

Nasalyk Roksolana B., Shkrobot Svitlana I., Sokhor Natalya R., Milevska-Vovchuk Lyubov S., Duve Khrystyna V., Nasalyk Bohdan H., Zukow Walery, Popovych Igor L. Characteristics of cerebral hemodynamics in patients with chronic brain ischemia. *Journal of Education, Health and Sport.* 2020;10(11):11-20. eISSN 2391-8306. DOI <http://dx.doi.org/10.12775/JEHS.2020.10.11.001> <https://apcz.umk.pl/czasopisma/index.php/JEHS/article/view/JEHS.2020.10.11.001> <https://zenodo.org/record/4247258>

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8. 2) and § 12. 1. 2) 22.02.2019.

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The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 23.10.2020. Revised: 29.10.2020. Accepted: 05.11.2020.

## CHARACTERISTICS OF CEREBRAL HEMODYNAMICS IN PATIENTS WITH CHRONIC BRAIN ISCHEMIA

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### Summary

**Background.** Literature sources report conflicting results regarding the severity of clinical symptoms of hydrocephalus (HC) and their association with cerebral arterial blood flow (CABF); most studies do not show a direct relationship, while some suggest a link between clinical severity and progressive decrease in CABF. The study of hemodynamic changes in the brain of patients with chronic brain ischemia (CBI), elucidation of their relationship with cognitive impairments helps to improve diagnostic approaches and optimize the prognosis of the disease. **The purpose** of this study is to investigate the state of cerebral hemodynamics and to establish its relationship with changes in cognitive functions in patients with CBI and HC. **Materials and Methods.** A comprehensive examination of 110 patients with CBI and HC was performed. The localization of the HC and results of Montreal Cognitive Test (MoCA scale) were taken into account. Computed tomography of the brain was performed with subsequent determination of morphometric parameters and indices. The state of cerebral blood flow and structural changes of blood vessels were studied using transcranial color-coded duplex ultrasonography (TCCS) of intracranial vessels and extracranial divisions. Microsoft Excel 2011 and Statistica were used for statistical processing of the results. **Results.** It was found that there was a significantly larger diameter of both common carotid arteries (CCA), thickness of complex intima-media (CIM), maximum systolic velocity (Vs), velocity at the end of the diastolic cycle (Vd), as well as peripheral resistance (IR) in left CCA ( $p < 0,05$ ) in patients with CBI and HC compared with patients with CBI without HC. Significantly larger diameter of left internal carotid artery (ICA), Vs, Vd and IR was revealed; Vs and Vd in the right ICA; Vd in the extracranial division of vertebral artery (VA) in patients with HC. A significant relationship was found between the following blood flow

parameters and the values of the MoCA scale: Vs MCA/MoCA ( $r = 0,45$ ,  $p < 0,05$ ), Vs ACA/MoCA ( $r = 0,38$ ,  $p < 0,05$ ), ICA diameter/MoCA ( $r = -0,51$ ,  $p < 0,05$ ). It was found that the diameter of CCA and the thickness of CIM were significantly larger in patients with CBI with internal HC in comparison with patients with CBI with external HC ( $p < 0,01$ ). Vs and Vd in patients with internal hydrocephalus were significantly lower, and IR was significantly higher compared with patients with external hydrocephalus ( $p < 0,01$ ). The diameter of ICA and IR in patients with CBI with internal HC was significantly higher, and Vs was significantly lower compared with patients with CBI and external HC ( $p < 0,01$ ). Significantly different values were found in Vs and IR meanings in middle cerebral artery (MCA) and Vs in anterior cerebral artery (ACA) in patients with CBI with internal HC compared with patients with CBI with external HC.

**Conclusions.** Functional parameters of blood flow and structural changes of cerebral arteries in patients with CBI with concomitant hydrocephalus differed significantly from those in patients without hydrocephalus and depended on the type and severity of hydrocephalus. ICA occlusion, ICA stenosis  $>50\%$ , and intracranial venous stasis were significantly more common in patients with severe and moderate HC compared with those with mild HC. A weak relationship was found between the structure of carotid arteries and blood flow in intracranial arteries in patients with CBI and HC. There was a significant relationship between blood flow indices and the values of the MoCA scale, which indicated the effect of changes in the vessels of the anterior circular basin on the cognitive functions and the lack of such connection with blood flow indices in vertebrobasilar basin.

