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Postmortem serotonin level in cerebrospinal fluid as a marker of the manner of death

Postmortalne vrednosti serotonina u cerebrospinalnoj tečnosti kao marker porekla smrti

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Abstract

Background/Aim. Serotonin [5-hydroxytriptanine (5-HT)], as a neurotransmitter in the central nervous system, is included in the regulation of autonomic and cognitive functions, sensory processing, motor activity, emotions, mood, and almost every kind of behavior. In forensic investigations of death, 5-HT has been studied in different body fluids, regarding the cause of death, particularly in suicides and drug abuse or as a marker of an acute stress response. The aim of this study was to establish 5-HT levels in cerebrospinal fluid (CSF) as a marker of its central activity during fatal event in deaths different in manner, particularly in cases where the victims were aware of the stressful event. **Methods.** Study sample consisted of 81 postmortem CSF obtained during autopsy. Concentrations of the 5-HT were established regarding natural versus violent (accidents, homicides and suicides) deaths. After preparation, samples were analyzed through the liquid chromatography-tandem mass spectrometry method. **Results.** Violent deaths had significantly higher 5-HT levels (U = 519.000; $p < 0.05$). Differences were found in mean values among different causes of death (higher in blunt injury, stabbing and intoxication, while lower in cardiac deaths and hypothermia) but without statistical significance. 5-HT levels significantly differed among age groups (χ^2 = 13.354; p = 0.001), with the tendency to decrease with age. No differences in 5-HT levels were observed regarding gender, length of agony period, and awareness of impending lethal outcome. The values tended to increase with postmortem interval albeit without significant differences. **Conclusion.** Serotonin could be a useful postmortem biochemical marker to distinguish natural and violent death, regardless of individual variability in concentrations.

Key words:
autopsy; biomarkers; cause of death; cerebrospinal fluid; chromatography, liquid; death; forensic medicine; serotonin.

Apstrakt

Uvod/Cilj. Serotonin [5-hidroksitriptamin (5-HT)], kao neurotransmiter u centralnom nervnom sistemu (CNS), uključen je u regulaciju autonomnih i kognitivnih funkcija, obrade senzornih informacija, motorne aktivnosti, emocija, raspoloženja i gotovo svakog oblika ponašanja. U sudskomedicinskoj istrazi, 5-HT je proučavan u različitim telesnim tečnostima u odnosu na uzrok smrti, a posebno u vezi sa samoubistvima, zloupotrebom droga ili kao marker akutnog odgovora na stres. Cilj ove studije bio je utvrđivanje nivoa 5-HT u cerebrospinalnoj tečnosti, kao markera njegove centralne aktivnosti tokom procesa umiranja, u smrtima različitog porekla, a posebno u slučajevima gde su žrtve bile svesne stresnog događaja. **Metode.** Istraživanje je sprovedeno na postmortalnim uzorcima 81 likvora uzetih tokom obdukcije. Koncentracije 5-HT su određivane u odnosu na prirodno i nasilno (zadesno, samoubilačko i ubilačko) poreklo smrti. Nakon pripreme, uzorci su analizirani metodom tečne hromatografije sa tandem masenom spektrometrijom. **Rezultati.** Vrednosti 5-HT su bile značajno više u slučajevima nasilnih smrti u odnosu na prirodne smrti (U = 519,000; $p < 0.05$). Utvrđene su razlike u srednjim vrednostima 5-HT između različitih uzroka smrti (usled trovanja, povređivanja dejstvom tupine, šiljka i oštrice, a niže u hipotermiji i srčanoj smrti). Vrednosti 5-HT značajno su se razlikovale među grupama (χ^2 = 13,354; p = 0.001, sa tendencijom sniženja sa godinama. Nisu utvrđene razlike u odnosu na pol, dužinu agonije i svesnost o nastupajućem smrtnom ishodu. Vrednosti su imale tendenciju rasta sa dužinom postmortalnog intervala, ali se to nije pokazalo statistički značajnim. **Zaključak**. Serotonin bi mogao biti koristan postmortalni biohemijski marker u razlikovanju prirodnih i nasilnih smrti, uprkos velikim individualnim varijacijama.

Ključne reči:
autopsija; biološki pokazatelji; smrt, uzrok; cerebrospinalna tečnost; hromatografija, tečna; smrt; medicina, sudska; serotonin.

