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Protective properties of HDL cholesterol and atherosclerotic plaque calcifications in advanced atherosclerosis in ischaemic stroke patients

Ochronne właściwości cholesterolu HDL i zwapnień w blaszce miażdżycowej w zaawansowanej miażdżycy u pacjentów z udarem niedokrwiennym mózgu

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SUMMARY

Introduction: Atherosclerotic changes in carotid arteries play an important role in the pathogenesis of ischaemic stroke. To a high extent there is evident asymmetry within the development of these changes, affecting just one artery.

The aim of the study was to determine the impact of the cardiovascular risk factors on the presence of haemodynamically significant atherosclerotic changes or occlusion only in one compared to both of the carotid arteries in patients with ischaemic stroke.

Material and methods: Patients diagnosed with ischaemic stroke were retrospectively assessed towards stenosis of ≥70% or occlusion in at least one of the internal or common carotid arteries. There were 104 patients enrolled in the study. Group I consisted of individuals with haemodynamically significant (≥70%) stenosis or occlusion in one carotid artery (n = 48). Group II consisted of patients with bilateral significant (≥70%) stenosis or occlusion in carotid arteries (n = 56).

Results: There were no changes found in the presence of non-modifiable stroke risk factors between the groups. In group I higher HDL level (45.7 vs 38.9 mg/dL, p = 0.038) and significantly more frequent calcifications in the atherosclerotic plaques of carotid arteries were found (p = 0.03). There were no differences in other tested factors between groups.

Conclusions: The protective properties of HDL cholesterol and the slow formation of more stable, calcified plaques play an important role only in the development of unilateral advanced atherosclerosis in carotid arteries. The role of HDL cholesterol in stroke pathomechanism needs further studies.

Key words: cerebral stroke, atherosclerosis, risk factors, high-density lipoproteins, carotid arteries.

STRESZCZENIE

Wstęp: Zmiany miażdżycowe w tętnicach szyjnych odgrywają istotną rolę w patogenezie udaru niedokrwiennego mózgu. W znacznym procencie przypadków stwierdza się wyraźną asymetrię w rozwoju tych zmian, które zajmują tylko jedną tętnicę. Celem pracy było ustalenie wpływu sercowo-naczyniowych czynników ryzyka na obecność istotnych hemodynamicznie zmian miażdżycowych lub niedrożności tylko w jednej tętnicy szyjnej w porównianiu do obustronnego ich zajęcia u pacjentów z udarem niedokrwiennym mózgu.

Materiały i metody: Pacjenci z rozpoznaniem udaru niedokrwiennego mózgu poddani zostali retrospektywnej ocenie w kierunku zwężenia ≥70% lub niedrożności, co najmniej w jednej tętnicy szyjnej wewnętrznej lub wspólnej. Do badania zostało włączonych 104 pacjentów. Grupę I stanowili chorzy z istotnym hemodynamicznie zwężeniem (≥70%) lub niedrożnością jednej tętnicy (n = 48). Grupę II stanowiły osoby z obustronnym istotnym hemodynamicznie zwężeniem (≥70%) lub niedrożnością tętnic szyjnych (n = 56).

Wyniki: Nie stwierdzono różnic w częstości występowania niemodyfikowalnych czynników ryzyka pomiędzy grupami. W grupie I stwierdzono istotnie wyższy poziom cholesterolu HDL (45,7 vs 38,9 mg/dL, p = 0,038) i istotnie częściej obecność zwapnień w blaszkach miażdżycowych tętnic szyjnych (p = 0,03). Nie stwierdzono różnic w częstości występowania innych badanych czynników pomiędzy grupami.

Wnioski: Ochronne właściwości cholesterolu HDL i wolniejsza dynamika powstawania bardziej stabilnych, uwapnionych blaszek miażdżycowych odgrywają istotną rolę w rozwoju tylko jednostronnych, zaawansowanych zmian miażdżycowych w tętnicach szyjnych. Rola HDL w patomechanizmie powstawania udaru mózgu wymaga dalszych badań.

Słowa kluczowe: udar mózgu, miażdżyca, czynniki ryzyka, lipoproteiny wysokiej gęstości, tętnice szyjne.

INTRODUCTION

Atherosclerosis is a generalized process of a multifactorial background, connected with the presence of the inflammation, blood lipids profile, hypertension, diabetes and exogenous factors, such as cigarette smoking, infective factors or mechanical forces [1, 2, 3]. The presence of advanced atherosclerosis in the carotid arteries increases the risk of ischaemic stroke and is responsible for about 15% of strokes. They are located most often in the bifurcation of the common carotid artery and carotid sinus [4, 5]. The classic, generalized risk factors of cardiovascular diseases result in the development

of atherosclerosis within the carotid arteries to a similar extent on both sides. Meanwhile, in many patients atheromatous changes develop in the carotid arteries asymmetrically, which results in the search for local agents such as differences in the geometry and shape of the arteries. Available results are contradictory. On one hand, it has been demonstrated that the asymmetry of atherosclerosis is positively correlated with the increased ratio of the surface area of the cross-section of the common carotid artery in relation to the internal carotid artery. On the other hand, no such relation was found when the size of the angle measured between the common carotid artery and both external and internal carotid arteries was compared [6]. It has also been demonstrated that carotid bifurcation geometry is an independent, albeit weak, predictor of its early wall thickening [7].