**Key words:** chronic brain ischemia; hydrocephalus; computed tomography; cerebral atrophy; transcranial color-coded duplex ultrasonography

## INTRODUCTION

Cerebrovascular diseases (CVD), among which there are acute and chronic (chronic brain ischemia (CBI)) forms, are one of the most important medical and social problems both in Ukraine and all over the world due to their high prevalence and severe consequences - loss of efficiency, disability, disorders of higher nervous activity [1, 2, 3].

One of the least studied manifestations of CBI is hydrocephalus (HC), although the combination of hydrocephalus and CBI is a fairly common phenomenon [4, 5]. On the one hand, such combination is possible with a combination of CBI and normal pressure hydrocephalus (NPHC), on the other - this is due to the morphological stages of CBI progression [6]. In practice, arterial hypertension and atherosclerosis have the greatest etiological significance in the development of CBI [7, 8, 9, 10, 11]. Patients with carotid artery disease can have cognitive impairments for a variety of reasons. Clinically obvious strokes, as well as silent brain infarcts as a result of carotid artery stenosis lead to memory impairment [12]. Among the patients with "asymptomatic" carotid stenosis, these data are of the greatest importance, because there are no established factors of cognitive impairment associated with stroke [13, 14, 15, 16, 17]. Isolated studies highlight the problem of how increased arterial diameters are related to the state of brain parenchyma and cognitive functioning. Under the physiological circumstances, the diameter of the arteries in the circle of Willis is directly proportional to the area of blood supply and the density of capillaries on it

[17]. Hereditary or acquired conditions that lead to increased blood flow or weakness of the arterial wall can lead to external remodeling [18].

Literature sources report conflicting results regarding the severity of clinical symptoms of HC and their association with arterial cerebral blood flow (ACBF); most studies do not show a direct relationship [19], while some indicate a link between clinical severity and progressive decrease in ACBF [20].

**The aim** of this study was to analyze the state of cerebral hemodynamics and to establish its relationship with cognitive changes in patients with CBI and HC.

## **MATERIAL AND METHODS**

We examined 140 patients (67 (47,86%) women and 73 (52,14%) men) with CBI, aged 44 to 82 years (average age ( $65,78 \pm 9,11$  years)), who underwent inpatient treatment in Ternopil Regional Municipal Clinical Psychoneurological Hospital. All patients had hypertension, which was combined with cerebral atherosclerosis in 77 (55,00%), coronary heart disease - in 28 (20,00%), angina pectoris - in 5 (3,57%), diabetes mellitus - in 10 (7,14%) people. Acute cerebrovascular accident in the anamnesis was observed in 34 (24,29%) patients. CBI of the I<sup>st</sup> stage (according to the common diagnoses in Ukrainian neurological practice) was diagnosed in 30 (21,43%), CBI of the II<sup>nd</sup> - in 90 (64,29%), CBI of the III<sup>rd</sup> - in 20 (14,29%) patients. All patients were divided into two groups. The first group consisted of 110 (78,57%) patients with CBI, who were diagnosed with the signs of internal HC - 37 (33,64%) – group 1A, external HC - in 26 (18,57%) – group 1B or mixed HC - in 47 (47,73%) patients – group 1C. Mild HC was found in 26 (23,64%), moderate - in 57 (51,82%), severe - in 27 (24,55%) patients. The average score according to the MoCA scale was  $15,65 \pm 4,11$  points. The second group (comparison group) included 30 (21,43%) patients with CBI without manifestations of HC. The average score according to the MoCA scale was  $20,64 \pm 2,59$  points. The state of cognitive functions was assessed according to the Montreal scale of cognitive functions (MoCA test). Neuroimaging was performed using spiral computed tomography (CT) scanning on an Asteion 4 (Toshiba) device. The thickness of the cross-sectional images was about 2-5 mm. In evaluating the results of the study, attention was paid to the following points: the state of the ventricular system, subarachnoid and perivascular spaces; the presence of cystic formations; differentiation of white and gray matter. The state of cerebral blood flow was studied using transcranial color-coded duplex ultrasonography (TCCS) of intracranial vessels and extracranial divisions of brachiocephalic vessels on the Philips HDI device: in common carotid arteries (CCA), external carotid arteries (ECA), internal carotid arteries (ICA), vertebral arteries (VA) - in segments V1 and V2, in the anterior cerebral arteries (ACA), middle cerebral arteries (MCA), posterior cerebral arteries (PCA), basilar artery (BA) and vertebral artery (VA).

