Estimation of DOPAMINE in the Human Brain Tissue: Post-Mortem Studies in a Group of Suicides and in a Control Group

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Abstract

Mind has managed to enthrall from common man on the street to researchers and scholars alike, especially when it approaches to mental diseases like Depression, Anxiety, Schizophrenia, Huntington, Alzheimer or to the Act Of Suicide. The stories of someone known or unknown committing suicide has stewarded its way to the morning newspaper headlines and has tended to hold our miniscule attention every so often. Without losing sight of the fact, we have endeavoured to dig out the biochemical relation behind suicide in our research.

Dopaminergic pathways of brain have long been panned out as the chief commander of reward, motivation and people' likes and dislikes psychology and also their association with major diseases like Schizophrenia, Parkinson disease, psychosis, Huntington disease been turned out to be a fact. Nucleus accumbens, neostriatum and substantia nigra's dopamine loss over time leads to anhedonia, which is one of the major risk factor of suicide.

The scientific experiments among rats, mice have corroborated the influence of stress, aggression, strong like, dislike psychology over Hypothalamo-Pituitary Axis, culminating to a deranged cortisol and dopamine secretion which incepts suicidal behaviour and eventually the act.

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

Keywords: Suicide, Dopamine, HPLC, HPA, Mental Illness.

Introduction

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

The basic unit of brain is neuron. Neuron communicates and addresses with its bordering neurons and axons with two basic modes of

communication and interaction, one is electrical impulse and other is chemical impulse. The electrical and chemical impulse and signals vary in their behaviour and arrangement. The point or nodal area where transmission of message befalls, either in the electrical or chemical mode is synapse, it can be pre synapse, from where the transmitter signals have sprung to post synaptic where they will be received with open arms
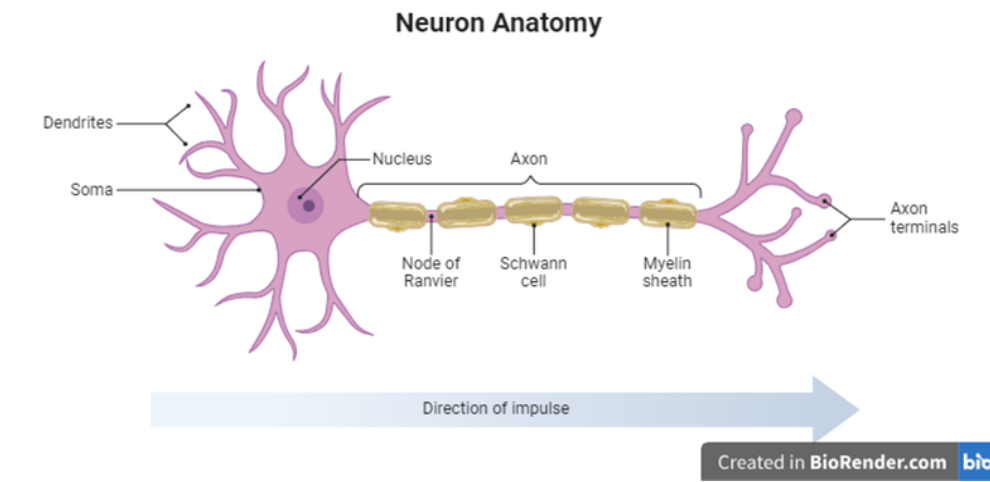
Figure 1: Anatomy of Neuron

There are billions of nerve cells in human brain, each possessing their own uniqueness in terms of action, shape and forms. The 10^{11} neurons constitute of thousands of collaborations among their fellow brothers or the ones belonging to disparate team for signal transmission.

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

CHARACTERISTICS OF SYNAPSE

ELECTRICAL SYNAPSE

- Transmission of signals volleys through gap junctions.
- Smaller in size, 0.2 nm.
- Transmission occurs at whirlwind speed
- There is no issue whatsoever with respect to low oxygen or pH.
- Most commonly occur in hippocampus, retina, olfactory bulb and cerebral cortex.

CHEMICAL SYNAPSE

- Transmission of signals volleys in one way with the presence of neurotransmitters
- Large in size, 10-20 nm.
- Transmission is sluggish, with tendency to be weary
- Quite problematic in absence or low quantity of oxygen
- Found in most of neuronal junctions.

NEUROTRANSMITTERS

Neurotransmitters are chemical messengers that transmit signals in the nervous system. They play a crucial role in the communication between nerve cells, or neurons, and are involved in various physiological and behavioural processes, including mood regulation, memory formation, and motor function. One such neurotransmitter is Dopamine which belong to the Monoamine (eg. Dopamine, Serotonin and Norepinephrine) class of neurotransmitters, is produced by substantia nigra, ventral tegmental area and hypothalamus of the brain.The DA system of the brain has a

numerous role in personality, behaviour, attitude, moods, reward seeking actions and the recent studies have proved that since DA system was last part of embryological origin to turn fully functional, its circuits are novel and their destabilization might be an answer to mental health related disorders like schizophrenia, Parkinson, Alzheimer etc.

To fully grasp the understanding of Dopamine, we revert to the basics and study the original part of the brain, where all the systems of brain originate from, The Prefrontal cortex.
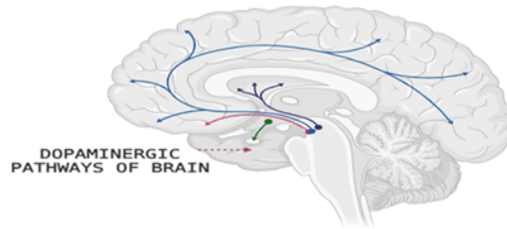

Figure 2: Dopaminergic Pathways of Brain

DOPAMINE PATHWAYS OF BRAIN

a)   Dopamine's role in reward, motivation and people' likes and dislikes psychology has been well demonstrated and understood in rat experiments.

b)   Their presence in nucleus accumbens and neostriatum and substantia nigra being the source of dopamine is well documented among humans too following extensive research over the decades.

c)   They have association with major diseases like Schizophrenia, Parkinson disease, psychosis, Huntington disease, and dopamine loss over time leads to anhedonia.
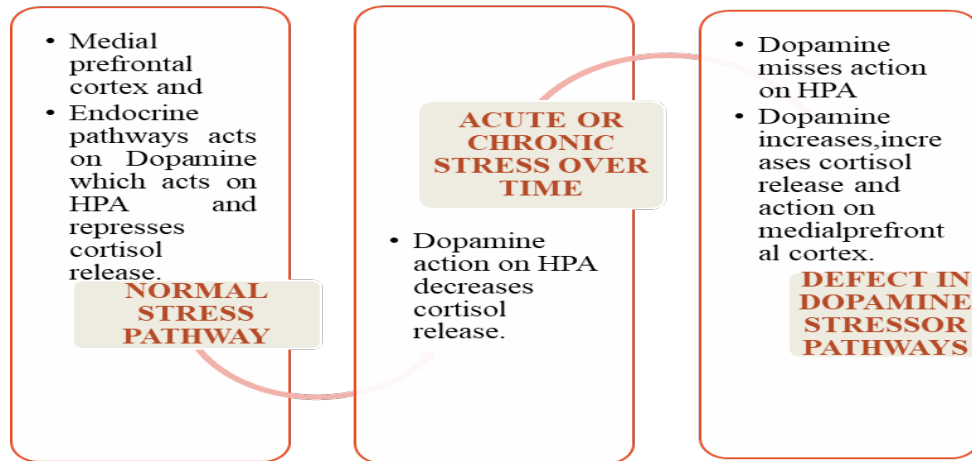
Table 1: Tabulated description of Dopamine action on Hypothalamo-Pituitary Axis

(d) Dopamine association with suicidal and aggressive behaviour is certified in rat experiments over time due to its influence in Hypothalamo -Pituitary Axis.

