MEAN PLATELET VOLUME IN DEPRESSION AND ANXIETY DISORDER-A HOSPITAL BASED CASE CONTROL STUDY

Depression and anxiety disorder are the common mental disorders. Serotonin (5-hydroxytryptamine [5-HT]) is well established neurotransmitter in the central nervous system (CNS). It has a role in the anxiety, depression, appetite, motor, cognitive and autonomic functions, platelet aggregation and regulation of vascular tone. As the CNS is difficult to access, peripheral platelet models are widely used as indicators of central 5-HT metabolism; moreover, they are known to reflect central serotonergic function. Mean platelet volume (MPV) is contemplated as the marker of platelet function. Mean platelet volume (MPV) is a measure of platelet size and a good indicator of platelet activity. In this backdrop the current study was carried out to evaluate the MPV in depression and anxiety disorder.

Method:

Consecutive 90- depressive disorder patients, 76- anxiety disorder patients diagnosed according to DSM V criteria and 49 healthy control subjects were recruited for the study. Hamilton rating scale for anxiety (HAM-A), Hamilton rating scale for depression (HAM-D), MPV and platelet count was measured in all subjects.

Results: MPV was more in Depression (9.73±1.23) and Anxiety disorder patients (9.84±1.32) compared to the controls (8.773±0.44) and this difference was statistically significant ($F=14.95$, $p<0.001$). There was no statistical difference in the MPV values between the Depression and anxiety patients. There was negative correlation between MPV and platelet count.

Conclusion: This study suggests increased MPV is associated with depression and anxiety disorder. Future research should be planned to investigate the effect of treatment of depression and anxiety disorder on MPV.

1. Introduction:

Depression and anxiety disorder are the common mental disorders. Serotonin (5-hydroxytryptamine [5-HT]) is an important neurotransmitter in the central nervous system (CNS) [1] and is considered to be influential in mediating mood and anxiety symptoms. Abnormalities in serotonin pathways are thought to play a pathophysiological role in depressive and anxiety disorder. [2] It also has a pivotal role in the vascular system for regulation of vascular tone and platelet aggregation. [3]

Researchers have recorded that a hyper serotonergic state resulting from impaired Serotonin transporter (5-HTT) function can cause fear response and depressive symptoms by stimulating the amygdala.[4] Investigators have suggested platelet activity is increased by emotional stress and hypothesized that the actions of stressors on platelets may be a primary trigger in coronary events such as myocardial infarction.[5]

The uptake, storage and metabolism of serotonin are similar in platelets and neurons [6] and the same gene encodes for the serotonin transporter in both cell types.[7] As more than 99% of the serotonin in the body is found in the dense granules of platelets[2] and the CNS is difficult to access, peripheral platelet models are widely used as indicators of central 5-HT metabolism; moreover, they are known to reflect central serotonergic function.[1,4]

Mean platelet volume (MPV) is a measure of platelet size and is a good indicator of platelet activity. Peripheral platelet models are usually used as pointers to reflect the serotonin changes in the brain as CNS is hard to approach. Increased MPV is considered to be closely linked with cardiovascular diseases (CVDs), like acute myocardial infarction (MI), ischemic heart diseases, congestive heart failure and a close affiliation exists between CVDs anxiety and depressive disorders.[8,9]

There are few studies done to evaluate platelet parameters in depression, schizophrenia, bipolar disorder and anxiety disorder which are inconclusive. Their findings were also limited because most of the studies were retrospective, poor inclusion and exclusion criteria, and lack of use of standardized scales.[9-15] To overcome these limitations, the present study primarily aimed to compare the MPV values in patients with Anxiety disorder, Depressive disorder and healthy controls.

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2. Methodology:

This was a hospital-based, descriptive, cross-sectional case control study, conducted in the outpatient department of psychiatry of The Oxford Medical College, Hospital and Research centre (T.O.M.C.H&R.C), Bangalore, India in the year 2016 for duration of 3 months. Consecutive 76 patients of anxiety disorder and 90 patients of depressive disorder in the age group of 18-65 years were included in the study. Age and gender matched 49 subjects who were hospital employees or relatives of the patients and did not have any psychiatric disease were taken as controls. Subjects who had seizure disorders, mental retardation, other psychiatric disorders, hypertension, hypercholesterolemia, acute or chronic physical illnesses, pregnancy, or a history of any drug use during the last month, smoking and alcohol use were excluded from the study. Written informed consent was taken from the cases and controls. They were administered a semi structured proforma to collect socio demographic details, height, body weight, Hamilton rating scale for anxiety (HAM-A) and Hamilton rating scale for depression (HAM-D) was assessed by the psychiatrist in the OPD. Complete Blood Count, MPV and lipid profile were measured and recorded for each subject. The study was approved by the Institutional ethics committee of "The Oxford Medical College, hospital and research centre".

