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Nontraditional Risk Factors in Carotid Artery Disease

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Abstract

Carotid atherosclerosis (AS) is one of the main risk factors for ischemic stroke. Our aim is to evaluate the nontraditional biochemical markers in asymptomatic and symptomatic patients with carotid artery plaque. This study was conducted on 55 patients: 43 with symptomatic and 12 with asymptomatic carotid artery disease. Lipoprotein (a) (Lp(a)), homocysteine, adiponectin, nitric oxide (NO), and tumor necrosis factor α (TNF-α) levels were measured in the plasma. The mean of total cholesterol, triglyceride, and homocysteine levels was significantly elevated in the symptomatic group as compared with the asymptomatic group ($P = .03$). In the asymptomatic group, adiponectin and NO levels showed elevations as compared with the symptomatic group but this increase was not significant ($P > .05$). Lipoprotein (a) and TNF-α levels acted inversely with adiponectin and NO. There was an insignificant decline in Lp(a) and TNF-α levels in the asymptomatic group as compared with the symptomatic group ($P > .05$).

Keywords

adiponectin, Lp(a), TNF-α, homocysteine, nitric oxide, carotid artery disease

Introduction

Cerebrovascular events are the second leading cause of death in the world, the third in most industrialized countries, and carotid artery disease is one of the main causes of stroke.[1] Extensive work on atherosclerotic plaque specimens from carotid endarterectomy consistently demonstrated that the composition of the plaque is a factor determining the risk of cerebral ischemia.[2,3] Despite traditional risk factors such as hypertension, smoking, diabetes, diet, and obesity, nowadays especially inflammatory molecules, cytokines, and homocysteine play an important role in the development of AS. Adiponectin is an adipocyte-specific protein, which is secreted by adipose tissue. It is abundantly present in human plasma and has been proposed to play an important role in the development of AS.[4-7] The antiatherogenic effects of adiponectin show that the plasma concentration is inversely correlated with the adipose tissue mass and has an anti-inflammatory effect on the vasculature, by inhibiting vascular smooth muscle cell proliferation and macrophage adhesion. Adiponectin also stimulates production of nitric oxide in vascular endothelial cells, resulting in the improvement of endothelial dysfunction.[8,9] In several studies, vascular endothelial nitric oxide (NO) biosynthesis is reported to be impaired in patients with AS.[10,11]

Nitric oxide is a free radical molecule known to play a significant role in the pathogenesis of inflammation.[12] Nitric oxide in blood is rapidly oxidized to nitrite and finally to nitrate by oxygenated hemoglobin, molecular oxygen, and superoxide anion.[13] Adiponectin and NO work together to have antiatherogenic properties, and hence hypoadiponectinemia might be associated with a higher incidence of vascular disease. Tumor necrosis factor-α (TNF-α) is another cytokine known to suppress the expression and secretion of adiponectin in adipocytes. It has reciprocal effects with adiponectin and plays an atherogenic role in the development of AS.[14]

Another pathological molecule for blood vessels, total homocysteine (tHcy), is a sulfur-containing amino acid formed during metabolism of methionine.[15] Numerous clinical studies have shown that hyperhomocysteinemia is an independent risk factor for coronary, cerebral, peripheral artery disease, and venous thrombosis.[16] The importance of hyperhomocysteinemia in the pathogenesis of AS is highlighted by the fact that 50% of all patients with genetic homocystinuria show

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Table 1. Lipid Parameters of the Patient Group

	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	LDL Cholesterol (mg/dL)	VLDL Cholesterol (mg/dL)	HDL Cholesterol (mg/dL)	N
Symptomatic	149.42 ± 38.93[a]	142.69 ± 96.46[b]	92.66 ± 30.29	28.54 ± 19.29	32.36 ± 7.70	43
Asymptomatic	124.33 ± 18.47[a]	86.42 ± 29.67[b]	100.42 ± 13.73	17.28 ± 5.33	54.58 ± 9.04	12

NOTES: HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein.
[a] $P = .046$.
[b] $P = .03$.

atherosclerotic changes before 30 years of age.[16,17] Elevated levels of tHcy are toxic to vascular endothelium, causing endothelial dysfunction, decreased bioavailability of nitric oxide, oxidative stress, protein modification, and endoplasmic reticulum stress[18,19] and thrombosis.[20] The accumulation of Hcy can also induce proinflammatory reactions[21-23] and contributes to the development of AS.

