

# **D-Aspartic Acid In The Human Brain Tissue: A Luminous Theory.**

## **ABSTRACT:-**

### **Objective**

Estimation of D-Aspartic Acid value in suicide victim's brain tissue and comparing it with accidental death patient's brain tissue samples and establishing DAA as a marker of suicide.

### **Methods**

This is a hospital-based prospective study. Subjects of suicide cases and road traffic victims have been taken into account (N=30) in cases and controls respectively. Apart from routine mandatory informed consent, BRAIN TISSUE (Pre-frontal cortex) has been obtained from aforementioned subjects who have committed suicide and victims of road accident. And before that ethical clearance has been approved from the Institutional Human Ethics Committee-PGR, following which the study has been initiated.

### **Results**

The output indicates that the mean for Control (Road traffic accidents) is 24.2737 and for the Case (Suicide group) is 19.5540. The mean of CONTROLS minus CASES equals 4.7197. 95% confidence interval of this difference is from 1.5361 to 7.9033. The standard deviation column shows a difference between cases and controls. Since, the p value equals as 0.0044, which is way less than 0.05, we can reject the null hypothesis which states that there is no association between the level of D-Aspartic acid and suicide and accept the alternative, which corroborates our idea that there is an association between DAA and suicide.

### **Conclusions**

Suicide was among top 20 causes of death in 2024. The exact number of suicide death is unknown, since there are ways to commit suicide and in many areas of either India or the globe, cases go undocumented. There is no treatment available to treat suicide since it is the surcease of life, but there are ways to modify or alter one's course of action if we get to realize, diagnose one's impending act. With this study, we aim to achieve the role of DAA as a prognostic marker, we have the achievement among the dead, but to modify among the living, we admit, it requires more brain storming and man power.

**KEYWORDS:** Suicide, D-Aspartic Acid, HPLC, Mental Illness.

## **INTRODUCTION**

Suicide, the act to kill oneself is an untoward act. According to WHO 2023 statistics, more than 800,000 people committed suicide in 2023, with suicide being among top 17 leading cause of death in 2020-2022. Post pandemic era has only noticed an upsurge in numbers. These numbers and statistics are documented ones, nobody has taken cognizance of undocumented numbers.

Statistics have been way higher in developing and third world countries as compared to developed nations. India statistics have observed that suicide rate was 12.4 in 2022, the rates have increased by 27% if we compare it from 2018.

39 With more than 50% population less than 25 years and more than 65% population being  
40 below the age of 35 years, it is imperative to act on matter. Statistics have established time  
41 and again that it is young and elderly who are more vulnerable to suicide. Risk factors and  
42 psychological autopsy over the time have demonstrated that what could be an insignificant  
43 matter or concern to a third party, for the ones going through the upheaval, it could pose as  
44 world turning upside down moment for them and hence they jump on to do the act.

45 Mind is both intricate and modest, callow and ripe, vital and languid, chap and adult,  
46 cognizant and ignorant and a truckload of many more things that we are oblivious about. It  
47 has attained the charisma to navigate emotions, thoughts, perceptions and ideas into  
48 enterprise.

49 There are billions of nerve cells in human brain, each possessing their own uniqueness in  
50 terms of action, shape and forms. The 1011 neurons constitute of thousands of  
51 collaborations among their fellow brothers or the ones belonging to disparate team for signal  
52 transmission.

53 Without these connections or associations, the electrical or chemical signal will be lost. These  
54 are the sustenance units of message transfer. The electrical signals cross over with the help  
55 of ions and chemical signal propels through neurotransmitters

56 Human brain, as a part of the Central nervous system, presides over our emotions,  
57 behaviours, actions, attitude, personality and human entity in its all glory. Neuron or nerve  
58 cell is the basic unit of central nervous system. The communication between the nerve cells  
59 or neurons can be either electrical or chemical based. The electrical or chemical signal  
60 proceeds among the neurons like passing a ball among two elite level players, where  
61 dropping of ball is negligible.

62 The point or nodal area where transmission of message befalls, either in the electrical or  
63 chemical mode is synapse, it can be pre synapse, from where the transmitter signals have  
64 sprung to post synaptic where they will be received with open arms.

65 One such novel neurotransmitter which has been going back and forth among synapses with  
66 ease but missed the researchers' sharp sight is D-Aspartic Acid, called DAA in our  
67 research. It has distinctively passed the test of neurotransmitter with flying colours. The  
68 DAA has been thoroughly examined and dissected for its varying actions among the rats  
69 and it has been established that DAA has a central mechanism of action when it comes to  
70 cognition, attitude, thoughts, sexual activities as well as tendency of depression among the  
71 rodents.

72 Keeping this piece of knowledge in our mind and curiosity to establish a similar association  
73 among the homo sapiens, we started on this voyage of quantitative analysis of the DAA  
74 among the suicidal group and road traffic accidents.