2.1Measurements:

2.1.1Hamilton rating scale for Anxiety (HAM-A):

HAM-A is one of the instruments frequently used to evaluate anxiety. It is a screening tool for anxiety symptoms with 14 items. Each item is rated on a 0 to 4 scale with a final item which rates behavior at interview. Score above 14 is considered as clinical anxiety present. Score below-14 no anxiety, 14-17 mild anxiety, 18-24 moderate anxiety, 25-30 severe anxiety. [16]

2.1.2 Hamilton depression rating scale (HAM-D):

HAM –D has 21 items and is an observer rated screening tool. It has 21 items and only 17 items are scored. Four additional items are used for diagnostic purpose. Ratings are made on the basis of clinical interview along with any additional available information such as nursing or family member report. Scores 7 and below is considered as normal, 8–13 as mild depression,14 – 18 as moderate depression, 19–22 as severe depression and 23 and above as very severe depression.[17]

2.1.3 Complete blood count and Biochemical analysis:

5 ml blood was obtained from medial cubital vein by venepuncture avoiding hemolysis. Blood samples were drawn from each subject after a fasting period of 12 hours. The first 2 ml venous blood was collected in sterile BD Vacutainer tube with 5.4mg of K2 Ethylene Diaminetetraacetic acid (EDTA) from BD Franklin Lakes NJ USA. Complete blood counts, including MPV, were determined using Sysmex XP -100: A1489 haematology analyser (Sysmex, India). In order to measure MPV reliably and to minimize the potential influence of anticoagulant [EDTA], blood samples were analysed within 60 minutes after venepuncture. MPV and platelet count were measured for all subjects. The reference range for MPV was between 6.9–10.8 fL. Remaining 3ml of blood samples was collected in gel Vacutainer. Samples were centrifuged after 30 minutes at 3000 rpm for 10 minutes. All the analysis was

carried on serum samples. Serum Cholesterol was measured by CHOD- PAP Method,[18,19] Triglycerides by GPO-PAP method, HDL by Phosphotungstic Acid method and LDL-C, VLDL-C were calculated by using Friedwald's Equations.[20] All the blood samples were analysed at the same laboratory.

2.2Statistical analysis:

The data was analysed using SPSS for Windows version 16.0 software (SPSS.INC Chicago ,IL, USA).Data were tested for normal distribution using Kolmogorov-Smirnov test. Results obtained were analysed using descriptive and inferential statistical methods. Chi square test was used for categorical data and student t test, ANOVA was used for continuous data. . Pearsons correlation was used to know the association of MPV, platelet count and anxiety scores and depressive scores.

3. Results:

There was no statistically difference in the socio-demographic details and body mass index of the cases and the controls (Table-1). HAM A and HAM D mean scores were higher in cases than controls and this difference was statistically significant (Table- 2). There was no statistically significant difference in the lipid profiles and haemoglobin levels between the groups. (Table-2) MPV was more in depressive disorder (9.73±1.23) and anxiety disorder (9.84±1.3) than in controls (8.77±0.44) and this difference was statistically significant ($p<0.001$) Platelet count was more in depression group and anxiety group than in control group and this difference was statistically significant. (Table-2). When we compared MPV and platelet count between Depressive disorder and anxiety disorder there was no statistically significant difference between the groups,(Table-3)

Among the 90 cases of depression 49(54.4%) were having Major Depressive disorder (MDD), 24(26.5%) were having Dysthymic disorder and 17(19.1%) were having Recurrent

Depressive disorder(RDD). Among the 76 cases of Anxiety disorder, 44(57.8%) Generalized anxiety disorder (GAD), 20(26.3%)-Panic disorder (PD) and 12(15.9%) -Social Anxiety disorder(SAD).

When we compared the MPV within the group the value in RDD was more than the MDD and Dysthymia but there was no statistical difference between the groups (Table-4). MPV value was higher in Social Anxiety disorder than in GAD and PD but there was no statistical significance(Table-5). There was negative correlation between MPV and platelet count (r value was - 0.067) and there was a positive correlation between HAM-A scores and MPV (r value was +0.245) and HAM-D scores and MPV (r value was +0.312).

