Original Article

The psychological autopsy: An overview of its utility and methodology

Divya Bhushan[1], Jayanthi Yadav[1], Abhijit Ramdas Rozatkar[2], Sangita Moirangthem[1], Arneet Arora[1]

Departments of [1]Forensic Medicine and Toxicology and [2]Psychiatry, All India Institute of Medical Sciences Bhopal, Madhya Pradesh, India.

ABSTRACT

Objectives: The objective of this study is to provide an overview of the psychological autopsy (PA) research method, including its methodology, uses, limitations, and ethical considerations.

Materials and Methods: The study conducted a PA investigation on 35 cases of suicide. Information was collected from multiple sources and reliable informants, including family members, friends, medical and mental health professionals, and other relevant individuals. Qualitative and quantitative research methods were used to analyze the collected information.

Results: The results indicated that several factors were associated with suicide, including mental health problems, life stressors, interpersonal conflicts, substance abuse, and history of previous suicide attempts. The findings have important implications for suicide prevention strategies, emphasizing the significance of addressing mental health issues and providing social support.

Conclusion: The PA is a valuable research method for investigating and understanding suicide. Despite challenges such as recall biases and methodological limitations, it provides insights into the psychological factors associated with suicide and informs suicide prevention strategies. However, conducting psychological autopsies requires careful consideration of ethical issues. Further research is needed to replicate and extend the findings of this study

Keywords: Suicidal case, Psychological autopsy, Autopsy, Post-mortem examination

INTRODUCTION

A psychological autopsy (PA) is the reconstruction of events leading to death; ascertainment of the circumstances of the death, including suicidal intent; and an in-depth exploration of other significant risk factors for suicide.[1-5] Psychological autopsies were originally designed to investigate, clarify, and help police enquiries into the mode of death in equivocal fatalities. However, in recent years, they have become more often utilized as a research tool for investigating risk factors for completed suicides. Suicide is a major public health concern worldwide, and it is responsible for a significant number of deaths annually. In India, suicide is a major public health issue with the national suicide rate 12.[6] The reasons for suicide are complex and multi-factorial and it is essential to identify the risk factors to develop effective preventive strategies.

PA is a valuable tool for understanding the complex and multidimensional nature of suicide.[7] It involves the systematic and comprehensive exploration of the deceased person's life to determine the psychological, social, and environmental factors that contributed to their suicide.[8] Mental diseases, drug abuse, psychological states, cultural, family and social situations, genetics, trauma or loss experiences, and nihilism are all factors that influence the likelihood of suicide.[9-12] Substance abuse and mental illness frequently coexist.[13] Other risk factors include having attempted suicide before,[14] having a means to end one's life readily available, having a family history of suicide, or having traumatic brain damage.[15] This method is particularly useful when the individual has not sought mental health treatment or when their medical history is unavailable.[16]

There are multiple researches on PA of suicide victims in our country. According to a study done in the North-west region of India on 101 suicidal cases, the majority of suicides were in the age group of 20–29 years and male outnumbered the female, hanging was the most common method used by suicide victims. Psycho-social stressors were found in 60% cases and psychiatric illness was found in 34% cases.[17]

Despite the fact that multiple PA studies have been published from India, no attempt has been made to thoroughly examine the existing literature. Lack of standardized techniques or methods of PA, informant bias, lack of interviewer training, recall bias, and issues with control selection are some of the major methodological flaws that have led to legal questions about the admissibility of PA evidence.[18,19] This study was conducted after reviewing previously done studies on PA with attempts to minimize the challenges faced in previous studies. PA on 35 cases of suicide was conducted using a structured, self-designed, and close-ended questionnaire to identify the common risk factors associated with suicide in this population.

MATERIALS AND METHODS

The study was approved by Institutional human ethics committee of All India Institute of Medical Sciences Bhopal (AIIMS) Bhopal vide LOP letter no 2020/PG/Jan/09. The study was funded by Indian Council of Medical Research. Cases included all eligible deceased bodies that arrived at Mortuary of AIIMS, Bhopal for autopsy from November 2020 to May 2022 and fulfilled the inclusion and exclusion criteria. The sampling method was convenient sampling, which resulted in 35 suicidal cases. Exclusion criteria included relatives not willing to give consent, decomposed bodies, and any case in which reliable informant was not available. The methodology for this study involved the use of a structured, self-designed, close-ended questionnaire to gather information from close relatives or next of kin of the deceased brought for medico-legal autopsy at the mortuary of AIIMS Bhopal. Informed consent was obtained beforehand, and if the next of kin or close relatives were not available, a telephonic call was arranged to obtain appropriate answers. The information was collected by more than one close relatives separately to avoid recall and informant bias. Efforts were made to collect information from persons who were living closely with the deceased. The questionnaire covered the general demographic details and tries to find out the type of psychiatric illness present along with the past history of suicidal attempt and drug abuse. A total of 29 questions were asked during the PA process [Table 1].

We have categorized the 29 questions into seven different categories with following categorization-

1. Diagnosed psychiatric disorder: This category includes questions related to the diagnosis of a psychiatric disorder, such as bipolar disorder, major depressive disorder, and schizophrenia. The questions in this category may ask about the presence of specific symptoms that are associated with a particular disorder or about the diagnosis itself
2. Past suicidal behavior: This category includes questions related to any previous suicidal thoughts,

plans or attempts. The questions in this category may ask about the frequency, intensity, and timing of these behaviors
3. Depressive symptoms: This category includes questions related to the presence and severity of depressive symptoms, such as feelings of sadness, hopelessness, and worthlessness. The questions in this category may also ask about changes in appetite and sleep patterns
4. Anxiety related symptoms: This category includes questions related to the presence and severity of anxiety-related symptoms, such as excessive worry, panic attacks, and social anxiety. The questions in this category may also ask about physical symptoms, such as sweating, trembling, and palpitations
5. Psychotic symptoms: This category includes questions related to the presence and severity of psychotic symptoms, such as hallucinations, delusions, and disorganized thinking. The questions in this category may also ask about changes in behavior, such as social withdrawal and agitation
6. Presence of stressor: This category includes questions related to the presence of a recent or ongoing stressful event, such as a death of a loved one, a divorce, or a job loss. The questions in this category may also ask about coping strategies and social support
7. Presence of substance abuse: This category includes questions related to the presence and severity of substance abuse, such as alcohol and drug use. The questions in this category may also ask about the frequency and quantity of substance use, as well as any negative consequences associated with use.

Statistical analysis

All the data collected from the questionnaire were entered in Microsoft Excel sheet and the results were computed from the spreadsheet.