Direct analysis of the diameter of carotid arteries asymmetrically affected by the process of atherosclerosis showed that the stenosis or occlusion of the internal carotid artery more often appears on the side where the diameter of the common carotid artery is smaller [8]. In contrast to the above study, it has been shown that the ratio of the diameter of the common carotid artery in relation to the internal carotid artery does not affect the asymmetrical development of atheromatous plaques [9].

It is being discussed that atherosclerotic asymmetry in the carotid arteries is connected with the forces affecting the artery wall: wall shear stress (WSS) and tensile stress (TS) [10]. The role of WSS and TS in atherogenesis in not yet clear. During blood flow the force of friction is exerted on the endothelium (shear stress). Meanwhile, tensile stress is induced by arterial pressure. Processing of the mechanical signals to biological ones is defined as mechanotransduction and takes place in the endothelium. Mechanoreceptors, the glycocalyx, transcription factors are involved in this process, and the activity of at least a few dozen genes is modified.

The lower value of shear stress promotes the development of atherosclerosis in arteries. Development of atherosclerotic changes in the vein by-pass, which suggests independence from type of the vessel, underlines the importance of mechanotransduction in the process of the formation of atherosclerosis [11, 12].

Some authors suggest that decrease in WSS extends the contact time of flowing blood with the endothelium, causing greater penetration of inflammatory molecules, leucocytes, thrombocytes, intensification of the local synthesis of mitogenic substances, oxidative stress, and matrix destruction, and in the end promotes excessive remodelling and greater intima-media thickness (IMT) [1, 13]. The results of later studies show that the increase in WSS also leads to endothelial dysfunction, but physiological values may protect against atherosclerosis [14, 15, 16]. The significantly increased value of WSS fosters the formation of perimural thrombotic changes as a result of the previously present artery stenosis [17]. On the other hand, it was shown that higher WSS can exert a beneficial effect by stimulation of the synthesis of nitric oxide [18].

Contradictory results of studies in the field of the geometry of the arteries may suggest that in the process of atherogenesis an important role is played by the classic risk factors of cardiovascular diseases. At the beginning it was shown that a history of ischaemic heart disease predisposes patients to the bilateral presence of atherosclerosis in carotid arteries [19]. Similarly, a history of myocardial infarct, peripheral vascular disease [20, 21] and older age is connected with the presence of bilateral compared to unilateral carotid artery atherosclerosis [1].

In our study, an attempt was made to identify the impact of the risk factors of cardiovascular diseases and other selected parameters on advanced atherosclerotic changes only within one of the carotid arteries.

METHODS

The retrospective analysis was carried out on consecutive patients diagnosed with ischemic stroke or transient ischemic attack (TIA) and hospitalized in the Department of Neurology of the Pomeranian Medical University in Szczecin (PMU) in the years 2009–2011. There were 104 patients enrolled in the study, in which atherosclerosis of ≥70% or occlusion in at least one of the internal or common carotid arteries was found. Group I consisted of patients with haemodynamically significant stenosis (≥70%) or occlusion within one of the carotid arteries (n = 48). Group II consisted of patients with bilateral haemodynamically significant stenosis or occlusion of carotid arteries (n = 56).

Ischaemic stroke and TIA were defined on the basis of the clinical and radiological picture [22]. Information about the presence of stroke risk factors were obtained from medical records, patients and family members.

Patients with carotid artery stenosis caused by artery dissection were excluded from the study. The presence of stenosis or occlusion of carotid arteries meant a stenosis ≥70% within the bifurcation of the common carotid artery or extracranial part of the internal carotid artery, which was assessed using Doppler duplex ultrasonography. The ultrasonography of carotid arteries was performed in the Department of Neurology of the PMU with the use of the GE LOGIQ Book XP camera, linear head 7,5 MHz. The appearance and permeability of arteries were assessed in longitudinal, transverse, antero-lateral and postero-lateral projections. The complex of intima-media was described as the distance between lines demarcating the artery lumen and inner wall (intima) to media and adventitia. Intima-media thickness was measured using the manual system on the distal wall of common carotid arteries in the distal part [23, 24]. Atherosclerotic plaques in carotid arteries were defined in accordance with the recommendations of the Manheim consensus [25]. The morphology of the plaques (echogenicity, heterogenicity, presence of calcifications) was assessed.

