Hydrocephalus: 5-HIAA and HVA in the Cerebrospinal Fluid

Mirsada Salihović[1(✉)] and Emin Sofić[2]

[1] Department of Natural Sciences in Pharmacy, Faculty of Pharmacy, University of Sarajevo, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina
mirsada.salihovic@ffsa.unsa.ba

[2] Department of Chemistry, Faculty of Science, University of Sarajevo, Zmaja od Bosne 33–35, 71 000 Sarajevo, Bosnia and Herzegovina

Abstract. The choroid plexus form cerebrospinal fluid (CSF) within four ventricles the brain. The composition of CSF is very similar to the of the brain's extracellular, and its composition depends on filtration and diffusion from the blood. Few conditions cause CSF volume and pressure to fall below normal (dehydration may be one), but many conditions can cause it to increase, sometimes under pressure. This may lead to blocked flow CSF from one or more ventricles, which causes an increase in intracranial pressure, and CSF buildup i.e. to hydrocephalus. Hydrocephalus results from one of three possible causes: over secretion of CSF, impaired absorption of CSF or obstruction of CSF pathways. The concentration of metabolites of the monoamines homovanillic acid (HVA) and 5-hydrocephalus acetic acid (5-HIAA) were determined with HPLC-ED in the CSF of 17 patients (children of 1 age) with hydrocephalus. Determined 5-HIAA concentrations are in the range of 0,99–113,89 ng/mL and HVA 0,98–165,92 ng/mL in CSF due to pressure changes occurring in hydrocephalus is increasing the turnover of serotonin and dopamine to its metabolites. The correlation no could be established between the levels of 5-HIAA and HVA and type of hydrocephalus, and the duration of disease.

Keywords: Hydrocephalus · 5-HIAA · HVA · Cerebrospinal fluid

1 Introduction

For more than 30 years, neurotransmitters such as noradrenaline (NA), dopamine (DA), and serotonin (5-HT) have been the main targets of neurochemical research for the development of therapies for neurological and psychiatric diseases Biosynthesis of catecholamines (CAs) DA, NA, and adrenaline (A) is performed in the brain, chromaffin cells of adrenal glands and in synaptic nerve endings. CAs are synthesized through a simple sequence of successive enzymatic reactions. The tyrosine hydroxylase (TH) enzyme catalyzes the synthesis of 3, 4-dihydroxyphenylalanine (DOPA) from tyrosine, which is synthesized from phenylalanine. DA is produced by the conversion of DOPA which is catalyzed by the aromatic amino acid decarboxylase enzyme. CAs break down very

A. Badnjevic and L. Gurbeta Pokvić (Eds.): CMBEBIH 2021, IFMBE Proceedings 84, pp. 403–412, 2021.
https://doi.org/10.1007/978-3-030-73909-6_46

quickly, approximately two minutes in the serum. The major metabolites relevant for laboratory testing are homovanillic acid (HVA), metanephrine (MN), and vanillylmandelic acid (VMA) [1].

5-HT mediates a number of central and peripheral functions, which makes its physiology very complex. Among other things, it is thought to be involved in the modulation of behavior and homeostatic processes in the body: mood regulation, cognitive and motor functions, and circadian and neuroendocrine rhythms such as regulation of breathing, sleep, cardiovascular function, metabolism, temperature, feeding, sexual activity and more [2, 3]. 5-HT is broken down by the action of the MAO enzyme and aldehyde dehydrogenase to 5-hydroxyindoleacetic acid (5-HIAA). Aside from the brain, this acid can be found in the cerebrospinal fluid (CSF), blood and urine [4]. Many literature reports indicate that low concentrations of 5-HIAA in CSF can be the cause of psychomotor agitation and positive schizophrenic symptoms, while high concentrations of 5-HIAA in CSF cause negative symptoms [5, 6].

