MEAN PLATELET VOLUME AND RISK OF THROMBOTIC STROKE

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ABSTRACT

BACKGROUND
Stroke is a major cause of long term morbidity and mortality. Several factors are known to increase the liability to stroke. Platelets play a crucial role in the pathogenesis of atherosclerotic complications, contributing to thrombus formation. Platelet size (mean platelet volume, MPV) is a marker and possible determinant of platelet function, large platelets being potentially more reactive. Hence an attempt has-been made to study the association if any between mean platelet volume and thrombotic stroke. The aim of this study was to determine whether an association exists between Mean Platelet Volume (MPV) and thrombotic stroke.

MATERIALS AND METHODS
The study is a case control study and data was collected at Government Medical College Hospital, Kottayam, Kerala a tertiary care referral centre. The study was carried out among fifty patients diagnosed with thrombotic stroke and presenting to the hospital within forty eight hours of onset of symptoms. Fifty age group and sex matched controls were also recruited. Mean platelet volume was obtained using a SYSMEX automated analyser.

RESULTS
This study has shown a statistically significant relation between mean platelet volume and risk of thrombotic stroke but no statistically significant correlation between clinical severity of stroke and mean platelet volume.

CONCLUSION
This study has shown an elevation of MPV in acute phase of thrombotic stroke. Platelet mass was found to be more or less a constant. This study did not find a statistically significant correlation between clinical severity of stroke and mean platelet volume.

KEYWORDS
Mean Platelet Volume, Thrombotic Stroke.

BACKGROUND
Cerebrovascular disease is one of the most common and devastating disorder. The incidence of cerebrovascular diseases increases with age and the number of strokes is projected to increase as the elderly population grows, with a doubling in stroke deaths in the United States by 2030.[1] The prevalence of stroke in India was estimated as 203 per 100,000 populations above 20 years, amounting to a total of about 1 million cases. Stroke is one of the major causes of human morbidity and mortality. It ranked as the sixth leading cause of disability-adjusted years (DALY; one DALY is one of the lost year of healthy life) in 1990 and is projected to rank fourth by the year 2020.[2] Ischaemic stroke accounts for about 85% of cases, primary Intracerebral Haemorrhage

(ICH) for 10% and Subarachnoid Haemorrhage (SAH) for the remaining.[3]

Stroke is associated with increased long term mortality, residual physical, cognitive, and behavioural impairments, recurrence, and increased risk of other types of vascular events.[4] several factors are known to increase the liability to stroke, and it is in this context that large-scale public health measures have had a substantial influence. The most important of these are hypertension, heart disease, atrial fibrillation, diabetes mellitus, cigarette smoking, and dyslipidaemia.[3] Platelet size is found to be elevated in individuals with hypertension and diabetes mellitus, both conditions that predispose to the development of vascular events. As this is such a huge public health problem, other risk factors and possible preventive measures need to be identified. It is in this context that this study has its significance.

Platelets play a crucial role in the pathogenesis of atherosclerotic complications, contributing to thrombus formation.[5] Platelets are anucleate cells and are heterogeneous regarding their size, density and haemostatic potential. Platelet size (Mean Platelet Volume, MPV) is a marker and possible determinant of platelet function, large

platelets being potentially more reactive. For example, large platelets contain more dense granules, undergo greater in vitro aggregation in response to agonists such as ADP and collagen, and release more serotonin and beta Thromboglobulin (b-TG). In normal individuals the platelet count is inversely proportional to MPV; platelet mass (the product of MPV and platelet count) is a near constant. Although platelets are incapable of de novo protein synthesis they are very active metabolically and respond rapidly to vascular injury or trauma by undergoing a series of reactions (adhesion, release of granule contents, shape change and aggregation), which ultimately result in the formation of a platelet-fibrin plug. Thus there is evidence that platelet function is accentuated in acute ischemic stroke.[6]

Though there have been quite a few studies which have demonstrated an association between myocardial infarction and platelet size, very few studies has looked at the association between platelet size and thrombotic stroke. Among them there has been discrepancy regarding the sample size, methodology used and the final result. There are only few documented studies in India comparing the association of mean Platelet volume with thrombotic stroke; hence an attempt has-been made to study the association if any between mean platelet volume and stroke in an Indian population.

MATERIALS AND METHODS
This is a case control study of fifty patients above 40 years diagnosed with thrombotic stroke and presenting to a Tertiary Care Center in the State of Kerala, India within forty eight hours of onset of symptoms. Fifty age group and sex matched controls were also recruited.

Inclusion Criteria- Cases of thrombotic stroke above 40 years of age presenting to hospital within 48 hours of onset of symptoms.

Exclusion Criteria- Thrombocytopenia, Peripheral smear showing platelet aggregates, large platelets. Patients with previous history of stroke, coronary/valvular heart disease, congenital heart disease, dilated cardiomyopathy, atrial fibrillation. Patients on antiplatelet. History suggestive of embolic stroke. Patients with carotid bruit. Imaging showing multiple territory involvement in ischemic stroke. Patients with malignancies.

