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ASSESSMENT OF ENDOTHELIAL FUNCTION IN PATIENTS WITH INITIAL MANIFESTATIONS OF CHRONIC CEREBRAL ISCHEMIA

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ABSTRACT

Introduction: Over the last decade, the number of patients with cerebrovascular diseases in Ukraine has grown by 1.5 times. This negative trend is based on the significant increase of vascular risk factors - arterial hypertension, dyslipidemia, sedentary lifestyle, excessive weight/obesity, diabetes mellitus, tobacco smoking, etc. - prevalence among the population of the country, their early development and combination of several vascular risk factors, which makes prevention of cerebrovascular diseases at their early stages a priority.

The aim: Study of endothelial vasomotor function in patients with initial symptoms of chronic cerebral ischemia.

Materials and methods: The study included 260 patients of the main group (mean age 50.6±70.9) with initial manifestations of CCI and 30 persons of similar age of the control group. All the patients underwent a clinical neurological examination, MRI of the brain, ultrasonography with the establishment of flow-mediated endotelium-dependent dilatation (FMD) index based on the results of brachial artery compression test, as well as laboratory tests of endothelin-1 and nitrite levels. The patients were subdivided into 3 groups based on their MRI of the brain data: without structural changes of the brain (group 1), with initial vascular structural changes (group 2), with initial structural changes and mild cerebral atrophy (group 3).

Results: Reliable differences of FMD index were established in the groups with different degrees of structural brain damage, and in comparison with the control group ($p<0.001$). Considerable downward trend of FMD index with increase of the number of existing vascular risk factors (VRF) was identified ($p<0.05$). An associative link with the presence of arterial hypertension (AH), its degree and FMD index ($p<0.05$) was established. It was shown that a thickened intima-media complex (IMC) of common carotid arteries >0.9 mm had a significantly lower predetermination to FMD ($p=0,001$). Statistically significant lower FMD indices were registered in persons with endothelial vasomotor function disorder according to the biochemical markers: with endothelin level increase and nitrite level decrease ($p<0.001$).

Conclusions: An associative link with the presence of endothelial dysfunction according to FMD index were established with different degrees of structural brain damage, with the presence of AH, with IMC of common carotid arteries >0.9 mm, with endothelial vasomotor function disorder according to the biochemical markers: with endothelin level increase and nitrite level decrease.

KEY WORDS: initial manifestations of chronic cerebral ischemia, flow-mediated dilatation, endothelial dysfunction, endothelin-1, nitrite

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INTRODUCTION

Over the last decade, the number of patients with cerebrovascular diseases (CVD) in Ukraine has grown by 1.5 times [1]. This negative trend is based on the significant increase of vascular risk factors (VRF) - arterial hypertension (AH), dyslipidemia, sedentary lifestyle, excessive weight/obesity, diabetes mellitus, tobacco smoking, etc. - prevalence among the population of the country, their early development and combination of several VRF, which makes prevention of CVD at their early stages a priority [2]. The vascular system disorders of target organs are latent (take a long time to develop) and are clinically manifested by potentially fatal and disabling vascular events [3].

The permanent influence of VRF suppresses the protection mechanisms of the vascular endothelium, which threatens the functional and structural integrity of the vascular system. There is significant evidence of endothelial dysfunction (ED) involvement in initiation and progression of atherosclerosis and its complications. ED is known to be a systemic syndrome involving several vascular pools, including the cerebral one [4].

ED is considered to be primarily caused by the loss of balance between the vasodilatory and vasoconstrictive factors, and later manifests itself as pro-inflammatory, pro-oxidant, proliferative, pro-coagulative and pro-adhesive changes [4]. That is why, it is the endothelial vasomotor function disorder that can have the highest prognostic value as it can be diagnosed at the earliest stages of vascular disruption to a blood vessel.

It is known that the functional and structural integrity of endothelium makes it possible to assess the degree of VRF influence and the risk of a vascular disease development in the corresponding vascular pool. There are no established standards either of ED diagnosis, or its treatment. The most frequently used methods of endothelial function assessment are as follows: flow-mediated endothelium-de-

pendent dilatation (FMD), plethysmography, angiography and determination of the concentrations of factors which are synthetized in endothelium, e.g. endothelin 1, nitrite (as NO metabolite), circulating endothelial cells and their fragments, assessment of NOS (NO-synthase) and antigenic structure of endothelium, etc. [5]. Notwithstanding a large amount of recent clinical and experimental studies, features of clinical, neurovisual and hemodynamic initial manifestations of the chronic cerebral ischemia (CCI) not fully understood, as well as mechanisms for the disorder of vasomotor endothelial functions [6].