The following hemodynamic parameters were determined: vascular internal diameter, linear blood flow velocity (LBFV) (maximum systolic velocity ( $V_s$ , cm / s), velocity at the end of the diastolic cycle ( $V_d$ , cm / s)), peripheral resistance (IR), and structural changes.

Statistical analysis was performed on a personal computer using Statistica v. 6.1.

## RESULTS

Functional parameters of extra- and intracranial arterial blood flow are presented in table 1.

**Table 1. Functional parameters of extra- and intracranial arterial blood flow in patients with CBI according to TCCS, (M ± m)**

Index	Group of patients			
	1 <sup>st</sup> group, n=110		2 <sup>nd</sup> group, n=30	
	right	left	right	left
ECA				
diameter	7,86±0,09*	7,40±0,07*	6,52±0,19	6,32±0,15
CIM	1,15±0,04*	1,09±0,02*	0,88±0,07	0,80±0,03
Vs	63,28±10,07	65,18±14,03	68,65±2,98	70,95±3,42
Vd	25,00±0,23*	23,00±0,18**	28,00±0,31	25,00±0,26
IP	0,79±0,19	0,83±0,23*	0,79±0,31	0,69±0,22
ICA (right)				
diameter	5,25±0,09	4,83±0,06**	5,31±0,22	4,13±0,14
Vs	58,29±3,1*	58,71±4,9**	78,35±6,11	70,49±4,69
Vd	14,86±1,89*	14,04±1,69**	26,00±1,69	22,4±1,56
IP	0,82±0,15	0,74±0,12*	0,78±0,23	0,52±0,19
VA (extracranial)				
diameter	3,9±0,23	3,7±0,15	3,8±0,22	3,4±0,19
Vs	41,15±2,36	35,18±1,95	46,17±1,20	40,22±2,05
Vd	12,43±0,56*	16,51±0,29	22,15±0,62	16,29±0,49
IP	0,91±0,13	0,73±0,17	0,82±0,21	0,76±0,19
ACA				
Vs	48,26±4,3*	54,94±9,50	67,85±13,26	61,75±9,14
Vd	37,15±2,15	41,29±1,69	39,36±1,22	43,55±1,32
IP	0,54±0,05	0,55±0,03	0,54±0,06	0,52±0,07
MCA				
Vs	76,58±5,62	70,62±4,98	81,00±5,33	77,00±4,37
Vd	33,12±0,98*	29,65±0,32*	43,28±0,29	41,62±0,35
IP	0,55±0,21*	0,53±0,17*	0,50±0,22	0,48±0,14
PCA				
Vs	51,15±8,75	41,85±7,25	58,69±8,41	50,51±7,59
Vd	23,15±1,43	21,97±1,11	25,11±2,65	22,19±1,65
IP	0,60±0,08	0,56±0,06	0,57±0,03	0,59±0,07
BA				
Vs	51,12±1,15		52,60±1,76	
Vd	23,28±0,87		24,22±0,78	
IP	0,55±0,02		0,56±0,06	
VA (intracranial)				
Vs	50,15±3,42	56,24±3,84	53,30±3,64	57,28±3,48
Vd	21,22±1,44	27,15±1,42	25,29±1,54	27,22±1,58
IP	0,58±0,04	0,54±0,08	0,57±0,03	0,55±0,07
angiospasm	95 (86,36 %)		30 (100,00%)	

Notes: 1. \* - the index is reliable compared with the similar index in the second group (p<0,01);  
2. \*\* - the index is reliable compared with the similar index in the second group (p<0,05).

It was found that the patients of the first group had a significantly larger diameter of both ECA, the thickness of Complex Intima/media (CIM), Vd, Vs, as well as the IR of the left ECA (p <0,05) compared with the patients of the second group. We have also detected

significantly larger diameter of the left ICA, Vs, Vd and IP; Vs and Vd in the right ICA; Vd in the extracranial division of VA in patients with HC. As for the other indicators, no significant difference was found. Significantly different were the values of Vd and IR in both MCA and Vs in right ACA ( $p < 0,05$ ). No significant difference was found in both PCA and in BA.