75 We in our research have exerted to decipher the puzzle among humans on the same theorem  
76 for D-Aspartic Acid by using pre-frontal cortex samples of suicide victims (N=30, CASES)  
77 and comparing it to pre-frontal cortex samples of road-traffic accident victims (N=30,  
78 CONTROLS).

## **Methods**

In accordance with the guidelines for the Case-Control study, both cases and controls had more than 15 parameters that is 30, hence we did not use non-parametric test like Mann-Whitney U test and do not have to worry about normality assumption, we can directly jump onto Unpaired/Independent-t test for parameter D-Aspartic acid. The quantitative estimation of DAA has been performed on HPLC (High Performance Liquid Chromatography) The two groups are independent by nature.

## **Eligibility Criteria**

Cases and Controls were selected for inclusion if they 1) were already brought within 24 hours of being declared dead ,2) death by hanging for suicides and road traffic accident death happen without under any toxic influence for controls, and 3) Informed consent was dictated and signed by next of kin under no external influence, 4) Age and gender can vary. Cases and controls were excluded if they 1) did have any metabolic disorder and 2) did not die by mentioned means, 3) next of kin refused consent, 4) admission in hospital for more than 24 hours in case of road traffic accidents.

## **Data Extraction And Analysis**

Deceased were enrolled for study at hospital mortuary for hospital autopsy. A psychological autopsy was conducted with the deceased kin regarding patient's mental history before attempting suicide. Another mandatory questionnaire regarding name, gender, ethnicity, profession, mental health history, recreational drug use history, alcohol intake, history of any previous treatment.

Brain tissue, about 400 mg from mainly prefrontal cortex has been collected in a micro centrifuge tubes of 1.5 ml capacity and been stored in -80°C ultradeep freezer, till further analysis.

## **HIGH-PRESSURE LIQUID CHROMATOGRAPHY:-**

### **(A) Extraction Of D-Aspartic Acid From Brain Tissues**

An ideal, quantitative, amino acid analysis combines speed and sensitivity with reliability of both derivatization reaction and analytical techniques.

These goals are achieved with automated, online derivatization using O-phthalaldehyde (Opa) for primary amino acids; the automated derivatization is then integrated with rugged HPLC analysis.

118 The analysis was performed using the Agilent 1220 HPLC connected to ECLIPSE PLUS C-  
119 18, 46\*150 mm, 5micron columns.

120 The OPA derivatives elute chromatographically. The derivatization process is fast and is  
121 easily automated using the AGILENT Autosampler.

122 Total analysis from the injection to injection can be achieved in as little as 14 minutes (10-  
123 min analysis time) on the 75-mm column.

124 On the 150-cm column total run time is 26 minutes (16-min analysis time) for all amino  
125 acids. Both analyses provide high sample throughput. Each run represents an individual  
126 derivatization and its chromatographic separation.

127 There producibility of the derivatization, represented by a peak area has an average of %  
128 relative standard derivation of 2.0.

129 For D-Aspartic acid, based on standard curve, the method file was run for 5 minutes with 0.5  
130 minutes post-run time.

131

## 132 **(B) ESTIMATION OF BRAIN D-ASPARTIC ACID**

### 133 **(1) Chemicals**

- 134 1. Citric Acid – used to prepare Phosphoric Citric Buffer for brain sample preparation.
- 135 2. Dibasic Sodium Phosphate ( $\text{Na}_2\text{HPO}_4$ ) - used to prepare Phosphoric Citric Buffer for brain  
136 sample preparation and for preparation of Buffer A
- 137 3. Sodium Borate ( $\text{Na}_2\text{B}_4\text{O}_7$ ) – used for preparation of Buffer A
- 138 4. Acetonitrile - used for preparation of Buffer B
- 139 5. Methanol - used for preparation of Buffer B
- 140 6. Ultrapure Water – was obtained using a Milli-Q filter system
- 141 7. HCl – to prepare 6N HCl
- 142 8. D-Aspartic Acid – for standard preparation
- 143 9. O-phthalaldehyde - for the derivatization of amino acids

144

145 **All reagents purchased were of HPLC grade.**

146

147

### 148 **(2) Brain Sample Preparation**

149 Postmortem brain was obtained from prefrontal complex and immediately stored at -80°C. At  
150 the time of processing, 300 mg of brain was mixed with 4 ml phosphoric-citrate buffer (pH -  
151 3.25).

This mixture was homogenized for 40s at moderate speed using homogenizer. The homogenate was then centrifuged (26,000 g, 20 min, 4°C). The supernatant was then filtered using syringe filter of 0.2 µm.

Further elimination of proteins was done using 10-kDa Ultra Centrifugal Filters (14,000 g, 20 min, 4°C) which were washed twice beforehand with mobile phase.

**(3) Instrumentation**

The measurement was performed on HPLC (Agilent Technologies) equipped with autosampler and variable wave length detector (VWD).

The chromatographic separation was achieved using C18 column (150 mm x 4.6 mm x 5 µm).

Instrument control, data acquisition and analysis were achieved with EZ Chrome Elite software.

