Monir *et al.*
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RESEARCH

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Role of osteoprotegerin rs3102735 gene polymorphism in acute ischemic stroke patients

Dina Monir[1], Ahmed Osama[1], Alaa Eldin Saad[2], Mohamed Negm[1] and Reda Abd El-Razek[1*]

Abstract

Background Ischemic stroke ranks third among leading causes of death and disability. Both endothelial and vascular smooth muscle cells generate osteoprotegerin (OPG). Ischemic stroke and its severity may be enhanced by the OPG rs3102735 gene polymorphism. Our research aims to investigate OPG rs3102735 gene polymorphism role in ischemic stroke risk and to assess its association with stroke severity at presentation and degree of vascular stenosis and evaluate its potential as a predictor of stroke severity. Fifty people with acute ischemic stroke as well as fifty controls were studied. The NIHSS and ASPECTS were utilized to evaluate stroke severity and the infarction size, respectively. All subjects underwent extracranial carotid duplex study and molecular assessment for genotyping of OPG rs3102735) gene polymorphism.

Results Stroke patients had markedly higher concentrations of OPG in the plasma than controls (311.60 ± 109.48 versus 240.20 ± 75.96 mmol/ml, $p = 0.001$). The optimal plasma OPG cutoff value for the predicting the occurrence of stroke was determined to be > 250 mmol/ml, the 95% confidence interval (CI) was (0.625–0.843), sensitivity was 68% and specificity was 72%. Ischemic stroke had a significantly different genotype distribution for the OPG rs3102735 gene polymorphism than did controls (36 CC, 13 CT, and 1 TT) versus (28 CC, 15 CT, and 7 TT) respectively. Stroke patients had a significantly greater CC + CT genotype than controls did (P = 0.041), also they had a higher propensity for carrying the C allele than the T allele (P = 0.017). Carotid intima medium thickness and the NIHSS both had positive correlations with OPG serum level (r = 0.39, p = 0.02 and r = 0.4, p = 0.02, respectively), whereas ASPECTS had an inversed correlation (r = − 0.65, p = 0.001).

Conclusions The current study shows that as an independent risk factor, increased plasma OPG level, may participate in the atherothrombotic ischemic stroke pathophysiology, in addition, genetic variants in the OPG gene (rs3102735) are a separate risk factor for large artery atherosclerosis and plasma OPG level can serve as a biomarker to determine the severity of a stroke.

Keywords Ischemic stroke, Osteoprotegerin, OPG rs3102735 gene polymorphism

Background

Ischemic stroke ranks third among leading causes of death and disability. Multiple variables, including genetics, chronic inflammation, and risky behaviors, combine to produce this condition [1, 2]. Osteoprotegerin (OPG) is a receptor for tumor necrosis, specifically a member of the 11B subfamily [3]. The soluble glycoprotein OPG serves as a trap receptor by attaching to and disabling the nuclear factor-B receptor activator and the TNF-related apoptosis-inducing ligand [4]. The primary producers of the OPG are endothelial cells and vascular smooth

*Correspondence:
Reda Abd El-Razek
Dr.reda_abdelrazek@yahoo.com
[1] Department of Neurology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt
[2] Department of Clinical Pathology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

muscle cells [5]. OPG abnormalities are linked to cardiovascular and cerebrovascular illness, according to numerous studies [6]. With more cerebral arteries affected by atherosclerosis, the plasma levels of OPG considerably increased, and this was thought to be a biomarker for cerebral atherosclerosis [7, 8]. Additionally, it has been determined that ischemic stroke frequency and severity are associated with the OPG rs3102735 gene polymorphism [9, 10]. Our research aims to investigate OPG rs3102735 gene polymorphism role in ischemic stroke risk and to assess its association with stroke severity at presentation and degree of vascular stenosis and evaluate its potential as a predictor of stroke severity.