It's known that CSF has several important roles in the functioning of the CNS. The cerebrospinal fluid protects the brain and spinal cord from serious injury in sudden movements and strokes. In subarachnoid spaces, the cerebrospinal fluid allows the brain to "float" which prevents the weight of the brain from pressing the roots of the cranial nerves and blood vessels against the inner surface of the skull. The cerebrospinal fluid enables the dilution and elimination of products of metabolism of brain cells and other substances coming from the blood into the brain. Furthermore, cerebrospinal fluid enables intracerebral transport [7, 8]. Excess CSF expands the brain chambers and creates pressure in the brain. The pressure created by an increase in the level of CSF associated with hydrocephalus can damage brain tissue and cause greater damage to brain functions.

Hydrocephalus is the enlargement of the cerebral chambers by excess CSF. Manifestations are head enlargement and brain atrophy. Increase in intracranial pressure causes irritability and bulging fontanelle in children. Diagnosis in infants is performed by ultrasound and in older children by CT or MRI [9].

Catecholamine and indolamine metabolites concentrations are usually determined by high-pressure liquid chromatography with electrochemical detection (HPLC-ED), gas chromatography (GC), or gas chromatography with mass spectrometry (GC-MS). For GC or GC-MS, the samples must be derivatized, while the HPLC method can analyze polar compounds without prior derivatization [10]. The advantage of HPLC methods is that they are fast and allow simultaneous determination of various catecholmines and indoleamine and their metabolites in the same sample.

The aim of this study is to determine the concentrations of 5-HIAA and HVA in CSF, by HPLC-ED method, in 17 patients diagnosed with hydrocephalus.

2 Materials and Methods

Biological Material: Liquor.

Reagents and Solutions: Perchloric acid ($HClO_4$) 60%, pro analysis, Merck; 5-HIAA minimum 98%, titration, Sigma; HVA, Sigma; Water Redistilled for Chromatography, Panreac; EDTA titriplex III disodium salt- dihydrate min 99%, pro analysis, Merck;

Sodium acetate anhydrous, for analysis, Merck; Acetic glacial-glacial acid ≥ 99,8%, pro analysis, Panreac; Methanol LiChrosolv, Merck.

Equipment and Accessories: Mixer VWR International, Germany AC 230 V 50 Hz 51 W 100–2400 1/min.; Microcentrifuge, Hettich; Micro 22R; Ultrasonic Water Bath, Sonis, Iskra; Vacuum Filtration Device, Supelco; Pair filter, nylon 66 membranes; 0,45 um × 47 mm, Supelco; HPLC, Shimadzu LCSOL SINGLE-LC EN, Made in Kyoto, Japan; Detector: ED, BAS Liquid Chromatography CC-5E, LC-4C; Pump: LC-20AT; Degasser: DGU-20A3; Oven: CTO-10Asvp; Working Electrode: Glassy carbon; model MF-1000; BASS; Reference electrode: Ag/AgCl; model MW-2030; BASS; Columns: ODS Hypersil 5 μm 200 × 4,6 mm Hewlett Packard.

2.1 HPLC-ED Analysis

Samples were stored at −80 °C. After thawing, the samples were diluted with $HClO_4$ (0,2 mol/L) at a ratio of 1:1. Such dilutions were centrifuged at 15,000 rpm for 30 min at 4 °C. After that, samples were decanted into clean and dry Eppendorf microcentrifuge tubes and re-centrifuged at 15,000 rpm for 10 min at 4 °C and decanted into pure Eppendorf microcentrifuge Tubes. These samples were injected (20 μl) in HPLC for analysis.

Standard Solutions: 5-HIAA and HVA 0,2 mol/L.

Mobile Phase: EDTA 372 mg/L; 0,1 M Sodium acetate (13,61 g/L); 50% Methanol (63,3 ml/L); 7,9 ml Acetate - glacial acid.

Table 1. Data for the calibration curve of standard solutions 5-HIAA and HVA

	5-HIAA	ng/mL		HVA	ng/mL
10	1171498 983528	10,628 8,959	10	548965 553693	9,513 9,305
75	8550778 8451767	76.183 75,304	20	1352981 1337587	21,090 20,866
100	11211557 11131136	99,820 99,106	100	6738233 6783594	100,222 99,561
R R^2 m	0,9998977 0,9997954 24914,1		R R^2 m	0,9998686 0,9997373 82350	

Conditions for HPLC-ED Analysis: Detector conditions; Range: 50 nA; Potential: + 0,75 V; Filter: 0,02 Hz; Flow rate = 0,9 mL/min; Column: ODS Hypersil 5 μm, 200 × 4,6 mm Hewlett Packard.