Analyzing the obtained results, we found that structural changes of vessels significantly prevailed in the patients of the first group: hemodynamically significant ( $> 50\%$ ) stenosis of ICA (in 41,82% of patients), compared with 6,67% of persons in the second group. ICA stenosis  $< 50\%$  was more common in the second group (63,33%) than in the first group (43,63%), but the difference between the groups was not significant ( $p > 0,05$ ). ICA occlusion was not common in patients of both groups (3,65% and 3,33% in the first and second group, respectively). Angiospasm of cerebral vessels occurred in almost all subjects and was recorded in 86,36% of patients in the first group and in 100,00% of patients in the second group. It is natural, in our opinion, that the phenomena of venous congestion were significantly more often recorded in patients of the first group than in the patients of the second group in 80,91% and 36,67% of patients, respectively. This indicates the role of impaired venous circulation in the development of hydrocephalus at CBI.

A significant relationship was found between the following blood flow parameters and the values of the MoCA scale: Vs MCA/MoCA ( $r = 0,45$ ,  $p < 0,05$ ), Vs ACA/MoCA ( $r = 0,38$ ,  $p < 0,05$ ), ICA diameter/MoCA ( $r = -0,51$ ,  $p < 0,05$ ). Reduced or significantly increased diameter of cerebral arteries is usually associated with the state of the brain parenchyma and its function and can have a direct impact on cognitive function. We found a relationship between the structure of the carotid arteries and blood flow through the intracranial arteries in patients of the first group: the larger diameter of ECA, the greater vascular resistance in the intracranial vessels and the lower velocity of blood flow through them. The relationship between the diameter of ECA and IR ICA ( $r = 0,30$ ,  $p < 0,05$ ) and between the diameter of ECA and IR MCA ( $r = 0,31$ ,  $p < 0,05$ ), between the diameter of ECA and Vs MCA ( $r = -0,34$ ,  $p < 0,05$ ).

There were correlations between changes in cerebral vessels and morphometric parameters of the brain: CIM/BFI (bifrontal index) ( $r = 0,54$ ,  $p < 0,05$ ), CIM/right LV ( $r = 0,62$ ,  $p < 0,05$ ), IR MCA/BCI (bicaudal index) ( $r = 0,58$ ,  $p < 0,05$ ).

The obtained data coincide with the results of the other researchers who indicated a nonlinear relationship between the mean diameter of left MCA, ICA and semantic memory, as well as between the diameter of left MCA and episodic memory, in patients without stroke. These results show partial confirmation of the comprehensive hypothesis that the arterial diameter of the brain can be an important biomarker of cognitive function of parenchyma [18]. In order to reveal the influence of the form of HC on the state of cerebral hemodynamics, we investigated the functional parameters of extracranial and intracranial arterial blood flow depending on the location of HC (Table 2).

**Table 2. Functional parameters of extra- and intracranial arterial blood flow in patients with CBI according to TCCS depending on the form of HC, (M±m)**

Index	Group of patients		
	1A group, n=37	1B group, n=26	1C group, n=47
<b>ECA</b>			
diameter	8,17±0,07 pA-B<0,05	6,7±0,07 pB-C<0,01	8,02±0,10
CIM	1,14±0,03 pA-B<0,01	0,97±10,02 pB-C<0,01	1,25±0,04
Vs	60,95±3,69 pA-B<0,01	68,48±3,48 pB-C<0,01	63,26±14,33
Vd	22,45±1,00 pA-B<0,01	28,16±2,07 pB-C<0,05	21,39±2,01
IP	0,83±0,18 pA-B<0,01	0,75±0,17 pB-C<0,05	0,85±0,28
<b>ICA right</b>			
diameter	5,29±0,07 pA-B<0,01 pA-C<0,05	4,78±0,08 pB-C<0,01	5,05±0,09
Vs	54,98±14,23 pA-B<0,05	69,93±11,12 pB-C<0,01	50,59±12,15
Vd	14,23±1,84	16,54±1,71	12,68±1,82
IP	0,82±0,16 pA-B<0,05	0,68±0,12	0,84±0,14
<b>VA (extracranial)</b>			
Diameter	3,82±0,21	3,78±0,08	3,8±0,28
Vs	37,65±2,24	41,57±1,98	35,29±2,26
Vd	13,98±0,56	14,98±0,29	14,45±0,44
IP	0,82±0,09	0,80±0,07	0,84±0,29
<b>ACA</b>			
Vs	45,78±9,86 pA-B<0,01 pA-C<0,05	58,49±8,69 pB-C<0,05	50,53±14,15
Vd	38,39±1,68	40,15±1,89	39,12±2,19
IP	0,58±0,06	0,53±0,02	0,54±0,04
<b>MCA</b>			
Vs	68,23±4,47 pA-B<0,01 pA-C<0,05	77,42±6,45	75,15±4,98
Vd	26,98±0,58	34,54±0,59	32,65±0,78
IP	0,59±0,28 pA-B<0,01	0,49±0,13 pB-C<0,05	0,54±0,16
<b>PCA</b>			
Vs	41,59±7,36 pA-B<0,05	51,93±9,15 pB-C<0,05	45,98±7,49
Vd	21,38±0,97	23,71±1,35	22,59±1,49
IP	0,60±0,09	0,55±0,05	0,57±0,07
<b>BA</b>			
Vs	48,25±0,81	52,69±1,25	52,42±1,39
Vd	21,58±0,91	24,68±0,89	23,58±0,91
IP	0,58±0,06	0,53±0,04	0,54±0,02
<b>VA (intracranial)</b>			
Vs	52,16±3,58	55,14±3,82	52,39±3,49
Vd	22,68±0,81	26,30±1,69	23,59±1,78
IP	0,58±0,05	0,54±0,07	0,56±0,06