Controls- Controls will be primarily hospital based. Each patient will have a control matched for age and sex.

Inclusion Criteria for Controls- Patients attending the hospital or outpatient clinic for other illness.

Exclusion Criteria for Controls- Individuals with previous history of stroke, coronary artery disease. Patients on antiplatelet History of malignancy Thrombocytopenia Peripheral smear showing platelet aggregates and large platelets.

Methods of Collection of Data- All stroke patients admitted to the hospital were screened during the time period described above. Each of them was entered into a stroke log. Patients fulfilling the criteria were enrolled into the study after obtaining an informed consent. Each patient was given a serial number and was formally included into the study as a case. Each patient was assessed and a Modified Rankin's Scale was assigned to them. A Blood sample was collected from the antecubital vein using a 5 cc syringe and transferred to an EDTA bottles. The samples were then taken to the laboratory within 2 hours of collection and analysed using the SYSMEX automated analyser using electrical impedance to measure the mean platelet volume. After the analysis, peripheral smear was done to look for platelet aggregates.[7] If platelet aggregates were found then such cases were excluded from the study. The same procedure was adopted in controls for taking the samples.

RESULTS
236 patients of strokes admitted to the medical wards were screened to get 50 cases meeting eligibility criteria. The major reason for exclusion was late presentation ie, patients presenting 48 hrs after the onset of stroke were excluded. The mean age for the cases was 58.10 ± 13.67 when compared to 57.50 ± 13.82 for the controls. The maximum number of cases in this study were in the age group between 51-60 which was followed by age group of 61-70. Females with stroke were having higher age 59.46 ± 9.12 when compared to males 55.86 ± 14.38. 74% of the cases and controls were males and 26% were females Out of the many risk factors for stroke metabolic syndrome was the most prevalent in this study group with a percentage of 74 among cases and 48 among controls with a significant p value of 0.008. Hypertension came second with percentage of 52 among cases and 34 among controls with a trend towards significance with a p value of 0.045.

Comparison of Blood Parameters in Cases and Controls
There was a trend for lower platelet counts in cases but this was not at all significant.

Blood Parameters	Cases	Control	Significance P value
Hemoglobin (g/dl)	13.37 ± 2.21	12.49 ± 1.79	0.85
Platelet count (lakh)	2.56 ± .58	2.69 ± .83	0.380
Neutrophil %	71.14 ± 12.02	73.47 ± 8.47	0.43
Lymphocyte %	23.98 ± 9.98	20.74 ± 11.11	0.128
Eosinophil %	3.20 ± 2.72	2.51±1.79	0.159
Table 1. Comparison of Blood Parameters			

Stroke- Clinical Severity Score
The clinical severity of stroke at presentation was determined by the Modified Rankin's scale and severe disability was seen with 20% of the cases. 28% of the cases had no significant disability.

Modified Rankin's Score	Number	Percentage
Score 1: No significant disability	14	28
Score 2: Slight disability	9	18
Score 3: Moderate disability	6	12
Score 4: Moderate severe disability	11	22
Score 5: Severe disability	10	20
Table 2. Clinical Severity Score		

MPV has got a statistically significant correlation with Ischemic stroke with a P value of 0.004 with an average MPV in cases being 7.45 ± 0.81 compared to controls who average 6.94 ± 0.59. The platelet mass being a product of platelet count and mean platelet volume is almost a constant. This was evident from the values in cases and controls; 18.85 ± 4.67, 18.52 ± 5.31 respectively. However there was no statistical correlation of platelet mass to thrombotic stroke.

The risk factors for stroke were compared with MPV to look for any positive correlation. Statistically significant correlation was found with hypertension and metabolic syndrome.

There was no statistically significant difference in MPV and severity of strokes there was no statistically significant difference in MPV and territory of strokes there was no statistically significant difference in platelet mass and severity of strokes, p value 0.712.

MPV Values			
Risk Factor	Present	Absent	Significance (P value)
Hypertension	7.64 ± 0.82	6.98 ± 0.57	0.046
DM	7.12 ± .78	6.94 ± 0.61	0.078
Smoking	7.49 ± .95	7.28 ± 0.56	0.081
Alcohol	7.12 ± 0.87	6.99 ± 0.63	0.0562
Metabolic syndrome	7.69 ± 0.45	7.02 ± 0.96	0.041
Table 3. Comparison Of MPV In Cases And Controls According To Risk Factors			

Association of MPV Quintiles and Stroke Severity

MPV was arranged into quintiles and compared with the stroke severity score which was further subdivided into two groups. Group 1 with a score of 0-2 being less severe and group 2 with a score of 3-6 being more severe. The p value obtained was 0.654.