Quantification of 5-HIAA and HVA in CSF: standard solutions of 5-HIAA and HVA in 0,2 N $HClO_4$ were analyzed by HPLC-ED method. Table 1 showed concentrations of 5-HIAA and HVA standard solutions and surfaces of peaks. Based on the data presented, a calibration curve for the standard 5-HIAA and HVA solutions was constructed.

Figure 1 showed a chromatogram of a mixture of standards 5-HIAA and HVA in 0,2 N $HClO_4$, whose concentration is 40 ng/mL, which showing retention times for 5-HIAA standards and HVA. By analyzing the linear correlation of 5-HIAA and HVA standards, the concentration of 5-HIAA and HVA in the CSF samples was determined.

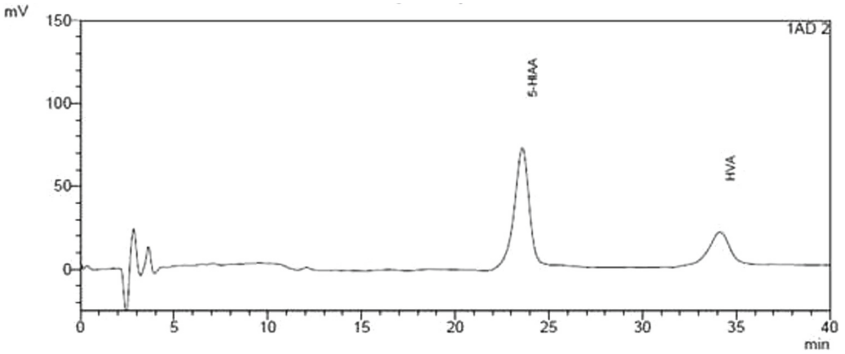


Fig. 1. Chromatogram of 5-HIAA and HVA standard solution (40 ng/mL)

3 Results

The concentration of 5-HIAA and HVA in CSF of children determined with high-pressure liquid chromatography with electrochemical detection using HPLC Shimadzu, Kyoto, Japan with BAS amperometric detector. Studies were carried out on CSF of children belonging to both sexes, with ages from few months to one year old. Patients were with hydrocephalus conditions.

Table 2 are shown the concentrations for 5-HIAA and HVA determined in 17 CSF samples in individuals up to 1 year of age, of different sex, diagnosed with hydrocephalus. The mean concentration of 5-HIAA is 38,13 (SEM ± 8,31) ng/mL, and the HVA is 47,25 (SEM ± 9,22) ng/mL. The detection limit for 5-HIAA in the CSF is 4,6 pg and for HVA it is 3,6 pg.

Table 2. The concentration of 5-HIAA and HVA in the CSF of persons of different sex and age of 1, diagnosed with hydrocephalus

Number	Sex	5-HIAA (ng/mL ± SD)	HVA (ng/mL ± SD)
1	Male	19,9 ± 0,90	23,54 ± 0,29
2	Male	7,95 ± 0,68	39,19 ± 0,97
3	Male	23,5 ± 0,75	32,32 ± 0,32
4	Male	9,6 ± 0,15	1,37 ± 0,46
5	Male	77,7 ± 50	59,5 ± 1,12
6	Male	113,1 ± 0,78	38,72 ± 0,38
7	Male	4,36 ± 0,13	44,46 ± 0,57
8	Male	60,88 ± 0,46	115,12 ± 1,13
9	Male	46,00 ± 0,68	165,92 ± 0,98
10	Male	25,68 ± 0,72	36,78 ± 0,86
11	Male	0,52 ± 1,01	15,20 ± 0,63
12	Male	0,38 ± 1,35	12,12 ± 0,88
13	Male	113,86 ± 0,23	29,50 ± 1,16
14	Female	32,73 ± 0,55	51,06 ± 0,77
15	Female	40,13 ± 1,43	48,23 ± 0,94
16	Female	36,42 ± 1,21	46,29 ± 0,32
17	Female	35,43 ± 0,73	43,94 ± 0,91

3.1 Determination of 5-HIAA Concentration in CSF

After HPLC-ED analysis of the CSF samples and the standard solutions of 5-HIAA and HVA, the concentrations of these metabolites were determined. The concentrations of 5-HIAA shown in Fig. 1 were determined based on the calibration curve of the standard solution 5-HIAA (example, Figs. 2 and 3).

