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Abnormal levels of serum anti-elastin antibodies in patients with symptomatic carotid stenosis

Plamen Tzvetanov[a], Vish Hegde[a], Jasem Y. Al-Hashel[c], Milena Atanasova[b], Aman P.S. Sohal[a], Rossen T. Rousseff[c,*]

[a] Department of Neurosciences, University Hospitals, Coventry and Warwickshire NHS Trust, Clifford Bridge Road, Coventry CV2 2DX, UK
[b] Department of Biology, Medical University of Pleven, Kliment Ohridski st 1, Pleven 5800, Bulgaria
[c] Department of Neurology, Ibn-Sina Hospital, pob 25427, Safat 13115, Kuwait

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ABSTRACT

Background and objective A correlation between the levels of antibodies to alpha-elastin (alpha-AEAb) and tropoelastin (tropo-AEAb) and the corresponding peptide concentration is found in human serum in health and disease. Serum elastin peptide and anti-elastin antibodies (AEAb) levels are age-related and vary with the stages of atherosclerotic vascular damage. This study aims to determine if elastin metabolism (assessed by the ratio of tropo-AEAb to alpha-AEAb) differs in patients with symptomatic carotid stenosis versus subjects with asymptomatic stenosis.
Patients and methods: Alpha-AEAb and tropo-AEAb were measured by ELISA in blood sera of 65 patients with ultrasound verified high-grade symptomatic carotid stenosis (resulting in stroke 1–7 days before measurement) compared to 51 patients with asymptomatic stenosis.
Results: Serum anti-alpha-elastin IgG levels are extremely increased in symptomatic versus asymptomatic carotid stenosis. The ratio of tropo-AEAb (reflecting elastin synthesis) to alpha-AEAb (a function of elastin degradation) was 3.7 in symptomatic stenosis versus 14.2 in asymptomatic stenosis ($p < 0.001$).
Conclusions: There is a significant difference in elastin metabolism in patients with symptomatic carotid stenosis versus asymptomatic stenosis. The ratio of tropo-AEAb to alpha-AEAb as an index of elastin synthesis/degradation proves useful in investigation of atherosclerotic lesions and may represent a new immunologic marker for carotid plaque destabilization.

1. Introduction

Elastin is a major component of the extracellular matrix. Changes of its metabolism are involved in pathophysiology of destructive lesions of elastin-rich organs, such as blood vessels, kidney, skin and lungs. Elevation of serum elastin-derived peptides (EDPs) levels is observed in emphysema, abdominal aortic aneurysm and atherosclerosis [1–3].

Mature atherosclerotic lesions develop a fibrous cap composed of dense extracellular matrix containing collagen and elastin. Degradation of elastin in arterial walls is a characteristic feature of atherogenesis [4,5]. The products of degradation are EDPs that have been detected and quantified in circulating blood [3,6]. Their serum levels are increased only in ulcerative, but not in occlusive atherosclerotic lesions [3].

Serum EDPs are immunogenic and provoke the synthesis of anti-elastin antibodies (AEAbs) [6]. These secondary immune and inflammatory responses to elastin might lead to further elastinolysis and production of more EDPs triggering a vicious circle which causes further degradation of the fibrous cap [4,5].

We undertook this study in an attempt to compare elastin turnover in stroke patients with symptomatic carotid stenosis (resulting in stroke 1–7 days before measurement) versus subjects with asymptomatic stenosis. We measured the absolute levels of serum tropo-AEAb (that correlates with elastin synthesis) and alpha-AEAb (that parallels with elastin degradation) and used the ratio of tropo-AEAb to alpha-AEAb as an index of elastin metabolism.

2. Patients and methods

2.1. Patients

The study enrolled 116 patients (mean age: 62.5; SD: 11.3; range: 28–85 years; 53 women) with ultrasound verified internal carotid stenosis as a part of research project. The study procedures are approved by a Local Ethic Committee. All the patients

* Corresponding author. Tel.: +96566379970; fax: +96524948226/96524849226.
E-mail address: rossentrousseff@yahoo.co.uk (R.T. Rousseff).

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or their representatives gave informed consent for participation in the study in accordance with the provision of Helsinki Declaration.

Exclusion criteria were infection, systemic diseases, neoplasm, emphysema and pneumonia.

All patients underwent CT investigations on a Siemens Somatom ARC. According to the protocol of our stroke unit, persons with normal CT on entry were repeatedly scanned between 3rd and 7th hospital day.

At the initial evaluation, colour duplex scan was performed by 2 operators using Phillips SONOS 5500 equipment. The patients and control subjects underwent colour duplex carotid scan on the right and left common and internal carotid arteries in a supine position. High resolution B-mode, colour Doppler and pulsed-wave Doppler were done with an ultrasound linear array 5–10 MHz transducer.

In accordance with the consensus for Doppler ultrasound criteria for the diagnosis of internal carotid stenosis [7], carotid stenosis severity was classified into: significant (>70%) identified by a peak systolic velocity >230 cm/sec and non-significant (<50%) with a peak systolic velocity <125 cm/sec. Plaque morphology was assessed using the modified Gray-Weale's criteria [8]. According to their structural appearance plaques were classified as heterogeneous and homogeneous [9]. Plaque surface morphology was also assessed and classified as regular (smooth) or irregular [10].

Clinical characteristics and biochemical parameters of patients are shown in Table 1.

According to clinical and imaging criteria [11–13] patients were divided into two groups.