**Pump Setting**

- (1) Flow: 2 mL/min
- (2) Stop time: 5 min
- (3) Post time: 0.5 min

**GRADIENT SETTINGS (Wavelength: 338 nm)**

Gradient (Time period)	Mobile Phase A(%)	Mobile Phase B(%)
0 Minutes	0	100
1.5 Minutes	20	80
2.5 Minutes	40	60
3.5 Minutes	80	20
4.5 Minutes	90	10

**(4) Buffer Composition:-**

**Buffer A: Borate Buffer for 2L**

Carefully weigh 2.84 gm of Na<sub>2</sub>HPO<sub>4</sub> and 7.63 gm of Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> in 2L of a bottle, add 1800 mL of Milli-Q water and mix it well, then adjust the pH to 6.0 using 6 N HCl freshly prepared, adjust the volume to 2L using measuring cylinder and subsequently filter the buffer with 0.2 µm filter.

#### **Buffer B:**

#### **ACN: MeOH: H<sub>2</sub>O (45:45:10) in 1 L**

In a measuring cylinder, mix 450 ml of acetonitrile, 450 ml of methanol and 100 ml of Milli-Q water and sonicate it for 10 min.

We prepared fresh buffer. Since there were 60 samples, buffers were used within 3 days from the date of preparation and freshly prepared subsequently.

Filter buffer (Buffer A) with 0.22 µm nylon filter and de-gas both buffers for 10 min on a sonicator to remove air bubbles.

#### **OPA Solution**

Prepare a fresh solution of 70 mg OPA and 1 ml Methanol and 95 ml Buffer A.

#### **Injection Diluent**

Take 100 ml mobile phase A and add 0.4 ml conc. H<sub>3</sub>PO<sub>4</sub> (Phosphoric Acid) in a 100 ml bottle, mix well and store at 4°C.

### **(5) Standard Preparation And Calibration Curve**

Preparation of D-aspartic acid stock (1M)

Accurately weighted D-aspartic acid and added 10 mL of Milli-Q water.

Mix well using a vortex mixture until dissolved.

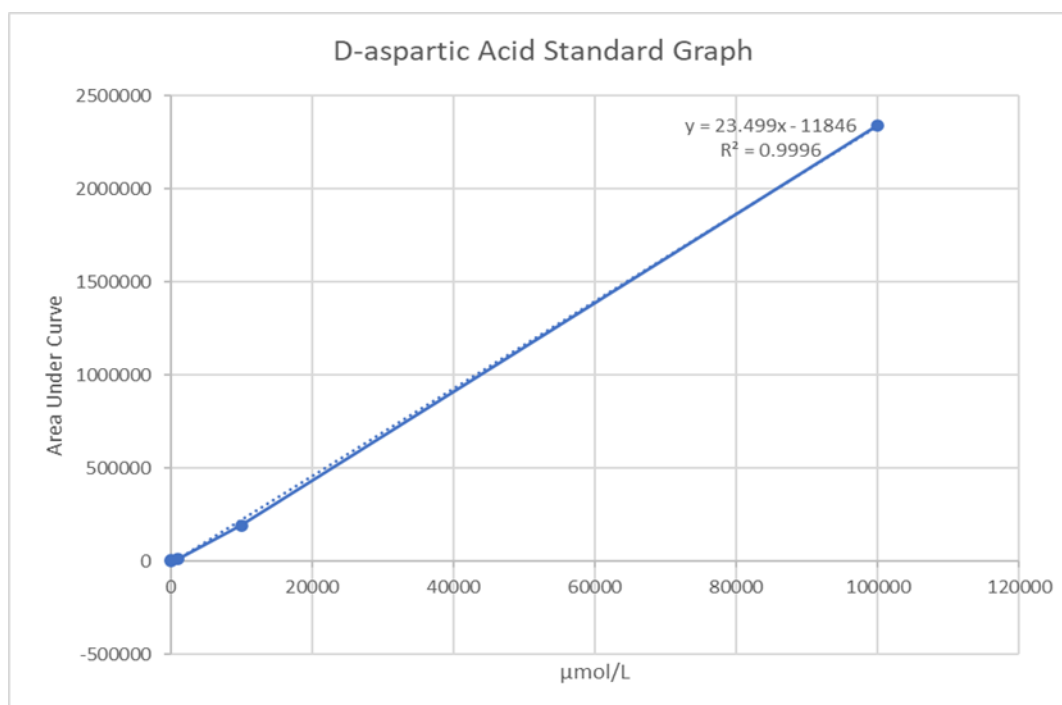
Prepare 1 mM working solution of the standard by using the formula:

$$M_1 \times V_1 = M_2 \times V_2$$

We worked with fresh standard stock. However, standard stock was used within 15 days from the date of preparation since stored at -20°C.

Serial dilution of first sample was done to prepare 5 standards.