THE AIM

Study of vasomotor endothelial function in patients with initial symptoms of chronic cerebral ischemia (CCI).

MATERIALS AND METHODS

The study included 290 patients (70.7 % (205/290) women and 29.3 % (85/290) men) aged 39 to 65 years. All the patients underwent a clinical, neurological examination, MRI of the brain, ultrasonography, laboratory tests, which were carried out in the hospital N9 in Kyiv in 2012-2016.

The main group consisted of 206 patients (mean age 50.6±70.9) with initial manifestations of CCI. One or more VRF were found in all patients – AH, dyslipidemia, excessive weight/obesity (body mass index 25-29 kg/m²), diabetes mellitus, positive history of acute vascular events in first-degree relatives up to 55 years of age for men and 65 for women – these factors were defined as eligibility criteria in the main group.

The study did not include patients with history of stroke or myocardial infarction, AH of the 3rd degree or IIIrd stage, persons with severe somatic pathology, oncological pathology, with mental illness.

30 persons, mean age 50.4±6.6 without VRF and clinical signs of CCI made up the control group.

Based on the MRI data, our study included persons without structural changes of the brain, as well as patients with initial vascular structural changes. Separate foci (gaps), no more than 3-5, up to 5 mm in diameter, not affecting the basal ganglia; local leukoaraiosis around the lateral ventricles and mild cerebral atrophy were allowable.

Ultrasonography examination included ultrasound (US) duplex scanning of the main neck and brain vessels, was performed according to standard methods using the device "Sonoline G-50" (Siemens, Germany) [7].

In order to determine the functional condition of endothelium, all patients had a flow-mediated endothelium-dependent dilatation (FMD) index assessment based on the results of brachial artery compression test using the US examination. The state of the vasomotor function of the endothelium was evaluated with the help of FMD index – increase of the brachial artery diameter (as %) during the reactive hyperemia test (Celermajer D. S. (1992)). Brachial artery diameter was measured using a 7-10 MHz US sensor; then the blood flow in brachial artery was blocked for 5 minutes using a compression cuff. After quick decompression the change in diameter was recorded in response to an increase in blood flow. Artery reaction to ischemia due to cuff compression leads to normal NO-synthase (eNOS) activation and to release of nitric oxide (NO). An increase in brachial artery diameter is more than 10% while maintaining vasomotor function of the endothelium. Vascular reaction is considered pathological in cases when the increase in diameter is less than 10%, or in cases of paradoxical vasoconstriction [8].

The patients were subdivided into 3 groups based on their clinical and neuroimaging findings. Persons with VRF and clinical manifestations of CCI without structural changes of the brain entered the 1st group (n=134). Patients with initial vascular structural changes based on their MRI of the brain data were assigned to the 2nd group (n=94). Structural changes in the 2nd group were characterized by the presence of isolated foci, no more than 3, in the white matter of cerebral hemispheres up to 5 mm in diameter, delated perivascular spaces (criblures) up to 3 mm in diameter, local leukoaraiosis, lack of signs of cerebral atrophy. Patients with above-mentioned structural changes and mild cortical and/or internal cerebral atrophy formed the 3rd group (n=32) [9].

Endothelin-1 (ET-1) levels were studied in 126 patients of the clinical groups and in 20 persons of the control group by enzime immunoassay using Labline-100 enzime immunoassay analyzer (WestMedica, Austria), using Endothelin-1 ELISA (EIA-3420) reagents, DRG Instruments Inc., USA. The sensitivity of the method was 0,06 ng/ml.

Nitrite levels were measured in 201 patients of the clinical groups by photometric method, using Griess reagent (Sigma-Alorich); Sph-46 spectrophotometer (Russia) with green filter and 520 nm optical density was also used. The content of nitrite ions was established using the calibration graph. Reference values for nitrite levels in healthy individuals of the appropriate age and gender were based on blood serum samples analysis in 20 persons of the control group.

