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ACCURACY OF CRANIAL ULTRASOUND IN THE ASSESSMENT OF HYDROCEPHALUS EVALUATION IN EXPERIMENTAL RATS AFTER HEMORRHAGIC STROKE

ДОСТОВІРНІСТЬ КРАНІАЛЬНОГО УЛЬТРАЗВУКОВОГО ДОСЛІДЖЕННЯ В ДІАГНОСТИЦІ ГІДРОЦЕФАЛІЇ В ЕКСПЕРИМЕНТАЛЬНИХ ТВАРИН ПІСЛЯ ГЕМОРАГІЧНОГО ІНСУЛЬТУ

Резюме. Субарахноїдальний крововилив є третім найбільш поширенішим підтипом інсульту. Серед його ускладнень найбільш суттєвими є гідроцефалія, повторна кровотеча, відстрочена ішемія, внутрішньомозковий крововилив та внутрішньошлуночковий крововилив. Постгеморагічна гідроцефалія може розвинутися у 66 % випадків з внутрішньошлуночковим крововиливом, і вона також пов’язана з гіршими функціональними результатами. Патофізіологія гідроцефалії після субарахноїдального крововиливу залишається незрозумілою. Експериментальні моделі на малих тваринах у фундаментальних і доклінічних науках є невід’ємною частиною перевірки нових гіпотез перед впровадженням у клінічну практику. Потреба в новітніх підходах до профілактики постгеморагічної гідроцефалії є актуальною, оскільки недоліки наявних методів (шунтування, ендоскопія) є очевидними. Ультразвукове дослідження головного мозку є неінвазивним методом з чудовою просторовою роздільною здатністю для візуалізації шлуночків головного мозку експериментальних тварин.
Мета дослідження. Аналіз валідності ультразвукового дослідження головного мозку при діагностиці комунікантної гідроцефалії в експериментальній моделі геморагічного інсульту у малих тварин.
Матеріал і методи. Піддослідні тварини були розподілені на дві групи. У першій групі (контрольна група) хірургічні втручання не виконувались. У другій групі (20 щурів) тваринам вводили 0,15 мл крові у велику потиличну цистерну з повторною ін’єкцією 0,15 мл крові через 48 годин. Під час експерименту було виконано 46 ультразвукових досліджень. Гідроцефалія дігностувалася при середніх показниках індекса Левіна > +3 стандартного відхилення у контрольних тварин.
Результати. Було виконано 37 операцій на 20 щурах. За даними ультразвукового дослідження, гідроцефалія в хірургічній групі розвинулася у 56 % щурів. У одинадцяти піддослідних тварин значення індексу Левін перевищували 3SD від середнього значення у контрольній групі; в хірургічній групі різниця в індексах Левіна до і після операції становила «+31 %» (p-value менше 0,0001).
Висновки. Ультразвукове дослідження мозку є валідним і точним методом оцінки постгеморагічної гідроцефалії у дрібних експериментальних тварин.
Ключові слова: ультразвукове дослідження мозку, субарахноїдальний крововилив, постгеморагічна гідроцефалія, геморагічний інсульт.