## 210 (6) D-Aspartic Acid Standard Graph



211 Using the standard graph formula,  $y = mx - c$ , calculations have been made

212 Where  $m$  = slope value/ gradient of the line

213 X and Y = coordinates of this line, C = intercept of the line

214 The coefficient of determination (R) is a number between 0 and 1 that measures how well a  
 215 statistical model predicts an outcome. The lowest possible value of  $R^2$  is 0 and the highest  
 216 possible value is 1. Put simply, the better a model is at making predictions, the closer its  $R^2$   
 217 will be to 1.

218 R is calculated by using correlation coefficient.

$$(R)^2 = (r)^2$$

220 Where  $r$  = Pearson correlation coefficient, putting all in the equation

221 **R value was 0.9996.**

222 The model perfectly predicts the outcome.

223 The value of  $m$  can be calculated from the angle which this line makes with the x-axis or a  
 224 line parallel to the x-axis. Putting our area under curve values in excel, the

225 **'m' value was 23.499.**

226 Based on the intercepting line on excel,

227 **'c' value was 11846.**

228 Putting all this into the equation for individual D-Aspartic acid levels of cases and controls,

Observed D-Aspartic acid value was calculated (per Litre in 300mg of brain).  
And for D-Aspartic acid level in mmol/l/g, the values were calculated by

$$\text{Observed D-Aspartic acid value} \times 3.3/1000$$

**For example, Case 1**

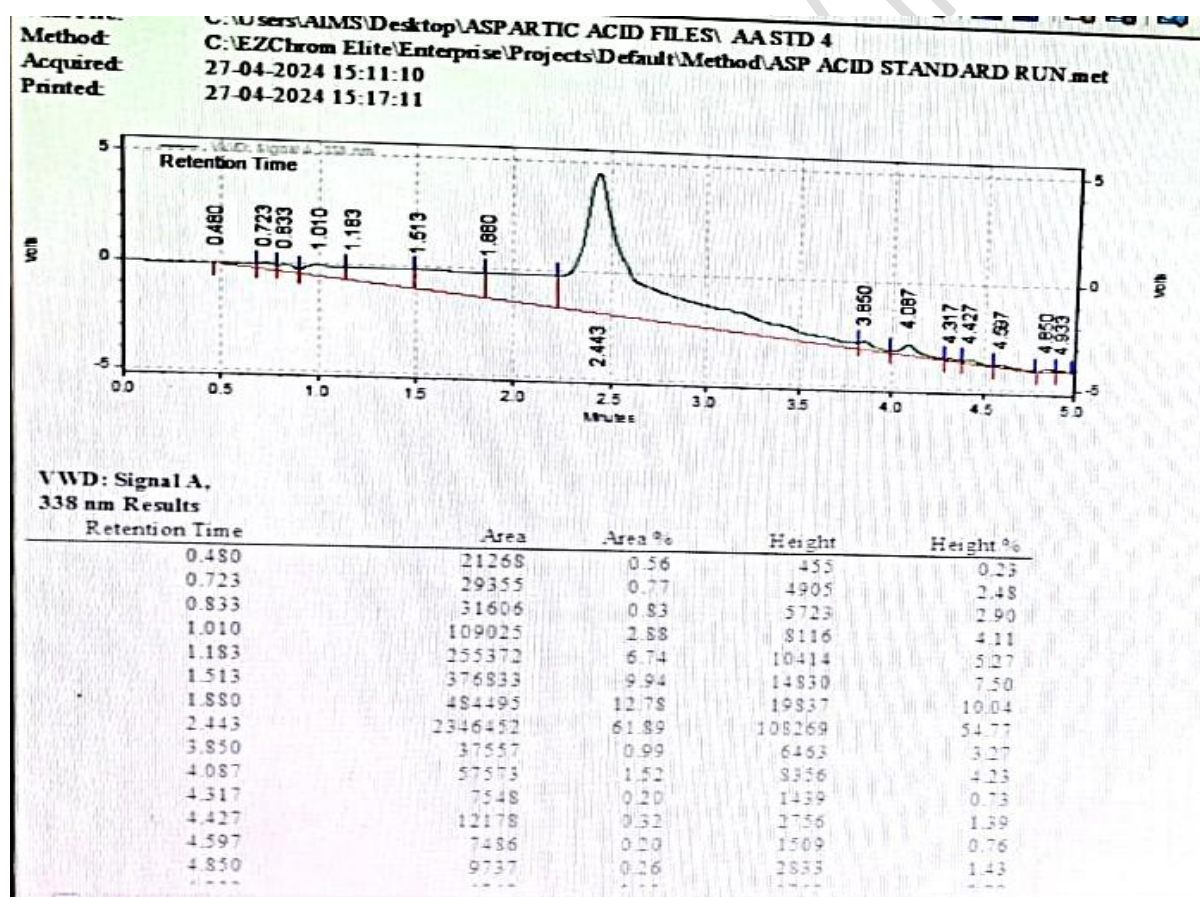
Area under curve (AUC) for D-Aspartic Acid = **74040**, after HPLC RUN with  
Retention time at **2.447 minutes** at **338nm**.