Methods

The Neurology and Clinical Pathology departments collaborated to carry out this case–control study between November 2017 to January 2021. Fifty adults with ischemic stroke of acute presentation of both sexes, categorized in accordance with the Trial of Org 10172 in Acute Stroke Therapy (TOAST) [11] as a large artery atherosclerosis (LAA) or a small vessel disease (SVD) were included. Patients with other subtypes of stroke, major cardiac, hepatic, renal, endocrinal disorders, malignant diseases, trauma, surgery, or chronic inflammatory illnesses, acute inflammatory or infectious problems or a history of fever lasting at least 15 days before the study, acute organ ischemia during the previous 3 months were excluded. Fifty matched controls without ischemic stroke history were included from the hospital's outpatient clinic. The study was authorized by the faculty of medicine's ethical committee and all participants completed informed consent forms.

Every individual was subjected to a thorough medical history and physical examination. To grade the severity of stroke, the National Institutes of Health Stroke Scale (NIHSS) [12] was utilized. For grading the infarction size in the anterior and posterior circulations, Alberta Stroke Program Early CT Score (ASPECTS) [13] and the posterior circulation ASPECTS (pc-ASPECTS) [14] were applied respectively. Clinically, the ischemic stroke was categorized total and partial anterior (TACI, PACI), lacunar (LACI), and posterior circulation (POCI) infarcts by the Bamford classification [15] are. At presentation, all patients had a non-contrast CT brain to rule out hemorrhagic stroke, followed by a brain magnetic resonance imaging (MRI). TOAST classification [11] was applied to categorize ischemic stroke into: LAA, SVD, cardioembolic (CE), and other determined and undetermined stroke, dependent on electrocardiography, Holter monitoring, transthoracic echocardiography, MRI of the brain and extracranial carotid duplex.

The studied individuals were subjected to the following:

Using the method described by [16, 17], an extracranial carotid duplex investigation was performed using a linear probe B-mode ultrasonography with great resolution (Philips HD11) (7 MHz).

A full blood count, glucose levels (both fasting and post-meal), lipids, C-reactive protein, coagulation profile, serum uric acid, and kidney and liver function tests.

Measurement of plasma OPG concentrations: a commercially available reagent (OPG DuoSet; RandD Systems, Abingdon, UK) was used as an enzyme-linked immunosorbent test according to the outlined procedure. Venous samples obtained in EDTA tubes at the time of admission, plasma is produced by centrifuging blood for 15 min at 1900×*g* at 4 °C, filtered plasma kept at 80 °C till analysis. This test had a detection range of 31.25–4000 pg/ml.

Molecular assessment for genotyping of osteoprotegerin (OPG rs3102735) gene polymorphism: genomic DNA was isolated from blood leukocytes utilizing *Qiagen's* QIA amp® DNA Mini Kit provided by (Qiagen, Germany).

To analyze genetic polymorphism, polymerase chain reaction (PCR) was employed. GenBank sequences were utilized as a reference. Using the following oligonucleotide primers, PCR was used to amplify the OPG rs3102735 promoter sequence: 5′-CTGGAGACATAT AACTTGAACA-3′ and 5′-CCATC ATCAAAGGGCTA TTGGT-3′. The following were utilized in a 25 µl reaction volume for PCR amplifications: forward and reverse primer (10 mol/l) of equal volumes (0.6 µl each), double distilled water (2.3 µl), LA Taq DNA polymerase (1 µl), 2 buffer solution (12.5 µl), genomic DNA (4 µl). The steps taken to complete the procedure were as follows: a 5-min denaturation at 94 °C is followed by 30 s cycles at 94 °C, 56 °C, and 72 °C, and then a 7-min extension at 72 °C. Following PCR amplification, AseI (New England Biolabs, Beijing, China) as a restriction enzyme was utilized to digest 4 µl of the product for an hour at 37 °C. The product was then examined using 2% agarose gel electrophoresis. The electrophoretic pattern visualized under ultraviolet light showed different lanes as follow (Fig. 1):

Statistical analysis

Data analysis was carried out on Windows with SPSS 22.0. In order to make comparisons, the average and standard deviation for continuous data and frequency (%) for categorical variables. When comparing quantitative and qualitative aspects, we employed the Student's t (t′) and the Chi-square tests. Fisher's exact and Pearson's 2 tests were used to compare genotypes and alleles. The