RESULTS

During the study duration, total 249 autopsies conducted at the mortuary of AIIMS Bhopal. Out of those 249 cases PA was done on total 35 suicidal cases; those met the inclusion criteria and rest 214 cases excluded on the basis of exclusion criteria. Out of the 35 cases of suicide, 24 (69%) cases were male and 11 (31%) cases were female. The age distribution of the cases was as follows: 15 cases (43%) were in the 19–30 years age group, 13 cases (36%) were in the 31–40 years age group, followed by 2 (6%) cases in each 41–50 years age group, 51–60 years age group, and in <18 years age group bracket. The least incidence was 1 case (3%) which was in more than 60 years age group. The median age of the suicidal

group was 30 years (interquartile range 21.50–34.50), ranging from 16 years to 65 years [Table 2].

Regarding occupation, 11 (32%) were involved in skilled work, and an equal number of 12 (34%) cases were involved in manual labor work. Students accounted for 5 (14%) cases, and 7 cases (20%) were unemployed. In terms of socioeconomic status, the majority of cases, 16 (46%), of suicide victims belonged to the middle class, 8 (23%) cases to the lower-middle class, 6 (17%) cases to the upper-middle class, 4 (11%) cases to the lower class, and only 1 (3%) case to the upper class [Table 2].

The majority of cases, 22 (63%) were married, whereas only 13 (37%) cases were unmarried. In terms of education, 12 (34%) cases were studied till primary or below primary level, 8 cases (23%) were of secondary level, 9 cases (26%) managed to complete their graduation and post-graduation level, and 6 cases (17%) were illiterate.

Out of the total cases of suicide studied, 30 (86%) persons had their home as their preferred place for the commission of suicide. The remaining only 5 (14%) persons had committed suicide at places other than home. Violent methods of suicide such as hanging were used by 24 (69%) persons. Non-violent methods were used by 11 (31%) persons who poisoned themselves. Out of total 35 cases of suicide that were studied, 16 (46%) cases committed suicide using cloth ligature such as saree and gamacha, 8 (23%) cases committed suicide using ligature material other than cloth such as wire, rope, and belt, and 11 (31%) cases committed suicide by ingestion of poison.

Out of the total cases investigated, 77% of the cases had one or more depressive symptoms. Substance abuse history was present in 51% of the cases, and 37% of the cases had a history of past suicidal behavior. A total of 11% of the cases had a diagnosed psychiatric illness, while 17% and 3%

Table 1: Questionnaire for psychological autopsy.
1. Was the deceased diagnosed with any psychiatric disorder/Chronic debilitating disease?
2. Was the deceased under any treatment for psychiatric disorder/Chronic debilitating disease?
3. Has the decreased ever taken treatment for his behavior problems?
4. Was there any history of previous suicide attempt?
5. Was there any circumstance evident of suicide plan?
6. Was there any history of the deceased hurting himself/herself in the past?
7. Did the deceased leave any suicide note?
8. Did the deceased have personal history of legal trouble?
9. Was there any History of withdrawal from family, friend or society?
10.Did the deceased have any history of abuse like Emotional/Physical/Sexual?
11.Did the deceased have any History of neglect by the family member/neighborhood/relatives/friends/teachers/work place?
12.Did the deceased have any history of broken relationship?
13.Did the deceased have any problem of sadness of mood or being irritable for minor/no reason in the past 2 weeks preceding the suicide?
14.Did the deceased show loss of pleasure in activities that he enjoyed in the past 2 weeks preceding the suicide?
15.Did the deceased express any unusual feeling of tiredness in the past 2 weeks preceding the suicide?
16.Was the deceased anxiousness for some reason with palpitations, sweating, tremors, or chocking sensation in the past 2 weeks preceding the suicide?
17.Did the deceased have any difficulty in concentration or recollection in the past 2 weeks preceding the suicide?
18.Did the deceased express guilt for events that affected his loved one in the past 2 weeks preceding the suicide?
19.Did the deceased ever complain of difficulty in getting sleep in the past 2 weeks preceding the suicide?
20.Did the deceased have any changes in his appetite in the past 2 weeks preceding the suicide?
21.Did the deceased ever mentioned that as an individual his life was a failure or that it is not possible for him to achieve anything in life, in the past 2 weeks preceding the suicide?
22.In the past 2 weeks preceding the suicide, did the deceased express desire that his/her life should end?
23.Did the deceased ever complain that he could hear voices in their ear but could not see who was talking in the past 2 weeks preceding the suicide?
24.Did the deceased express unusual thoughts that some people are planning to get him killed or harm him in some way in the past 2 weeks preceding the suicide?
25.Did the deceased express unusual thoughts that some people are watching him wherever he goes or that events around are happening with reference to him in the past 2 weeks preceding the suicide?
26.Did the deceased express unusual thoughts that people around could know what he was thinking without ever speaking to them/or that his thoughts were known to all people or that thoughts of others could enter into his mind in the past 2 weeks preceding the suicide?
27.Did the deceased consume excess amount of alcohol and/or sleep medications in the past 2 weeks preceding the suicide?
28.Did the deceased consume excess amounts of drugs like cannabis/opium etcin the past 2 weeks preceding the suicide?
29.Did the deceased consume excess amount of nicotine products in the past 2 weeks preceding the suicide?

of the cases had anxiety-related symptoms and psychotic symptoms, respectively.

In addition, 31% of the cases had a history of stressors related to suicide, these include relationship problems, financial problems and employment issues, legal problems, and health issues [Table 3].

These findings suggest that there is a need for mental health support and intervention for individuals experiencing depressive symptoms, substance abuse, and suicidal behavior. In addition, identifying and addressing life stressors, particularly relationship and financial problems, may reduce the risk of suicide. The results also highlight the importance of promoting suicide prevention and mental health awareness.

DISCUSSION

The present study provides a comprehensive analysis of 35 cases of suicide through a PA approach. This study was conducted after reviewing previously done studies on PA with attempts to minimize the challenges faced in the previous studies. In this study, a self-designed structured close ended questionnaire was designed with the help of

Table 2: Age distribution and socioeconomic status in suicidal cases.

Age distribution	Age (in years)	No. cases (%)
	<18	2 (6)
	19–30	15 (43)
	31–40	13 (36)
	41–50	2 (6)
	51–60	2 (6)
	>60	1 (3)
Socioeconomic status	Class	No. of cases (%)
	Lower class	4 (11)
	Lower middle class	8 (23)
	Middle class	16 (46)
	Upper middle class	6 (17)
	Upper class	1 (3)

Table 3: Psychological autopsy.