Using formula,  $y = mx - c$ ,

Where  $y = 74040$ ,  $m = 23.499$  and  $c = 11846$ ,

we get x/ Observed D-Aspartic acid value (/L in 300 mg Brain) as **3654.87**

The desired D-Aspartic acid level (mmol/l/g) is  $3654.87 \times 3.3/1000 = 12.17$



**FIGURE 01: The above D-Aspartic acid standard describing Retention time of 2.447 minutes with a wavelength at 338 nm.**

## (7) Validation Of Method



Analytical method validation is performed by regulated laboratory, and deals with the testing of significant method characteristics to ensure that under routine use, the analytical method is accurate, precise, specific, reproducible, and rugged over the whole specified range, for which an analyte (s) is determined.

The validation of chromatographic methods should be performed before the first routine use of the procedure, and a validation of methods of analysis is crucial in all phases of analyte development.

Validation is an important step in determining the reliability and reproducibility of the method because it is able to confirm that the intended method is suitable to be conducted on a particular system using ICH guidelines.

The parameters to be investigated include system suitability, linearity, precision, accuracy, specificity, the limit of detection (LOD), limit of quantification (LOQ), and robustness.

## **(8) Statistical Analysis Plan**

Data was entered in MS-Excel and analysis was done in SPSS (Version 21 software). Distribution of numerical values would be examined by plotting histogram and pie chart as and when required. The statistical significance of differences between two means of two different groups was evaluated by unpaired t-test. The level of significance has been set at  $p < 0.05$ . The association between low levels of cholesterol and D-Aspartic acid with suicide and high levels of dopamine association with suicide was determined by Spearman's rank test.

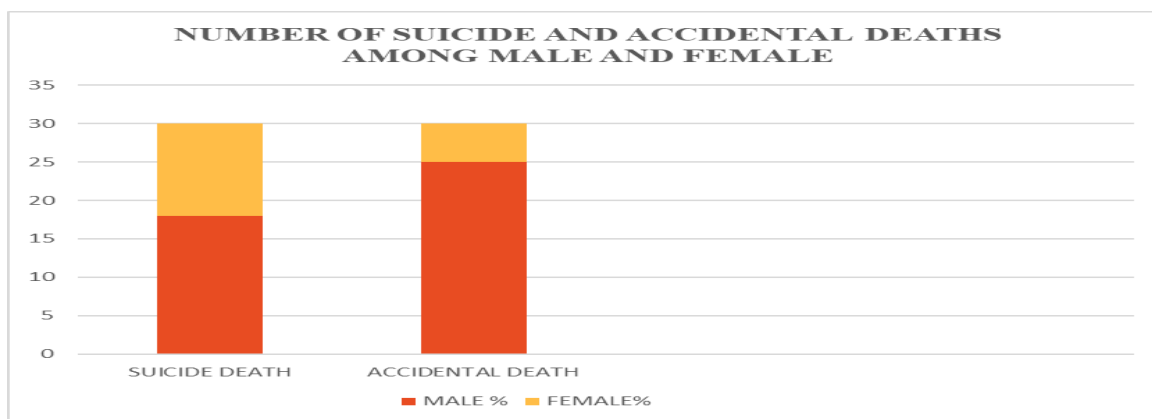
## **RESULTS**

Among the enrolled for case samples, out of 30, 18 were males and 12 were females, which make it 60% males and 40% females with the youngest one committing suicide was a 12 year old female and the oldest being 52 year old female and the rest fall in between and for control youngest age is 13 year old male and oldest is 76 year old male.

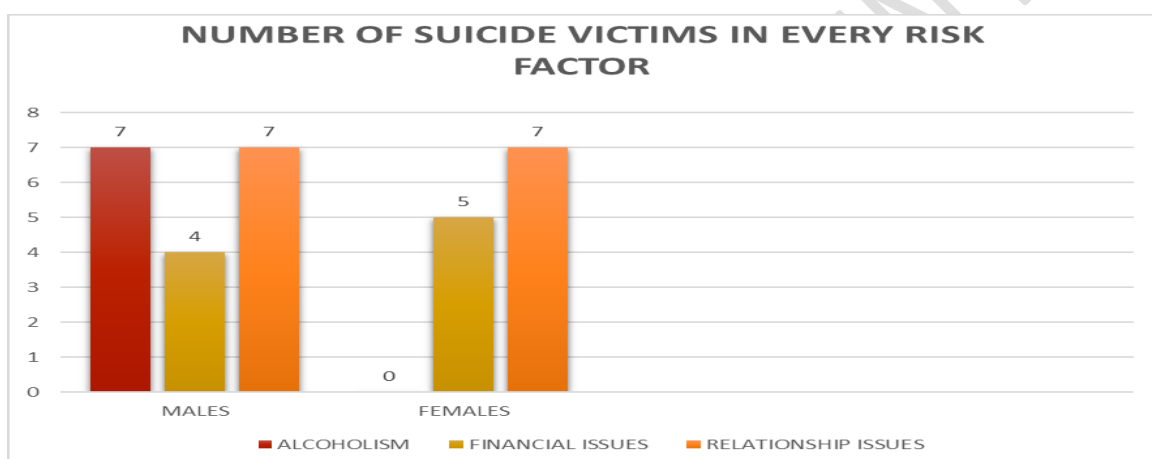
Mean age of suicide victim being 27.3 years and for accidental death, the mean age is 42.26 years.