4. Discussion:

MPV has been defined as a decisive factor in platelet function. It has been shown that platelet size, measured as MPV, correlates with platelets' reactivity.[21] Serotonin neurotransmission is considered to be important in mediating positive affect and mood. Abnormalities in serotonin pathways are thought to play a pathophysiological role in major depression and anxiety. This takes on importance when considering platelet function because most of the serotonin in the body is found in the dense granules of platelets.[2] The storage and metabolism of serotonin are similar in platelets and CNS.[7] Depression and anxiety disorders are one of the important factors in the aetiology of mortality in CVDs.[22] It has been suggested that platelet activity is influenced by emotional stress and coronary events such as MI may be provoked by these stressors.[5]

In the present study, we found increased MPV levels in patients with depressive disorder and anxiety disorder compared to controls. There are few studies that have investigated MPV in psychiatric populations. Ataoglu et al reported that MPV was found to be elevated in 15

patients with MDD and after 8 weeks of treatment with escitalopram, it was observed that MPV levels were statistically significantly lower than baseline in 15 patients.[10] Canan et al in a population-based study, 289 patients with major depression were found to have increased MPV levels in comparison with control subjects[11]. Kokacya et al and Asoglu et al has shown increased MPV levels in patients with PD.[12,13] Almis and Aksoy have found increased MPV levels in GAD patients compared to healthy controls.[14] Gul et al and Ransingh et al contrary to our findings have found lower MPV levels in PD patients compared to the control group. They speculated that abnormal 5-HT metabolism, such as specific alterations of the 5-HT receptor functional state in platelets of PD patients, could lead to decreased MPV.[9,15] But they could not explain the exact mechanism of or reason for the decreased MPV in PD patients. Stressful life events, anxiety, depression, or disruptive behavior disorder have increased catecholamine levels, sympathetic activity, and cortisol secretion. [23,24] Vizioli et al have shown that increased sympathetic activity can also cause higher MPV values.[25]

On the basis of these reports, some investigators have postulated that the sympathoadrenal activation may stimulate platelets via 2-adrenoreceptor activation, which in turn induces shape change and thereby increases MPV.[26] Anxiety and depressive disorders are also associated with increased inflammatory cytokine levels, endothelial dysfunction, and platelet reactivation. As in the central nervous system, plasma platelets play a role in serotonin synthesis, secretion, and reuptake. Serotonin not only has a pivotal role in the pathophysiology of depression and anxiety disorder, but also participates in hemostasis by affecting platelet aggregation. [6] It has been reported that patients with anxiety disorder and depressive disorder have increased platelet reactivation related to serotonin. [27,28]

The following mechanisms have been suggested by Nemeroff and Musselman leading to platelet abnormalities in major depression: altered platelet function by increased plasma concentrations of 5-HT and epinephrine, affected platelet function by increased intraplatelet calcium mobilization, upregulation of 5-HT2A receptors or a-adrenoreceptors, downregulation of 5-HT transporter number, altered second messenger signal transduction, or altered intraplatelet concentrations of monoamines and catecholamines.[29]

Patients with major depression have been shown to exhibit alterations of multiple platelet parameters, including reduction of serotonin transporter [3H]-imipramine binding sites in platelets,[30] as well as increase in 5-HT2 receptor binding sites on the platelet surface compared with controls.[31] Platelet monoamine oxidase activity has been shown to be elevated in depressed patients.[32] Additionally, there are several reports indicating decreased platelet activity after treatment of depression especially with selective serotonin reuptake inhibitors.[17,18]

In our study there was no difference in the MPV values of depression and anxiety disorder and this can be explained as both the disorders share common pathological mechanisms and both are treated by similar drugs(SSRI).[12]

In the current study there was a negative correlation between MPV and platelet count. It has been previously reported that larger platelets have a greater mass, denser granules and are more active than smaller platelets, enzymatically and metabolically.[33] They have a greater thrombotic potential caused by higher levels of intracellular thromboxane A2 and also express more procoagulant surface proteins such as P Selectin and Gp IIb/IIIa.[34] Additionally, larger platelets aggregate more rapidly than smaller platelets. Increase in platelet volume are often associated with decrease in platelet count perhaps as a result of small platelets being consumed in order to maintain a constant platelet functional mass.[35]

When we compared the MPV within the Depression group the value in RDD was more than the MDD and Dysthymia but there was no statistical difference between the groups ~~(table 3).~~ MPV value was higher in Social Anxiety disorder than in GAD and PD but there was no statistical significance. There was positive correlation between the HAM-A score and MPV and also between HAM-D and MPV. Depression and anxiety disorders have been suggested to be the main risk factors in the etiology of mortality in CVD.[36] At pathophysiological levels it has been hypothesized that biological mechanisms may be affected by stress-related conditions, resulting in worsening of cardiovascular functions. Platelet activity increases with the emotional stress, and coronary events such as myocardial infarction can be induced. The MPV has been thought as a determinant marker of the platelet function. Furthermore, increased MPV levels may indicate either increased platelet activation or an increased number of large and hyperaggregated platelets.[37,38] Our results showed that this increase may reflect the abnormality of platelets rather than increase in their counts.