Statistical data processing was performed using the program SPSS 20.0. Nonparametric statistics methods were used. The Mann-Whitney U-test was used to compare quantitative indicators in two independent groups. The Kruskel-Wallis ANOVA was used to compare persons in more than two independent groups. The significance level of differences was $p<0.05$, or $p<0.017$ in pairwise comparison of three groups, $p<0.013$ when pairwise comparing four groups.

RESULTS

Clinical signs of initial manifestations of CCI in patients showed headache complains, dizziness, general fatigue, absent-mindedness, wabbliness when walking without focal neurological symptomatics.

Patients in clinical groups had lower FMD rates according to the results of the compression test of the brachial artery in comparison with the control group. Significant differences from the control group in terms of Kruskel-Wallis

Table 1. Brachial artery FMD indices in the examined cohorts

Groups	Brachial artery diameter		FMD index (%)
	Baseline	After compression	
Control (n=30)	3.5 (3.4–3.7)	4.2@* (4.0–4.5)	17.4 ^*&*@* (14.6–19.6)
Group 1 (n=134)	3.4** (3.1–4.0)	4.0 (3.4–4.4)	13.8** (10.0–17.6)
Group 2 (n=94)	3.7 (3.1–4.0)	4.0 *** (3.4–4.4)	9.7*** (7.3–12.1)
Group 3 (n=32)	3.3 (3.0–3.4)	3.6# (3.3–4.2)	7.3#* (5.8–9.4)

Notes: materiality level value $p<0.05$ ($_*$ – $p<0.013$) for Mann-Whitney U-test for the relevant indicator:
* - comparing patients of groups 1 та 2 ;
# - comparing patients of groups 1 та 3;
** - comparing patients of groups 2 та 3;
^ - comparing patients of group 1 and control group;
&- comparing patients of group 2 and control group;
@ - comparing patients of group 3 and control group.

Table 2. FMD indices in patients with a different numbers of VRF

VRF	Quantity (n)	FMD index (%)
0	30	17.4 (14.6–19.6)
1	91	12.0 (9.8–17.1)*
2	93	10.0 (7.3–12.9)*
3	58	9.7 (7.7–11.1)*
4	16	9.4 (7.4–10.0)*
5	2	7.4 (6.7–8.1)*

Notes: * — materiality level value for Mann-Whitney U-test p=0.01 when comparing indicators vertically with patients without VRF.

index ($p=0{,}01\cdot10^{-4}$): FMD index in control group was Me (Q_1-Q_3): 17.4% (14.6–19.6%), in patients of the 1st group – 13.8% (10.0–17.6%), remaining within the normal range, while in patients of the 2nd group – 9.7% (7.3–12.1%), in 3rd group – 7.3% (5.8–9.4%). So, 17 (53.1 %) patients of the 3rd group had reduced FMD indices.

Analysis of FMD indices allowed us to distinguish a group of patients with diffuse ED. It was found that the presence of ED according to the compression test results was reliably associated with degree of structural damage to the brain in initial CCI, namely, $X^2_{(2)} = 81.66$; Cramer's V = 0.517 ($p<0.001$). The FMD indices in the groups were distributed according to the degree of structural changes to the brain are presented in the table 1.

At the same time a statistically significant association was discovered between signs of ED and the presence of small foci of vascular character in the white matter of cerebral hemispheres ($X^2=47.0$; Cramer's V = 0.393, $p<0.001$), with relative risk of small vascular foci being 2.7 times higher (CI 2.0–3.7) in persons with diffuse ED. A weak association was established between the presence of leukoaraiosis and diffuse ED ($X^2=26.8$; Cramer's V = 0.296, $p<0.001$). The relative risk of the occurrence of leukoaraiosis alongside ED according to the compression test results was 2.2 times higher (CI 1.7–2.8).

The presence of mild cerebral atrophy was significantly associated with diffuse ED ($X^2=44.3$; Cramer's V=0.381, $p<0.001$), and relative risk of the development of cerebral atrophy due to ED was 2.6 times higher (CI 2.0–3.3). The presence of changes in vascular origin on MRI in patients with initial signs of CCI was also significantly associated with diffuse ED with strong density bond ($X^2=59.7$; Cramer's V=0.442, $p<0.001$). The risk of MRI changes due to ED according to the compression test results was 3.4 times higher (CI 2.4–4.8).