(e) In humans, during stress, its release from substantia nigra following medial pre-frontal cortex and action of endocrine pathways leads to

dopamine stimulating HPA axis and squeezing cortisol to deal with stress.

(f) Defect in stress pathways, its receptors or due to acute or chronic stress over an abnormal time period affects the normal stress pathway and causes overt raise of cortisol and loss of inhibition of dopamine, which deranges HPA axis and makes the individual and more likely suicidal.

WHO and The National Crime Record Bureau (NCRB) data report 133,623 committed suicides, with a rate of 10.6/100,000 in 2015. NCRB report also shows lesser suicidal rate compared to the WHO report. This discrepancy has led to serious criticism that NCRB data are not true representative of suicidal rates. This is because for NCRB data systematic verbal autopsy is not used, but a passive surveillance is done. The data are mainly gathered from the First Information Report (FIR) from the police.The reporting of suicide faces many challenges such as stigma attached to suicide deaths, post-mortem procedure and legal issues involved in completed suicide. However, the new Mental Healthcare Act 2017, Section 115, has decriminalized suicide, facilitating help seeking.
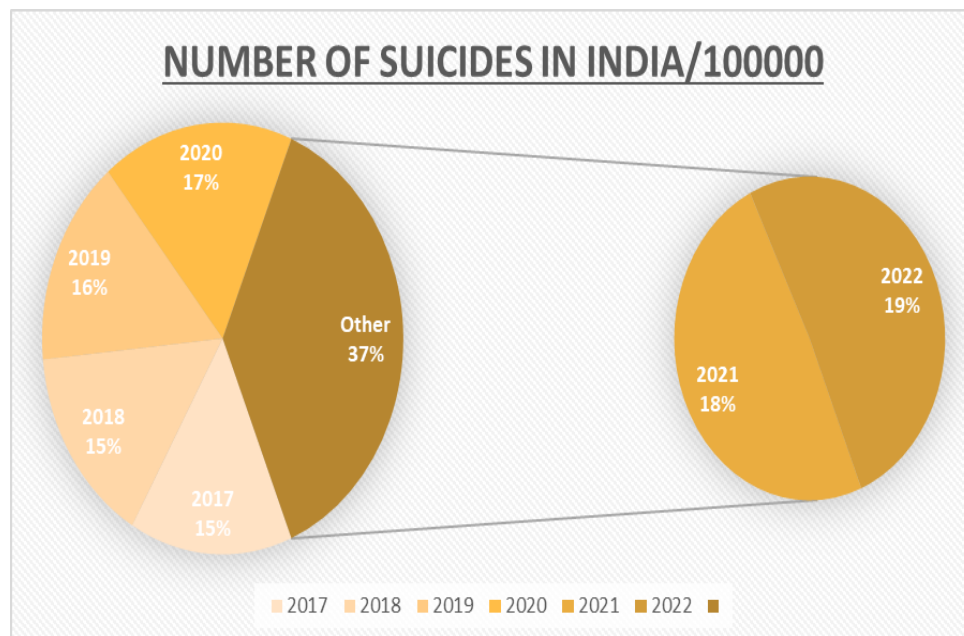
Figure 3: Number of Suicides in India /100000 From 2017-2022

Association Of Neurotransmitters with Suicide

a)   Right from early 19th century, when research regarding psychological disorder was initiated, there has been hue and cry over the source of menace.

b)   Following centuries of research and study on animals and human alike, there has not been a single soul to take the blame.

c)   Many neurotransmitters have been extensively studied, but there is not a concrete proof in our hands.

d)   Serotonergic pathway and dopaminergic pathway affect our personality, behaviour, thought process, reward behaviour, motivation and that has a proven alliance to irrational act like suicide.

e)   Hence, the role of neurotransmitters is a pressing point with respect to act of suicide.

MATERIALS AND METHODS

1. STUDY DESIGN

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 of ALL INDIA INSTITUTE OF MEDICAL SCIENCES, BHOPAL following which the study has been initiated.

2. CASES

A. Inclusion criteria

1. Deceased who have committed suicide and brought to AIIMS-BHOPAL for post-mortem.
2. Death due to suicide by hanging.
3. Deceased who have brought to AIIMS-BHOPAL mortuary within 24 hours of death.
4. Age above or below 18 years of age based on availability.

B. Exclusion criteria

1. Deceased whose next kin refuse to give consent.
2. Death due to pesticide poisoning.
3. Any other recreational drug use by victim
4. Any victim suffering from metabolic disorders or any systemic disorders.

3. CONTROLS

C. Inclusion criteria

1. Deceased who had accidental death.
2. Deceased who have brought to AIIMS-BHOPAL mortuary within 24 hours of death.
3. Age above or below 18 years of age based on availability.

D. Exclusion criteria

1. Deceased whose kin refuse to give consent.
2. History of recreational drug use by victim.
3.History of neurodegenerative diseases, stroke etc.

4. SAMPLE SIZE:

To correlate the levels of biochemical markers with the patients who have committed suicide, factors t-test [mean difference between two independent means (two groups) will be used]. Sample size has been calculated manually. Total sample size has been calculated with confidence interval 95%.

5. Data Assumptions:

N= NUMBER OF CASES
Z=DEGREE OF CONFIDENCE (FOR 95% CONFIDENCE INTERVAL, Z=1.96)
P=PREVALENCE=10.42
D=PRECISION OF ERROR=6%
$N=Z2*P(1-P)/D2$
$N= (1.96)2*0.1042(1-0.1042)/(0.06)2$
N=99.60, which is equal to 100
Though, the calculated sample size is 50 each, the collected sample size based on availability and ruling out internal and external criteria has reached 30 each. Hence, our **N=30 for cases and controls each**.