Precipitating factor	No. of cases			Percentage of cases
	M	F	Total	
Diagnosed psychiatric disorder	2	2	4	11
Past suicidal behavior	10	3	13	37
Depressive symptoms	18	9	27	77
Anxiety-related symptoms	4	2	6	17
Psychotic symptoms	1	0	1	3
Presence of stressor	8	3	11	31
Presence of substance abuse	18	0	18	51

psychiatrist and the same questionnaire was administered to all the participants to remove. The information was collected by more than one close relatives separately to avoid recall and informant bias. Efforts were made to collect information from persons who were living closely with the deceased so that accurate answers could be obtained. All the interviews were taken by a single person to avoid interviewer's bias. Males made up 69% of the subjects who committed suicide in the present study, compared to females who made up 31%. In our analysis, the third decade had the highest rate of suicides (43%), followed by the fourth decade. Similar results have been obtained by Indian researchers Chavan *et al.*[17] and others.[20-25] Ponnudurai *et al.*[26] came to the conclusion that the second and third decades of life appear to be the most vulnerable time for suicides after evaluating 12 papers on suicidology from various regions of India.

The results of the study revealed that a high proportion of the individuals who died by suicide had depressive symptoms, which is consistent with previous research indicating that depression is a major risk factor for suicide.[27,28] There is lack of seeking psychiatric help for depression as state of depression is not readily observed by family members and the patient is not in a state to seek medical help by himself. Substance abuse history and past suicidal behavior were also identified as risk factors for suicide.[29,30]

Interestingly, a relatively small proportion of the individuals who died by suicide in this study had a diagnosed psychiatric illness. This may suggest that individuals who experience suicidal ideation or behavior may not always seek or receive mental health treatment, or may not receive an accurate diagnosis.[31] In addition, a higher proportion of the individuals had stressors related to suicide, indicating that adverse life events may play a role in the development of suicidal ideation and behavior.[32,33] The majority of the individuals who died by suicide were male and married.[34]

The study also found that hanging was the most common method of suicide, followed by ingestion of poison.[35] Overall, the results of the study suggest the need for early identification and treatment of depressive symptoms, substance abuse, and past suicidal behavior, as well as effective management of stressors related to suicide. In addition, it highlights the importance of targeted suicide prevention strategies for high-risk groups such as young adults and men. The study provides valuable insights into the risk factors associated with suicide, which can inform the development of effective suicide prevention and intervention strategies. The PA has been used to investigate a range of questions related to suicide, including the identification of risk factors, the effectiveness of prevention strategies, and the evaluation of mental health services. It can also be used to provide information to the family and friends of the deceased, which can aid in the grieving process and help to prevent future suicides.

Limitations

The accuracy and validity of the information gathered through the PA can be influenced by a number of factors, including biases, limitations of the data sources, and methodological limitations. For example, family members and friends may have incomplete or biased information about the person's life, and there may be limitations to the information that can be gathered from medical and mental health records. Limited number of the participants was also one of the limitations of study.

CONCLUSION

The present study sheds light on the various risk factors associated with suicide. The majority of cases had a history of depression and substance abuse, highlighting the importance of timely screening and treatment for these conditions. Past suicidal behavior was also found to be a risk factor, emphasizing the need for follow-up and monitoring of individuals with a history of suicidal behavior. The study also highlights the importance of identifying and addressing stressors related to suicide.

The study also highlights the need for improving mental health literacy and access to mental health services, particularly in lower socioeconomic groups and those with lower educational levels. The study findings underscore the need for a comprehensive and multidisciplinary approach to suicide prevention, including screening and treatment of mental health conditions, identification and management of stressors, and awareness campaigns to improve mental health literacy. However, the accuracy and validity of the information gathered through this method can be affected by biases and methodological limitations. Furthermore, conducting psychological autopsies requires careful consideration of ethical issues and concerns. Future research is needed to identify effective interventions and strategies for suicide prevention, particularly in high-risk populations. Overall, the PA remains a valuable tool for investigating and understanding suicide.

Declaration of patient consent

Institutional Review Board (IRB) permission obtained for the study.

Financial support and sponsorship

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Khan FA, Anand B, Devi MG, Murthy KK. Psychological autopsy of suicide-a cross sectional study. Indian J Psychiatry 2005;47:73-8.
2. Beautrais AL, Joyce PR, Mulder RT. Risk factors for serious suicide attempts among youths aged 13 through 24 years. J Am Acad Child Adolesc Psychiatry 1996;35:1174-82.
3. Värnik P. Suicide in the world. Int J Environ Res Public Health 2012;9:760-71.
4. Cooper J. Ethical issues and their practical application in a psychological autopsy study of suicide. J Clin Nurs 1999;8:467-75.
5. Kelly TM, Mann JJ. Validity of DSM-III-R diagnosis by psychological autopsy: A comparison with clinician ante-mortem diagnosis. Acta Psychiatr Scand 1996;94:337-43.
6. NCRB Report 2021: Suicides on the Rise, Experts on what can be Done. Available from: https://indianexpress. com/article/lifestyle/health/ncrb-report-2021-death-by-suicide-rate-increase-mental-health-experts-realistic-tips-awareness-8120292 [Last accessed on 2023 Mar 14].
7. Isometsä ET. Psychological autopsy studies--a review. Eur Psychiatry 2001;16:379-85.
8. Linehan MM, Goodstein JL, Nielsen SL, Chiles JA. Reasons for staying alive when you are thinking of killing yourself: The reasons for living inventory. J Consult Clin Psychol 1983;51:276-86.
9. Risk and Protective Factors; 2021. Available from: https://www.cdc.gov/suicide/factors/index.html [Last accessed on 2022 Apr 15].
10. Hawton K, Saunders KE, O'Connor RC. Self-harm and suicide in adolescents. Lancet 2012;379:2373-82.
11. Zalsman G, Hawton K, Wasserman D, van Heeringen K, Arensman E, Sarchiapone M, *et al.* Suicide prevention strategies revisited: 10-year systematic review. Lancet Psychiatry 2016;3:646-59.
12. Suicide. In: Wikipedia; 2022. Available from: https://en.wikipedia. org/w/index.php?title=suicide&oldid=1082485574 [Last accessed on 2022 Apr 15].
13. Vijayakumar L, Kumar MS, Vijayakumar V. Substance use and suicide. Curr Opin Psychiatry 2011;24:197-202.
14. Chang B, Gitlin D, Patel R. The depressed patient and suicidal patient in the emergency department: Evidence-based management and treatment strategies. Emerg Med Pract 2011;13:1-23; quiz 23-4.
15. Simpson G, Tate R. Suicidality in people surviving a traumatic brain injury: Prevalence, risk factors and implications for clinical management. Brain Inj 2007;21:1335-51.
16. Sveen CA, Walby FA. Suicide survivors' mental health and grief reactions: A systematic review of controlled studies. Suicide Life Threat Behav 2008;38:13-29.
17. Chavan BS, Singh GP, Kaur J, Kochar R. Psychological autopsy of 101 suicide cases from northwest region of India. Indian J Psychiatry 2008;50:34-8.
18. Snider JE, Hane S, Berman AL. Standardizing the psychological autopsy: Addressing the Daubert standard. Suicide Life Threat Behav 2006;36:511-8.
19. Hjelmeland H, Dieserud G, Dyregrov K, Knizek BL, Leenaars AA. Psychological autopsy studies as diagnostic tools: Are they methodologically flawed? Death Stud 2012;36:605-26.
20. Hegde RS. Suicide in rural community of North Karnataka. Indian J Psychiatry 1980;22:368-70.