Out of 30 suicide cases, 18 being male and 12 being females and out of 18 males, 7 had issues of alcoholism and drug addicts as mentioned in psychological autopsy by family members, 7 had relationship issues as confirmed by police during cell phone examination and interrogation of family members and friends while 4 victims family members mentioned financial issues as the cause of concerned act.

Among 12 females committing suicide, 0 had alcoholism or drug issues, 5 had financial issues and they belonged to lower socio-economic strata while 7 had relationship issues as mentioned by police and family members during confiscated cell phone examination and interrogation respectively.



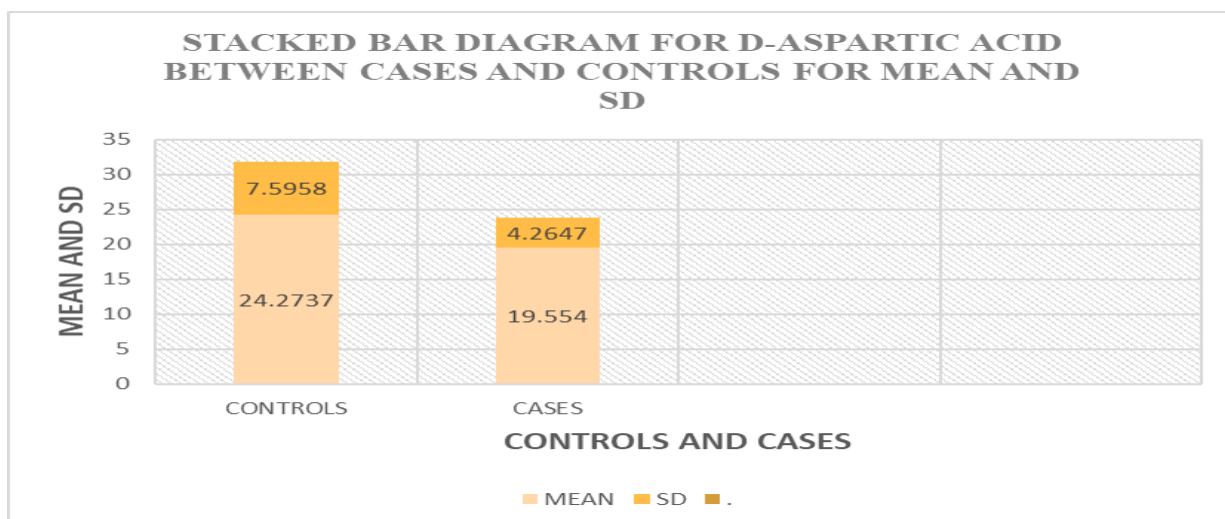
**FIGURE 02: Stacked column showcasing number of males and females among suicide and accidental deaths**



**FIGURE 03: Clustered column depicting number of suicide victims based on risk factor**

**TABLE 1: Tabulated Description of Mean, Sd, SEM And N Difference Of Controls And Cases With Respect To Brain D-Aspartic acid**

GROUP	CONTROLS	CASES
MEAN(MMOL/ML/G)	24.2737	19.5540
SD	7.5958	4.2647
SEM	1.3868	0.7786
N	30	30



The output indicates that the mean for Control is 24.2737 and for the Case is 19.5540. (Table 1). The mean of CONTROLS minus CASES equals 4.7197. 95% confidence interval of this difference is from 1.5361 to 7.9033. The standard deviation column shows a difference between cases and controls. Since, the p value equals as 0.0044, which is way less than 0.05, we can reject the null hypothesis which states that there is no association between the level of D-Aspartic acid and suicide and accept the alternative.

The alternative hypothesis proves that brain D-Aspartic acid has an effect in the act of suicide and its values show a large drop before the act of suicide is commenced as compared to road traffic accidents which correlates with the study where D-AA was studied as a novel endogenous neurotransmitter in case of mental health disorders in humans, though never studied on suicide victims, likely getting its reference from research occurring on rats where it was discovered that D-AA plays a role in cognition, personality development, thought process, aggression.

The other study which was related to humans and signified the role of D-AA in reproduction, where low D-AA leads to infertility and depression and hence providing the much needed proof of role of D-AA in mental health disorders and suicide, and subsequently to the act of suicide.

Thus, our aim to estimate D-Aspartic acid level and the relationship between suicide and the neurotransmitter is beneficial for the future endeavours.