6. IMPLEMENTATION PLAN:

Deceased were enrolled for study at AIIMS Bhopal hospital mortuary for hospital autopsy by Forensics And Toxicology Department. A psychological autopsy was conducted with the deceased kin regarding patient's mental history before attempting suicide by myself under the guidance of Department Of Psychiatry. Another mandatory questionnaire regarding name, gender, ethnicity, profession, mental health history, recreational drug use history, alcohol intake, history of any previous treatment.

6.1. Brain Tissue Retrieval Following Autopsy

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 -80ºC ultradeep freezer, at Department of Biochemistry till further analysis.

For each suicide or accidental death, centrifuge tubes were labelled for parameter Dopamine and portions of retrieved pre-frontal cortex were inserted in respective microcentrifuge tubes following hospital autopsy at AIIMS, BHOPAL Hospital Mortuary.

6.2. HIGH-PRESSURE LIQUID CHROMATOGRAPHY:-

(A) <u>Extraction Of Dopamine from Brain Tissues</u>

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.

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

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

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

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

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

Since, we have decided to run single desired amino acids called DOPAMINE, we did not require 26 minute or 14 minutes run time.

For dopamine based on our standard graph, value was estimated under 4 minutes and post -run time being 0.5 minutes.
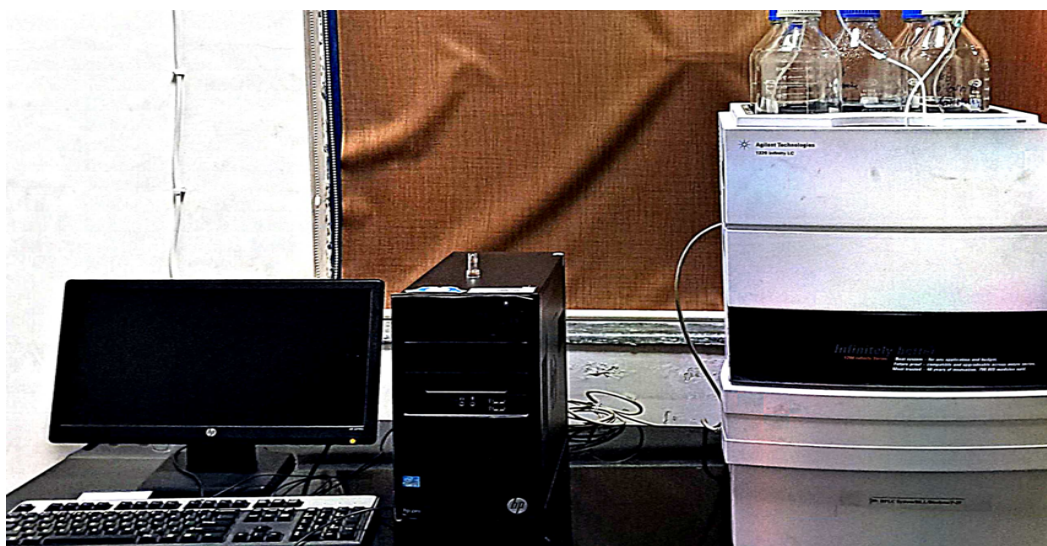

Figure 4: Figure showcasing installed HPLC Machine in HPLC Room at Department of Biochemistry, AIIMS BHOPAL

7. ESTIMATION OF BRAIN DOPAMINE
(A) Chemicals
1. Citric Acid – used to prepare Phosphoric Citric Buffer for brain sample preparation.
2. Dibasic Sodium Phosphate (Na2HPO4) - used to prepare Phosphoric Citric Buffer for brain sample preparation.
3. Potassium Phosphate Monobasic (KH2PO4) – used for preparation of mobile phase
4. Acetonitrile - used for preparation of mobile phase
5. Ultrapure Water – was obtained using a Milli-Q filter system

6. Dopamine HCl – for standard preparation
All reagents purchased were of HPLC grade.
(B) Brain Sample Preparation
Postmortem brain was obtained from prefrontal complex and immediately stored at -80ºC. At the time of processing, 300 mg of brain was mixed with 4 ml phosphoric-citrate buffer (pH 3.25).

This mixture was homogenized for 40s at moderate speed using homogenizer. The homogenate was then centrifuged (26,000 g, 20 min, 4ºC).

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, 4ºC) which were washed twice beforehand with mobile phase.

Since, the two parameters are Dopamine and D-AA, both being amino acids, for the processing same sample was used, hence total amount of brain tissue taken was 400mg, out of which 300 mg was used for Dopamine and DAA.

(C) Instrumentation

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

The autosampler was set to take 20 μL of this solution. 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.

(D) Chromatographic Separation

The chromatographic separation was performed using a mobile phase A containing 50 mM Potassium Phosphate Monobasic (KH2PO4) buffer and mobile phase B containing acetonitrile at a flow rate of 1 mL/min.

The column temperature was set at 30ºC and monitoring wavelength at 280 nm. To quantify the amount of dopamine the area under curve (AUC) was used.

Before using mobile phase, it was first filtered through 0.45 μm membrane filter and buffer 0.20 μm membrane filter and using vacuum filtration and then degassed using sonicator to remove air bubble.

Gradient Program

(1)   0 – 5 min: MP A: 95-65

(2)   Since the standard showcased the dopamine peak at retention time of 2.2 seconds during five serial dilutions of standard sample, our dopamine sample processing method file was set at under 5 minutes and mobile phase A and B being 95 and 5% each during the course of run of each sample.

(E) Standard Preparation And Calibration Curve

The highest standard was prepared by dissolving 1 M of dopamine HCl in 10 mL of deionised water.

This solution was then serially diluted in mobile phase to generate the different points of standard curve ranging from 10 to 100000 μmol/L.

Retention time was observed at 2.2 seconds.

(F) Dopamine Standard Graph
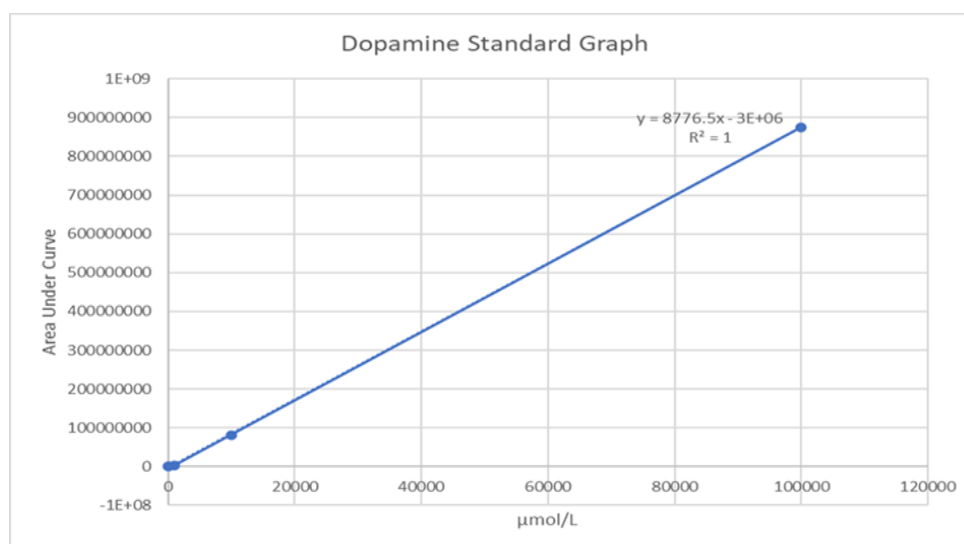
Using the standard graph formula, $y = mx + c$, calculations have been made

Where m = slope value/ gradient of the line

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

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

$(R)2 = (r)2$

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

R value was 1.