21. Shukla GD, Verma BL, Mishra DN. Suicide in Jhansi city. Indian J Psychiatry 1990;32:44-51.

22. Ponnudurai R, Jayakar J. Suicide in Madras. Indian J Psychiatry 1980;22:203-5.

23. Sathyavathi K, Murti Rao DL. A study of suicide in Bangalore. Indian J Soc Work 1973;34:101-6.

24. Rao AV, Mahendran N, Gopalakrishnan C, Reddy TK, Prabhakar ER, Swaminathnan R, *et al.* One hundred female burns cases: A study in suicidology. Indian J Psychiatry 1989;31:43-50.

25. Jha P, Ramasundarahettige C, Landsman V, Rostron B, Thun M, Anderson RN, *et al.* 21st-century hazards of smoking and benefits of cessation in the United States. N Engl J Med 2013;368:341-50.

26. Ponnudurai R, Patnaik KA, Sathianathan R, Subhan K. A study on the venues of suicide. Indian J Psychiatry 1997;39:34-6.

27. Cavanagh JT, Carson AJ, Sharpe M, Lawrie SM. Psychological autopsy studies of suicide: A systematic review. Psychol Med 2003;33:395-405.

28. Hegerl U, Althaus D, Schmidtke A, Niklewski G. The alliance against depression: 2-year evaluation of a community-based intervention to reduce suicidality. Psychol Med 2006;36:1225-33.

29. Borges G, Nock MK, Abad JM, Hwang I, Sampson NA, Alonso J, *et al.* Twelve-month prevalence of and risk factors for suicide attempts in the World Health Organization World Mental Health Surveys. J Clin Psychiatry 2010;71:1617-28.

30. Lesage AD, Boyer R, Grunberg F, Vanier C, Morissette R, Ménard-Buteau C, *et al.* Suicide and mental disorders: A case-control study of young men. Am J Psychiatry 1994;151:1063-8.

31. Beautrais AL, Joyce PR, Mulder RT. Psychiatric contacts among youths aged 13 through 24 years who engage in multiple episodes of self-harm. Psychiatr Serv 1999;50:1619-21.

32. Hawton K, van Heeringen K. Suicide. Lancet 2009;373:1372-81.

33. Mann JJ, Apter A, Bertolote J, Beautrais A, Currier D, Haas A, *et al.* Suicide prevention strategies: A systematic review. JAMA 2005;294:2064-74.

34. Luoma JB, Martin CE, Pearson JL. Contact with mental health and primary care providers before suicide: A review of the evidence. Am J Psychiatry 2002;159:909-16.

35. Vijayakumar L, Satheesh-Babu R, Nagaraj K. Suicide prevention: The need of the hour. Indian J Psychiatry 2010;52:153-7.

◀ (authordash.php)

Title: Validated Ultraviolet High Performance Liquid chromatography method for Post-mortem 5-hydroxy-indoleacetic acid measurement in human cerebrospinal fluid.

Decision correspondence

Decision: **Accept**
Date of decision: 2023-08-01
Decision email title: Accept manuscript
Decision email text: Manuscript: JLP-2023-3-4/R1 RESUBMISSION - (1654) - Validated Ultraviolet High Performance Liquid chromatography method for Post-mortem 5-hydroxy-indoleacetic acid measurement in human cerebrospinal fluid.

Date submitted: 2023-07-31

Dear Dr. Yadav

Congratulations! It is a pleasure to inform you that your manuscript is now acceptable for publication in Journal of Laboratory PhysiciansIndia. However, in order to proceed further with the publication of your article, we would kindly request you to submit the completely filled and duly signed Copyright Form, if not done already. Please note that this is a mandatory step without which, we won't be able to move your article into production. The Copyright Form can be downloaded from the journal login page of the submission portal jlp.manuscriptmanager.net. Kindly send the Copyright form to Aditi Saxena at aditi.saxena@thieme.in.

We hope you will continue to submit work from your group to the Journal of Laboratory Physicians in the future and thank you for your valuable contribution .

Sincerely,

Prof. Sarman Singh
Editor-in-Chief
sarman.singh@gmail.com
Journal of Laboratory Physicians

Company
About (https://www.ManuscriptManager.com/#aboutus)
Offices (https://www.ManuscriptManager.com/#aboutus)

Support
ONLINE HELP (https://manuscript-manager.elevio.help/en)
ASK A QUESTION (contactform.php)
0.004653

Session
Jayanthi Yadav
Role: Author
[Sign out] (logout.php)

Title - **Validated Ultraviolet High - Performance Liquid chromatography method for Post-mortem 5-hydroxy-indoleacetic acid measurement in human cerebrospinal fluid**

Authors

1. **Dr. Divya Bhushan MD**
   Senior resident , Department Forensic Medicine And Toxicology, AIIMS Bhopal, Madhya Pradesh. Email- bhushandivya1993@gmail.com
2. Dr. Jayanthi Yadav, MD
   Professor Department Forensic Medicine And Toxicology, AIIMS Bhopal, Madhya Pradesh. Email-jayanthi.fmt@aiimsbhopal.edu.in
3. Dr Arneet Arora MD
   Professor and head Department Forensic Medicine and Toxicology, AIIMS Bhopal, Madhya Pradesh. email-head.fmt@aiimsbhopal.edu.in
4. Dr. Dhruv Agrawal PhD
   Assistant Professor. Department of Medical Laboratory Sciences, LPU, Phagwara, Punjab. Email- dhruvagrawal782@gmail.com
5. Dr. Achala Apte PhD
   Research Scholar. Department of Biological Sciences, IISER, Bhopal, Madhya Pradesh. email-apte1achala@gmail.com

Corresonding author – Dr. Jayanthi Yadav, MD, Professor Department Forensic Medicine and Toxicology, AIIMS Bhopal, Madhya Pradesh.
Address -Department Forensic Medicine and Toxicology, 3rd rd floor , Sardar Vallabh Bhai Patel Bhawan, AIIMS Bhopal,462020 Madhya Pradesh. India
email-jayanthi.fmt@aiimsbhopal.edu.in Phone no +91- 9009825352

No of fig – 2

No of words in manuscript -2086

Author's contribution

1. **Dr. Divya Bhushan – inception of idea and carrying out the work and manuscript preparation**
2. Dr. Jayanthi Yadav - responsible for inception of idea, Guidance and supervision and writing the manuscript
3. Dr Arneet Arora -Supervision, and facilitation for work and editing the manuscript
4. Dr. Dhruv Agrawal- guidance and supervision in analyzing the data
5. Dr. Achala Apte - carrying out the lab work and analyzing data .