**TABLE 02: Correlation of Mean, SD and P value between controls and cases among three parameters**

MEAN AND SD	CONTROL	CASE	P VALUE
D-ASPARTIC ACID	24.2737 ± 7.5958	19.5540 ± 4.2647	0.0044

## DISCUSSION

The present study was conducted during the period of September 2022 to May 2024. The study was initiated only following proper documentation and letter of permission by Institutional Human Ethics Committee-Postgraduate Research was received on 12th September, 2022. Case and control samples (N=30 each) were received at Hospital Mortuary and following Informed consent from next of kin and nullifying inclusion and exclusion criteria, we moved forward to procedural hospital autopsy, following which the prefrontal brain cortex was retrieved in an Eppendorf tube measuring 1.5 ml measuring 400 mg for parameter D-Aspartic acid for every case and control.

Risk factors were evaluated in history taking and psychological autopsy and any documented systemic illness were considered exclusion criteria and the same sample has been neglected as they play a tremendous role in thoroughly altering the brain chemistry and that would have been a major drawback in our study.

The overall aim was to estimate the values of parameter D-Aspartic acid which has been estimated by HPLC method.

Our youngest suicide victim was a 12 year old female, and based on psychological autopsy the family members doubted either a relationship issue or could be a victim of sexual assault.

The oldest case aged 52 year old female and her psychological autopsy presented a mindset clogged by fear, anxiety related to an impending event. According to the relatives and friends, she was worrisome over the fact that she is unable to find a good groom for her highly qualified daughter.

All these encircle an unanswered question that lingers all around us in our day to day life and pans out the existing truth that the matters that could be a trivial issue to a third party, to the sufferers of psychological issues, they pose as the end of the world.

And, hence any biomarker which could fall as a blessing to the psychological issues patients in saving their lives, could benefit the clinician in prognosis and diagnosis and hence, shall be researched rigorously.

Our initiative to illustrate the main parameter D-Aspartic acid has measured up to being a parameter of concern since its values dropped down drastically in suicide victims and hence it could be delivered as a potential biomarker by measuring it in serum or urine level.

The overall understanding illustrates that underlying mental health issue which can stem up in the form of fear of unknown, anxiety, abandonment issues, anger, jealousy, hatred, addiction of drugs or alcohol, could result in uncontrolled behaviour and outcome which alters the personality, perception and action of the individual.

## Conclusion

According to results achieved by us D-Aspartic acid has showcased a correlation with the suicide, the data has proved low level among the suicide victims with respect to accidental death. The previous studies have ruled the association of amino acid like D-AA with steroidogenesis, for eg. testosterone formation in humans and rats both, moving further and

taking this biochemical relation into the account and correlating with previous studies we can presume that since low testosterone plays a significant role in personality, perception and attitude, hence D-AA could be the biomarker associated with suicide.

### **Limitations and Recommendations for Future Studies**

Further studies are needed to alter the course to a better world for people who need to ameliorate their worries, fear, anxiety, depression and much more on a familiar basis.

Though every other day we come across the news and gory details of people committing suicide, by a common man on the street to a well decorated celebrity, still the lack of consciousness regarding the matter and absence of mental hygiene is flabbergasting.

With only less than 3% of tax payer's money being exhausted on public healthcare in a developing and overpopulous country like India, one necessitates a modest approach towards the matter and regarding that D-Aspartic acid could be promoted as a diagnostic, prognostic biomarker. It could be explored in a simple blood or urine test to advertise against the mental health agony, to rescue the sufferers, to refine the approach and to upgrade the quality of life for many.

### **Conflict of Interest**

The author declare that they have no conflict of interest.

