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INFLUENCE OF INFLAMMATORY AGENT ON STRUCTURE OF CAROTID ATHEROSCLEROTIC PLAQUES

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ABSTRACT

The sonographical, morphological, and immunohistochemical Chlamydia pneumoniae characteristics of the atherosclerotic plaques from 200 patients with asymptomatic (A=59) and symptomatic (S=141) courses of carotid stenosis (A/S-1/2.4) were studied. 34 (17 %) homogeneous (HO) or stable and 166 (83%) heterogeneous (HE) or unstable plaques were found morphologically. According to the number of their histopathological signs and prevalence of inflammatory cell type, the plaques were subdivided into 3 HO and 5 HE subtypes with different sonographical characteristics. Complicated plaques, in both A and S patients, have signs of the activation of chronic inflammation (PMN), of phagocytosis (foreign body macrophages), and of atheromatosis (foam cells) all together in HE plaque subtypes (HE2, HE5, HE4) and only of atheromatosis in HO plaques (HO2). Disruptures, intraplaque haemorrhages, and thrombi were associated with the C. pneumoniae, as the inflammatory agent, found in every kind of phagocytes located in the fibrous cap, atheroma, and mainly at the boundary between the fibrous cap and atheroma. The complications strongly correlate with the abundance of C. pneumoniae ($p < 0.001$) and so with a greater risk of a cerebral ischaemic stroke as in A and in S patients with carotid stenosis. Reliable correlation between C. pneumoniae and the degree of carotid stenosis and plaque thickness ($p < 0.06 - 0.07$) as well as sufficient correlation between plaque thickness and the disease's symptomaticity ($p < 0.01$) were found.

Key words:

atherosclerosis, carotid stenosis, morphology, immunohistochemistry, inflammatory signs, Chlamydia pneumonia

Introduction

The risk of ischaemic stroke increases with the degree of atherosclerotic carotid stenosis, which diminisches cerebral perfusion throughout the narrowed main artery, or with the embolisation of the smaller arteries. Ulcerated and fissured or disrupted atherosclerotis plaques from the inner carotid wall frequently become unstable and the main source of microemboli (1, 2). Therefore, the correlation of the morphological and clinical characteristics of unstable carotid plaques is very important for evaluating cerebral vascular disturbances before the onset of an acute cerebral stroke and can contribute to preventing hazardous disabilities and death, especially in patients with the asymptomatic course of the disease. It was recently suggested that infective pathogens `such as Chlamydia pneumoniae might play an important role in the pathogenesis of atherosclerosis (3, 4, 5, 6, 7, 8, 9). The purpose of this study was to: 1) investigate carotid atherosclerotic plaque morphology and immunohistochemistry (IHC) in respect to localization sites of *Chlamydia pneumoniae* IHC signal, 2) find correlations between plaque morphology and clinical sonographical characteristics (the disease's asymptomaticity or symptomaticity, degree of carotid stenosis, and plaque thickness), and 3) determine the correlation between the abundance of *C. pneumoniae* IHC signals, and the complexity of the plaque.

Materials and Methods

Patients and biopsies

200 patients with A (n=59) and S (n=141) courses of carotid stenosis were studied (A/S ratio: 1 / 2.4). The patients got into the Neurology and Neurosurgery Clinic of Vilnius University Emergency Hospital for TIA or acute ishaemic stoke were averaged 66.8 years old, men being 5 years younger and outnumbering women 3.7 : 1. Double scanning of the narrowed carotids was performed using a Toshiba ultrasonic scanner before surgical operation. Atherosclerotic plaques were obtained by carotid endarterectomy. For IHC the atherosclerotic plaques were incubated with primarily monoclonal anti-Chlamydia pneumoniae antibody clone RP402, code no. M6600, diluted 1 : 50 (DAKO).

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Correlation was made of the clinical (symptomaticity, asymptomaticity), sonographical (plaque thickness, degree of stenosis) and morphological – immunohistochemical (*C. pneumoniae)* characteristics of the atherosclerotic plaques in 200 patients with carotid stenosis. Only several studies were done with so high count of surgically obtained atherosclerotic plaques in patients with carotid stenosis.

Results

34 (17 %) homogeneous (HO), or stable, and 166 (83 %) heterogeneous (HE), or unstable, plaques were devided into 3 HO and 5 HE subtypes according to the number of histopathological signs and prevalence of inflammatory cell types, having different sonographical characteristics and plaque thickness. Complex plaques of both A and S patients have activation signs for chronic inflammation, phagocytosis and atheromatosis: all three of these in HE plaque subtypes, and only for atheromatosis in HO plaques.

IHC study revealed that 89.9 % of atherosclerotic plaques in A and 88.7 % in S patients had *C. pneumoniae* IHC signals.