Lipoprotein (a) (Lp(a)) has many similarities with low-density lipoprotein cholesterol (LDL-C). Lipoprotein (a) contains a unique protein apolipoprotein (a) (Apo (a)), which is structurally different from other apolipoproteins. Apolipoprotein (a) is linked to Apo B through a single disulfide bond connecting their C terminal regions and has a high degree homology with plasminogen.[24] Raised levels of Lp(a) have been acknowledged as an inherited risk of both premature and advanced AS at different vascular sites.[25] Lipoprotein (a) is an important risk factor for cardiovascular disease and is associated with ischemic heart disease, age, dyslipidemia, body fat, and Framingham score.[26]

Little data are available on the effects between circulating cytokines and carotid AS.

Our aim is to evaluate the nontraditional biochemical markers in asymptomatic and symptomatic patients with carotid artery plaque.

Materials and Methods

Patients

Patients were recruited at the Cerrahpasa Medical School, Department of Heart and Vessel Surgery, University of Istanbul. The upper age limit was set at 70 years. Prior to the start of the study, blood samples were taken from 12-hour fasted patients and collected in Vacutainer tubes with citrate. Samples were immediately centrifuged at 1300*g* for 10 minutes. Plasma was removed and stored at −80°C until analysis.

A total number of 55 patients were included in the study: 43 were symptomatic and 12 were asymptomatic. The average patient age was 67 in the symptomatic group and 47 in the asymptomatic group. We compared the results according to the patient's symptoms.

Patients who have blurred or loss of vision in one or both eyes, weakness/numbness of the arm, leg or face on one side of their body, slurring of speech, difficulty talking or comprehending, loss of coordination, dizziness or confusion, and trouble swallowing were considered symptomatic patients. Other patients who lack these symptoms but have plaques on carotid vessels were considered asymptomatic patients.

Laboratory Methods

Nitric oxide levels were studied with the Nitric Oxide Kit (Cat No: 917-010; Assay Designs, Ann Arbor, Michigan). The kit involves the enzymatic conversion of nitrate to nitrite by the enzyme nitrate reductase, followed by the colorimetric detection of nitrite as a colored azo dye product of the Griess reaction that absorbs visible light at 540 nm.

Enzyme-linked immunosorbent assay (ELISA) was used to determine plasma adiponectin (Cat No: CY-8050; MBL, Massachusetts-USA) and TNF-α levels (Cat No: ET2010-1; Assaypro, St Charles, Missouri). The levels of serum homocysteine were measured using competitive chemiluminescent enzyme immunoassay (Immulite 2000-BIODPC, Los Angeles, California).

Plasma Lp(a) levels were determined by solid phase capture sandwich ELISA (Cat No: 1740-9; Diagnostic Automation, Inc, Calabasas, California)

Lipid parameters were analyzed at the Central Research Laboratory of the Cerrahpasa Medical School (Abbott C8000i).

Statistical Analysis

Statistical analyses were conducted using Unistat 5.1 software. All numerical values were reported as means ± SD. A comparison of variables between the 2 groups was performed using Student *t* test. Pearson correlation coefficient (*r*) was determined where appropriate.

P values less than .05 were considered significant.

Results

Table 1 shows the lipid parameters of the symptomatic and asymptomatic groups. Serum NO, TNF-α, adiponectin, homocysteine, and Lp(a) levels are presented in Table 2. Tables 3 and 4 show the Pearson correlation test applied to pairs of variables in symptomatic and asymptomatic groups, respectively. Mean total cholesterol and triglyceride levels were significantly elevated in the symptomatic group as compared with the asymptomatic group ($P = .046$, $P = .03$). We found serum homocysteine levels were elevated in the symptomatic group as compared with the asymptomatic group ($P = .03$). In the

Table 2. Serum NO, TNF-α, Homocysteine, Adiponectin, and Lp(a) Levels in Patients With Carotid Artery Disease

	Symptomatic	Asymptomatic
NO (μmol/L)	6.72 ± 2.27	15.81 ± 4.91
TNF-α (pg/mL)	40.02 ± 8.32	22.00 ± 7.05
Homocysteine (μmol/L)	15.22 ± 6.01[a]	9.53 ± 2.91[a]
Adiponectin (μg/mL)	32.28 ± 17.53	56.41 ± 10.3
Lp(a) (mg/dL)	2.17 ± 0.62	1.58 ± 0.56
Age	67.36 ± 10.34	46.92 ± 12.33
N	43	12

NOTES: Lp(a) = lipoprotein (a); NO = nitric oxide; TNF-α = tumor necrosis factor α.
[a] P = .03.

Table 3. Pearson Correlation Test Applied to Pairs of Variables in Symptomatic Group

	Serum LDL		Serum Cholesterol		Triglyceride	
	r	*P*	*r*	*P*	*r*	*P*
Homocysteine	.447	<.01	.362	<.05	.075 (−)	>.05
Age	.168 (−)	>.05	.273 (−)	>.05	.603 (−)	<.01

NOTES: LDL = low-density lipoprotein; *r* = correlation coefficient.

asymptomatic group, adiponectin and NO levels showed elevations as compared with the symptomatic group but this increase was not significant ($P > .05$). Lipoprotein (a) and TNF-α levels acted inversely with adiponectin and NO. There was an insignificant decline in Lp(a) and TNF-α levels in the asymptomatic group as compared with the symptomatic group ($P > .05$).

