, descriptive statistics), independent group comparison (Unpaired t-test, One-Way ANOVA), Pearson Correlation analysis, and Reliability analysis (Cronbach's alpha) were used in the study.

Descriptive statistics such as frequency, percentage, arithmetic mean, standard deviation, minimum, maximum, etc., were used in the data analysis. Parametric tests were used in the analysis of the data. An unpaired t-test was used to compare the means of 2 independent groups, and the result of the One-way ANOVA test was used to compare more than two independent groups. In the ANOVA test, if variance homogeneity was provided for group differences, then Tukey HSD was used; otherwise, the Tamhane multiplex group comparison test was used. Pearson correlation coefficient was calculated in the correlation analysis of the scales. All test results were evaluated at $p \leq 0.05$ significance level.

RESULTS

In the study, 45% of the individuals in the patient group were female, and 55% were male. The mean age was 37.80 ± 7.29 years, and the mean BMI was 26.47 ± 3.72 kg/m^2. The body mass index of 41.7% of the individuals was normal, 46.7% were slightly overweight, and 11.6% were obese.

When the systolic BP group of the individuals in the patient group was analyzed, 35% were normotensive, 50% were pre-HT, and 15% were HT patients. In the diastolic BP group, 41.7% were normotensive, 48.3% were pre-HT, and 10% were HT patients.

In the patient group, 78.3% had normal diabetes assessment, 15% had prediabetes, and 6.7% had DM; 81.7% had no carotid plaque, 13.3% had a plaque in the ICA, and 5% had ICA occlusion. 48.3% were active smokers, 10% had smoked in the past, 95% did not drink alcohol, and 92% did not have any chronic disease.

In the study, 42.5% of the individuals in the control group were female, and 57.5% were male. The mean age was 37.05 ± 7.78 years, and the mean BMI was 25.73 ± 3.15 kg/m2. The body mass index of 52.5% of the individuals was normal, 42.5% were slightly overweight, and 5% were obese.

When the systolic BP group of the individuals in the control group was analyzed, 27.5% were normotensive, 52.5% were pre-HT, and 20% were HT patients. When the diastolic BP group was analyzed, 32.5% were normotensive, 52.5% were pre-HT, and 15% were HT patients.

In the control group, 80% of the individuals were normal; 12.5% had pre-DM; 7.5% had DM; 92.5% had no carotid plaque, and 7.5% had a plaque. Forty-five percent of the individuals were active smokers, 82.5% did not drink alcohol, and all (100%) did not have any chronic disease.

In addition, no significant difference was found between the patient and control groups in terms of socio-demographic characteristics and ischemic stroke risk factors ($p>0.05$).

In the study, when the telomere lengths between the patient and control groups were evaluated by unpaired T-test, the mean telomere length of the patient group was 7.52 ± 3.29 kb, which was significantly higher than the mean telomere length of the individuals in the control group by 5.01 ± 1.71 kb.

In the study, telomere length of the individuals in the patient group did not show a significant difference between socio-demographic characteristics (age, gender) and ischemic stroke risk factors (systolic BP, diastolic BP, DM, BMI, smoking, alcohol use, chronic disease) ($p>0.05$).

Our study indicates that the telomere length of the individuals in the control group does not show a significant difference between socio-demographic characteristics (age, gender) and risk factors of ischemic stroke (systolic BP, diastolic BP, DM, BMI, smoking, alcohol use, chronic disease) ($p>0.05$).

In the study, telomere length of the entire study population did not show a significant difference between socio-demographic characteristics (age, gender) and ischemic stroke risk factors (systolic BP, diastolic BP, DM, BMI, smoking, alcohol use, chronic disease) ($p>0.05$).

DISCUSSION

In the cardiovascular field, many studies have been conducted on telomere length, and the relationship between telomere length and hypertension[5], atherosclerosis[6], type 2 diabetes[7], obesity[7-8],

Table 1: Characteristics of socio-demographic and ischemic stroke risk factors in patient and control groups