Discussion

Although the degree of stenosis was more severe in symptomatic (S) patients but about 61 % of A and S patients had a high or critical degree of carotid stenosis (Table 2). Thicker atherosclerotic plaques (3-7 mm) were found in S and thinner (1-3 mm) in A patients (except very thick plaques in A patients with diffuse atherosclerosis). Besides

this we found a statistically sufficient correlation between the disease symptomaticity and plaque thickness ($p < 0.01$). It is known that the thickness of the fibrous cap and atheroma with lipid core may be the same A and S patients, but the core's position and local thinning of the fibrous cap and thickening of the atheroma, which is always wider, softer, and located nearer the fibrous cap in unstable HE plaque may become a reason for deformations, ulcerations, and disruptions and may create real conditions for intraplaque haemorrhages (2, 1, 14, 13). Thus, a clinical-sonographical evaluation of patients according to plaque thickness and degree of carotid stenosis is very significant when selecting patients for surgical treatment.

Table 1.

Frequency of histopathological signs in atherosclerotic plaques of asymptomatic and symptomatic patients with carotid stenosis

Histopathological signs in atherosclerotic plaques		A patients n=59	S patients n=141	A/S ratio 1 / 2.4
Fibrous cap (FC)		59 (100 %)	141 (100 %)	1/1
Atheroma (AM)		57 (97 %)	133 (94 %)	1/1
Intraplaqueal hemorrhages (thrombus, hemosiderine, siderophages)		22 (37 %)	65 (46 %)	1/1.2
Ruptures and fissures		19 (32.76)	52 (36.88)	1:1
Inflammatory cells:	Lymphocytes (L)	48 (81.4 %)	102 (72 %)	1.1/1
	Macrophages (M)	32 (54 %)	69 (49 %)	1/1.1
	Foreign bodies macrophages	2 (3.4 %)	6 (4.3%)	1/1.3
	Foam cells (F cell)	28 (47.5%)	49 (35 %)	1.4/1
	Plasma cells (PL)	1 (1.7 %)	9 (6.4 %)	1/3.8
	PMNs	1 (1.7 %)	2 (1.4 %)	1.2/1
	Eosinophils	0	1 (0.7%)	0/0.7
Angiomatosis (neovascularisation)		0	3 (2.1 %)	0/2.1
Scores of calcifications	0	8 (13.6%)	6 (4.2 %)	3.2/1
	1 (5 - 40 %)	16 (27.1 %)	28 (19.9 %)	1.4/1
	2 (50 – 70 %)	17 (28.8 %)	70 (49.7 %)	1/1.7
	3 (80 – 100 %)	18 (30.5 %)	37 (26.2 %)	1.2/1

The 12 histopathological signs in atherosclerotic plaques, distributed numerically almost the same in A and S patients with carotid stenosis, were attributed to 34 (17 %) homogenous (HO) and 166 (83 %) heterogeneous (HE) plaques (Table 1). HO plaques have fibrous cap, atheroma, and foam cells with no inflammatory component are so-called stable plaques. HE plaques with a fibrous cap, atheroma (necrotic core) and many inflammatory cells are so-called unstable plaques. Thus the specific weight of the histopathological signs in HO and HE plaques was not the same. According to number of histopathological signs and prevalence of inflammatory cell type, the HO plaques were subdivided into 3 and HE into 5 subtypes. The HO subtypes have only 3 histopathological signs – fibrous cap and atheroma and foam cells. So HO 3 subtype was judged to have activation sign of atheromatosis (foam cell).HE subtypes have different inflammatory cells (lymphocytes, macrophages, plasma cells, PMNs, foreign body cells and foam cells) and were judged to have activation signs of chronic inflammation (PMN), phagocytosis (foreign body macrophages), and atheromatosis (foam cells). Some heterogeneous plaques subtypes (HE2, HE4, and HE5) were thick (2.6 mm in A and 3.27 mm in S patients) with nearly critical or critical stenosis (about or over 90 %). Thus, the subtypes of HO and HE atherosclerotic plaques together with their sonographical characteristics somewhat reflect not only the plaque development sequence, but the inflammation sequence typical of chronic inflammation and its activation (PMNs), activation of phagocytosis (foreign body macrophages) and atheromatosis (foam cells) of HE plaques. Subtypes of atherosclerotic plaques does not mean age related sequence to the development of atherosclerotic lesion as it was show in Stary classification (12), because we doesn't divide our patients into age groups. Besides we think that the development of atherosclerosis is not all the same in every patient. Blood vessels of many patients even of the advanced age may have no any atherosclerotic lesions.

About 46.5 % of both HO and HE carotid plaques were *complicated* somewhat more in S (47.5 %) than in A (44 %) patients and more frequently among subtypes having signs of the activation of chronic inflammation, phagocytosis, and atheromatosis in HE plaques subtypes. Complicated plaques were either very thin (from 0.5 to 1.5 mm) mostly in the HO1 and HE1 subtypes or thick (over 3 mm) in the HE2, HE3, HE4, and HE5 subtypes with nearly critical (over 83 % in A) or critical (90% in S patients) degree of carotid stenosis. What initiates and activates the inflammatory process in atherosclerotic plaque and induces complications has only recently begun to be explained.