The study was approved by Institutional human ethics committee of AIIMS Bhopal vide LOP letter no 2020/PG/Jan/09 dated Nov 21, 2020
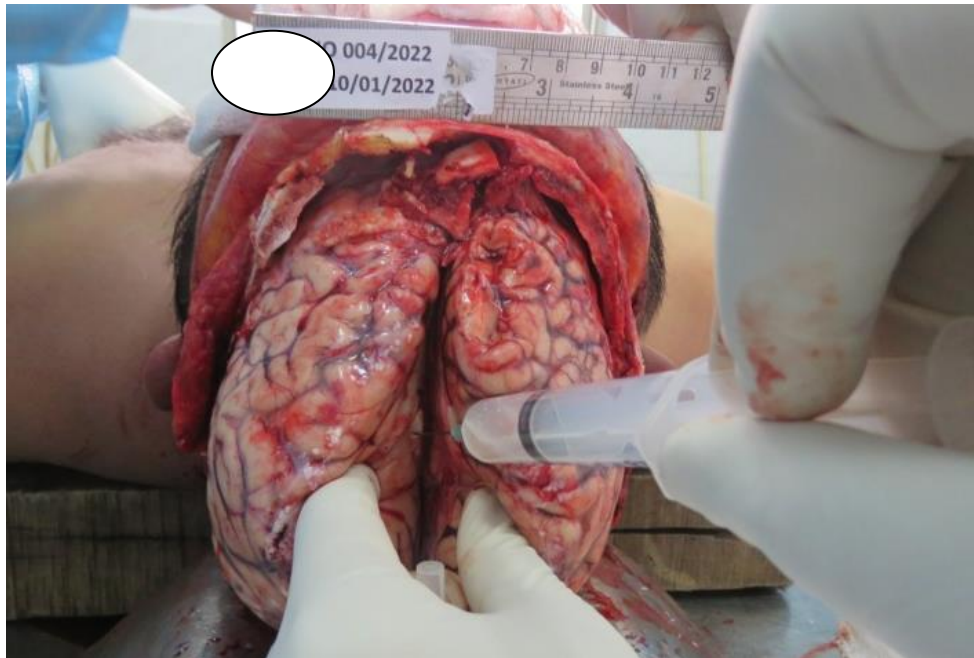
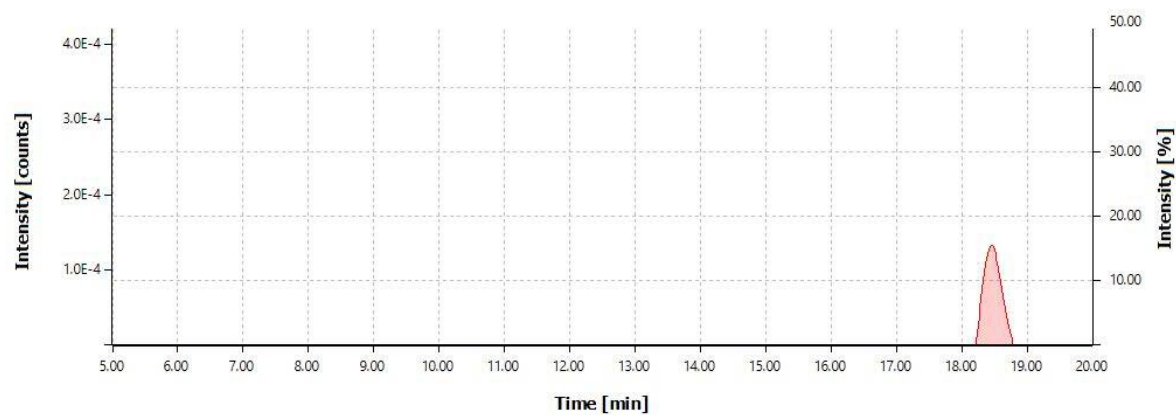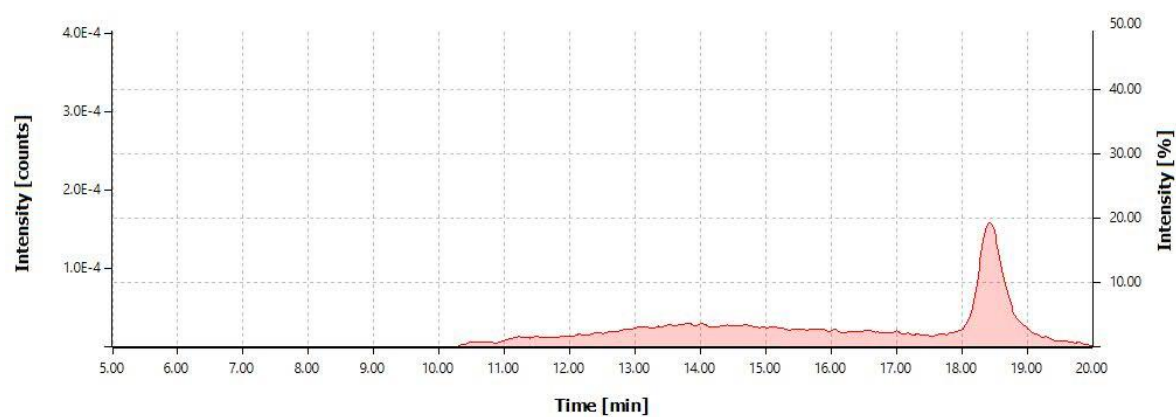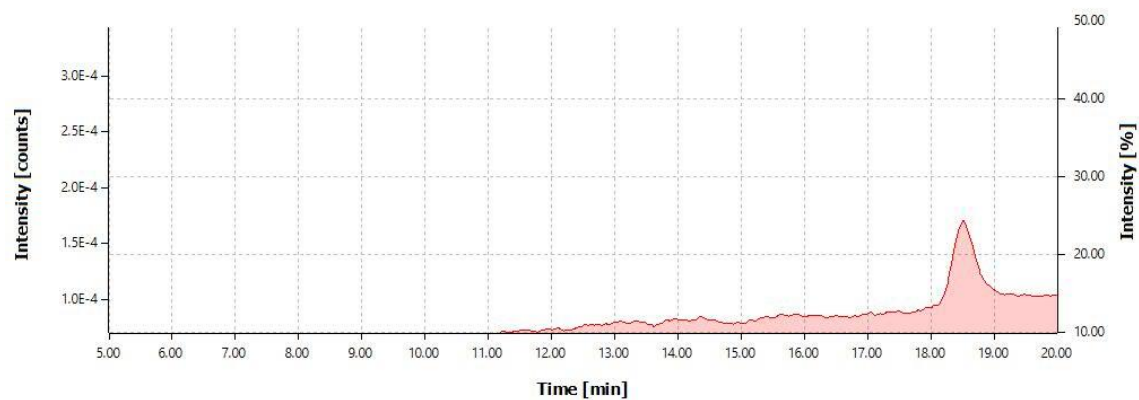**Figure1: Collection of CSF during autopsy after opening the skull**

Fig 2. 5 HIAA concentration graph at 100, 150 and 200 nM.