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Introduction

Serotonin, a biogenic monoamine, chemically identified as 5-hydroxytryptamine (5-HT), acts both as a neurotransmitter in the central nervous system (CNS) and a local hormone in the peripheral tissues [1]. Through the projections, 5-HT is involved in the regulation of sleep, appetite, sexual activity, body temperature, circadian rhythms, autonomic and cognitive functions, sensory processing, motor activity, emotions, mood, i.e. almost every kind of behavior. In forensic investigation of death, 5-HT has been studied in different body fluids, regarding the cause of death – particularly suicides and drug abuse or as a marker of an acute stress response [2–7] with a significant contribution.

Briefly, stress is a biological response to real/potential threat to body integration and homeostasis, which initiates numerous autonomic, endocrine, cognitive and affective processes, aimed to neutralize the source of danger, through different behaviors such as fight or flight response, aggression, escape, etc. Involved in all these, monoamines and 5-HT act very rapidly, just within seconds after the onset of a stressor [3, 8–10]. Our hypothesis was that this rapid change in 5-HT levels could be detected and used for different forensic purposes.

In the presented study we investigated postmortem serotonin concentrations in cerebrospinal fluids (CSF) as a marker of its central activity in different manners of death, particularly in cases where the victims were aware of the stressful event.

Methods

Autopsy material

Our sample consisted of 81 autopsy cases, examined in the Institute of Forensic Medicine, Faculty of Medicine, University of Belgrade. The inclusive parameters for the analysis were: older than 18 years of age, with a postmortem interval (i.e. time from estimation of death until autopsy and sampling) within 72 h. Case profiles are presented in Table 1. Further inclusive parameters were: death at the scene without cardiopulmonal resuscitation and critical medical care before death; no documented history of either acute or chronic neuronal psychiatric or metabolic disorder, terminal diseases (as malignancy, e.g., and drug abuse or psychotropic therapy. Regarding surviving interval, determined as time between onsets of symptoms/injury to time of death, the distinction was made between very short and delayed (i.e. up to several minutes and up to several hours). Awareness of stressful life-threatening event was presented as dichotomy yes/no. In this regard, we divided the subjects to those who had been aware of impending lethal danger (either the one who was drowning or facing an attacker equipped with a gun or knife) and those who had no sense of it (the ones who died of heart failure during sleep or pedestrian hit from behind). The causes, manner of death and other data were determined through autopsy and toxicological findings while demographic data, circumstances and other data originated from police reports and heteroanamnesis.

Table 1

Demographic data and case profiles

Cause of death	Case number n (%)	Manner of death* N/A/S/H n (%)	Male/female n (%)	Survival time very short/delayed n (%)	Awareness of the stressful event yes/no n (%)
Violent death	50 (61.7)				
mechanical injury	17 (34)			13/4	17/0
blunt	7 (14)				
jump from a height	2	S (2)	2/0	2/0	2/0
traffic traumatism	4	A (4)	4/0	3/1	4/0
blows	1	H (1)	0/1	0/1	1/0
firearm	8 (16)	S (5)/H (3)	5/3	7/1	8/0
stabbing	2 (4)	h (2)	1/1	1/1	2/0
mechanical asphyxia	18 (36)				18/0
drowning	5	S (5)	3/2	5/0	5/0
hanging	13	S (13)	10/3	13/0	13/0
environmental injury	8 (16)				0/8
hypothermia	5	A (5)	1/4	0/5	0/5
electrocution	3	A (3)	3/0	3/0	0/3
CO intoxication	7 (14)	A (7)	4/3	5/2	3/4
Natural death					
acute cardiac death	31 (38.3)	N (31)	25/6	13/18	19/12
Total	81 (100)	31/19/25/6 (38.3/23.5/30.9/7.4)	58/23 (71.6/28.4)	52/29 (71.6/28.4)	57/24 (70.4/29.6)

***Manner of death: N – natural (38.3%); A – accidental (23.5%), S – suicidal (30.8%), H – homicidal (7.4%), out of total of violent deaths (61.7%).**
CO – carbon monoxide.

In order to evaluate relevance of CSF 5-HT levels, we related its values in central CSF with those from lumbar CSF as well as with its femoral serum levels. The specimens of CSF and serum were obtained during standard autopsy procedure. CSF samples were taken either after removing of calvaria and dura while brain was still *in situ* by needle puncture of ventral horn of a lateral ventricle through *corpus callosum* [11–13], while lumbar CSF samples were taken through ventral approach to lumbar cistern, puncturing intervertebral space L4/L5 after removal of abdominal content [7]. The samples of blood were taken through puncture of femoral vein, and then centrifuged to separate sera. Samples of the CSF and sera were immediately frozen and stored at -20°C until analyzed. To avoid influence of blood contamination or hemolysis only clear bright CSF and serum samples were analyzed, while those with cloudy or pinkish impurities were excluded.