Reliable data that blood vessel immune inflammations may be induced by micro-organisms, such as *Chlamydia pneumoniae, adenovirus, herpes simplex virus type 1 , Cytomegalovirus,* by carrying infective agents into the blood vessel wall and so participating in the pathogenesis of the atherosclerosis have appeared (3, 6, 7, 9, 15, 16). Microbes

Table 2.

The thickness of atherosclerotic plaques of different subtypes and degree of carotid stenosis in asymptomatic and symptomatic patients

Subtype	*Degree of stenosis %*				*Thickness of plaques mm*	
	n	*x ± m*	*n*	*x ± m*	*x ± m*	
		S patients		*A patients*	*S patients*	*A patients*
HO1	***3***	***86.7*** *± 3.3*	***2***	***65*** *± 15*	***2.0*** *± 0*	***4.1*** *± 1*
HO2	***20***	***82.5*** *± 3.2*	***6***	***78.3*** *± 9.4*	*3.1 ± 0.23*	*3.2 ± 0.48*
HO3	***3***	***80*** *± 0*	***-***	*-*	*3.7 ± 1.2*	*-*
HE1	***4***	***70*** *± 4.1*	***-***	*-*	*2.0 ± 0*	*-*
HE2	***55***	***89.1*** *± 1.7*	***19***	***87.4*** *± 2.7*	*3.65 ± 0.17*	*3.05 ± 0.33*
HE3	***8***	***93.7*** *± 5.0*	***1***	***60*** *± 0*	*3.0 ± 0.62*	*2.0 ± 0*
HE4	***13***	***91.5*** *± 2.2*	***7***	***91.4*** *± 4.6*	*3.2 ± 0.36*	*2.6 ± 0.2*
HE5	***35***	***90.3*** *± 1.9*	***24***	***89.6*** *± 1.8*	*3.26 ± 0.17*	*2.75 ± 0.16*

may infect the blood vessel wall, persist, and induce latent and relapsing infections. *C. pneumoniae* may infect circulatory components (PMN as we have shown), which may attach to endothelium and smooth muscle cells and kill them by apoptosis (11). This attachment is defined by the genetic tropism of *C. pneumoniae* for some tissues (10). It is the first step to an inflammatory response. Now it is presumed that a genetic variant of the toll-like 4 receptor determines the differences in the inflammatory reactions types to different microbe polysaccharides and is connected with the development of atherosclerosis (8). Our study shows that 89.8 % of atherosclerotic carotid plaques in A and 88.7 % in S patients have *C. pneumoniae* IHC signals, particularly abundant (2 and 3 scores) in some HE subtypes. The morphological signs of complications *strongly* correlates with the *Chlamydia pneumoniae* IHC signal ($p < 0.001$) in our study. Such a high percent of *C. pneumoniae* IHC signal in carotid atherosclerotic plaques in our study may be explained by several causes. First, by time-sensitivity of DAKO En-vision system solutions which being prepared ex-tempore increases sensitivity to specific anti-*Chlamydiae* antibodies (DAKO protocol), and second, by our patients cohort who were admitted to the Emergency Hospital of Vilnius University for TIA or acute ischaemic cerebral stroke. *C. pneumoniae* IHC signal were seen in histiocytes between edematous collagen fibres by the inflammatory infiltrations and small calcifications of fibrous cap; phagocytosed and released by macrophages, large foreign body cells, and foam cells (especially abundant) at the fibrous cap /atheroma boundary,even in PMNs at the site of fresh thrombi and by lipids in the atheroma. Almost the same distribution of *C. pneumoniae* in coronary artery atherosclerotic plaques was noticed by pathologists who used IHC for *C. pneumoniae* and ultrastructural techniques (5). We suppose that even the accumulation of foam cells with *C. pneumoniae* IHC signals at the fibrous cap/ atheroma boundary shows its importance in fibrous cap thinning and in atheroma thickening together with deposition of released fats and proteins from cell and take part in growing the necrotic core and in the thinning the fibrous cap of atherosclerotic plaque.

Some authors suggest that *C. pneumoniae* is specific to atherosclerotic lesions because it is not found in normal artery tissues (3, 4, 7, 9). The finding in our study of macrophages, foreign body cells, and foam cells loaded with *C. pneumoniae* in atherosclerotic carotid plaques shows a specific inflammatory response and may be identified as a sign of plaque instability and complications.

Conclusions

According to the number of histopathological signs and prevalence of inflammatory cell type, the intensity of the inflammation in the atherosclerotic plaques did not determine a patient's *symptomaticity* because stronger inflammation were frequently found in patients with asymptomatic carotid stenosis.

Heterogeneous atherosclerotic plaques with disruptures, intraplaque haemorrhages, and thrombi were associated with the *C. pneumoniae* IHC signals found in every king of phagocyte, located in the fibrous cap/ atheroma, are much more dangerous than homogenous ones for the development of complications as in asymptomatic and symptomatic patients. The complications rate, so plaque instability strongly correlate ($p < 0.001$) with the abundance of *C. pneumoniae* IHC signals which have morphological activation signs for chronic inflammation.

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