Subarachnoid hemorrhage (SAH) is the third most common subtype of stroke [1]. According to the most recent World Health Organization report, low- to middle-income countries experience a disproportionately high burden (two-fold) of SAH incidence compared to their higher-income counterparts [2]. Early case fatality remains high, as up to one-quarter of patients with aneurysmal SAH do not reach the hospital or die in the emergency room [3]. Although SAH accounts for a relatively small percentage (~15 %) of strokes, those suffering from SAH are younger compared to the mean age of all stroke patients. This fact typically indicates a massive loss of life quality resulting from neurologic deficits experienced by these SAH patients [4]. Among complications, the most important include hydrocephalus, rebleeding, delayed ischemia, intracerebral hemorrhage, intraventricular hemorrhage, increased intracranial pressure, seizures, left ventricular systolic dysfunction, and myocardial infarction. Posthemorrhagic hydrocephalus (PHH) can develop in as many as 66 % of adults with IVH, and it is also associated with worse functional outcomes [5]. The current treatment for hydrocephalus mainly involves surgeries, such as the insertion of a shunt endoscopic third ventriculostomy, which carries a high risk of infection and failure [6-8]. Pathophysiology of hydrocephalus after SAH remains unclear, but it is generally attributed to an obstruction of arachnoid granulations by blood extravasated in the subarachnoid space. This would prevent the CSF outflow to cranial sinus veins [9]. Recently, this «CSF outflow» hypothesis has been questioned in another form of hemorrhagic stroke, intraventricular hemorrhage (IVH). A study has shown that post-hemorrhagic hydrocephalus (PHH) is related to CSF hypersecretion by the choroid plexus (CP) and not to a decrease in CSF outflow [10]. This CSF hypersecretion was associated with an inflammatory response to blood in the ventricular system [11]. Small animal models in basic and preclinical sciences constitute an integral part of testing new hypotheses before translation to clinical practice [12-14]. Ultrasound (US) measurements of the lateral ventricles play an essential role in the early recognition of post-hemorrhagic ventricular dilation [15]. Computed tomography, magnetic resonance imaging, positron emission tomography, single photon emission, and optical coherent tomography have been developed and used for small animal imaging. However, their cost-effectiveness and real-time capability are still significant issues [16]. High-frequency US imaging provides a non-invasive method of superior spatial resolution for imaging small animals [17]. Therefore, the current study evaluated the accuracy of cranial US in the assessment of hydrocephalus evaluation in experimental rats after hemorrhagic stroke.

Material and methods. Thirty adult Wistar rats (Biological Research Center, Lithuanian University of Health Sciences, Kaunas, Lithuania), approximately aged 2-6 months, fifteen males and fifteen females, weighing 250 to 500 g., were enrolled in the study. The rats were bred and maintained at the Lithuanian University of Health Sciences animal house under controlled conditions. The experiments were approved by the State Food and Veterinary Service (Vilnius, Lithuania), following European (2010/63/UE) regulations for the care and use of laboratory animals.

The model of SAH was performed as previously described [18]. The surgery was performed in sterile conditions under general anesthesia with 3 % sevofluran (Baxter, USA). The first group (control group – CG) was without surgery. In the second group, a 0.15 ml blood injection into cistern magna was followed by a 0.15 ml blood injection 48 hours later.

To calculate the ventricle sizes, rats of all groups were each subjected to a real-time non-invasive US examination with a 4-MHz transducer (SP5-1s, Mindray M7 Premium, China) on the 1st day (n=30) and rats from the surgical groups after 20th-day post-operation (n=16). A coronal US view of the lateral ventricles was obtained (Figure 2), and the Levene index (LI) was calculated as the distance between the falx and the lateral wall of each anterior horn in the coronal plane.