		Patient (n=60)	Control (n=40)	P
		$\overline{X}$ ± *S.S.*	$\overline{X}$ ± *S.S.*	
Age[1]		37.80 ± 7.29	37.05 ± 7.78	0.625
BMI[1]		26.47 ± 3.72	25.73 ± 3.15	0.303
Systolic BP[1]		123.75 ± 14.69	125.25 ± 14.45	0.616
Diastolic BP[1]		79.67 ± 9.29	81.62 ± 9.29	0.304
LDL[1]		98.87 ± 29.82	111.83 ± 36.19	0.054
Pulse[1]		73.43 ± 6.9	72.38 ± 9.18	0.513
Cigarette (pack/year)[1]		19.62 ± 13.6	13.22 ± 9.25	0.086
Hemoglobin[1]		14.38 ± 2.49	14.79 ± 2.14	0.392
Haematocrit[1]		43.44 ± 7.11	45.32 ± 6.31	0.181
		n (%)	n (%)	P
Gender[2]	Female	27 (45.0)	17 (42.5)	0.967
	Male	33 (55.0)	23 (57.5)	
BMI[2]	Normal	25 (41.7)	21 (52.5)	0.389
	Slightly overweight	28 (46.7)	17 (42.5)	
	Obese	7 (11.6)	2 (5.0)	
Systolic BP[2]	Normotensive	21 (35.0)	11 (27.5)	0.669
	Pre-hypertensive	30 (50.0)	21 (52.5)	
	Hypertensive	9 (15.0)	8 (20.0)	
Diastolic BP[2]	Normotensive	25 (41.7)	13 (32.5)	0.573
	Pre-hypertensive	29 (48.3)	21 (52.5)	
	Hypertensive	6 (10.0)	6 (15.0)	
Diabetes Mellitus[2]	Normal	47 (78.3)	32 (80.0)	0.933
	Pre-diabetic	9 (15.0)	5 (12.5)	
	DM patient	4 (6.7)	3 (7.5)	
ICA carotid plaque[2]	N/A	49 (81.7)	37 (92.5)	0.215
	Present	8 (13.3)	3 (7.5)	
	Occlude	3 (5.0)	0 (0.0)	
Smoking[2]	N/A	25 (41.7)	22 (55.0)	0.084
	Ex-smoker	6 (10.0)	0 (0.0)	
	Active user	29 (48.3)	18 (45.0)	
Alcohol use[2]	N/A	56 (95.0)	33 (82.5)	0.083
	Social drinker	3 (5.0)	5 (12.5)	
	Active user	0 (0.0)	2 (5.0)	
Chronic disease[2]	N/A	55 (92.0)	40 (100.0)	0.081
	present	5 (8.0)	0 (0.0)	

[1]Unpaired T test, [2]Chi-square correlation test
BMI: Body mass index, BP: Blood pressure, LDL: Low-density lipoprotein, ICA: Internal carotid artery, DM: Diabetes Mellitus

Table 2: Comparison of telomere length between patient and control groups

	Group	n	$\overline{X}$ ± S.S.	t	p
Telomere length	Patients	60	7.52 ± 3.29	4.997	**0.000***
	Control	40	5.01 ± 1.71		

[1]Unpaired T test, *p≤0.05

coronary artery disease[9-10], renal dysfunction[11], sleep apnea syndrome[12], hyperhomocysteinemia[13] and overall vascular disease risk has been demonstrated.

Many studies show an association between telomere length and cardiovascular diseases. However, the relationship between cerebrovascular disease and telomere length is controversial.

Table 3: Comparison of telomere length in the patient group according to socio-demographic and ischemic stroke risk factors

		n	Telomere length	
			$\overline{X}$ ± S.S.	p
Age[1]	30 and under	8	7.79 ± 2.91	0.423
	Between 31-40	26	6.89 ± 2.38	
	41 and above	26	8.08 ± 4.09	
Gender[2]	Female	27	7.79 ± 3.79	0.571
	Male	33	7.3 ± 2.86	
Systolic BP[1]	Normotensive	21	7.9 ± 4.14	0.724
	Pre-hypertensive	30	7.18 ± 2.79	
	Hypertension	9	7.79 ± 2.81	
Diastolic BP[1]	Normotensive	25	7.31 ± 3.16	0.878
	Pre-hypertensive	29	7.75 ± 3.47	
	Hypertensive	6	7.34 ± 3.41	
Diabetes Mellitus[1]	Normal	47	7.36 ± 3.28	0.509
	Pre-diabetic	9	8.68 ± 2.89	
	DM	4	6.86 ± 4.53	
ICA carotid plaque[1]	N/A	49	7.66 ± 2.87	0.779
	Present	8	7.06 ± 5.75	
	Occlude	3	6.53 ± 1.72	
BMI[1]	Normal	25	6.89 ± 2.98	0.280
	Slightly overweight	28	8.25 ± 3.34	
	Obese	7	6.86 ± 3.99	
Smoking[1]	N/A	25	7.83 ± 3.88	0.750
	Ex-smoker	6	7.89 ± 2.29	
	Active user	29	7.19 ± 2.96	
Alcohol use[2]	N/A	56	7.53 ± 3.3	0.917
	Social drinker	3	7.33 ± 4.51	
Chronic disease[2]	N/A	55	7.62 ± 3.32	0.445
	Present	5	6.44 ± 2.97	