Analytical procedure

In 100 μL of either CSF or serum sample, 20 μL of benzylamine, as internal standard, was added and briefly vortexed. Subsequently 30 μL of ethanol:pyridine solution (75:25, v/v) and 30 μL of reagent solution consisting of a mixture of ethylchloroformate, chloroform and n-hexane (in the ratio 20:70:10, v/v) was added for derivatization [14, 15]. The newly formed derivative was extracted with 500 μL of ethyl acetate, and then centrifuged (14,000 × g/15 min). Supernatant was transferred to a fresh vial and evaporated to dry under nitrogen flow. Dry extract was reconstituted with 60 μL of acetonitrile and placed into insert vial for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, conducted using Nexera UHPLC system coupled to liquid chromatography-mass spectrometry (LCMS)-8030 triple quadripole mass spectrometer (Shimadzu, Japan).

Statistical analysis

Group comparisons were obtained using parametric tests (Analysis of variance) and nonparametric (Mann-Whitney *U* test and Kruskall-Wallis test). The relationship between two variables was tested using Spearman's correlation. Receiver operating characteristic (ROC) analysis was used to estimate significant diagnostic test. Youden index was used to obtain an optimal cut-off point. All *p* values less than 0.05 were considered significant. The results are presented depending on data type as count (percent), medians (range) and mean (standard deviation - SD), in tables and box-plot (50% of the data are summarized in the box; the line in each box shows the median; lines outside of each box represent the 90% confidence interval). The obtained data were statistically analyzed using SPSS 20.0 (IBM corp.) and R for windows 3.3.0 (CRAN project) statistical software.

Results

The range of measurement for 5-HT concentrations was 0.08–143.6 ng/mL in all samples. The medians (range) and means ± SD of the 5-HT levels (ng/mL) for CSF and serum are presented in Table 2. There was no correlation between central CSF and peripheral serum levels, and central and lumbar CSF 5-HT levels (Table 3). Statistical significance was not found between the levels of the latter.

No gender-related differences of central CSF 5-HT levels were detected ($U = 553.000$; $p = 0.232$) (Table 3.). The subjects were 51.77 ± 14.26 years old (range 18-87 years). The differences in 5-HT levels among age groups were highly statistically significant ($\chi^2 = 13.354$; $p = 0.001$), with tendency to decrease with age. Even though levels of 5-HT increased with postmortem interval (Table 3), no statistically significant difference was detected ($\chi^2 = 1.572$; $p = 0.456$). The difference was not statistically significant between very short and delayed agony interval ($U = 708.000$, $p = 0.650$, Table 4). There was no statistically significant difference in 5-HT levels among subjects with and without awareness of lethal event ($U = 711.000$; $p = 0.775$, Table 4).

Table 2

Serotonin levels (ng/mL) in the cerebrospinal fluid (CSF) (central and lumbar) and serum

Body fluid	Median	Range (min–max)	Mean ± SD
Central CSF	2.75	0.08–6.13	2.86 ± 1.43
Lumbar CSF	3.01	1.44–7.36	3.26 ± 1.39
Serum	26.89	3.42–143.57	34.34 ± 29.72

SD – standard deviation.

Table 3

Correlations of serotonin levels (ng/mL) in the cerebrospinal fluid (CSF) and serum

Body fluid	Valid N	r	*p*
Central/lumbar CSF	17	- 0.015	0.955
Central CSF/ serum	46	- 0.095	0.530

Table 4

Serotonin levels in the cerebrospinal fluid (CSF) with regard to gender, age, postmortem interval, agony interval and awareness of the stressful event

Parameter	Serotonin levels in the central CSF (ng/mL)				*p* value
	valid N	median	range (min–max)	mean ± SD	
Gender					
male	58	2.84	0.08–6.13	2.97 ± 1.45	> 0.05
female	23	2.42	0.14–5.17	2.58 ± 1.36	
Age (years)					
< 40	16	3.66	1.85–6.03	3.81 ± 1.20	
41–65	52	2.75	0.08–6.13	2.81 ± 1.39	< 0.05
> 65	13	1.71	0.14–4.89	1.16 ± 1.71	
Postmortem interval (h)					
< 24	58	2.76	0.14–6.13	2.95 ± 1.40	> 0.05
25–48	15	2.75	0.08–3.62	2.33 ± 1.09	
49–72	8	3.42	0.61–5.23	3.22 ± 2.03	
Agony interval					
very short	52	2.72	0.14–6.13	2. 32 ± 1.5	> 0.05
delayed	29	2.84	0.08–5.59	2.73 ± 1.29	
Awareness of the stressful event					
yes	29	2.63	0.14–5.64	2.87 ± 1.51	> 0.05
no	51	2.88	0.08–6.13	2.9 ± 1.38	

SD – standard deviation.