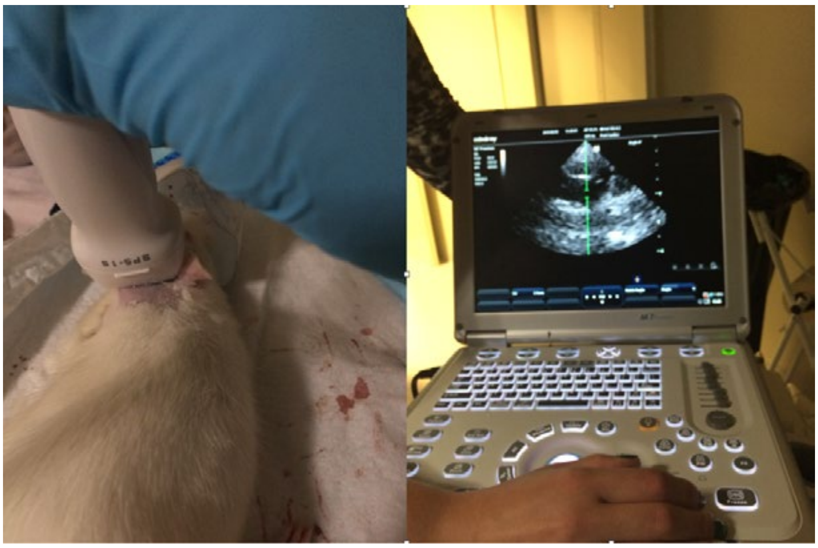

Fig. 1. Ultrasound investigations on experimental animals

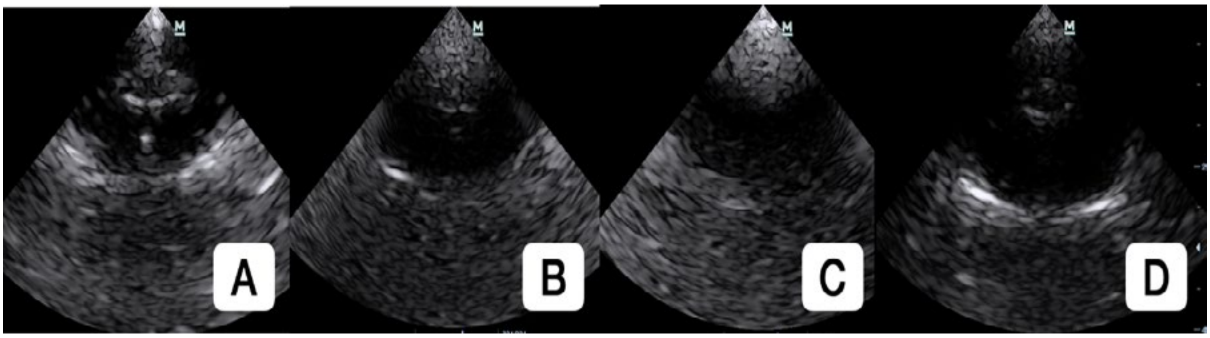

Fig. 2. Ultrasound images of SAH animals with progressive ventricular dilatation (A to D)

Hydrocephalus was defined as a Levene index over +3SD of the mean in sham and control animals.

Statistical Analysis. Ventricle sizes were analyzed by repeated measures of two-way ANOVA, and post-hoc comparisons of control vs. SAH animals were Bonferroni-corrected. All analyses were done using GraphPad Prism 5.00 software (GraphPad Prism Software Inc., San Diego, CA, USA). Values were presented as mean±SD. The statistical significance level is set at $p<0.05$.

Results and discussion. Thirty-seven surgical interventions were performed on 20 research animals. The preoperative and postoperative medium LI in surgical groups was 1.000 versus 1.333 (+33 %). P value <0.0001.

The induction of hydrocephalus was 56 %. In eleven experimental animals, Levene index values were above 3SD of the mean in control animals (Figure 3).

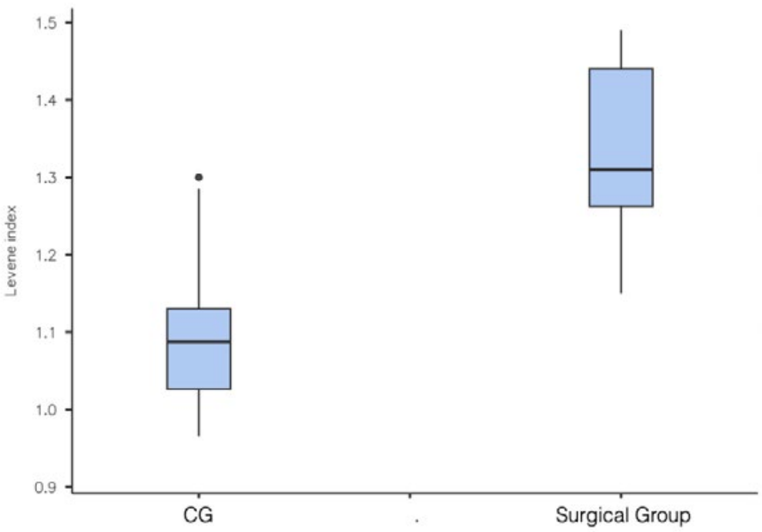

Fig. 3. Hydrocephalic experimental animals in surgical group above 3SD of the mean in control animals