Hypertension and type 2 diabetes were identified on the basis of the patient's history, or diagnosed during hospitalization. Hypertension had been diagnosed before admission or when at least 2 measurements revealed blood pressure of ≥140/90 mmHg. Type 2 diabetes had already been diagnosed

or detected during hospitalization when at least 2 results showed fasting blood glucose of ≥126 mg/dL or non-fasting blood glucose of ≥200 mg/dL. The abuse of alcohol was diagnosed if the person admitted to the consumption of more than 40 g of ethyl alcohol per day in men (>4 standard doses of spirit), and over 20 g of ethanol for women (>2 standard doses) [26].

Atrial fibrillation meant both chronic and paroxysmal – diagnosis given in the history or established during hospitalization.

The use of antiaggregants meant receiving acetylsalicylic acid, ticlopidin or clopidogrel, and the use of oral anticoagulants meant receiving acenocumarol or warfarin. New oral anticoagulants were not used.

The groups were compared with the use of the Mann–Whitney U-test. The nominal variables analysis was carried out by the χ^2 test and Fisher's bilateral test. Stepwise linear and multiple regression were performed to evaluate the factors connected with the atherosclerosis distribution. As statistically significant, p value was considered less than 0.05. Analyses were made using Statistica v. 7.1 software.

RESULTS

Non-modifiable risk factors of stroke

Group I (n = 48) consisted of 26 men aged 43–92 years (median 72 years), and group II (n = 56) consisted of 34 men aged 50–89 years (median 72 years). There were no significant differences between the groups in terms of sex, age and previous stroke/TIA (20 patients in group I and 17 in group II).

Modifiable risk factors of stroke

The presence of haemodynamically significant stenosis/occlusion of carotid arteries in relation to modifiable stroke risk factors is shown in Tables 1–3.

No differences were found between the study groups in terms of other investigated factors (age, NIHSS score, temperature on admission, peripheral vascular disease, previous treatment with statins, antiaggregants, oral anticoagulants and hypotensives, education, occupation; type of stroke according to TOAST classification, blood platelet count, fibrinogen).

No relationship was found between the presence of unilateral advanced atherosclerotic changes in the carotid arteries and the risk of death during hospitalization (4 individuals in both group I and II, p = NS).

TABLE 1. Modifiable stroke risk factors in relation to uni- or bilateral carotid artery stenosis

Parameter	Group I (n = 48)	Group II (n = 56)	p
Hypertension	37	47	NS
Ischaemic heart disease	21	32	NS
Type 2 diabetes	16	18	NS
Atrial fibrillation	12	9	NS
Present cigarette smoking	16	15	NS
Excessive alcohol consumption	19	14	NS

NS – statistically non-significant.

TABLE 2. Additional test results in study groups

Parameter	Group I – mean (min.–max.)	Group II – mean (min.–max.)	p
IMT (mm)	0.09 (0.06–0.17)	0.09 (0.008–0.12)	NS
BMI (kg/m²)	26 (21–55)	28 (20–46)	NS
Blood CRP (mg/L)	9.8 (0.5–209)	21.7 (0.5–246)	NS
Triglycerides (mg/dL)	141.1 (67–363)	136.5 (41–409)	NS
LDL (mg/dL)	125.7 (48–255)	122.5 (45–196)	NS
CH (mg/dL)	202.2 (125–348)	191.9 (105–289)	NS
Glycaemia on admission (mg/dL)	113.7 (75–225)	117.7 (74–412)	NS
SBP on admission (mmHg)	154 (110–230)	145 (83–210)	NS
DBP on admission (mmHg)	84 (60–30)	83 (50–120)	NS

p – Mann–Whitney U-test; IMT – intima-media thickness; BMI – body mass index; CRP – C-reactive protein; LDL – low density lipoprotein; CH – total cholesterol; SBP – systolic blood pressure; DBP – diastolic blood pressure; NS – statistically non-significant.

DISCUSSION

In the conducted study the authors were looking for the factors promoting the development of haemodynamically significant atherosclerotic changes in only one of the carotid arteries compared to bilateral involvement. Classic risk factors of stroke were analyzed, as well as ultrasound characteristics of the atherosclerotic plaques.