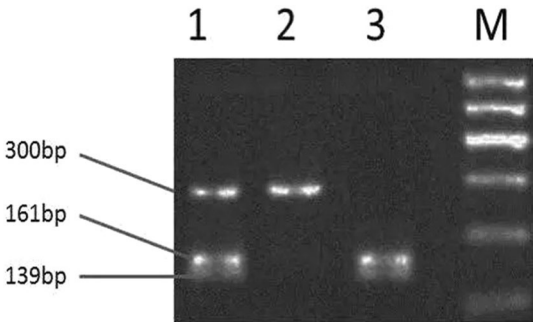

Fig. 1 OPG rs3102735 polymorphism agarose gel electrophoresis. 1: CT; 2: CC; 3: TT; M: DNA marker

cutoff for a significant difference, as determined by the P-value, was set at 0.05.

Results

Patients and controls demographics and clinical characteristics (Table 1). Bamford classification of strokes shows more affection of anterior circulation 43 (86%) than posterior circulation 4 (8.0%) and lacunar stroke 3 (6.0%) and according to the TOAST classification, 29 patients (58%) had large artery atherosclerosis and 21 patients (42%), small vessel disease. Stroke severity was recorded as follows: 44 (88%) moderate stroke, 6 (12%) moderate to severe stroke (Table 2).

Stroke patients had markedly higher concentrations of OPG in the plasma than controls (311.60 ± 109.48 versus 240.20 ± 75.96 mmol/ml, $p = 0.001$) (Table 3).

Ischemic stroke had a significantly different genotype distribution for the OPG rs3102735 gene polymorphism than did controls (36 CC, 13 CT, and 1 TT) versus (28 CC, 15 CT, and 7 TT) respectively. Stroke patients had a significantly greater CC + CT genotype than controls did (P = 0.041), also they had a higher propensity for carrying the C allele than the T allele (P = 0.017) (Table 4).

The optimal plasma OPG cutoff value for the predicting the occurrence of stroke was determined to be > 250 mmol/ml, the 95% confidence interval (CI) was (0.625–0.843), sensitivity was 68% and specificity was 72%, according to analysis of ROC curve (Table 5 and Fig. 2).

Patients with posterior circulation syndrome (POCS) stroke had substantially higher plasma OPG values than those with other types (432.50 ± 51.23, p = 0.010), in large artery atherosclerosis stroke than in small vessel disease stroke (332.80 ± 116.56, p = 0.049) and in moderate to severe stroke patients than in those with moderate strokes (410.0 ± 70.0 versus 305.3 ± 109.0 mmol/ml, p = 0.048) (Table 6). Patient OPG rs3102735 polymorphism was not associated with Bamford classification, TOAST classification, or stroke severity (P value > 0.05). (Table 7).

Table 1 Patients and controls demographics and clinical characteristics

	Patients (n = 50)	Controls (n = 50)	Test of sig.	p
Age (years)				
Mean ± SD	56.26 ± 6.81	56.14 ± 7.0	t = 0.087	0.931
Median (Min.–Max.)	56.0 (44.0–73.0)	55.0 (44.0–73.0)		
Sex	n (%)	n (%)		
Male	18 (36.0%)	22 (44.0%)	$\chi^2 = 0.667$	0.414
Female	32 (64.0%)	28 (56.0%)		
Risk factors	n (%)	n (%)		
Smoking	32 (64.0%)	11 (22.0%)	17.993*	< 0.001*
Previous stroke	7 (14.0%)	0 (0.0%)	7.527	^{FE}p = 0.012*
Previous ischemic heart disease	8 (16.0%)	2 (4.0%)	4.0*	0.046*
Hypertension	32 (64.0%)	11 (22.0%)	17.993*	< 0.001*
Diabetes mellitus	27 (54.0%)	8 (16.0%)	15.868*	< 0.001*
Lipid profile (mg/dl)	Mean ± SD	Mean ± SD		
Triglyceride	259.60 ± 67.09	108.70 ± 29.55	t = 14.555*	< 0.001*
Total cholesterol	261.08 ± 42.17	193.64 ± 16.98	U = 26.50*	< 0.001*
Low density lipoprotein	172.26 ± 14.20	132.40 ± 18.54	U = 71.0*	< 0.001*
High density lipoprotein	38.34 ± 8.67	43.74 ± 5.15	t = 3.787*	< 0.001*
Serum uric acid	6.52 ± 1.79	3.67 ± 1.24	U = 278.0*	< 0.001*
C-reactive protein (mg/dl)	9.69 ± 2.62	3.75 ± 1.21	U = 79.0*	< 0.001*