On the chromatogram of the CSF sample No. 1, shown in Fig. 3 it can be seen that the retention times for 5-HIAA (t_R = 23,933) and HVA (t_R = 34,435) correspond to the retention times of the standard solutions of 5-HIAA and HVA, i.e. for 5-HIAA t_R = 24,268, and for HVA t_R = 33,556.

Figure 3 shows that the concentration of 5-HIAA in sample CSF No.1 was determined based on the calibration curve of standard solution 5-HIAA and the peak area of 5-HIAA in sample CSF No.1.

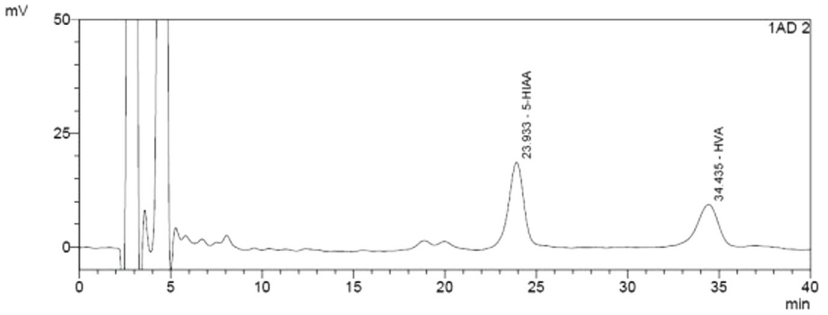


Fig. 2. Chromatogram of liquor sample No.1

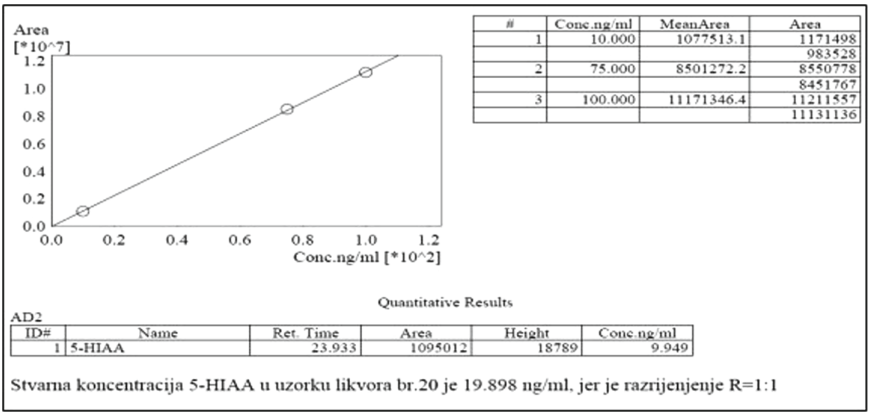


Fig. 3. Calibration curve of standard 5-HIAA solution

3.2 Determination of HVA Concentration in CSF

The HVA concentrations shown in Fig. 2 are based on the calibration curve of the standard HVA solution (for example, Figs. 4 and 5).