[1]One-Way ANOVA Variant Analysis, [2]Unpaired T Test
BMI: Body mass index, BP: Blood pressure, ICA: Internal carotid artery, DM: Diabetes Mellitus

Table 4: Comparison of telomere length in the control group according to socio-demographic and ischemic stroke risk factors

		n	Telomere length	
			$\overline{X} \pm S.S.$	p
Age[1]	30 and under	9	4.75 ± 1.32	0.873
	Between 31-40	18	5.07 ± 1.84	
	41 and above	13	5.11 ± 1.86	
Gender[2]	Female	17	4.99 ± 1.65	0.955
	Male	23	5.02 ± 1.78	
Systolic BP[1]	Normotensive	11	5.38 ± 1.42	0.684
	Pre-hypertensive	21	4.93 ± 1.68	
	Hypertensive	8	4.72 ± 2.22	
Diastolic BP[1]	Normotensive	13	5.2 ± 1.33	0.551
	Pre-hypertensive	21	5.1 ± 1.78	
	Hypertensive	6	4.31 ± 2.26	
Diabetes Mellitus[1]	Normal	32	5.31 ± 1.78	0.078
	Pre-diabetic	5	3.68 ± 0.49	
	DM	3	4.03 ± 0.25	
ICA carotid plaque[2]	N/A	37	5.1 ± 1.74	0.238
	Present	3	3.88 ± 0.43	
BMI[1]	Normal	21	5.02 ± 1.62	0.805
	Slightly Overweight	17	5.09 ± 1.93	
	Obese	2	4.23 ± 0.28	
Smoking[2]	N/A	22	5.03 ± 1.9	0.494
	Active user	18	4.98 ± 1.48	
Alcohol use[1]	N/A	33	4.97 ± 1.76	0.943
	Social drinker	5	5.18 ± 1.56	
	Active user	2	5.29 ± 2.04	

1One-Way ANOVA Variant Analysis, 2Unpaired T Test
BMI: Body mass index, BP: Blood pressure, ICA: Internal carotid artery, DM: Diabetes Mellitus

In the study by Ding *et al*., 1,309 patients and 858 stroke patients and the same number of controls were observed for five years, and telomere length, stroke risk, and post-stroke outcomes were evaluated. It was reported that telomere length was shorter in stroke patients compared to the control group, short telomere length was associated with increased stroke risk in both patient and control groups, and short telomere length was associated with all-cause mortality in follow-up.[14]

Toupance *et al*. observed 154 patients for 9.5 years to evaluate telomere dynamics and carotid plaque development. They found no correlation between carotid plaque formation and telomere attrition. In addition, they reported a strong association between carotid plaque formation at a young age and short telomere length.[15]

In a prospective study conducted by Biotti *et al*. in France to investigate the relationship between telomere length and clinical and biological risk factors of ischemic stroke in 215 patients, a significant correlation was reported between telomere length and age, homocysteinemia, triglycerides and antiphospholipid antibodies in ischemic stroke patients.[16] However, in our study, we could not show such a correlation between telomere length and age, gender, systolic and diastolic blood pressures, BMI, pulse rate, blood glucose level, LDL, hemoglobin and hematocrit in young ischemic stroke patients.

In a prospective study on stroke and telomere length in women by Schürks et al. with 1008 cases and controls, no results were obtained to support an association between telomere length and stroke.[17] Similarly, another controlled prospective study in male patients found no evidence of an association between telomere length and stroke.[18]

In a prospective study examining Parkinson's disease and telomere length in a neurodegenerative disease group, 408 patients and 809 controls were