SD standard deviation, *t* student t test, *U* Mann Whitney test, χ^2 chi square test, *FE* fisher exact test

*Statistically significant at p ≤ 0.05

Table 2 Clinical and radiological classification and stroke severity among patients

	n (%)
Bamford classification	
Total anterior circulation stroke	19 (38.0%)
Partial anterior circulation stroke	24 (48.0%)
Posterior circulation syndrome	4 (8.0%)
Lacunar syndrome	3 (6.0%)
TOAST classification	
Large artery atherosclerosis	29 (58%)
Small vessel disease	21 (42%)
Cardioembolic	0 (0%)
Undetermined	0 (0%)
Stroke severity (NIHSS)	
Mild	0 (0%)
Moderate	44 (88%)
Moderate to severe	6 (12)
Severe	0 (0%)

NIHSS National Institutes of Health Stroke Scale, *TOAST* Trial of Org 10172 in Acute Stroke Treatment

Table 3 Patients' and controls' serum levels of osteoprotegerin (OPG)

	Patients (n = 50)	Controls (n = 50)	U	p
Serum OPG				
Mean ± SD	311.60 ± 109.48	240.20 ± 75.96	755.50*	0.001*
Median (range)	305.0 (160.0–600.0)	250.0 (140.0–410.0)		

OPG osteoprotegerin, *SD* standard deviation, *t* student t test, *U* Mann Whitney test

*Statistically significant at $p \leq 0.05$

Table 4 Patients' and controls' different types of osteoprotegerin (OPG) rs3102735 polymorphism

OPG rs3102735 polymorphism	Patients (n = 50)	Controls (n = 50)	Test of sig. χ^2	p
CC	36 (72.0%)	28 (56.0%)	2.778	0.096
CT	13 (26.0%)	15 (30.0%)	0.198	0.656
TT	1 (2.0%)	7 (14.0%)	5.029*	0.031*
CC + CT	49 (98.0%)	43 (86.0%)	4.891*	0.041*
C allele	85 (85.0%)	71 (71.0%)	5.711*	0.017*
T allele	15 (15.0%)	29 (29.0%)		

OPG osteoprotegerin, *C* cytosine nucleotide, *T* thiamine nucleotide, χ^2 Chi square test

*Statistically significant at $p \leq 0.05$

When compared to controls, stroke patients' carotid intima media thickness (IMT) (1.26) was statistically substantially larger than that of controls (0.91) (P value < 0.001) (Table 8). Carotid intima medium thickness and the NIHSS both had positive correlations with OPG serum level ($r = 0.39$, $p = 0.02$ and $r = 0.4$, $p = 0.02$, respectively), whereas ASPECTS had an inversed correlation ($r = -0.65$, $p = 0.001$) signifying that larger infarctions are associated with higher serum OPG levels (Table 9).

Discussion

Our research aims to investigate OPG rs3102735 gene polymorphism role in ischemic stroke risk and to assess its association with stroke severity at presentation and degree of vascular stenosis and evaluate its potential as a predictor of stroke severity.

Our research showed that acute ischemic stroke patients' plasma OPG levels were much greater than those of controls. Previous research has shown that having a high OPG level is strongly linked to increased ischemic stroke risk [7, 18, 19].

According to the findings of this study, a patient's risk of suffering an ischemic stroke may increase if they have the CC genotype of OPG rs3102735. Additionally, the C allele constituted a separate risk factor. Previous research demonstrated a strong connection between the with the OPG rs3102735 CC + CT genotypes and ischemic stroke [9, 10].