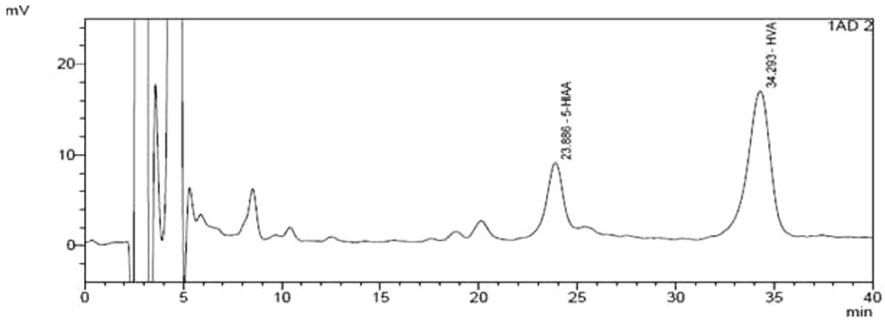
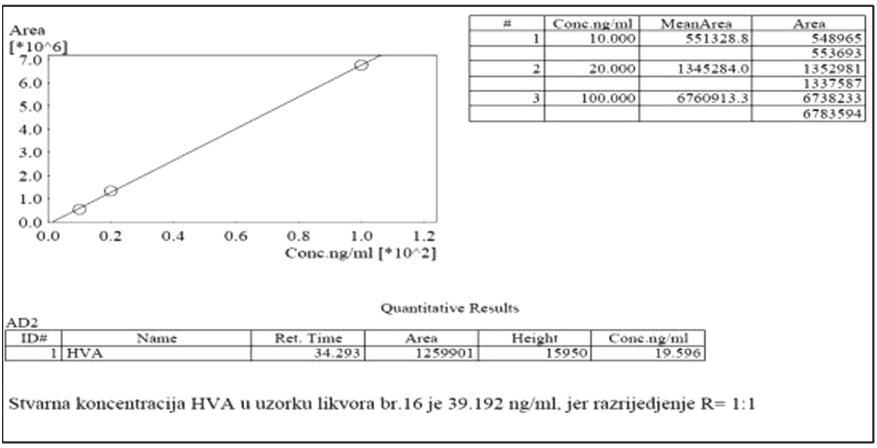


Fig. 4. Chromatogram of liquor sample No.2



#	Conc.ng/ml	MeanArea	Area
1	10.000	551328.8	548965
			553693
2	20.000	1345284.0	1352981
			1337587
3	100.000	6760913.3	6738233
			6783594

Quantitative Results

AD2

ID#	Name	Ret. Time	Area	Height	Conc.ng/ml
1	HVA	34.293	1259901	15950	19.596

Stvarna koncentracija HVA u uzorku likvora br.16 je 39.192 ng/ml, jer razrijedjenje R= 1:1

Fig. 5. Calibration curve of standard HVA solution

On the chromatogram of sample CSF No.2 showed in Fig. 5 shows that the retention times for 5-HIAA (t_R = 23,886) and HVA (t_R = 34,293) correspond to the retention times of standard solutions 5-HIAA and HVA, i.e. 5-HIAA t_R = 24,268, and for HVA t_R = 33,556.

Figure 4 shows that the HVA concentration in the CSF sample No.2 was determined based on the calibration curve of the standard HVA solution and the HVA peak area in the CSF sample No.2.

3.3 The Influence of Gender on the Concentration of 5-HIAA and HVA in CSF

The obtained results of the 5-HIAA and HVA concentrations in 17 samples of CSF, subjects different gender were divided into two groups:

- Group 1 - Thirteen male examiners
- Group 2 - Four female respondents

In these two groups, the influence of gender on the concentration of 5-HIAA and HVA in CSF was analyzed. Mean concentration values for 5-HIAA are shown in Fig. 6 and mean concentration values for HVA are shown in Fig. 7.

Figure 6 shows that the mean value of 5-HIAA concentrations in CSF in male subjects is higher than the mean value of 5-HIAA concentrations in CSF in female subjects. Whereas in Fig. 7, the mean value of HVA concentrations in CSF in male subjects is slightly higher than the mean value of HVA concentration in CSF in female subjects.