Table 5: Comparison of telomere length according to socio-demographic and ischemic stroke risk factors in the whole study population

		n	Telomere length	
			$\overline{X}$ ± *S.S.*	p
Age[1]	30 and under	17	6,18 ± 2,65	0,324
	Between 31-40	44	6,15 ± 2,33	
	41 and above	39	7,09 ± 3,76	
Gender[2]	Female	44	6,71 ± 3,41	0,575
	Male	56	6,37 ± 2,70	
Systolic BP[1]	Normotensive	32	7,04 ± 3,63	0,503
	Pre-hypertensive	51	6,25 ± 2,62	
	Hypertensive	17	6,34 ± 2,93	
Diastolic BP[1]	Normotensive	38	6,59 ± 2,84	0,696
	Pre-hypertensive	50	6,64 ± 3,15	
	Hypertensive	12	5,82 ± 3,18	
Diabetes Mellitus[1]	Normal	79	6,53 ± 2,94	0,676
	Pre-diabetic	14	6,89 ± 3,37	
	DM	7	5,65 ± 3,55	
ICA plaque[1]	N/A	86	6,56 ± 2,75	0,933
	Present	11	6,20 ± 5,04	
	Occlude	3	6,53 ± 1,72	
BMI[1]	Normal	46	6,04 ± 2,61	0,269
	Slightly overweight	45	7,06 ± 3,26	
	Obese	9	6,28 ± 3,65	
Smoking[1]	N/A	47	6,52 ± 3,39	0,502
	Ex-smoker	6	7,89 ± 2,29	
	Active user	47	6,34 ± 2,70	
Alcohol use[1]	N/A	89	6,58 ± 3,08	0,74
	Social drinker	8	5,99 ± 2,90	
	Active user	2	5,29 ± 2,04	
Chronic disease[2]	N/A	95	6,52 ± 3,04	0,951
	Present	5	6,44 ± 2,97	

[1]One-Way ANOVA Variant Analysis, [2]Unpaired T Test
BMI:Body mass index, BP:Blood pressure, ICA:Internal carotid artery, DM: Diabetes Mellitus

studied. In the outcome analysis of the study, it was reported that the risk of Parkinson's disease may be reduced in individuals with age-adjusted short telomere length.[19] It was stated that this result might be explained by the method of the study, the country where the study was conducted, the sample size, the age of the study population, the methods of investigating telomere length, and tissue-related differences.

Studies on telomere length and cancer development are also contradictory. In one study, it was reported that short telomere length increased the risk of developing colorectal cancer[20], in another study, short telomere length was associated with an increased risk of cervical cancer in HPV-positive women[21], and another study, short telomere length was associated with an increased risk of basal cell carcinoma of the skin in arsenic exposure.[22] In contrast to these studies, there are two different studies indicating that long telomere length is associated with an increased risk of soft tissue sarcoma, and similarly, long telomere length is associated with an increased risk of developing breast and pancreatic cancer.[23-24]

It has been reported that long telomere length is associated with poor survival in breast cancer and renal cell carcinoma and may be a poor prognostic factor.[25] In a study conducted on hepatitis B patients, it was reported that long telomere length was associated with the risk of

hepatocellular carcinoma.[26]

When the literature was reviewed, no study was available in which long telomere length was associated with increased stroke risk in studies examining the relationship between ischemic stroke risk and telomere length. In this sense, our study is the first to support this association. Differences in study design, country of study, ethnicity, sample size, age of the study population, telomere length methods, and tissue-related differences may partly explain this. Firstly, telomere length is determined by both genetic and environmental factors. Telomerase is an enzyme that protects telomeres from shortening.[27] Telomerase activity is regulated in response to various factors such as cell-cycle stage[34], stress hormones[35] and inflammation[36-37] which, in turn, may introduce bias based on time of day of blood sample collection[38], current infections[36-37], physical exercise[39] or acute stress exposure status.[35] Kim *et al*. found significantly increased DNA telomere length and high telomerase activity in 24 different cancer types compared to normal subjects.[28-29-30-31] Similarly, some studies suggest that basal telomerase expression/activity is upregulated in autoimmune diseases, with differences between active and inactive disease[32] and early and advanced diseases.[33] If such a process occurred in young ischemic stroke patients, this could explain the presence of longer telomeres. In both studies that did not find a significant association between stroke and telomere length, the participants were relatively younger with a mean age of 61 years.[17-18] The mean age of our study was 37 years. We think that telomerase activity may be increased in young ischemic stroke patients, and this may be the reason for this unexpected result.

In our study, telomere length was studied in 100 individuals, including 60 patients and 40 controls. The fact that we studied young ischemic stroke patients was the most important factor that reduced the study population. Compared to previous studies, having a smaller sample group can be considered an important limitation of our study. Another limitation of our study is that we included volunteers with infrequent episodic tension headaches as a control group. In the literature, no study examines the relationship between headache and telomere length. This factor may be the reason for the unexpected result. To better understand the relationship between telomere length and stroke risk, studies with larger participation and younger patient groups are clearly needed.

DISCLOSURES

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Conflict of interest: None

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