According to the findings of this study, the higher plasma OPG levels the more severe stroke and larger infarction size at presentation. Similar outcomes were noted in earlier research [18, 20, 21]. However, no correlation between the incidence of ischemic stroke and OPG levels was discovered in one investigation [22].

Our research showed that plasma OPG level could serve as a biomarker to determine the severity of a stroke. The ideal plasma OPG level cutoff value was > 250 based on our AUC study. At this cutoff point, the specificity was 72%, while the sensitivity was 68%.

The assessment of stroke severity is the most typical clinical application of NIHSS scores. These patients' OPG plasma levels may provide a critical objective indicator for assessing the severity of a stroke. Consequently, a potential method of assessing the severity of a stroke might be using plasma OPG levels. Additionally, some stroke patients' NIHSS scores may be lower when they are admitted, but they increase as soon as their impairment gets worse in the first few days after admission. OPG levels in plasma may be an important indicator of how these people's strokes are progressing [21]. However,

Table 5 Area under the curve for analysis of serum osteoprotegerin (OPG) for differentiating stroke event from controls

Variable	Area	Stand. Error	p-value	Cut-off point	Sensitivity	Specificity
serum OPG	0.698	0.052	**0.001***	> 250	68.00	72.00

OPG osteoprotegerin

*Statistically significant at $p \leq 0.05$

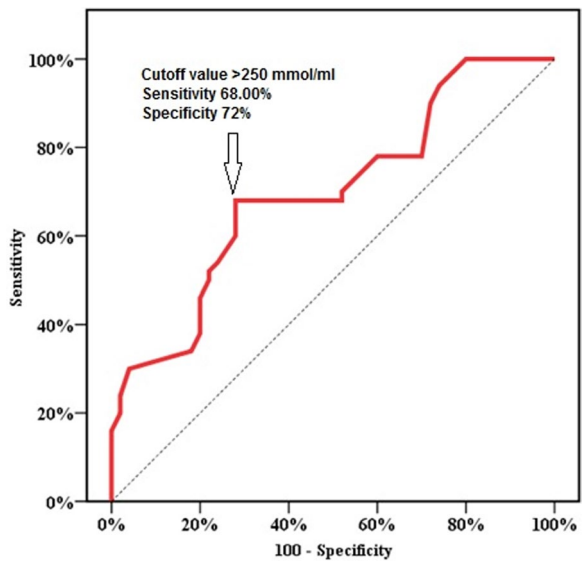

Fig. 2 ROC analysis based on plasma OPG and the occurrence of stroke

more research is necessary to determine whether OPG is a reliable indicator of stroke progression a few days after the stroke's onset.

Our results showed a significant association between the OPG rs3102735 CC + CT genotype and LAA ischemic stroke, as in other previous studies [9, 10].

All this information encourages the idea that stroke risk could be related to genetic variations in OPG. Previous research suggested that the OPG serum level and the CC/CT genotype of rs3102735 were positively correlated [23]. Many studies have linked inflammation to an increased risk of LAA stroke [1]. According to some research, increased plasma OPG levels enhance the inflammatory response, increase the likelihood of ischemic stroke, and may aggravate stroke severity, elevated plasma OPG levels could amplify the inflammatory response, increase the threat of ischemic stroke, and worsen its severity [24]. The exact way of involvement of OPG in these vascular processes is not well understood.

Table 6 Relationship between serum osteoprotegerin (OPG) level and Bamford and TOAST classifications and stroke severity among patients