The model perfectly predicts the outcome.

The value of m can be calculated from the angle which this line makes with the x-axis or a line parallel to the x-axis. Putting our area under curve values in excel, the **'m' value was 8776.5**.

Bases on the intercepting line on excel, **'c' value was 3*(10)6.**

Putting all this into the equation for individual Dopamine levels of cases and controls,

Observed Dopamine value was calculated (per Litre in 300mg of brain). And for Dopamine level in mmol/l/g, the values were calculated by **Observed Dopamine value*3.3/1000**

For example, Case 1

Area under curve (AUC) = 4573095 for Dopamine, after HPLC RUN with Retention time at **2.2 minutes** at **280nm**.

Using formula, y = mx + c,

Where **y = 4573095**, **m = 8776.5** and **c = 3000000**,

we get x/ Observed Dopamine value (/L in 300 mg Brain) as **862.88**

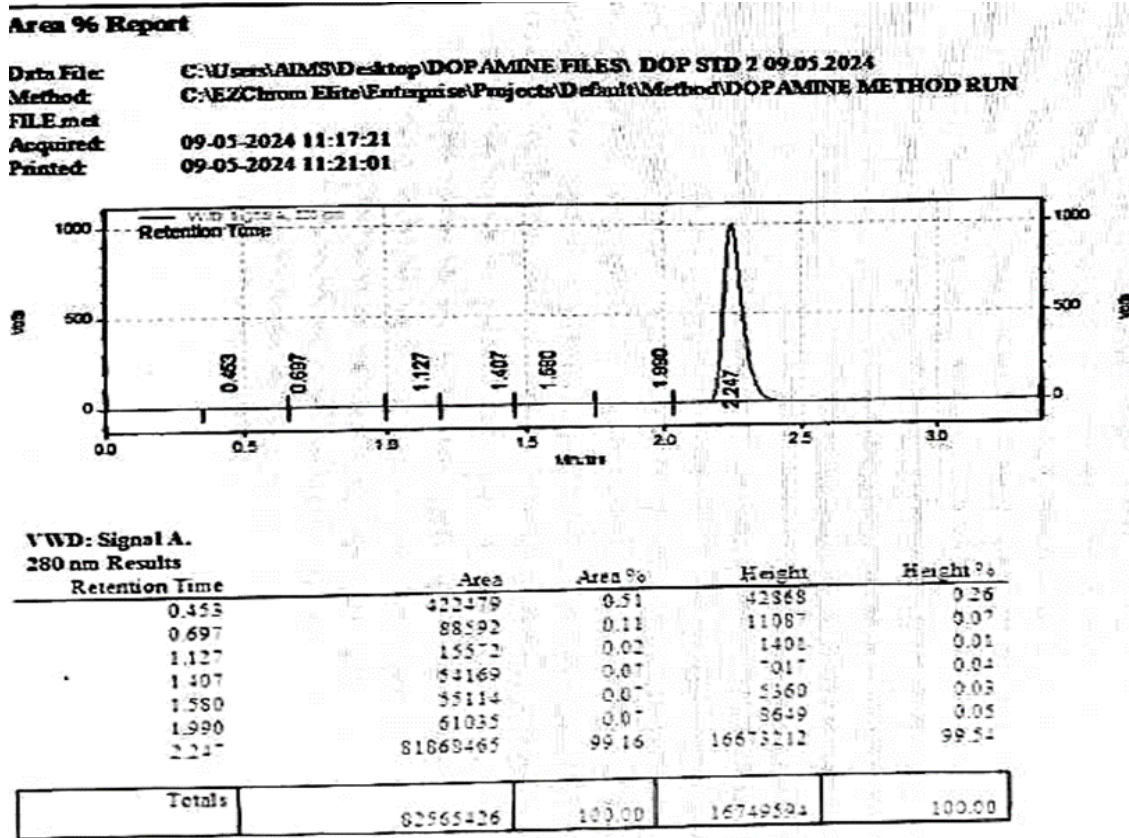The desired Dopamine level (mmol/l/g) is 862.88*3.3/1000 = **2.84**

Figure 5: The above Dopamine standard describing Retention time of 2.2 minutes with a wavelength at 280nm

(G) VALIDATION OF DOPAMINE 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.

I. Stock Solution

The reagent procured by us was Dopamine HCL which has a molecular weight of **189.64g/mol** and hence to make standard stock solution, we required approximately 0.38 mg weighed accurately in an analytical balance and transferred in 10ml of fresh falcon tube, carrying 2ml of ultra-pure water as a diluent. This is defined as 1M.

The solution was vortexed well and filtered using 0.45μm pore size membrane filter before being utilized for serial dilutions to create standard graphs.

II. Standard Solution

To create five standard graphs, five serial dilutions were carried out.

In first standard, 990 μl of ultra-pure water was taken as diluent and 10μl of standard stock was put in a fresh 1.5 ml micro centrifuge tube, and credited as 0.1M.

In second standard, 10μl of stock was taken from first standard and 990μl of ultra-pure water as water as diluent and put in a fresh 1.5 ml micro centrifuge tube. It is credited as 0.01M.

In third standard, 10μl of stock was taken from second standard and 990μl of ultra-pure water as water as diluent and put in a fresh 1.5 ml micro centrifuge tube. It is credited as 0.001M.

In fourth standard, 10μl of stock was taken from third standard and 990μl of ultra-pure water as water as diluent and put in a fresh 1.5 ml micro centrifuge tube. It is credited as 0.0001M.

In fifth standard, 10μl of stock was taken from fourth standard and 990μl of ultra-pure wateras water as diluent and put in a fresh 1.5 ml micro centrifuge tube. It is credited as 0.00001M.

All were labelled as 1,2,3,4 and 5 respectively and were vortexed well to be run as dopamine standards in HPLC utilizing HPLC micro tubes having capacity of 1.0ml.

III. Linearity

The linearity of a method is defined as its ability to provide measurement results thatare directly proportional to the concentration of the analyte, or are directly proportional after some type of mathematical transformation.

Standard calibration has been prepared using five standard solutions within the concentration range of 38-100 μg m/L. In optimised chromatographic conditions, each standard solution was chromatographed for 10 minutes five times.