In this experimental study, we evaluated the accuracy of cranial US in hydrocephalus evaluation in experimental rats after hemorrhagic stroke. The research's key findings include that cranial US is a valid and precise method for assessing posthemorrhagic hydrocephalus in small experimental animals. US has been widely used for diagnostic and therapeutic purposes due to its easy accessibility, non-ionizing, and non-invasive nature with the possibility of real-time observation. Additionally, high resolution could be added to these merits [19, 20]. The US has not been often used for imaging small animals in research as compared to other in vivo molecular imagers such as micro magnetic resonance imaging (micro-magnetic resonance imaging, micro-MRI), micro-computed tomography (micro-computed tomography, micro-CT), and optical imagers [20] their cost-effectiveness and real-time capability still need to be improved [16]. Typical frequency ranges from 3-5 MHz up to 30-50 MHz between adjacent tissue layers; the higher the delivered US frequency, the higher the tissue spatial resolution achieved at the expense, however, of higher attenuation by tissue, resulting in more superficial penetration [21, 22]. Considering the previous facts, we obtained US with a 4 MHz probe balancing excellent visualization and enough penetration in this study, which was also observed in other studies with the same purpose and US frequency [23, 24].

US measurements of the lateral ventricles play an important role in the early recognition of post-hemorrhagic ventricular dilation [15]. Assessment of cerebral ventricular size is essential in the clinical management of hydrocephalus. Ventricular size is often estimated on US, CT, or MR images using linear indices such as Levene or Evans' frontal horn and bicaudate indexes. [25-27]. The ventricle index, better known as the Levene, is the distance from the falx to the lateral boundary of the lateral ventricle. This is measured in the coronal plane just posterior or at the level of the foramen of Monro. It is the most commonly described method and has the largest reference database [15]. During research in the current study, we performed 36 US investigations in experimental groups, creating pretreatment normal values of ventricle sizes and investigating changes in LI after SAH or minocycline treatment.

Conclusion. Based on the findings, cranial ultrasound is a valid and precise method for assessing posthemorrhagic hydrocephalus in small experimental animals.

Perspectives of further research. Ultrasound imaging is a practical investigation that, due to its non-invasiveness and perfect structural efficiency, has many potential implications in experimental brain research, particularly in neurooncology and functional neurosurgery. One of the most promising directions is investigating the neuromodulation effects of transcranial ultrasound stimulation.

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ACCURACY OF CRANIAL ULTRASOUND IN THE ASSESSMENT OF HYDROCEPHALUS EVALUATION IN EXPERIMENTAL RATS AFTER HEMORRHAGIC STROKE

Abstract. Subarachnoid hemorrhage (SAH) is the third most common subtype of stroke. Among complications, the most important include hydrocephalus, rebleeding, delayed ischemia, intracerebral hemorrhage, and intraventricular hemorrhage. Post-hemorrhagic hydrocephalus (PHH) can develop in as many as 66 % of adults with intraventricular extension of blood, and it is also associated with worse functional outcomes. The pathophysiology of hydrocephalus after SAH remains unclear. Small animal models in basic and preclinical sciences constitute an integral part of testing new hypotheses before translation to clinical practice. The need for newer procedures for PHH prevention is imperative, as the limitations of traditional treatment methods (e.g., shunting, endoscopy) have become increasingly apparent. High-frequency brain ultrasound imaging provides a non-invasive method of superior spatial resolution for imaging brain ventricles in small animals.
Objectives. Cranial ultrasound in an experimental small animal hemorrhagic stroke model of cisterna magna double-injection was tested to see its validity in the assessment of communicative hydrocephalus.

Methods. Experimental animals were divided into two groups. The first group (control group – CG) was without surgery (10 rats). In the second group (20 rats), a 0.15 ml blood injection into cistern magna was followed by a 0.15 ml blood second injection 48 hours later. During the research 46 US, we performed. We defined hydrocephalus as Levene index on ultrasound, which was > +3 SDs above the mean in control animals.
Results. Thirty-seven operations were done on 20 rats. Hydrocephalus in the surgical group occurred in 56 % of rats, according to the ultrasound investigations. In eleven experimental animals, Levene index values were above 3SD of the mean in the control animal; in the surgical group, the difference in Levene indexes between pre- and post-operation was «+31 %» (p-value less than 0,0001).
Conclusion. Based on the findings, cranial ultrasound is a valid and precise method for assessing posthemorrhagic hydrocephalus in small experimental animals.
Key words: Cranial ultrasound, subarachnoid hemorrhage, posthemorrhagic hydrocephalus, hemorrhagic stroke.

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