The symptomatic stenosis group included 65 patients (mean age: 55.7; SD: 9.7; range: 28–74 years; 28 women) with clinically diagnosed and CT proven stroke involving the homolateral brain hemisphere. The asymptomatic stenosis group included 51 subjects without brain infarction (mean age: 54.8; SD: 14.6, range: 22–80 years, 25 women). In 15 patients the stenosis was significant. Ten of patients had multiple non-significant stenosis of extracranial arteries.

2.2. Measurement of anti-elastin antibodies

In symptomatic stenosis group, serum samples were obtained between days 1 and 7 (mean 3 days) after the strokes.

Using the method described by Mecham and Lange [14], alpha-elastin was prepared from human cadaver aortas. Tropoelastin was prepared from porcine aorta and purified from copper-deficient swine by modified method proposed by Sandberg et al. [15].

Detection of alpha-AEAb and tropo-AEAb in serum samples was carried out by enzyme-linked immunosorbent assay (ELISA). Ninety six microliter well plates have been coated with 100 μl of alpha- or tropoelastin antigens which were dissolved in a carbonate buffer (pH 9.6) at a concentration of 15 μg/ml. Then 100 μl of 0.1% bovine serum albumin in phosphate buffered saline (PBS, pH 7.2) was added for blocking the unoccupied binding sites and placed in adjoining wells as a control for each antigen, and 100 μl carbonate buffer was added as an assay control. The plates were sealed and incubated overnight at 4 °C. The plates were washed three times with PBS-Tween (0.05%) before adding 100 μl of patient serum diluted at 1:100 with PBS that contained 10% heat inactivated fetal calf serum. Phosphate buffered saline buffer (100 μl) was added to all control wells. Plates were sealed and incubated overnight at 4 °C. The wells were washed three times with PBS-Tween. Goat anti-human IgG alkaline phosphatase (Sigma; St. Louis, MO) secondary Ab was diluted with PBS-Tween at 1:1000 and added to all wells and incubated at 37 °C for 1 h. The wells were again washed three times with PBS-Tween and then 100 μl of p-nitrophenyl phosphate (substrate) made up in diethanolamine buffer (pH 9.6) at a concentration of 1 mg/ml, was added to all wells and incubated at a room temperature for 15 min to 2 h depending on maximum colour development. The results were recorded as absorbance A405 nm for the conversion of the substrate to p-nitrophenol expressed as the difference between the test sample and the serum response to carbonate buffer alone. All sera were assayed simultaneously on a Titerteck Multiskan MC ELISA reader. The specificity of the immunoconjugates was verified using a competitive version of this ELISA [16]. Optical density readings were selected from the linear portion of the time curve for colour development of alkaline phosphatase.

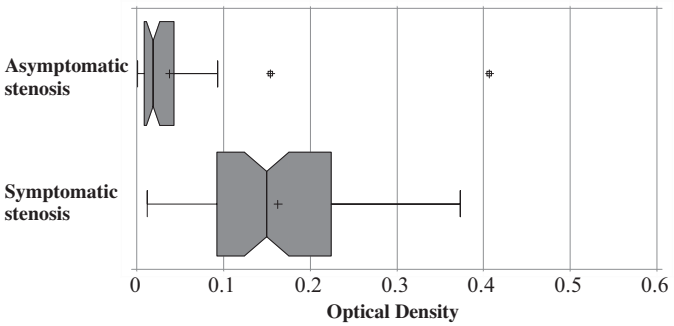

Fig. 1. Serum levels of anti-alpha-elastin antibodies in asymptomatic and symptomatic carotid stenosis.

2.3. Statistical methods

The Statgraphic Plus Version 2.1 statistical system was used for data analysis. For categorical data the $\chi 2$ and Kruskal–Wallis tests and one-way ANOVA for dispersion analysis were applied. For numerical data along with descriptive methods we used regression analysis (Pearson's r correlation coefficient). Difference between diseased and controls were considered significant for P values less than 0.05.

3. Results

Comparison of clinical, demographical and biochemical parameters between the patient groups is presented in Table 1.

Thirty five (54%) symptomatic patients had significant carotid stenosis, while the remaining 30 patients (46%) had multiple non-significant stenotic changes of extracranial arteries. Six patients (9.6%) had large infarctions (with involvement of at least 80% of the superficial territory or superficial/deep lesions) and the rest 59 patients (90.4%) had non-large infarction [11,12].

According to the Oxford Community Stroke Project classification [13] 5 of the symptomatic patients (8%) suffered total anterior circulation stroke, 46 (71%) had partial anterior circulation strokes, and 14 (21%) had lacunar stokes.

In 15 (30%) of asymptomatic patients the stenosis was significant. Ten (19%) patients had multiple non-significant stenoses of extracranial arteries.

ELISA-measured levels of antibodies to alpha-elastin were significantly higher ($p < 0.0001$) in symptomatic patients than in asymptomatic patients (Fig. 1). There was no difference in serum levels of anti-tropoelastin antibodies between the two groups (Fig. 2).

No significant correlation was found between either the number or the size of ischemic lesions and the levels of anti-elastin antibodies ($p > 0.05$).

The ratio of synthesis to degradation was 14.2 in patients with asymptomatic stenosis and 3.7 in symptomatic stenosis ($p < 0.001$) (Table 2). The abnormally lower ratio reflects the increased degradation of elastin fibers in patients with symptomatic carotid stenosis.