The least squares linear regression analysis of the average peak area versus concentration data were used to evaluate the linearity of the method using the formula

$y = mx + c$.

Where m = slope value/ gradient of the line

C = intercept of the line

IV. Accuracy

The accuracy of an analytical method is given by the extent by which the value obtained deviates from the true value. In this method, recovery experiments were conducted to confirm the accuracy of the proposed method.

V. Precision

Precision was analysed by calculating variations of the method in intraday (repeatability performed by analyzing standard solution on the

same day) and inter-day (repeatability carried out by analyzing standard solution on three different days). These analyses were performed by injecting six times standard solution.

VI. Selectivity/ Specificity

A method is said to be 'specific' if it provides a response for only a single analyte, while the term 'selective' means that the method provides responses for a number of chemical entities that may be distinguished from each other.

Selectivity also implies the ability to separate the analyte from degradation products, metabolites, and co-administrated drugs and 'specificity' as the ability to assess the analyte unequivocally in the presence of other components such as impurities, degradation products, and the matrix. The chromatogram of mobile phase/blank solution was compared with chromatogram of standard solution to evaluate the selectivity of the proposed method. Retention time (Rt) was calculated to demonstrate the chosen method.

VII. LOD AND LOQ

The LOD is defined as the lowest concentration of an analyte that can be detected under the analytical conditions to be used. The presence of analytes can be seen at the LOD; however, their concentrations cannot be quantitatively measured.

The LOQ is the lowest concentration that can be determined with acceptable accuracy and precision under the analytical conditions. Generally, the LOQ can be estimated as three times that of the LOD.

These values were determined using the standard error (s) and the slope of regression line (m).

LOD = 3:3 s/S

LOQ = 10 s/S

VIII. Robustness/Ruggedness

The robustness of an analytical method can be defined as a measure of the capability of the method to remain unaffected by small, but deliberate, variations in method parameters. The parameter therefore provides an indication of the method reliability during normal usage.

The ruggedness of a method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different lot of reagents, different days, etc. Our standard solution has passed that test.

IX. Stability In Matrices

For bioanalytical studies, the stability of analyte(s) in appropriate biological matrices should be evaluated, since it is important to verify that no degradation takes place between the time of sample collection and their analysis. Analyte instability can arise from various factors, such as interaction with container surfaces, reaction with air, thermal decomposition, evaporation of volatiles, or photolysis.

To minimize degradation, some treatment of the sample can be made, such as lowering the temperature or freezing the samples, protecting from light, adding some stabilizing agent, adjusting the pH, making a chemical derivatization, or adding enzyme inhibitors.

In our case, we did it by protecting from light and stabilizing the temperature.

The validation report has concluded that the Dopamine standard samples have passed the validity of the method and hence cases and controls Dopamine values are valid according to our knowledge and expertise.

5.0 RESULTS:

To fulfil the objective of estimating Dopamine value in suicide victim's brain tissue and compare it with accidental death patient's brain tissue samples, we have taken informed consent from 30 suicide victims next of kin and 30 accidental deaths next of kin, and all the victims fulfilled our inclusion and exclusion criteria.

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.**

Sample size about 400 mg was collected in 1.5 ml micro centrifuge tubes following informed consent and psychological autopsy from both suicide cases and accidental deaths.

Based on psychological autopsy there have been many risk factors ranging from alcoholism, financial issues, career issues, and marital issues among suicide victims.

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.

What we could not ascertain via psychological autopsy is that whether alcohol, drug consumption, financial worries or relationship issues were a result of underlying depression, psychosis or vice-versa.

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.