According to Pearson correlation analysis, significant (+) correlations were found between NO and adiponectin in the asymptomatic group ($r = .05$, $P < .05$) and between homocysteine and LDL in symptomatic group ($r = .447$, $P < .01$).

Discussion

We found an increase in serum triglyceride and total cholesterol and decreased HDL levels in the symptomatic group as compared with the asymptomatic group. Serum homocysteine levels were higher in the symptomatic group than in the asymptomatic group. In addition, we found a strong correlations between serum LDL and homocysteine levels in the symptomatic group. Triglyceride and homocysteine levels were weakly correlated.

Homocysteine contributed to arterial plaque history, causing endothelial damage to the vascular wall. Marked elevation of Hcy results in vascular injury and premature atherothrombosis,[29] with alteration in the coagulation process.[30] In our study, homocysteine levels were significantly elevated in the symptomatic group as compared to the asymptomatic group. High homocysteine levels may partially contribute to progression of the disease. Further studies with changes in ultrasound imaging and a control group will be required to evaluate the relationship between homocysteine and disease progression.

Table 4. Pearson Correlation Test Applied to Pairs of Variables in Asymptomatic Group

	Adiponectin	
	r	*P*
NO	.50	<.05

NOTES: NO = nitric oxide; *r* = correlation coefficient.

Lipoprotein (a) has a high degree of homology with plasminogen. Atherogenic hypothesis involves competition between plasminogen and Lp(a) for fibrin-binding sites. Successful competition by Lp(a) will block plasminogen, thus forming clots, which will not dissolve. So Lp(a) attenuates fibrinolysis and promotes coagulation.[25] Lipoprotein (a) has been associated with endothelial dysfunction.[27] The complex structural homology of Lp(a) with LDL and plasminogen suggest that Lp(a) might represent the ideal bridge between the fields of AS and thrombosis in the pathogenesis of vascular disease.[25] Lipoprotein (a) facilitates its oxidative modification and promotes the formation of foam cells and fatty streaks.[28] We could not find any significant differences between symptomatic and asymptomatic carotid artery disease ($P > .05$). In both groups, atheromatous plaques were found. It showed genetic predisposition for AS.

Adiponectin is an adipocyte-specific plasma protein, which is an anti-inflammatory and antiatherosclerotic cytokine.[31] In this respect, adiponectin diminishes the expression of adhesion molecules in endothelial cells,[32] suppresses macrophage to foam cell transformation,[6] and inhibits smooth muscle cells proliferation and migration.[33]

In several studies, plasma adiponectin levels were found to be decreased in patients with CAD as compared with the control group.[33-35]

In our study, we found that adiponectin levels diminished in the symptomatic group when compared with the asymptomatic group, but this decline was not significant. Circulating adiponectin levels are high (5-30 μg/mL), accounting for approximately 0.01% of total plasma protein.[36-39] Higher adiponectin levels make the defense system stronger in asymptomatic patients.

In this context, our results are in agreement with data reported on another inflammatory marker, the TNF-α. Tumor necrosis factor α is a major cytokine that is synthesized and released by wounding[40-42] and is secreted into the bloodstream.[42-46] It acts inversely with the adiponectin and shows atherogenic effects. In the asymptomatic group, we found a small decline in TNF-α levels as compared with the symptomatic group. High TNF-α levels in the symptomatic group is a marker of endothelial activation.[47,48]

Adiponectin also stimulates NO production in the arterial wall. Our findings supported this fact. Nitric oxide levels were significantly higher in the asymptomatic group as compared with the symptomatic group. We found middle powered correlation between the NO and the adiponectin levels ($r = .05$, $P < 05$). All data in our study were in agreement with literature.

Conclusion

In the symptomatic group, hyperhomocysteinemia caused endothelial damage, resulting in diminished NO production by the endothelium. Tumor necrosis factor α inhibited adiponectin released that has antiatherosclerotic effects. Lipoprotein (a) shows genetic predisposition to plaque formation. We can say, there is a higher risk of stroke in the symptomatic group as compared with the asymptomatic group in carotid artery disease.

The pathogenesis of AS involves the complex interaction between lipoproteins, coagulation and growth factors, immune and inflammatory components, and cytokines. All these factors such as adiponectin, TNF-α, homocysteine, and Lp(a) are in balance in the organism. When the balance is disrupted, many irreversible changes occur on and within the vascular wall.

Declaration of Conflicting Interest

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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