## REFERENCES :-

- 1) Hyman SE. Neurotransmitters. *Curr Biol*. 2005 Mar 8;15(5):R154-8. doi: 10.1016/j.cub.2005.02.037. PMID: 15753022.
- 2) Lovinger DM. Communication networks in the brain: neurons, receptors, neurotransmitters, and alcohol. *Alcohol Res Health*. 2008;31(3):196-214. PMID: 23584863; PMCID: PMC3860493.
- 3) Gottfries CG. Neurotransmitters in the brain. *Arzneimittelforschung*. 1989 Aug;39(8A):1025-9; discussion 1029-30. doi: 10.1007/978-3-642-74615-4\_20. PMID: 2573362.
- 4) Mayer EA. Neuronal communication. *Biol Signals*. 1993 Mar-Apr;2(2):57-76. doi: 10.1159/000109478. PMID: 8261018.
- 5) Steven E. Hyman, *Neurotransmitters, Primer*, Harvard University, Cambridge, Massachusetts 02138, USA.
- 6) Čechová B, Šlamberová R. Methamphetamine, neurotransmitters and neurodevelopment. *Physiol Res*. 2021 Dec 31;70(S3):S301-S315. doi: 10.33549/physiolres.934821. PMID: 35099249; PMCID: PMC8884400.
- 7) Zarrindast MR. Neurotransmitters and cognition. *EXS*. 2006;98:5-39. doi: 10.1007/978-3-7643-7772-4\_2. PMID: 17019881.
- 8) Stuart JN, Hummon AB, Sweedler JV. The chemistry of thought: neurotransmitters in the brain. *Anal Chem*. 2004 Apr 1;76(7):121A-128A. PMID: 15101371.
- 9) Da Y, Luo S, Tian Y. Real-Time Monitoring of Neurotransmitters in the Brain of Living Animals. *ACS Appl Mater Interfaces*. 2023 Jan 11;15(1):138-157. doi: 10.1021/acsami.2c02740. Epub 2022 Apr 8. PMID: 35394736.
- 10) Serrano CC, Dolci GF. Suicide prevention and suicidal behaviour. *Gac Med Mex*. 2021;157(5):547-552. English. doi: 10.24875/GMM.M21000611. PMID: 35104269.
- 14) Hawton K, Hill NTM, Gould M, John A, Lascelles K, Robinson J. Clustering of suicides in children and adolescents. *Lancet Child Adolesc Health*. 2020 Jan;4(1):58-67. doi: 10.1016/S2352-4642(19)30335-9. Epub 2019 Oct 9. PMID: 31606323.
- 15) Pirkis J, John A, Shin S, DelPozo-Banos M, Arya V, Analuisa-Aguilar P, Appleby L, Arensman E, Bantjes J, Baran A, Bertolote JM, Borges G, Brečić P, Caine E, Castelpietra G, Chang SS, Colchester D, Crompton D, Curkovic M, Deisenhammer EA, Du C, Dwyer J, Erlangsen A, Faust JS, Fortune S, Garrett A, George D, Gerstner R, Gilissen R, Gould M, Hawton K, Kanter J, Kapur N, Khan M, Kirtley OJ, Knipe D, Kolves K, Leske S, Marahatta K, Mittendorfer-Rutz E, Nezanov N, Niederkrotenthaler T, Nielsen E, Nordentoft M, Oberlerchner H, O'Connor RC, Pearson M, Phillips MR, Platt S, Plener PL, Psota G, Qin P, Radeloff D, Rados C, Reif A, Reif-Leonhard C, Rozanov V, Schlang C, Schneider B, Semenova N, Sinyor M, Townsend E, Ueda M, Vijayakumar L, Webb RT, Weerasinghe M, Zalsman G, Gunnell D, Spittal MJ. Suicide trends in the early months of the COVID-19

435 pandemic: an interrupted time-series analysis of preliminary data from 21 countries. *Lancet*  
436 *Psychiatry*. 2021 Jul;8(7):579-588. doi: 10.1016/S2215-0366(21)00091-2. Epub 2021 Apr 13.  
437 Erratum in: *Lancet Psychiatry*. 2021 Jun 4;; Erratum in: *Lancet Psychiatry*. 2021  
438 Nov;8(11):e21. PMID: 33862016; PMCID: PMC9188435.

439 16) Carballo JJ, Akamnonu CP, Oquendo MA. Neurobiology of suicidal behaviour. An  
440 integration of biological and clinical findings. *Arch Suicide Res*. 2008;12(2):93-110. doi:  
441 10.1080/13811110701857004. PMID: 18340592; PMCID: PMC3773872.

442 17) John W. Henderson, Robert D. Ricker, Brian A. Bidlingmeyer, and Cliff  
443 Woodward, Rapid, Accurate, Sensitive, and Reproducible HPLC Analysis of Amino Acids  
444 Amino Acid Analysis Using Zorbax Eclipse-AAA Columns and the Agilent 1100 HPLC

445 18) Debby Van Dam, Yannick Vermeiren, Tony Aerts, Peter Paul De Deyn. Novel and  
446 sensitive reversed-phase high-pressure liquid chromatography method with electrochemical  
447 detection for the simultaneous and fast determination of eight biogenic amines and  
448 metabolites in human brain tissue. *J Chromatogr A* 2014; Aug 1:1353:28-39

449 19) Beyza Sultan Aydin, Ibrahim Bulduk. A validated HPLC-UV method for detection of  
450 dopamine HCl in injectable Solutions *Eurasian J Bio Chem Sci* 2020; 3 (2): 116-120

451 20) Henderson JW, Ricker RD, Bidlingmeyer BA, Woodward C. Rapid, Accurate,  
452 Sensitive and Reproducible HPLC analysis of amino acids. 2000 Agilent Technologies Inc

453 21) Dubey A, Joshi S, Upadhyay K, Mahato A, Rathore AS. Automated method for  
454 quantification of 20 amino acids in cell culture media during biopharmaceuticals  
455 development. *Biotechniques* 2024; 76 (1): 27-36

456 22) Zhang Qi. Fast UHPLC method for the simultaneous determination of free D-aspartic  
457 acid and D-serine in brain tissue extracts. Thermo Fisher Scientific

458 23) Yufei Lu. Analysis of Amino Acids Derived online using an Agilent Advance Bio  
459 AAA column 2017 Agilent Technologies Inc

460 24) D'Aniello A, Di Fiore MM, D'Aniello G, Colin FE, Lewis G, Setchell BP. Secretion of  
461 D-aspartic acid by the rat testis and its role in endocrinology of the testis and  
462 spermatogenesis. *FEBS Lett* 1998;436:23-7.