Since both cases and controls had more than 15 parameters that is 30 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 each parameter like Cholesterol, D-Aspartic acid and Dopamine. The two groups are independent by nature.
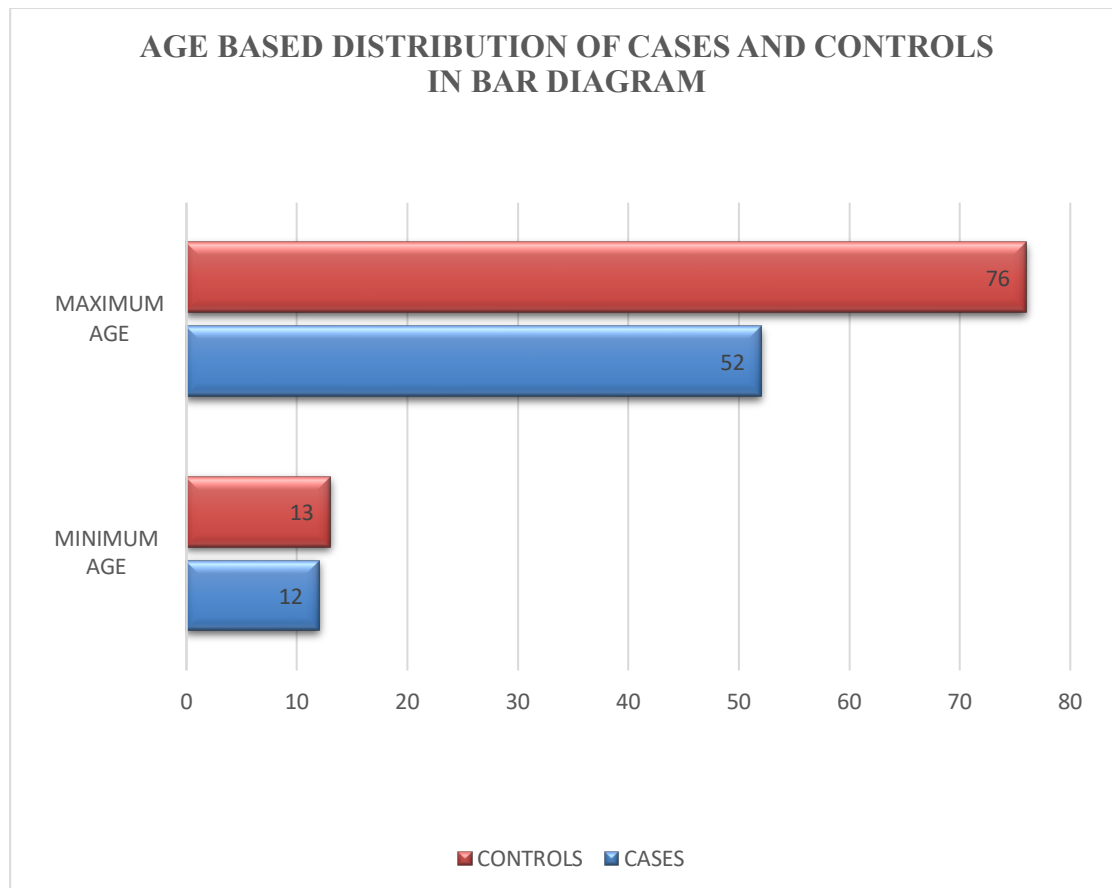
Figure 6: Age Based Distribution Between Cases and Controls
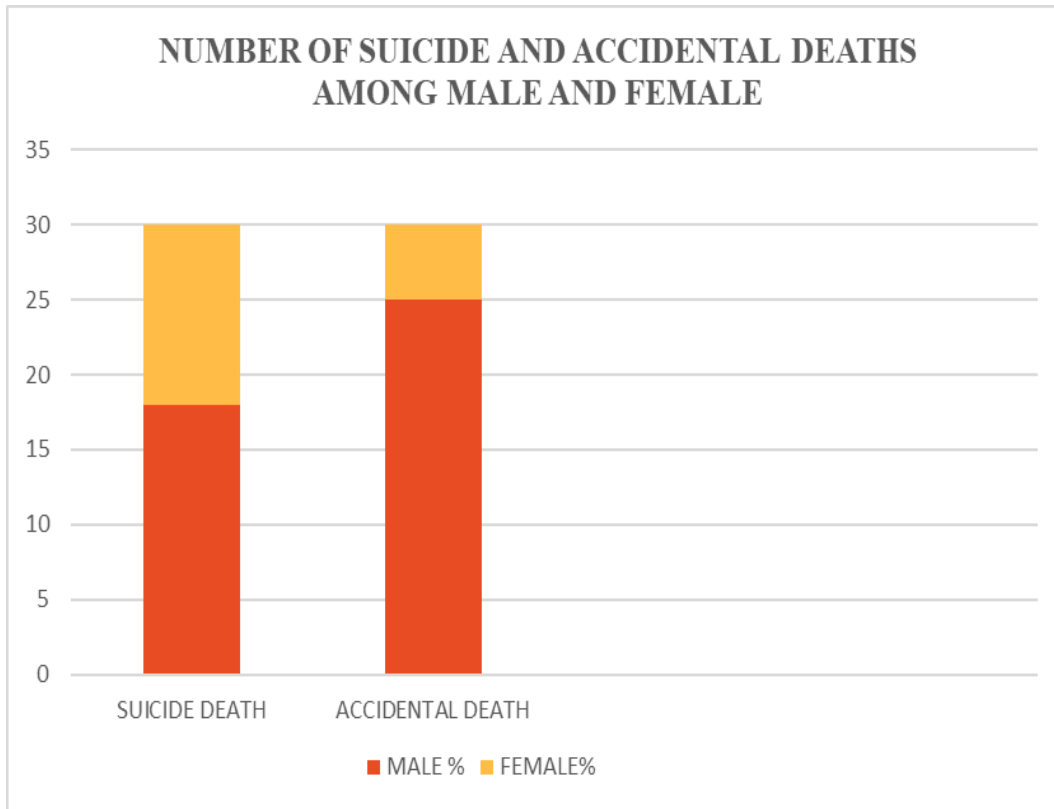
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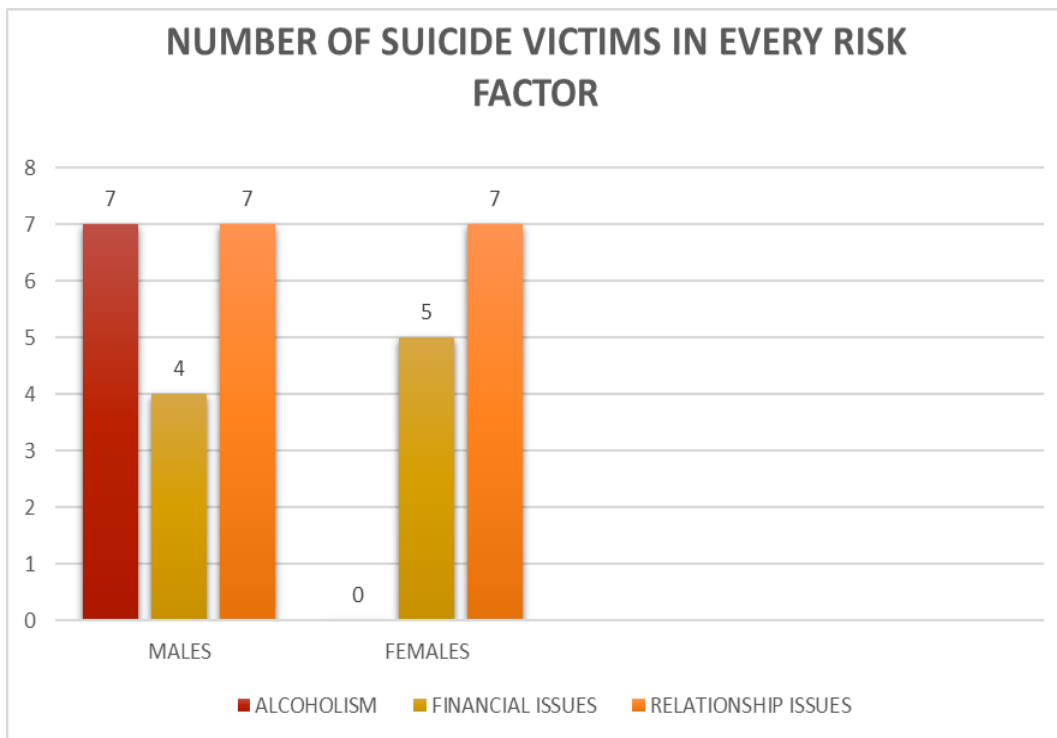
Figure 8: Number of Suicide Deaths in Every Risk Factor

Table 2: Tabulated description of calculated Brain Dopamine level of controls by HPLC method

SR. NO.	POST-MORTEM NUMBER	AREA UNDER CURVE	OBSERVED DOPAMINE VALUE (MMOL/LITRE IN 300 MG BRAIN)	DOPAMINE LEVEL (MMOL/ML/G)
1	22/2023	701790	421.78	1.40
2	27/2023	469071	395.26	1.31
3	51/2023	556072	405.18	1.34
4	32/2023	585242	408.5	1.36
5	43/2023	567855	406.52	1.35
6	46/2023	606844	410.96	1.36
7	49/2023	710843	422.81	1.40
8	50/2023	2348583	609.42	2.02
9	61/2023	922083	446.88	1.48
10	64/2023	703262	421.95	1.40
11	85/2023	722519	424.14	1.41
12	172/2023	714212	423.2	1.40
13	179/2023	578783	407.76	1.35
14	188/2023	825850	435.91	1.45
15	187/2023	1195415	478.02	1.59
16	191/2023	884595	442.61	1.47
17	483/2023	1428352	504.56	1.68
18	536/2023	1339237	494.41	1.64
19	32/2024	1835014	550.9	1.83
20	35/2024	2040342	574.29	1.91
21	61/2024	1906422	559.04	1.86
22	103/2024	1892983	557.5	1.85
23	120/2024	2381605	613.18	2.04
24	138/2024	2327338	607.00	2.02
25	140/2024	408024	388.31	1.29
26	146/2024	1578941	521.72	1.73
27	153/2024	1367242	497.60	1.65
28	179/2024	2493501	625.93	2.08
29	273/2024	506173	399.49	1.33
30	277/2024	667190	417.84	1.39