Variables	n (%)	Serum OPG Mean ± SD	Test of sig. U	p p-value
Bamford classification				
Total anterior circulation stroke (TACS)	19 (38.0%)	316.92 ± 142.33	231.50	0.842
Partial anterior circulation stroke (PACS)	24 (48.0%)	301.90 ± 104.79	116.0	0.176
Posterior circulation syndrome (POCS)	4 (8.0%)	432.50 ± 51.23	23.50*	0.010*
Lacunar syndrome (LACS)	3 (6.0%)	293.33 ± 124.69	110.0	0.530
TOAST classification				
Large artery atherosclerosis	29	332.80 ± 116.56	23.0*	0.049*
Small vessel disease	21	290.40 ± 100.30		
Cardioembolic	0	–		
Undetermined	0	–		
Stroke severity (NIHSS)				
Mild	0 (0%)	–		
Moderate	44 (88%)	305.3 ± 109.0	25.0*	0.048*
Moderate to severe	6 (12)	410.0 ± 70.0		
Severe	0 (0%)	–		

TOAST Trial of Org 10172 in Acute Stroke Treatment, *SD* standard deviation, *U* Mann Whitney test, *OPG* osteoprotegerin, *NIHSS* National Institutes of Health Stroke Scale

*Statistical significance at $P < 0.05$

Table 7 Relation between Bamford and TOAST classifications and stroke severity and osteoprotegerin (OPG) rs3102735 polymorphism

	Bamford classification				**χ^2**	**^{MC}p-value**
	TACS (n = 19)	**PACS (n = 24)**	**POCS (n = 4)**	**LACS (n = 3)**		
OPG rs3102735 polymorphism						
CC	14	18	3	1	2.377	0.481
CT	4	6	1	2	3.025	0.396
TT	1	0	0	0	3.523	0.524
	TOAST classification					
	LAA (n = 29)	**SVD (n = 21)**	**CE (n = 0)**	**Undetermined (n = 0)**		
OPG rs3102735 polymorphism						
CC	20 (70.0%)	14 (66.7%)	0	0	0.029	1.000
CT	8 (27.6%)	5 (23.8%)	0	0	0.009	0.923
TT	1 (3.4%)	2 (9.5%)	0	0	0.669	1.000
	Stroke severity (NIHSS)					
	Mild	**Moderate**	**Moderate to severe**	**Severe**		
OPG rs3102735 polymorphism						
CC	0	24 (54.5%)	4 (66.7%)	0	2.367	0.186
CT	0	13 (29.5%)	2 (33.3%)	0	2.927	^{FE}p = 0.117
TT	0	7 (15.9%)	0 (0%)	0	3.573	^{FE}p = 0.088

χ^2 Chi square test, *MC* Monte Carl, *FE* Fisher Exact, *OPG* osteoprotegerin, *C* cytosine nucleotide, *T* thiamine nucleotide, *TOAST* Trial of Org 10172 in Acute Stroke Treatment, *TACS* total anterior circulation stroke, *LAA* large artery atherosclerosis, *PACS* partial anterior circulation stroke, *SVD* small vessel disease, *POCS* posterior circulation syndrome, *CE* cardioembolic, *NIHSS* National Institutes of Health Stroke Scale, *LACS* lacunar syndrome

Table 8 Patients' and controls' carotid artery evaluation

	Patients (n = 50)	**Controls (n = 50)**		**p**
Intima-media thickness (IMT)				
Mean ± SD	1.26 ± 0.33	0.91 ± 0.45	t = 4.435*	< 0.001*
IMT > 1 mm	35 (70%)	20 (40%)	χ^2 = 9.091*	0.003*
IMT < 1 mm	15 (30%)	30 (60%)		
Presence of plaque	35 (70%)	16 (32%)	χ^2 = 14.446	< 0.001*
Plaque location				
Common carotid	12 (34%)	5 (31.5%)	χ^2 = 3.473	0.062
Carotid bulb	14 (40%)	9 (56%)	χ^2 = 1.412	0.235
Internal carotid	9 (26%)	2 (12.5%)	χ^2 = 5.005*	0.025*
Plaque echogenicity				
Echogenic	22 (63%)	4 (25%)	χ^2 = 6.297*	0.012*
Echolucent	13 (37%)	12 (75%)		
Plaque homogeneity				
Homogeneous	26 (74%)	10 (62%)	χ^2 = 0.735	0.510
Heterogeneous	9 (26%)	6 (38%)		
Plaque surface				
Smooth	17 (48%)	11 (68%)	χ^2 = 1.786	0.181
Irregular	11 (32%)	5 (32%)	χ^2 = 2.679	0.102
Ulcerated	7 (20%)	0 (0%)	χ^2 = 7.527*	0.012*
Stenosis degree				
< 70%	23 (66%)	14 (87.5%)	χ^2 = 5.617	0.017*
> 70%	12 (34%)	2 (12.5%)		