Title - Validated Ultraviolet High-Performance Liquid chromatography method for Post-mortem 5-hydroxy-indoleacetic acid measurement in human cerebrospinal fluid

Abstract:

Objective: The objective of this study was to develop a UV-HPLC method for the measurement of 5-hydroxyindoleacetic acid (5-HIAA) in human cerebrospinal fluid (CSF) as a potential biomarker for neurological and psychiatric illnesses, including depressive disorders with suicidal behavior.

Methods: The study utilized CSF samples from individuals brought for medico-legal autopsy. The 5-HIAA concentration was measured using a UV-HPLC method with 3 mobile-phase solvents. The most effective mobile phase solvent was then used to measure 5-HIAA in the CSF samples.

Materials: The materials used in the study included CSF samples obtained from brought for medico-legal autopsy, UV-HPLC equipment, and mobile phase solvents, including 5-Hydroxyindole-3-acetic acid (Merck/sigma), Acetonitrile, Concentrated formic acid, Concentrated Acetic acid, Methanol, phosphoric acid.

Statistical analysis: This was done using R Studio (version 4.2.0).

Results: The study found that the UV-HPLC method utilizing formic acid (0.05-0.1%): acetonitrile in a 1:9 was the most effective for measuring 5-HIAA in human CSF. The method exhibited excellent linearity, accuracy, and precision.

Conclusion: The study concludes that the developed UV-HPLC method is reliable and effective for measuring 5-HIAA in human CSF. Measuring 5-HIAA levels in CSF can serve as a potential biomarker for neurological and psychiatric illnesses, including depressive disorders with suicidal behavior. This method is promising for clinical and forensic practice to diagnose suicidal cases. Further research is needed to determine the clinical significance of these findings and the potential for broader application in psychiatry.The paper helps to give a practical, cost-effective methodology to detect CSF 5 HIAA.

Introduction:-

The cerebrospinal fluid (CSF) is a natural route by which many transmitter metabolites leave the brain(1). Cerebrospinal fluid is a clear fluid formed as an ultrafiltrate of plasma. The spinal and intracranial compartments both contain CSF. It circulates along CSF channels in the subarachnoid space of the brain and spinal cord. The choroid plexus continually secrete it consistently inside the brain's ventricles. The average adult's CSF contains about 140 mL and 500–700 mL of CSF is secreted daily, or 0.2–0.7 mL per minute. The primary function of the CSF is to reduce the buoyancy of the brain. Additionally, it provides nutrients and helps eliminate several substances, including cells, neurotransmitters, amino acids, and metabolic waste materials.

Several studies have suggested that CSF metabolites, in particular homovanillic acid and 5-hydroxyindoleacetic acid (5-HIAA), reflect the turnover of their parent amines in the brain(2)(3). 5-Hydroxyindoleacetic acid (5-HIAA) is the final metabolite of serotonin(4). The CSF compartment, which is anatomically connected to the brain, is a valuable source for tracking the number of vital biomarkers that are known to have a role in the pathophysiology of CNS illnesses(5) and suicidal cases (6). Reversed-phase high-performance liquid chromatography (rp HPLC), microdialysis, positron emission tomography tracers, etc., are widely used methods for analysing the metabolites in CSF(7). Measurement of serotonin

44 metabolite in CSF is used for the diagnosis of various neurological and psychiatric illnesses(8). Analysis

45 for CSF from autopsy samples requires unique methods for collection and analysis. The present study

46 involves the development of the HPLC-UV method for the estimation of CSF 5- HIAA, along with the

47 collection and optimisation of the sample preparation technique. For the measurement of ventricular 5-

48 HIAA, we have used high-performance liquid chromatography with UV detection using Waters 2489

49 UV/Visible detector.

Materials:

51 Autopsy instruments, 10 ml syringe with needle, 5 ml Eppendorf tubes, centrifugation machine,

52 Acrodiscsterile syringe filter having a pore size of 0.22 µm and 25 mm diameter (Pall Corporation,

53 United States), compatible freezer (-80oC), 5-Hydroxyindole-3-acetic acid (Merck/sigma), Acetonitrile

54 (Sigma-Aldrich, United States), Concentrated formic acid, Concentrated Acetic acid, Methanol,

55 phosphoric acid, ultrafilter (Ultra 370 series RIONS INDIA), Electronic balance machine, 1000ml

56 measuring cylinder, Micropipettes, High-performance liquid chromatography(Waters 2489 UV/Visible

57 detector).

Collection of CSF:

59 CSF was collected by direct visualisation method during autopsy. After opening the skull and removing

60 the dura, under direct vision, the two cerebral hemispheres are slightly parted using the index and middle

61 fingers. Then a sterile needle with 10 ml syringe attached to it was inserted into the dependent and

62 posterior part of the brain's lateral ventricle at around 1.5 cm depth. The needle was directed posteriorly

63 and downwards in each ventricle, and CSF was aspirated. (Fig -1) Around 4-5 ml of CSF was collected

64 from the brain's lateral ventricles and transferred to the 5 ml Eppendorf tubes. Then the CSF was

65 centrifuged upon collection at 15000 rpm for 15 min at 4oC and filtered with a sterile nylon syringe filter

66 with a pore size 0.22µm. Then CSF sample was stored at -80°C freezer until analysis.