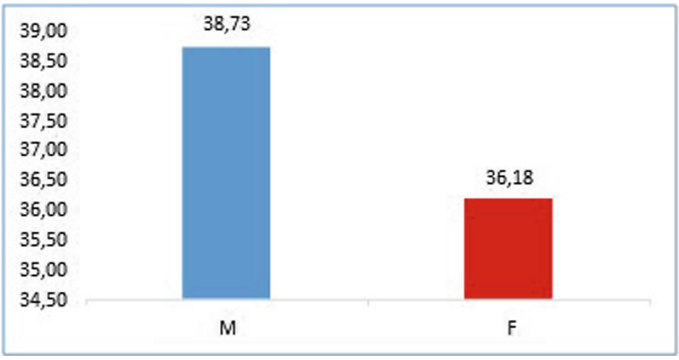


Fig. 6. Mean values concentrations of 5-HIAA female and male subjects

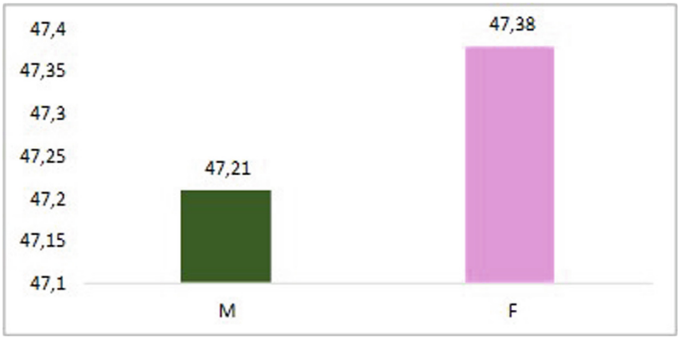


Fig. 7. Mean values concentrations of HVA female and male subjects

4 Discussion

The major metabolites of 5-HT and DA i.e. 5-HIAA and HVA can be found except in the brain and the CSF, plasma, serum, and urine [4]. Since CSF is in close contact with CNS structures, data on biochemical changes in the brain and spinal cord could be collected by CSF analysis, which could be used in the diagnosis and treatment of various diseases [11, 12].

The HVA concentration determined in CSF and plasma is used to study the neurotransmission of DA in neurological and psychiatric diseases [13]. Many literature data indicate that low concentrations of 5-HIAA in CSF may be the cause of psychomotor agitation and positive schizophrenic symptoms, while high concentrations of 5-HIAA in CSF cause negative symptoms [14]. Concentrations of CA and indolamine metabolites are usually determined by HPLC-ED, GC or GC-MS. For GC or GC-MS, samples must be derivatized, while HPLC can analyze compounds without first derivatization [10]. The advantage of HPLC methods is that they are fast and allow simultaneous determination of various CA and indoleamine and their metabolites in the same sample. The method used in this study relies principally on HPLC-ED methods for the determination of biogenic amines and their metabolites in biological samples, as well as in tissue

homogenates, cerebrospinal fluid, urine and serum of humans and animals [4, 15]. In our work, concentrations of 5-HIAA and HVA in CSF were determined by HPLC Shimadzu apparatus, Kyoto, Japan, with a BAS amperometric detector and a 20 μl manual volume injector. The obtained results showed that the male subjects have a slightly higher mean concentration of 5-HIAA, and a lower mean value of the HVA concentration in the CSF compared to the female subjects. However, further study of CSF on a larger number of subjects is needed to establish certain irregularities. CA and 5-HT deficiency and excess cause various diseases, such as adrenal carcinoid or other chromaffin tissues [4].

A tumor derived from argentaffin cells intensively produces 5-HT that converts to 5-HIAA, so patients excrete large amounts of 5-HIAA, which in urine can grow more than 100 times normal. Tumors most commonly occur in the intestinal tract, but can also occur in other organs containing argentaffin cells (biliary tract, pancreas, etc.) [16].

5 Conclusions

The concentrations of 5-HIAA and HVA in the CSF of 17 subjects up to one year of age and of different sex were determined. The mean concentration of 5-HIAA is 38,13 ± 8,31 ng/mL and the HVA is 47,25 ± 9,22 ng/ml. The detection limit of 5-HIAA is 4,6 pg and the HVA is 3,6 pg. The provided HPLC-ED method is suitable for the quantification of very low concentrations of 5-HIAA and HVA in one complex biological sample such as CSF. However, the Shimadzu apparatus, made in Kyoto, Japan with an ED detector, BAS has an injector calibrated to 20 μl, which is a major technical drawback of this HPLC-ED system. The mean value concentration of 5-HIAA in CSF in male subjects was 38,73 ± 10,87 ng/mL, and in female subjects was 36,18 ± 1,33 ng/mL. The mean value concentration of HVA in CSF in male subjects was 47,21 ± 12,07 ng/mL, and in female subjects was 47,38 ± 1,31 ng/mL. Male subjects had a slightly higher mean concentration of 5-HIAA and a lower mean value of HVA in the CSF compared to female subjects. Further studies of CSF on a higher number of subjects are needed to establish certain rightness.