Comparison of brachial artery compression test results with the number of VRF in patients established moderate correlation between the number of VRF and the FMD indices ($r=-0.373$, $p<0.001$). The patients with a different number of VRF were statistically significantly different in terms of FMD (by Kruskel-Wallis test $X^2_{(6)}=46.4$; $p=0.03\cdot10^{-3}$). Data on the distribution of the FMD index depending on the number of VRF represented in table 2.

One of the most common VRF in patients with initial signs of CCI was AH.

The data analysis revealed a low density association between the presence of AH and signs of ED according to the compression test results ($X^2_{(1)} = 13.7$; Cramer's V = 0.212, $p<0.001$). Statistical processing of data on FMD index dis-

Table 3. FMD indices depending on changes in biochemical markers

Vasomotor function of endothelium	Unchanged		With changes		p
	Me	Q_1–Q_3	Me	Q_1–Q_3	
ET-1 level	Within normal limits (n=84)		Increased (n=42)		<0.001
FMD index (%)	14.3	10.0–19.6	10.0	9.1–14.3	
Nitrite level	Within normal limits (n=118)		Outside the norm (n=83)		<0.001
FMD index (%)	16.1	10.0–20.0	10.0	8.6–12.5	

tribution in patients without AH (57 / 21.9%) and with AH (171 / 65.8%) showed a statistically significant difference between cohorts of patients in ability to FMD, namely Me (Q_1-Q^3): 13.3% (10.0 – 17.9%) та 10.0% (7.5 – 12.5%), p<0.05 for the Mann-Whitney U-test. Office systolic blood pressure was moderately inversely correlated with FMD indicator(r= -0.306).

Besides, it was discovered that patients with 2nd degree AH (66 / 38.5%) had a statistically significant lower FMD rates – Me (Q_1-Q_3): 9.7% (6.7 – 12.0%) in comparison with 1st degree hypertension patients (105 / 61.4%) – 10.0% (8.6 – 13.3%), p<0.05 for the Mann-Whitney U-test [8].

A significant marker of subclinical arterial damage is the thickening of the intima-media complex (IMC) in common carotid artery > 0.9 mm, its disorganization. It was established that in persons with IMC ≤0,9 mm (162 / 62.3%) the FMD index median (Me) was 11.8 % (9.4–17.2 %). In patients with IMC >0.9 mm (98 / 37.7%) FMD index was lower – 9.8 % (7.3–11.9 %). Wherein the patients in groups were statistically significant different for this FMD index for the Mann-Whitney U-test ($p=0.004 \cdot 10^{-2}$).

It was found that the thickening of the IMC moderately associated with diffuse ED according to the compression test results (X^2=10.1; Cramer'V = 0.239, p=0.001), wherein the relative risk of diffuse ED in patients with thickened IMC was 1.8 times higher (CI 1.3–2.6).

Assessment of biochemical parameters of vasomotor function of endothelium showed statistically significant differences between the groups of patients, which confirms the participation of this factors in the pathogenesis of the initial manifestations of CCI [10]. So, signs of vasomotor endothelial dysfunction revealed by biochemical markers were found in 91 patients. In 83 / 201 / 41.3% patients endothelial dysfunction was due to a deviation in the nitrite level. The levels of this index (Me (Q_1-Q_3), micromole/l) significantly differed in clinical and control groups: 5.24 (4.79 – 6.77) in the 1st group, 3.57 (2.41–4.16) in the 2nd, 2.28 (1.82–4.16) in the 3rd and 4.61 (4.18–5.02) in the control group, materiality level value p<0.0125 for Mann-Whitney U-test. An increase in endothelin-1 (ET-1) levels occurred in 42 / 126 / 33.3% patients in the 2nd and 3rd groups, with a significant difference in indices relative to control (Me (Q_1-Q_3), ng/ml): 0.478 (0.311 – 1.38) in the 2nd group, 2.02 (0.566 – 2.46) in the 3rd group and 0.211 (0.176 – 0.258) in control group, materiality level value p<0.0125 for Mann-Whitney U-test. There were no significant differences in ET-1 levels in the 1st group (0.222 (0.163– 0.395) ng/ml) in comparison with the control group.

Relationship between oppositely directed vasomotor factors of endothelium (ET-1 and nitrite) showed significant inversely correlated density (r=-0.865, $p=0.03 \cdot 10^{-3}$), and the connection significantly decreased with the onset of structural brain damage in the presence of VRF (r= -0.368, $p=0.012 \cdot 10^{-2}$). This indicated the importance of an imbalance between vasomotor endothelial factors with progression of CCI.