Notes. p<0,01, p<0,05 – significant difference between groups

It was found that the diameter of ECA and the thickness of CIM were significantly larger in patients of the 1A group compared with the patients of 1B group ( $p < 0,01$ ). Vs and Vd in patients with internal hydrocephalus were significantly lower, and IR was significantly higher compared with the patients with external hydrocephalus ( $p < 0,01$ ). The diameter of ICA and IR in patients of group 1A were significantly higher, and Vs was significantly lower in comparison with patients of group 1B ( $p < 0,01$ ). Significantly different values of Vs and IR in MCA and Vs in ACA in patients of group 1A in relation to patients of group 1B were revealed. There was no significant difference between the functional parameters of blood flow in the vertebrobasilar basin (in intra- and extracranial divisions of VA, PCA and BA). It was found that patients with internal and mixed hydrocephalus significantly more often had hemodynamically significant stenosis and occlusion of ICA, as well as intracranial venous stasis compared with patients of group 1B ( $p < 0,05$ ).

Thus, the analysis of functional parameters of extracranial and intracranial arterial blood flow showed significantly worse changes in patients with internal hydrocephalus compared to patients with external hydrocephalus. This difference was applied only to the vessels of the carotid basin.

A significant relationship was found between the following blood flow parameters and the values of the MoCA scale: Vs MCA/MoCA ( $r = 0,45$ ,  $p < 0,05$ ), Vs ACA/MoCA ( $r = 0,38$ ,  $p < 0,05$ ), ICA diameter/MoCA ( $r = -0,51$ ,  $p < 0,05$ ). Our data coincide with the results of other studies that show that diseases of the cerebral arteries, which are associated with either a very small or very large diameter of the lumen of cerebral arteries, will reduce cognitive processes [18]. For example, Alzheimer's disease is often associated with atherosclerosis. It has also been shown that aging of cerebral arteries, such as dilatation of arteries and loss of elastin, is also associated with Alzheimer's disease, regardless of age, brain infarction and atherosclerosis of large arteries, and the diameter of cerebral arteries is nonlinearly correlated with MMSE results [17,18].

The results of studied state of cerebral blood flow depending on the progression of HC are presented in table 3.

**Table 3. Functional parameters of extra- and intracranial arterial blood flow in patients with CBI according to TCCS depending on the severity of HC, (M±m)**