Table 5

Serotonin levels in the cerebrospinal fluid (CSF) with regard to manner and cause of death

Parameters	Serotonin levels in the central CSF (ng/mL)				*p* value
	valid N	median	range (min–max)	mean ± SD	
Manner of death					
natural	31	2.50	0.08–4.72	2.32 ± 1.17	< 0.05
violent	50	3.13	0.14–6.13	3.2 ± 1.50	
accidental	20	3.56	0.14–5.64	3.38 ± 1.66	
suicidal	24	2.72	0.5–6.13	2.91 ± 1.3	> 0.05
homicidal	6	3.18	1.45–6.03	3.76 ± 1.73	
Cause of death					
cardiac	31	2.50	0.08–4.72	2.32 ± 1.13	
hanging	13	2.92	0.50–6.13	2.92 ± 1.52	
drawning	5	3.54	2.04–4.50	3.22 ± 0.99	
blunt injury	7	3.93	1.27–5.18	3.57 ± 1.20	
firearm injury	8	2.67	1.32–6.03	2.79 ± 1.55	> 0.05
stabbing	2	4.37	3.16–5.6	4.37 ± 1.72	
hypothermia	5	2.11	1.23–5.17	2.84 ± 1.56	
electrocution	3	3.51	2.63–5.30	3.82 ± 1.36	
intoxication	7	4.60	0.14–5.64	3.44 ± 2.16	

Concerning the manner of death, CSF 5-HT levels were significantly higher in violent deaths (U = 519.000, p = 0.013). The cut-off value for 5-HT levels in deaths from violent manner was 3.27 ng/mL, with a sensitivity of 46% and specificity of 87.1% (AUC = 0.665 (0.548–0.783); p = 0.013).

There was no statistically significant differences among subgroups of the violent manners (χ^2 = 1.765; p = 0.414), although a lower level of 5-HT was observed in the suicides (Table 5). Regarding the cause of death (Table 5, Figures 1 and 2), 5-HT levels were higher in blunt injury, stabbing and intoxications, while lower in cardiac deaths and hypothermia, however without statistically significant difference (F = 1.478, p = 0.180).

Fig. 1 – Box-plot representing differences in serotonin levels with regard to manner of death (natural/violent).

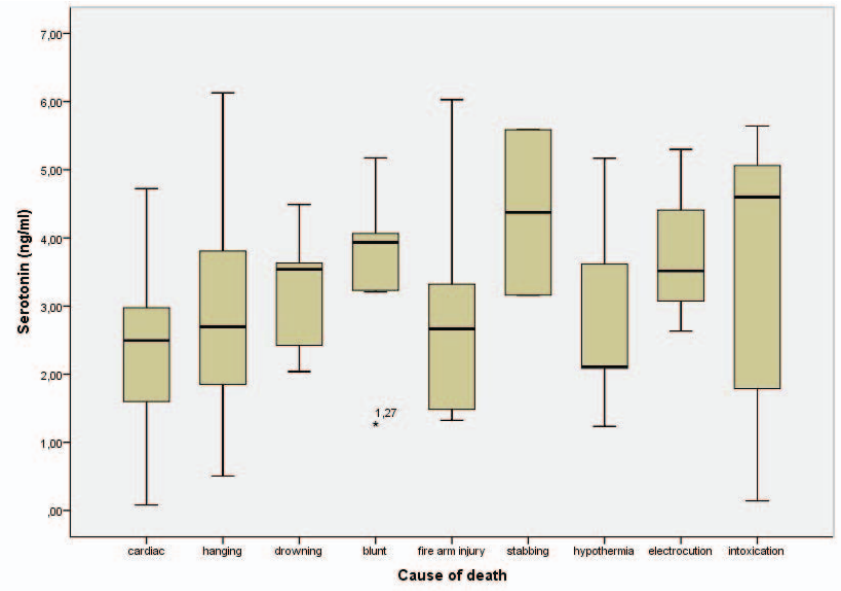

Fig. 2 – Box-plot representing differences in serotonin levels with regard to cause of death.