The study showed that patients with unilateral advanced atherosclerotic changes in the carotid arteries had significantly high-density lipoprotein (HDL) cholesterol levels in the blood. Lower levels of HDL cholesterol were associated with the bilateral location of advanced atherosclerotic changes in carotid arteries. This effect may result from the protective properties of HDL in the prevention of cardiovascular diseases. The relative risk of stroke is reduced by 11–15% for each increase in HDL of 10 mg/dL [27]. A high level of HDL reduces the risk of cardiovascular diseases by means of reverse cholesterol transport from the macrophages of endothelial cells to the liver, as well

TABLE 3. Factors significantly different between groups (multivariate analysis)

Parameter	Group I	Group II	p
HDL (mg/dL)	mean 45.7 (min 22; max 91)	mean 38.9 (min 20; max 109)	0.038 (OR = 1.036, 95%CI: 1.002–1.072)
Presence of calcification in the atheromatous plaques	n = 11 (23%)	n = 3 (5%)	0.015 (OR = 5.9, 95%CI: 1.42–24.6)

HDL – high-density lipoprotein.

as by preventing oxidation of low-density lipoprotein (LDL), and anti-inflammatory and antithrombotic effects. Such indirect action through LDL may explain why a high level of HDL does not directly inhibit the development of atherosclerotic plaque [28, 29]. The lower level of HDL is an independent risk factor of stroke and increases the growth of IMT, so that is why there may be promotion of the more generalized development of atherosclerosis. Such a mechanism might be responsible for the presence of bilateral atherosclerotic changes in the analysed patients. Physiologically higher levels of HDL are found in women, and its protective effect fades in the age of menopause [30]. The higher levels of HDL in group I were not, however, connected with sex, because there were no differences within the sex distribution between the groups. The majority of the examined women were of postmenopausal age. Thus, the protective effect of HDL related to age was not found. The more frequently observed calcifications within the atherosclerotic plaques in patients with unilateral advanced changes in carotid arteries may reflect a connection between the asymmetrical distribution of plaques and their slower development, which would result in the formation of more stable plaques [28, 29].

There may be a pathogenetic association in the presented relationship between the asymmetrical location of atherosclerosis, blood HDL, and the presence of calcifications in the carotid arteries. Increase in LDL and a reduction in HDL levels have been associated with low bone mineral density in postmenopausal women. Some authors suggest that oxidized LDL promotes arterial calcification and its accumulation in the subendothelial space by activating osteoblasts in the arterial pool [31]. Thus, the calcification process may combine the influence of local and systemic cardiovascular risk factors. Calcifications are present in sites affected by chronic inflammation. Calcified atherosclerotic plaque may be more prone to rupture due to the change in the distribution of forces affecting the plaque surface and because of increased activity of the matrix-degrading enzymes (matrix metalloproteinases), but it is noteworthy that less evident calcification was also detected in unstable plaques [28, 32].

We did not find any relationship between the asymmetrical involvement of carotid arteries and other modifiable or non-modifiable risk factors of stroke. This seems to be reasonable because almost all of the tested factors, besides blood pressure, affect both carotid arteries to a similar extent. In the analysed groups, the values of arterial blood pressure, both systolic and diastolic, were similar. Other authors have presented similar results. No impact of diabetes mellitus, hypertension, hyperlipidemia or cigarette smoking on the asymmetrical development of atherosclerosis was found [10]. Among the classic risk factors of cardiovascular diseases, older age and the higher values of blood pressure are also associated with the bilateral location of atherosclerotic changes in internal carotid arteries in comparison to unilateral changes [1]. The study demonstrated the link between the classic risk factors of cardiovascular diseases and local factors which play a role in an intensification of atherosclerosis development. This may indicate the cumulative effect of both local and systemic factors. Systolic blood pressure and cigarette smoking are negatively correlated with WSS, while HDL and impaired fasting glucose are positively correlated with WSS. Decrease in WSS related to aging may increase sensitivity to damage associated with systemic cardiovascular risk factors. In patients with low cardiovascular risk IMT and atherosclerosis in carotid arteries are connected with lower WSS. In patients with high cardiovascular risk the influence of WSS is masked, making age the only factor promoting the bilateral development of atherosclerosis in carotid arteries [32]. The accumulation of risk factors was associated with further deterioration of mechanical forces [1].

We may presume that local mechanisms in the arterial wall, not yet determined, promote the asymmetrical development of atherosclerosis in the carotid arteries. Cardiovascular risk factors may only accelerate it. This may potentially have clinical implications. The HDL role in modulating the risk of ischaemic stroke is unclear, and according to the stroke prevention guidelines this lipoprotein level is not a goal for dyslipidemia treatment with only the LDL cholesterol used in the guidelines [33]. Our results suggest that increase in HDL may inhibit the formation of atherosclerotic plaques and limit their number – in our patients to unilateral lesions. Hence, HDL should be treated as an important protector against cerebrovascular events.

Limitations

Our study may be biased because the carotid ultrasound examination was performed by 2 ultrasonographists, and the sample size was relatively small.

CONCLUSIONS

The factors connected with only unilateral stenosis or occlusion of the carotid artery in stroke patients are high blood HDL level and the presence of calcifications in the plaques. The protective properties of HDL and the slow formation of stable calcified plaques may play a role in the development of unilateral atherosclerosis in carotid arteries. The role of HDL cholesterol in stroke pathomechanism needs further study.

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