SD standard deviation, *χ^2* Chi square test, *IMT* intima-media thickness, *t* Student t-test

*Statistical significance at P < 0.05

Table 9 Correlation analysis between serum osteoprotegerin (OPG) level and carotid intima-media thickness, NIHSS and ASPECT score among patients

	Serum OPG level r_s	p-value
Intima-media thickness (IMT)	0.39	0.02*
NIHSS	0.4	0.02*
ASPECT score	−0.65	< 0.001*

NIHSS National Institutes of Health Stroke Scale, r_s Spearman coefficient, *ASPECT* Alberta stroke program early CT, *OPG* osteoprotegerin

*Statistical significance at P < 0.05

OPG may participate in ischemic stroke occurrence by increasing dendritic cells and T lymphocytes invasion into atheromatous plaque, stimulating angiopoietin-2 synthesis, and encouraging the adherence of endothelial cells [25, 26].

In our study the plasma OPG levels were also positively correlated to CIMT values. Previous studies also demonstrated that OPG levels were linked to the severity and development of carotid artery disease [20]. OPG has also been shown to be crucial in the calcification of atherosclerotic plaque and endothelial dysfunction [27]. According to Ohmori et al. [28], there was no difference in the vascular stenosis grade depending on the OPG rs2073617 gene polymorphism variant [28]. In contrast, vascular stenosis was associated with a substantial increase in plasma OPG levels [7]. The vascular stenosis grade depends on OPG plasma levels and gene variation. These variations may exist because different OPG gene loci may influence plasma OPG levels depending on the vascular stenosis degree [9].

There are various limitations in this study, we focused on the polymorphism at the OPG rs3102735 gene locus, which may possibly not fully reflect the gene. Furthermore, the study's sample size is small. A more sample size and more comprehensive research on this issue are required across more gene loci and to make sure of the results. The long-term outcome of the stroke was not investigated because we did not follow the patients.

Conclusions

The current study shows that as an independent risk factor, increased plasma OPG level, may participate in the atherothrombotic ischemic stroke pathophysiology, in addition, genetic variants in the OPG gene (rs3102735) are a separate risk factor for large artery atherosclerosis and plasma OPG level can serve as a biomarker to determine the severity of a stroke.

Abbreviations

ASPECTS	Alberta stroke program early CT score
AUC	Area under the curve
C	Cytosine nucleotide
CE	Cardioembolic
CIMT	Carotid intima-media thickness
LAA	Large artery atherosclerosis
LACS	Lacunar syndrome
NIHSS	National Institutes of Health Stroke Scale
OPG	Osteoprotegerin
PACS	Partial anterior circulation stroke
pc-ASPECTS	Posterior circulation Alberta stroke program early CT score
PCR	Polymerase chain reaction
POCS	Posterior circulation syndrome
SVD	Small vessel disease
T	Thiamine nucleotide
TACS	Total anterior circulation stroke
TOAST	Trial of Org 10172 in Acute Stroke Treatment

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Author contributions

MD carried out the Study conception and design, participated by acquisition of data and performed the statistical analysis and drafted the manuscript. OA carried out the design and conception of the study, the analysis and interpretation of data and helped to draft the manuscript. SA carried out the design and conception of the study, participated in the sequence alignment, laboratory data collection interpretation of data and drafting of manuscript. NM carried out the design and conception of the study, the analysis and interpretation of data and helped to draft the manuscript. AR carried out the study conception and design, participated in its design, and drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data can be publicly available at the Faculty of Medicine, Suez Canal University.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics committee of Suez Canal Faculty of medicine on November 21, 2017. Committee Number: 3294. An informed written consent was taken from all the participants in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests (financial or non-financial).

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