To the best of our knowledge this is the first study in India to examine the relationship of MPV, depressive disorder and anxiety disorder. The other studies done previously have not used scales to measure the severity of the depressive and anxiety symptoms and they could not correlate the severity of the disorder and the MPV and had quoted as the limitation in their studies. Our analysis did not include individuals with conditions such as hypertension, coronary artery disease, diabetes mellitus, malignancy, dyslipidemia, stroke, drug use, smoking and alcohol abuse which are known to affect platelet activity. Earlier studies have investigated in either depressive disorder or anxiety disorder, we have studied in both the disorders with controls.

Despite the strengths of the study, there were certain limitations. As it is a hospital based case control study with the small sample size, it could not be generalised to community. This study was a single centre study and not a multicentre study.

Conclusion

Increased MPV is associated with depressive disorder and anxiety disorder. There was no statistically significant difference in the MPV of Anxiety disorder and Depressive disorder and this further supports that they have common pathological mechanisms. There was a positive correlation between MPV and severity of depressive and anxiety symptoms. Further research for the estimation of MPV as a tool for neuropsychiatry and psychopharmacology to examine how certain mental diseases and medications influence the central nervous system is required. Studies to investigate the effect of MPV, anxiety disorder and depressive disorder on CVDs and the effect of treatment on MPV need to be carried out.

Table -1: Socio-demographic details

Variables		Depression N= 90	Anxiety N=76	Controls N=49	Statistical analysis
Age		37.02±9.869	35.07±9.22	34.69±6.84	F=1.433 p=.241
Gender	**male**	18	20	12	x^2=0.975 p=0.614
	female	72	56	37	
Marital status	**married**	72	64	38	x^2=3.25 p=0.916
	unmarried	18	8	11	
education	**illiterate**	38	36	22	x^2=0.443 p=0.80
	literate	52	40	27	
Socioeconomic status	**upper**	2	01	01	x^2=0.32 p=0.988
	middle	41	35	21	
	lower	47	40	27	
BMI		24.6±4.86	23.15±4.96	22.04±4.14	F=2.239 p=0.11

Table 2: HAM A ,HAM D and Biochemical variables in cases and controls

Variables	Depressive disorder N= 90	Anxiety disorder N=76	Controls N=49	Statistical analysis
HAM A	15.22±5.312	21.5±4.07	9.93±3.53	F=100.84 p<0.001*
HAM-D	18.64±4.25	16.57±5.98	11.6±2.53	F=35.702 p<0.001*
MPV fL	9.73±1.23	9.84±1.32	8.773±0.44	F=14.95 p<0.001*
Platelet count $10^{3/}$µl	311.6±59.89	312.8±59.49	276.3±23.68	F=8.435 p<0.001*
Haemoglobin g/dl	12.09±0.9	11.9±0.5	11.78±1.78	F=1.4701 p=0.2322
Lipid profile				
Total cholesterol	187.15±25.16	184.1±28.33	190.2±23.17	F=0,8427 p=0.4320
Triglycerides	167.84±75.6	168.78±75.8	167.99±74.9	F=0.035 p=0.996

HDL cholesterol	46.25±2.54	45.78±3.4	44.93±3.9	F=2.6988 p=0.0696
LDL cholesterol	103.54±31.62	104.54±30.44	103.17±32.68	F=0.0339 p=0.966
VLDL cholesterol	33.85±15.62	32.95±16.16	33.72±15.99	F=0.0720 p=0.9306

*significant

Table-3 :Platelet count and MPV in Depressive disorder and Anxiety disorder

Parameters	**Depressive disorder N=90**	**Anxiety disorder N=76**	Statistical analysis Df=164
MPV fL	9.73±1.23	9.84±1.32	t=0.5551 p=0.5996
Platelet count 103/µl	311.6±59.89	312.8±59.49	t=0.1290 p=0.8979

Table-4 :Platelet count and MPV in Depressive disorder

Parameters	**MDD (N=49)**	**Dysthymia (N=24)**	**RDD (N=17)**	Statistical analysis Df=2,87
MPV fL	9.3796±1.052	9.933±0.7811	10.58±1.747	F=0.0001 P=0.999
Platelet count 103/µl	318.73±61.177	309.5±63.062	294±50.2	F=1.0982 P=0.3382

Table 5:MPV and platelet count in anxiety and depression

Parameters	**GAD (N=44)**	**PD (N=20)**	**SAD (N=12)**	**Statistical analysis Df=2,73**
MPV fL	9.9455±1.48	9.5±0.842	10.05±1.35	F=0.9456 P=0.3932
Platelet count 10^{3}/µl	311.55±55.56	327.90±57.40	292±74.27	F=1.3421 P=0.2677

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