References

1. Sasaki, T., Fukushima, T., Ohishi, M., Toyo´oka, T.: Development of 6-hydroxychroman-based derivatization reagent: application to the analysis of 5-hydrxytryptamine and catecholamines by using high-performance liquid chromatography with electrochemical detection. Biomed. Chromatogr. **22**, 888–899 (2008)
2. Meltzer, H.Y., Arora, R.C.: Platelet serotonin studies in affective disorders: evidence for serotogenic abnormality. U: 5-Hydroxytryptamine in Psychiatry, A Spectrum of Ideas, Oxford University Press, pp. 50–89 (1991)
3. Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Humphrey, P.P.: International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol. Rev. **46**(2), 157–203 (1994)
4. Sofić, E.: Metabolic turnover of biogenic amines in physiological fluids: diagnostic significances. Turk. J. Biochem. **32**(3), 120–129 (2007)
5. Salihović, M., Sofić, E.: 5-HIAA and HVA in the coma cerebri, hydrocephalus and tumor cerebri. In: CMBEBIH 2017, pp. 396–401. Springer, Singapore (2017)

6. Nordström, P., Samuelsson, M., Åsberg, M., Träskman-Bendz, L., Åberg-Wistedt, A., Nordin, C., Bertilsson, L.: CSF 5-HIAA predicts suicide risk after attempted suicide. Suicide Life-Threat. Behav. **24**(1), 1–9 (1994)
7. Klarica, M.: Uloga osmolarnosti likvora u patofiziologiji intrakronijalnog tlaka. Disertacija, Medicinski fakultet Sveučilišta u Zagrebu, Zagreb (1992)
8. Juretic, A., Spagnoli, G.C., Schultz-Thater, E., Sarcevic, B.: Cancer/testis tumour-associated antigens: immunohistochemical detection with monoclonal antibodies. Lancet Oncol. **4**(2), 104–109 (2003)
9. Relkin, N., Marmarou, A., Klinge, P., Bergsneider, M., Black, P.M.: Diagnosing idiopathic normal-pressure hydrocephalus. Neurosurgery **57**(suppl_3), S2–S4 (2005)
10. Lionetto, L., Lostia, A.M., Stigliano, A., Cardelli, P., Simmaco, M.: HPLC-mass spectrometry method for quantitative detection of neuroendocrine tumor markers: Vanillylmandelic acid, homovanillic acid and 5-hydroxyindolacetic acid. Clin. Chim. Acta **398**, 1–4 (2008)
11. Sherman, J.L., Citrin, C.M.: Magnetic resonance demonstration of normal CSF flow. Am. J. Neuroradiol. **7**(1), 3–6 (1986)
12. Saunders, N.R., Habgood, M.D., Dziegielewska, K.M.: Barrier mechanisms in the brain, I. Adult brain. Clin. Exp. Pharmacol. Physiol. **26**(1), 11–29 (1999)
13. Ramirez-Bermudez, J., Ruiz-Chow, A., Perez-Neri, I., Soto-Hernandez, J.L., Flores-Hernandez, R., Nente, F., Montes, S., Rios, C.: Cerebrospinal fluid homovanillic acid is correlated to psychotic features in neurological patients with delirium. General Hosp. Psychiatry **30**, 337–343 (2008)
14. Csernansky, J.G., Wrona, C.T., Bardgett, M.E., Early, T.S., Newcomer, J.W.: Subcortical dopamine and serotonin turnover during acute and subchronic administration of typical and atypical neuroleptics. Psychopharmacol. **110**(1–2), 145–151 (1993)
15. Sperk, G.: Simultaneus determination of serotonin, 5-hidroxyindoleacetic acid, 3, 4-dihydroxyphenylacetic acid and homovanillic acid by hight performance liquid chromatography with electrochemical detection. J. Neurochem. **38**, 840–843 (1982)
16. Csernansky, J.G., King, R.J., Faustman, W.O., Moses, J.A., Poscher, M.E., Faull, K.F.: 5-HIAA in cerebrospinal fluid and deficit schizophrenic characteristics. Br. J. Psychiatry **156**, 501–507 (1990)