HPLC-UV method Development for 5-HIAA:-

HPLC analysis to detect the 5-HIAA in CSF samples was carried out using an HLPC pump system (Waters 515) fitted with Waters 2489 UV/Visible Detector and reverse phase SunFireTM C-18 column, 5 μm, 4.6×25 mm was used. The sample auto-injector used was fitted with an in-line degasser. The screening of different mobile phases, including: [acetic acid (0.01-0.1%): Methanol in 1:9 ratio], [phosphoric acid (0.01-0.1%): Methanol in 1:9 ratio] and [formic acid (0.05-0.1%): Acetonitrile in 1:9 ratio] was carried out at flow rate ranging from 0.5 ml min-1 to 1.5 ml min-1. The resolution of 5-HIAA was carried out at three different wavelengths, including 220 nm, 280 nm and 320 nm. The mobile phase containing formic acid (0.05%) and Acetonitrile in 1:9 at flow rate of 1.0 ml/min determined at 280 nm were found to be optimum to resolve the peak of 5-HIAA with a running time of 20 min at various dilution of 50, 100, 150, & 200 nM (Fig2); these parameters obtained were further used to analyse all the clinical samples. The peak from other mobile phases [acetic acid (0.01-0.1%): Methanol in 1:9 ratio], [phosphoric acid (0.01-0.1%): Methanol in 1:9 ratio] did not yield satisfactory results.

Analysis of CSF 5-Hydroxyindoleacetic acid by HPLC

CSF samples were removed from the deep freezer (-80 °C) and subjected to thawing on ice (4°C). After thawing, the CSF samples were centrifuged again at 15,000 rpm at 4°C for 15 min and filtered using a syringe filter (Pall Corporation, United States); 90 µl of this filtered CSF sample was directly injected into a reverse phase HPLC column fitted with UV detector, using an auto-injector.

The standard solution of 100 nM 5-HIAA (Merck, India) was used to calculate the concentration of 5-HIAA in all the clinical samples. All the runs were carried out in triplicates to check the repeatability of the results. We calculated the CSF 5-HIAA concentration from HPLC analysis quantitatively by comparing it with the concentration of the standard sample. A comparison of the area under the curve for

90   the unknown sample (cases) and that of the known sample was used to determine the amount of CSF 5-

91   HIAA in samples.

Statistical Analysis:-

93   All the statistical analysis was carried out using R Studio (version 4.2.0). Continuous data were expressed

94   as mean, SD, or median (IQR) depending on the distribution.

Results:

96   The study was approved by our institute's Institutional Human Ethics Committee vide LOP letter no

97   XXX. A total of 71 CSF samples were collected for 5-HIAA assessment from 71 dead bodies after

98   obtaining due consent from the next of kin.

99   Out of the three mobile phases used for HPLC- UV, the one containing formic acid (0.05%) and

100 Acetonitrile in 1:9 at a flow rate of 1.0 ml/min determined at 280 nm were found to be optimum to

101 resolve the peak of 5-HIAA with a running time of 20 min at various dilution of 50, 100, 150, & 200 nM.

102 The peak with the standard solution was obtained at 18.4 min at different dilutions mentioned. This

103 mobile phase was then used for quantification of the 5-HIAA level from the CSF in all the samples.

104 Out of the 71 cases studied, the median of 5-HIAA level in CSF was found to be 97.46 nMol/lit with an

105 interquartile range 80.86 -114.97 nMol/lit. The minimum level of 5-HIAA in CSF was 12.85 nMol/lit,

106 and the maximum level of 5-HIAA in CSF was 527.51 nMol/lit.

Discussion:

108 The present work contributes a new and straightforward method for estimating a serotonin metabolite, i.e.

109 5-HIAA, in CSF samples using HPLC-UV. The normal psychotic function is disturbed by the fluctuation

110 of neurotransmitters since serotonin plays a significant part in sustaining ordinary psychosis(7)(8). A

111 change in the concentrations of brain serotonin in disorders like Depression, schizophrenia, panic

112   disorder, and Parkinson's disease is already established (8), indicating the need for developing simple,

113   sensitive, and reliable methods for estimating serotonin metabolite in CSF samples. We used a sample of

114   CSF to determine the amount of serotonin since the level of serotonin in blood differs from that in the

115   brain(9). CSF, being in close proximity to the brain and a clear fluid with less protein content (10), is the

116   best-suited biological fluid for the estimation of 5-HIAA by HPLC. However, the relevant literature on

117   estimating 5- HIAA in biological samples using HPLC-UV is limited. The CSF neurotransmitter

118   composition is consistent under normal circumstances. However, the composition, quantity, and pressure

119   can change in a variety of neurological diseases. Since serotonin degrades quickly to 5-HIAA, this

120   parameter is used to measure the brain's serotonin levels (11). There are advanced methods like LCMS,

121   HPLC with fluorescence detection(12), HPLC with electrochemical detection, Gas chromatography-mass

122   spectrometry, Enzyme-linked immunosorbent assay (ELISA), Capillary electrophoresis, which can detect

123   these compounds at lower levels (13). However, it requires expensive equipment, solvents and extensive

124   sample preparation. Although these methods are faster and more sensitive than UV HPLC (14.15), they

125   require more expensive instruments, preparation and skill and are only feasible in high-end laboratories.

126   Although this study did not focus on assessing the sensitivity of this method, there are studies in the

127   literature with a detection limit of 0.2 mg/L in 5-HIAA by HPLC in urine (16). In our study, the minimum

128   value of 12.85 nMol/lit was detected in one of the samples with a history of Depression, which is

129   comparable.

130   HPLC –UV is a sensitive method which detects CSF 5-HIAA levels quickly with precision and accuracy,

131   using inexpensive chemicals and requires significantly less sample preparation. We used three different

132   sample preparation methods, and the best one was ascertained based on the accuracy of the result using a

133   standard solution of 5-Hydroxyindole-3-acetic acid (Merck/sigma).

134   During the analysis of 5-HIAA with HPLC, many problems like baseline drift, poor peak resolution,

135   ghost peak, and system leak were faced. Contamination in the sample is also a big problem, which can

136   interfere with the accuracy and specificity of the measurements. The possible sources of contamination

137   include Impurities in the solvents or reagents, Poor cleaning or sterilisation of the equipment or

138   containers, Cross-contamination from other samples or sources, and Poor sample handling or storage. To

139   avoid contamination-related issues, high-quality solvents and reagents were used, and the equipment was

140   cleaned and sterilised by flushing the equipment and containers thoroughly and adequately and handling

141   the samples carefully. The CSF samples were centrifuged just after collection and once more before

142   analysis. Centrifugation removes particulate matter such as cells, debris, and other insoluble material from

143   the sample, which can clog the HPLC column and affect the accuracy of the results. It also removes large

144   molecules, such as proteins or lipids, that may interfere with separating the analytes of interest in the

145   HPLC column (17). This improved the sensitivity and reproducibility of the analysis. In order to avoid or

146   resolve problems in UV HPLC, it is crucial to maintain the system and components, follow proper

147   operating procedures, and optimise the experimental conditions.