Index	HC stage of severity		
	Mild, n=26	Moderate, n=57	Severe, n=27
<b>ECA</b>			
diameter	6,68±0,06 pA-B<0,05 pB-C<0,05	7,99±0,08	8,22±0,10
CIM	1,01±0,02 pA-C<0,05	1,09±0,03 pB-C<0,05	1,26±0,04
Vs	68,85±12,59 pA-C<0,01	64,69±11,48 pB-C<0,05	59,15±13,43
Vd	27,32±0,00	23,28±0,00	22,40±0,01
IP	0,74±0,18 pA-C<0,05	0,83±0,19	0,86±0,26
<b>ICA right</b>			
diameter	4,86±0,08 pA-B<0,05 pA-C<0,01	5,01±0,07	5,25±0,09
Vs	66,02±12,22 pA-B<0,05 pA-C<0,05	59,33±12,12	50,15±13,16
Vd	15,56±1,82 pA-C<0,05	15,54±1,71 pB-C<0,05	12,25±1,84
IP	0,71±0,16 pA-C<0,01	0,77±0,12 pB-C<0,05	0,86±0,14
<b>VA (extracranial)</b>			
diameter	3,78±0,21	3,84±0,10	3,78±0,26
Vs	38,77±2,22	39,55±1,98	36,19±2,28
Vd	13,80±0,56	16,39±0,30	13,22±0,43
IP	0,81±0,09	0,80±0,07	0,85±0,29
<b>ACA</b>			
Vs	61,37±9,88 pA-C<0,01	49,21±8,66	44,22±14,16
Vd	40,55±1,68	39,54±1,88	37,57±2,20
IP	0,51±0,06 pA-C<0,01	0,55±0,02	0,59±0,04
<b>MCA</b>			
Vs	79,18±4,27	72,50±6,65	69,12±4,98
Vd	38,30±0,58 pA-C<0,05	29,65±0,60	26,22±0,77
IP	0,47±0,28 pA-B<0,01 pA-C<0,01	0,55±0,15	0,60±0,14
<b>PCA</b>			
Vs	41,59±7,36 pA-B<0,01	51,93±9,16	45,98±7,48
Vd	24,19±0,97	23,13±1,35	20,36±1,49
IP	0,55±0,08 pA-C<0,01	0,58±0,05	0,61±0,08
<b>BA</b>			
Vs	48,25±0,71	52,69±1,35	52,42±1,39
Vd	21,58±0,90	24,68±0,90	23,58±0,91
IP	0,58±0,06	0,53±0,04	0,54±0,02
<b>VA (intracranial)</b>			
Vs	55,18±3,58	54,39±3,82	50,12±3,49
Vd	24,96±0,81	24,98±1,69	22,63±1,78
IP	0,55±0,05	0,56±0,07	0,57±0,06



Functional parameters of blood flow were significantly different in patients with mild HC and in patients with severe HC. In patients with mild HC we found a significantly smaller diameter of the ECA, the thickness of CIM and IR of ECA and significantly larger Vs. ICA parameters in patients with mild hydrocephalus were significantly different from those in patients with severe and moderate degree of hydrocephalus (diameter, Vs). Blood flow rates in VA and BA did not differ significantly in patients with different degrees of hydrocephalus severity. In ACA and PCA, a significant difference was found in Vs and IR indices in patients with mild and severe hydrocephalus ( $p < 0,01$ ), in MCA - a significant difference was found in Vd and IR indices.

Decreased blood flow through the cerebral arteries with increasing degree of hydrocephalus can be associated with increased intracranial pressure, vascular compression as a result of increased ventricles and metabolic disorders. The force acting on the ventricles is transmitted centrifugally, compressing the brain and increasing the difference between ventricular pressure and convex pressure. The result is a global decrease in cerebral perfusion due to the fact that most of the arterial cerebral blood flow occurs centripetal, that means from the subarachnoid space to the center of the brain.

It was found that stenosis of ICA  $> 50\%$ , occlusion of ICA and intracranial venous stasis was significantly more often in patients with severe and moderate HC compared with the patients with mild HC ( $p < 0,05$ ).

## CONCLUSIONS

1. Functional parameters of blood flow and structural changes of cerebral arteries in patients with CBI with concomitant hydrocephalus differed significantly from those in patients without hydrocephalus and depended on the type and severity of hydrocephalus. ICA occlusion, ICA stenosis  $> 50\%$ , and intracranial venous stasis were significantly more common in patients with severe and moderate HC compared with those with mild HC.
2. A weak relationship was found between the structure of carotid arteries and blood flow in intracranial arteries in patients with CBI and HC: between the diameter of ECA and IR ICA ( $r = 0,30$ ,  $p < 0,05$ ), and IR MCA ( $r = 0,31$ ,  $p < 0,05$ ), and Vs MCA ( $r = -0,34$ ,  $p < 0,05$ ).
3. There was a significant relationship between blood flow indices and the values of the MoCA scale: Vs MCA/MoCA ( $r = 0,45$ ,  $p < 0,05$ ), Vs ACA/MoCA ( $r = 0,38$ ,  $p < 0,05$ ), diameter ICA/MoCA ( $r = -0,51$ ,  $p < 0,05$ ), which indicated the effect of changes in the vessels of the anterior circular basin on the cognitive functions and the lack of such connection with blood flow indices in vertebrobasilar basin.

## ACCORDANCE TO ETHICS STANDARDS

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

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