The level of biochemical markers of vasomotor endothelial function had a statistically significant association of moderate strength with FMD indices: ET-1 level inversely correlated with FMD index (r= -0.345, $p=0.02 \cdot 10^{-4}$); nitrite level directly correlated with FMD index on Spearman coefficient (r= 0.319, $p=0.008 \cdot 10^{-2}$).

The presence of ED according to the compression test results was poorly associated with increased ET-1 levels (X^2=6.2; Cramer's V = 0.222, p=0.013), the relative risk of the development of diffuse ED amid rising ET-1 level was 1.5 times higher (DI 1.1–2.0). The presence of diffuse ED was poorly associated with deviation of the nitrite level (X^2=6.5; Cramer's V = 0.18, p=0.011) and the relative risk of the coexistence of these changes increased 1.2 times (DI 1.1–1.5).

Given the possibility of deviations of the nitrite level both above and below the normative values, a relationship was found between nitrite level deviation and ED according to the compression test results, a moderate strength relationship was identified ($X^2_{(2)}$=20.3; Cramer's V = 0.319, p<0.001).

Taking into consideration the poors relationship between FMD indices and biochemical markers of vasomotor endothelial function, analysis of the FMD index depending on the presence or absence of vasomotor dysfunction was performed (table 3).

The data presented in the table 3 indicated that FMD indices according to the brachial artery compression test results were statistically significantly lower in persons with ET-1 and nitrite levels deviations.

DISCUSSION

Overall, patients in clinical groups had lower FMD indices according to the compression test for reactive hyperemia

in the brachial artery in comparison with the control group [11].

It was found out that the presence of common ED according to the compression test results was significantly associated with the degree of structural damage to the brain in initial manifestations of CCI ($X^2_{(2)}$ = 81.66, p<0,001). The relative risk of MRI changes due to diffuse ED was 3.4 times higher (CI 2.4–4.8) [12].

Persons with thickened IMC >0.9 mm had signs of diffuse ED according to the compression test results. It was found out that the thickening of the IMC was moderately associated with common ED (X^2=10.1; Cramer's V = 0.239, p=0.001) [11, 13].

A study of biochemical markers of of vasomotor function of endothelium has established that nitrite level in patients of the 1st group was significantly higher in comparison to persons in control group and groups 2 and 3. While ET-1 levels progressively increased in clinical groups, the control group and group 1 without structural brain damage were an exception for the ET-1 index, which confirms the value of this marker for the formation of clinically significant structural brain damage. At the same time, patients with structural brain damage (groups 2 and 3) did not differ in nitrite levels; NO concentration was important at the early stages of CCI.

The correlation between ET-1 and nitrite was inverse and had a significant density (r=-0.865, $p=0.03\cdot10^{-3}$), the connection density significantly decreased as brain damage appeared alongside vascular risk factors (r=-0,368, $p=0{,}012\cdot10^{-2}$). This indicates the significance of imbalance between vasomotor factors in endothelium with CCI progression. In other words, as ET-1 levels increased and nitrite levels decreased, a downward trend in FMD index was observed [10].

The dynamism of the synthesis of biologically active substances in the endothelium in healthy persons and in pathological conditions was observed earlier, however, it requires further study [14, 15].

Further research may be directed at finding other factors affecting the endothelial function in patients with initial manifestations of CCI and determination of their prognostic significance for clinical progression of CCI.

CONCLUSIONS

1. Study results confirm that diffuse ED has a part in the development of the initial manifestations of CCI.
2. According to the brachial artery compression test, the presence of diffuse ED is significantly associated with the degree of structural damage to the brain.
3. It has been found out that combinations of VRF contribute to the occurrence of diffuse ED thus promoting a greater probability of vascular events. The tendency to the decrease of FMD indices due to the increased number of simultaneously existing VRF was determined.
4. FMD indices were significantly lower in patients with grade 2 AH in comparison to those with AH 1 and without hypertension (p<0.05).
5. The presence of diffuse ED was associated with structural changes in the carotid arteries.
6. FMD indices were significantly lower in persons with impaired vascular function of the endothelium according to biochemical markers when the levels of ET-1 or nitrite were deviant from a norm, drawing close to ED borderline.

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The Authors declare no conflict of interest.

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