148   Numerous illnesses exhibit a pronounced decrease in 5-HIAA levels, according to research that has been

149   undertaken. Decreased CSF serotonin levels have also been associated with suicidal deaths (6) and can be

150   further researched to corroborate suicidal deaths. Serotonin is thus a possible biomarker for many

151   neurological and psychiatric illnesses, opening more possibilities for future research into the creation of

152   alternative biomarkers in forensics and medicine.

153   Conclusion:

154   A novel, sensitive, precise, and reliable HPLC-UV method for separating and quantifying 5-HIAA in

155   human CSF samples has been developed. CSF is a suitable sample type for the 5-HIAA measurement by

156   UV-HPLC method and can be used in diagnosing and monitoring patients with neuroendocrine tumours

157   (NETs) and various psychiatric diseases. Their use in this context should be adopted routinely as an

158   alternative to other methods. Commercial kits and methods other than UV-HPLC for analysing 5-hydroxy

159   indole acetic acid (5-HIAA) require extensive sample preparation. In contrast, using UV-HPLC, the

160   serotonin metabolites, i.e. 5-HIAA, can be measured in a quick analysis. This method applies to clinical

161   5-HIAA UV-HPLC analysis in CSF and provides the benefits of both fast analysis times and low-cost

162   sample preparation procedures.

168   Declaration of conflicting interests:

169   The author(s) declared no potential conflicts of interest concerning the research, authorship, and

170   publication of this article.

171   References:

172   1.Vernau W, Vernau KA, Sue Bailey C. Cerebrospinal Fluid. Clinical Biochemistry of Domestic

173   Animals. 2008;769–819.

174   2. Bennett A. Shaywitz, Donald J. Cohen, Malcolm B. Bowers Reduced cerebrospinal   fluid 5-hydroxy

175   indole acetic acid and homovanillic acid in children with epilepsy; Neurology Jan 1975, 25 (1) 72; DOI:

176   10.1212/WNL.25.1.72

177   3.Mathew SJ, Coplan JD, Smith ELP, Scharf BA, Owens MJ, Nemeroff CB, et al. Cerebrospinal Fluid

178   Concentrations of Biogenic Amines and Corticotropin-releasing Factor in Adolescent Non-human

179   Primates as a Function of the Timing of Adverse Early Rearing. Stress. 2002 Jan;5(3):185–93.

180   4.Lenchner JR, Santos C. Biochemistry, 5 Hydroxyindoleacetic Acid. 2022 May 8. In: StatPearls

181   [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 31869148.

182   5.Menon KN, Steer DL, Short M, Petratos S, Smith I, Bernard CCA. A Novel Unbiased Proteomic

183   Approach to Detect the Reactivity of Cerebrospinal Fluid in Neurological Diseases. Mol Cell Proteomics.

184   2011 Jun;10(6):M110.000042.

185   6.Carlborg A, Jokinen J, Nordström AL, Jönsson EG, Nordström P. CSF 5-HIAA, attempted suicide and

186   suicide risk in schizophrenia spectrum psychosis. Schizophrenia Research. 2009 Jul 1;112(1):80–5.

187   7.Jayamohananan H, Manoj Kumar MK, T P A. 5-HIAA as a Potential Biological Marker for

188   Neurological and Psychiatric Disorders. Adv Pharm Bull. 2019 Aug;9(3):374–81.

189   8.Correlation Between Salivary, Platelet and Central Serotonin Levels in Children | Canadian Journal of

190   Neurological Sciences | Cambridge Core [Internet]. [cited 2022 Nov 3]. Available from:

191   https://www.cambridge.org/core/journals/canadian-journal-of-neurological-sciences/article/   correlation-

192   between-salivary-platelet-and-central-serotonin-levels-in-children/

193   98A7C394671DBF747CF97E88B944C6D2

194   9. Collins CM, Kloek J, Elliott JM. Parallel changes in serotonin levels in the brain and blood following

195   acute administration of MDMA. J Psychopharmacol. 2013 Jan;27(1):109–12.

196   10. Hrishi Ap, Sethuraman M. Cerebrospinal Fluid (Csf) Analysis And Interpretation In Neurocritical

197   Care For Acute Neurological Conditions. Indian J Crit Care Med. 2019 Jun; 23(Suppl 2): S115-S119.

198   Doi: 10.5005/Jp-Journals-10071-23187. PMID: 31485118; PMCID: PMC6707491.

199   11.Takada A, Shimizu F, Masuda J. Measurement of Plasma Tryptophan Metabolites: Clinical and

200   Experimental Application for Depression and Stress States Assessment. Melatonin - Molecular Biology,

201   Clinical   and   Pharmaceutical   Approaches   [Internet].   2018   Nov   21;   Available   from:

202   http://dx.doi.org/10.5772/intechopen.78560

203   12. Fujino K, Yoshitake T, Kehr J, Nohta H, Yamaguchi M. Simultaneous determination of 5-hydroxy

204   indoles and catechols by high-performance liquid chromatography with fluorescence detection following

205   derivatisation with benzylamine and 1,2-diphenyl ethylenediamine. J Chromatogr A. 2003 Sep

206   19;1012(2):169–77.

207   13. Vaarmann A, Kask A, Mäeorg U. Novel and sensitive high-performance liquid chromatographic

208   method based on electrochemical coulometric array detection for simultaneous determination of

209   catecholamines, kynurenine and indole derivatives of tryptophan. J Chromatogr B Analyt Technol

210   Biomed Life Sci. 2002 Mar 25;769(1):145–53.

211   14. Miller, A. G., Brown, H., Degg, T., Allen, K., & Keevil, B. G. (2010). Measurement of plasma 5-

212   hydroxy indole acetic acid by liquid chromatography-tandem mass spectrometry—Comparison with

213   HPLC methodology. Journal of Chromatography B, 878(7-8), 695–699.

214   doi:10.1016/j.jchromb.2010.01.01

215   15. González, R., Fernández, R., Vidal, J., Frenich, A., and Pérez, M. (2011). Development and validation

216   of an ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS)

217   method for simultaneously determining neurotransmitters in rat brain samples. J. Neurosci. Methods 198,

218   187–194. doi: 10.1016/j.jneumeth.2011.03.023

219   16. Koel M, Nebinger P. Determination of urinary 5-hydroxy-3-indoleacetic acid by an automated HPLC

220   method. Biomed Chromatogr. 1989 May;3(3):114-7. doi 10.1002/bmc.1130030305. PMID: 2475195

221   17. Chapter 3: Investigating Proteins – Chemistry [Internet]. [cited 2023 Feb 16]. Available from:

222   https://wou.edu/chemistry/courses/online-chemistry-textbooks/ch450-and-ch451-biochemistry-defining-

223   life-at-the-molecular-level/chapter-3-investigating-proteins/