Discussion

Even if the role and importance of 5-HT were recognized decades ago, still its determination represents a great challenge in clinical and postmortem researches. Apart from interindividual variability in 5-HT activity, the postmortem 5-HT level depends upon many physiological body characteristics such as age, gender, weight, height of subjects, circadian rhythms of the 5-HT secretion, as well as postmortem conditions like postmortem interval, sampling and analytical methods, in detail summarized by Musshoff et al. [6]. For these reasons, there is still no consistency in referent 5-HT levels in literature, especially for its postmortem body fluids` values.

As a hydrophilic substance, 5-HT does not pass lipophilic blood-brain barrier readily, unlike its precursor tryptophan and metabolite 5-hydroxyindoleacetic acid. Hence, 5-HT in CSF mainly originates from the brain, while serum 5-HT is mainly derived from platelets [1, 16]. These mutually independent syntheses could explain the lack of correlation between central CSF and serum 5-HT levels in our results. Moreover, it could also mean that serum 5-HT levels do not reflect brain 5-HT neuroactivity.

The central and lumbar CSF compartments represent parts of a unique liquor system which circulates rostrocaudally through brain ventricles, central and spinal subarachnoidal space [17, 18]. For that reason, the lumbar approach to CSF system is widely used as a clinical diagnostic tool to reflect brain function [19]. Because of the rostrocaudal concentration gradient, in postmortem investigation of brain 5-HT, lumbar approach should not be the exclusive rule – two previous studies [6, 7] both revealed significantly higher values in central CSF. Our study showed no significant correlation between 5-HT central and lumbar levels, moreover, the median and mean levels in lumbar compartment were slightly higher. Excitation and firing activity in 5-HT neurons along the spinal cord, where it is involved in pain control [1, 20, 21], could contribute to such results. Further, our results could be due to differences among sampling sites in regard to postmortem degradation and/or because of diffusion from the surrounding tissues, certain in the process of postmortem decomposition.

What happens with 5-HT through the course of postmortem interval, apart from degradation/diffusion, is actually unclear – our study revealed the tendency of an increase in 5-HT levels, however without statistical significance. Previously, Quan et al. [5] observed the same, but with statistical significance.

The postmortem brain content of neurotransmitters can generally be determined through autopsy on two levels: in samples of brain tissue from exact brain areas and in samples of CSF which can be reached in its central compartments or by lumbar puncture [19]. It should be kept in mind, that CSF concentrations present only an average of neurotransmitters' levels from all brain regions in the moment of sampling and therefore cannot obtain information about functions of exact brain areas and neuronal groups. Even though the serotonergic system is involved in stress and worrying [22], and firing begins almost immediately**,** the previous might explain why 5-HT levels in CFS samples observed in the groups of very short and delayed interval between the onset of injury and moment of death, did not show any difference.

What we additionally tried to investigate herein is whether the magnitude of stress (caused by acute life threatening event) could somehow influence the 5-HT levels; for this purpose we divided subjects to those who were/were not aware of impending lethal danger. No differences were observed, moreover, mean values were very similar.

This study revealed significantly higher 5-HT levels in violent deaths than in natural ones. For this purpose, the cut-off value for distinguishing higher levels in violent manner was estimated at 3.27 ng/mL 5-HT. The area under the curve was small, but statistically significant. However, for the fur-

ther distinction among the groups of violent manner (i.e. accidental, homicidal and suicidal) our statistics did not reveal any significant differences, although with lower levels in suicides. Musshoff et al. [6] reviewed statistical significant decrease in 5-HT levels in suicides, while Kauert et al. [7] reviewed different.

Even though forensic approach to death clearly distinguishes mechanism of dying through different causes of death, our study on 5-HT CSF levels failed to prove these distinctions. Albeit the results apparently reveal lower 5-HT levels in cardiac death and hypothermia, higher in blunt injury and stabbing and the highest in carbon monoxide (CO) intoxication, there was a huge overlapping among these values. Unlike ours, previous studies showed remarkable and large differences among different causes of death [4–6].

Conclusion

The presented study determined central CSF as a body fluid of choice for postmortem measurements of serotonin neuronal activity. It also demonstrated that serotonin levels are markedly increased in violent deaths compared to natural ones, therefore serotonin could be a postmortem marker to distinguish natural and violent deaths, regardless of individual variability in concentrations.

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