Table 3: Tabulated description of calculated Brain Dopamine level of cases by HPLC method

SR. NO.	POST-MORTEM NUMBER	AREA UNDER CURVE	OBSERVED DOPAMINE VALUE (MMOL/LITRE IN 300 MG BRAIN)	DOPAMINE LEVEL (MMOL/ML/G)
1	57/2023	4573095	862.88	2.87
2	76/2023	4455575	849.49	2.82
3	88/2023	4380993	840.99	2.80
4	102/2023	4613417	867.47	2.88
5	189/2023	4991501	910.55	3.03
6	208/2023	5268485	942.11	3.17
7	271/2023	5782657	1000.7	3.33
8	348/2023	5093023	922.12	3.07
9	366/2023	3337596	722.10	2.40
10	367/2023	4980807	909.33	3.02
11	383/2023	7383506	1183.10	3.93
12	410/2023	4706062	878.03	2.92
13	439/2023	4769181	885.22	2.98
14	468/2023	4866763	896.34	2.98
15	482/2023	5148032	928.39	3.09
16	534/2023	4896101	899.68	2.99
17	552/2023	4419789	845.41	2.81
18	553/2023	4275116	828.93	2.76
19	567/2023	4927307	903.24	3.00
20	245/2024	3976178	794.87	2.64
21	612/2023	1152954	1655.5	5.51
22	623/2023	4469802	851.11	2.83
23	006/2024	5918268	1016.15	3.38
24	95/2024	3856143	781.19	2.60
25	102/2024	4019035	799.75	2.66
26	104/2024	7838400	1234.93	4.11
27	130/2024	9166317	1386.23	4.61
28	150/2024	4497485	854.26	2.84
29	152/2024	4745480	882.52	2.93
30	228/2024	4468543	850.97	2.83

INDEPENDENT/UNPAIRED T-TEST ANALYSIS:

P value and statistical significance:

The two-tailed P value is less than 0.0001

By conventional criteria, this difference is considered to be extremely statistically significant.

Confidence interval:

The mean of CONTROLS minus CASES equals-**1.5467**

95% confidence interval of this difference: **From-1.7987 to -1.294**
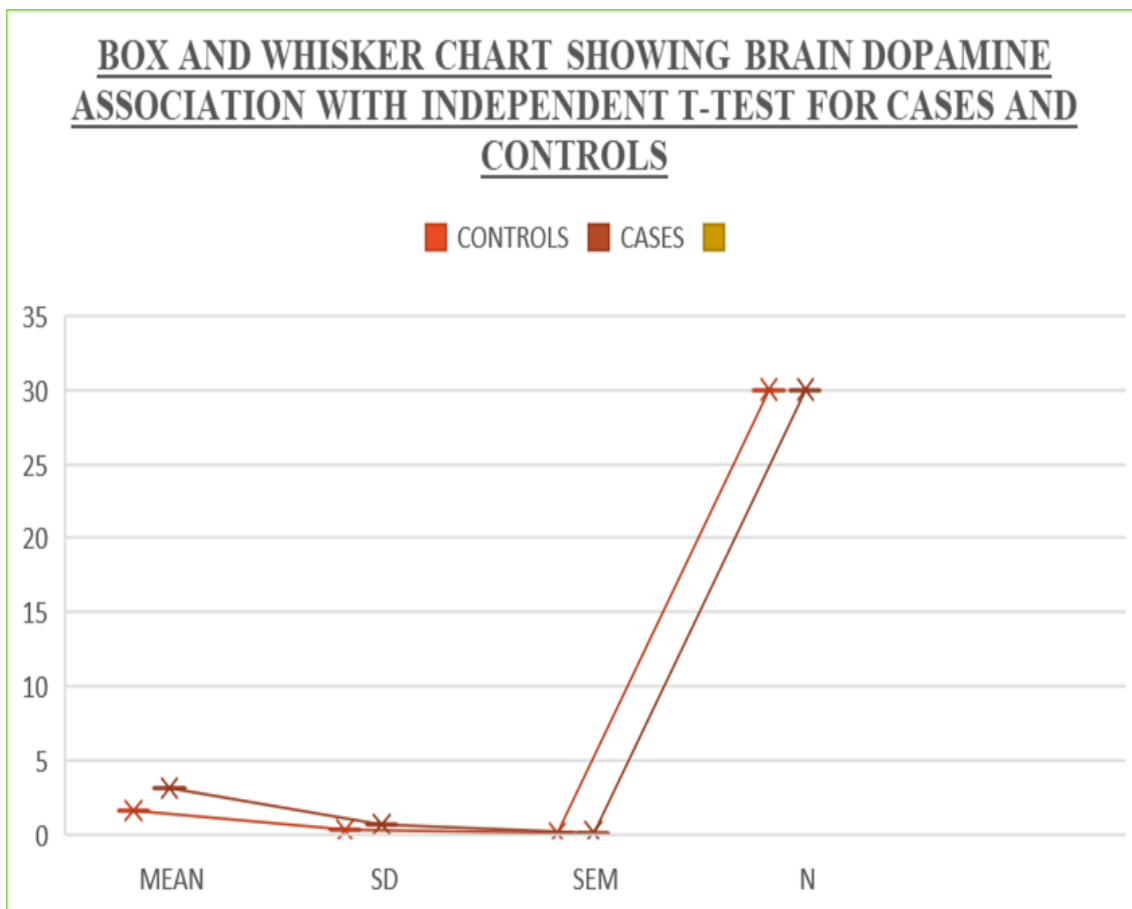
Intermediate values used in calculations:

t = 12.2845

df = 58
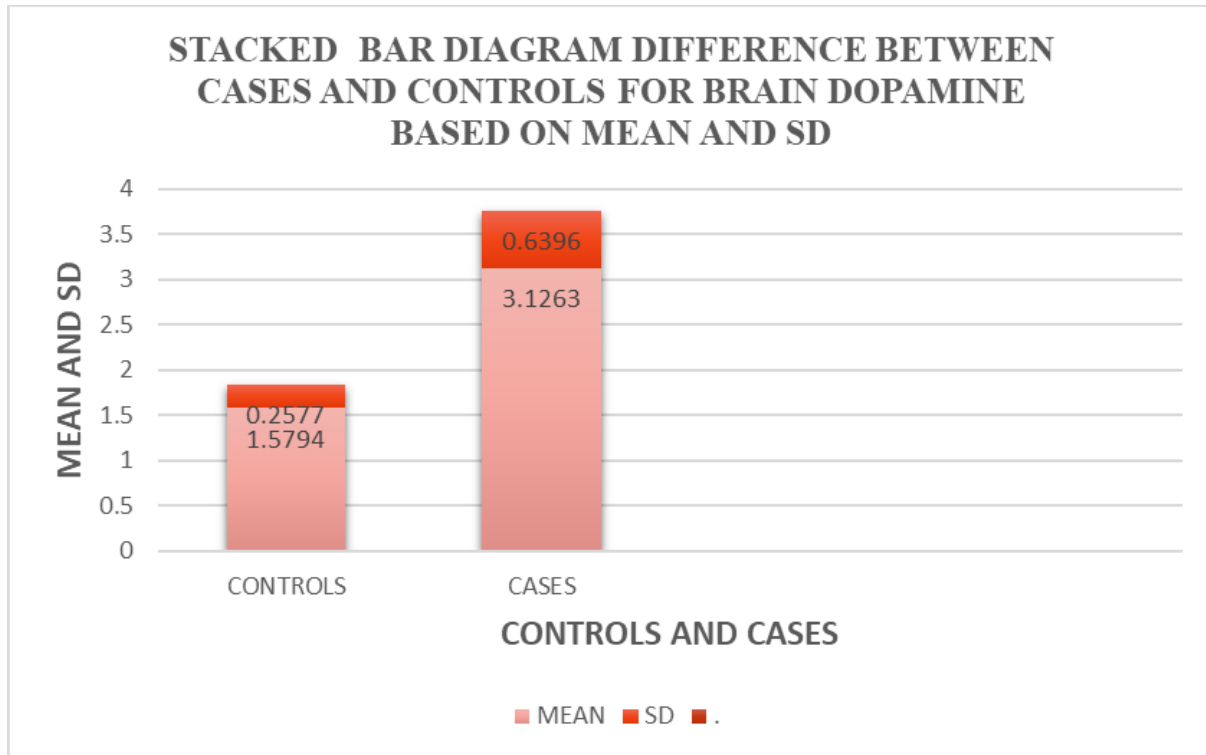
standard error of difference = **0.126**

Table 4: Tabulated Description of Mean, Sd, SEM And N Difference between Controls And Cases With Respect To Brain Dopamine

GROUP	CONTROLS	CASES
MEAN(MMOL/ML/G)	1.5797	3.1263
SD	0.2577	0.6396
SEM	0.0470	0.1168
N	30	30

Figure 9: Box And Whisker Plot for Brain Dopamine In Independent T-Test

Figure 10: Stacked Bar diagram For Brain Dopamine In Independent T-Test

The output indicates that the mean for Control is **1.5797** and for the Case is **3.1263**. The mean of CONTROLS minus CASES equals **-1.5467**. 95% confidence interval of this difference is from **-1.7987** to **-1.294**. The standard deviation column shows a not much difference between cases and controls with control values **0.2577** and case value being **0.6396**. Since, the p value has a steeped value of less than **0.0001**, which is less than 0.05 we can reject the null hypothesis which states that there is no association between the level of dopamine and suicide and accept the alternative.

The alternative hypothesis proves that brain dopamine has an effect in the act of suicide and its values show a large raise before the act of suicide is commenced as compared to road traffic accidents and that corroborates the previous studies where brain dopamine has proven an association with mental health disorders and the act of suicide.

The different studies have pointed out the dual role of dopamine as both excitatory and inhibitory neurotransmitter and has commenced the fact about its action on brain hypothalamo-pituitary axis.

This study also corroborates that fact where during the ongoing depressive or any mental health episode dopamine might be low but before commencing the suicidal act, the level shoots up.

Thus, our aim to estimate the value of dopamine and the first objective to correlate the act of suicide with high brain dopamine level and showcasing its association has been proved.

6.0. DISCUSSION:

The present study was conducted in All India Institute Of Medical Sciences, Bhopal by Department of Biochemistry in collaboration with Department of Forensics and Toxicology and Department of Psychiatry 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 at AIIMS, Bhopal which 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 Dopamine 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 parameters Dopamine by HPLC method.

Further, the objective was to compare the mentioned parameter between the suicidal victims (Cases) and road-traffic accident victims (Controls) which has been done by Unpaired/Independent t-test where we evaluated mean, standard deviation, SEM and N of every case sample with respect to our control sample and have documented that there is a sumptuous difference for the, p value= 0.0001 for Dopamine which delivered a successful result that Dopamine values have accelerated in positive direction for suicide victims as compared to accidental deaths.

It was a dawn of the day moment, as we expected all the values to fall in suicide as compared to accidental deaths.

Evaluation of risk factor has shed a light on how alcohol, drug consumption leads to change in behaviour and eventually the act of suicide based on psychological autopsy.

It has also brought to our notice that mean age of suicide being 27.3 years as compared to 42.26 years for road traffic accidents which showcases the loss of young lives to the evil and economically, socially, emotionally,

psychologically leaving an everlasting impact on the survivors and crippling them forever.

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 or rise can serve as a blessing to the psychologically affected patients in saving their lives, and could benefit the clinician in prognosis and diagnosis and hence, shall be researched rigorously.

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.

According to results achieved by us,for Dopamine, the results underline the fact of involvement of Brain Hypothalamo-pituitary axis and outplays the dual role of dopamine as both excitatory and inhibitory neurotransmitter. It underlines the fact that right before the act of suicide, there is an escalation in the level of dopamine due to excitatory nature, biochemical basis stems from the down regulation of dopamine receptors, releasing more cortisol from the prefrontal cortex, but during the course of mental health disorder, the levels of dopamine

are usually low due to inhibitory nature as has been proved in previous 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.

CONCLUSION:

Suicide was among top 20 causes of death in 2019. 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. So far, the risk factors that we have run into during the course of research are alcohol, drug addiction especially among young males, financial/ career crisis, relationship issue, lack of marital bliss according to people's perception, and ego clashes, an unidentified case of sexual assault and future anxiety. There are 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.

The aim of the study was to determine and estimate the level of Dopamine for suicide victims and road traffic accidents which have been achieved conclusively.

With the study, we realised the dramatic effects of drugs and alcohol on one's personality but we surely need more brain storming over the matter.

Strengths of the study:

The research was conducted at All India Institute of Medical Sciences, Bhopal which belongs to central state of India called Madhya Pradesh and it is one among the top 10 suicide states of the country.

Madhya Pradesh accounted for 15,386 total suicide cases out of 1,70,924 documented cases in year 2019. There have been 14 reports of mass/ family suicides in year 2020 from this part of country. The study was novel in the approach that we set on an endeavour to perceive Dopamine as a potential biomarker for which the first step was

to evaluate its level among the suicide victims and to demonstrate its association with the suicide victims, and which has been accomplished.

Future Prospective:

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 mentioned parameter 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.

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