MPV Quintiles	Stroke Severity Score		P value
	Score: 0-2	Score: 3-6	
6.50-7.20	4	10	0.654
7.21-7.60	2	4	
7.61-7.86	5	3	
7.87-8.60	8	6	
8.61-10.00	4	4	
Total	23	27	-
Table 4. MPV Quintiles and Stroke Severity			

DISCUSSION

Study	O'Malley et al	Butter worth hh et al	Bath et al	Pikija et al	Muscari et al	Current Study
No of cases recruited	58	137	301	81	137	50
Age in years	79.5 ± 6.5	71.9 ± 10.8	65 ±9	76	78	58.1 ± 13.6
Women	39 (67%)	55 (40%)	87 (29%)	49 (60%)	65 (47%)	13 (26%)
Men	19 (33%)	82 (60%)	214 (71%)	32 (39%)	72 (52%)	37 (74%)
Table 5. Comparison of Demographic Data of the Current Study with Western Literature						

Thus the mean age in our study was much lower (58.10 ± 13.67) compared to the other studies. There was a clear male preponderance in the cases of stroke recruited in this study. Similar patterns were seen in all the other studies compared except for O'Malley et al and Pikija et a[8] where a female preponderance was seen.

An Indian study by Shah et al[9] comprised of 100 cases with 59 males and 41 females with mean age of 58.

Risk Factors for Stroke

Out of the many risk factors for stroke metabolic syndrome was prevalent in this study group with a percentage of 74 among cases and 48 among controls. Hypertension came second with percentage of 54 among cases and 34 among controls. Diabetes mellitus constituted 28 percent of cases and 24 percent of controls. However hypertension was the most prevalent risk factor in most other studies (84.7% in A. Muscari et al[10] and 82.7% in Pikija et al.). Diabetes mellitus had a representation of 28% of the cases which was

higher when compared to the other study. Most previous studies have not included metabolic syndrome.

Drug History

There have been previous studies with aspirin and MPV and it showed no interference in vitro and in vivo. The same cannot be affirmatively said about the other antiplatelet drugs. But this study excluded all patients on aspirin and other antiplatelet. The other drugs used were predominantly antihypertensives with 32% of the hypertensive being on calcium channel blockers followed by beta blockers, ACEI and ARBs in various combinations.

Study	O'Malley et al	Butterworth et al	Bath et al	Pikija et al	Muscari et al	Current Study
No of cases recruited	58	137	301	81	137	50
Risk Factors						
Previous stroke	19 (33%)		217 (72%)	23 (28.4%)		NA
Hypertension	17 (29%)	.	-	67 (82.7%)	116 (84.7%)	27 (54%)
Atrial fibrillation	15 (26%)			26 (32.1%)	44 (32.1%)	NA
Previous myocardial infarction Infarction	15 (26%)		24 (8%)	3 (3.70%)	17 (12.4%)	NA
Angina pectoris	16 (28%)					NA
Alcohol	10 (17%)	.	.	-	-	4 (8%)
Peripheral vascular disease	7 (12%)			2 (2.47%)		NA
Current smoking	8 (14%)		60 (20%)	4 (4.9%)	20 (14.6%)	7 (14%)
Diabetes	5 (8.6%)	.	39 (13%)	15 (18.5%)	29 (21.2%)	14 (28%)
Table 6. Comparison of Risk Factor Profile of Current Study with Western Literature						

Study	O'Malley et al	Butter Worth et al	Bath et al	Pikija et al	Muscari et al	Current Study
No of cases recruited	58	137	301	81	137	50
Drug history						
Antiplatelet	19 (33%)	-	211 (70%)	33 (40.7%)	59 (43.1%)	NA
6-Blocker	3 (5%)	-	-	-	-	10 (20%)
Diuretic	24 (41%)	-	-	-	-	6 (12%)
Nitrate	7 (12%)	-	-	-	-	NA
Digoxin	11 (19%)	-	-	-	-	NA
Calcium antagonist	4 (6.8%)					16 (32%)
Statins	6 (10.2%)		-	6 (7.4%)	-	8 (16%)
ACI and ARB	7 (12%)		-	2 (2.5%)	-	7 (14%)
Table 7. Comparison of Drugs in Current Study with Western Literature						

Platelet Parameters and Stroke

Platelet parameters assessed were MPV, Platelet mass, and platelet count. The main parameter studied was MPV. MPV has got a statistically significant correlation with ischemic stroke with a p value of 0.004 with an average MPV in cases being 7.45 ± 0.81 compared to controls who average of 6.94 ± 0.59.

MPV assessment using EDTA and Citrate was done only in one study i.e. Butterworth et al and the values were 8.04 ± 1.04 (7.69 ± 0.83) for EDTA and 7.35 ± 1.05 (7.09 ± 0.74) for citrate. The values were comparable even though these studies were done in different populations. The other studies have used EDTA as the anticoagulant and there is no uniformity in the collection method, time of analysis, transport of the specimen or the storage. O'Malley study had performed the test after 24 hrs of storage in room temperature. Most of the other studies have performed the test after 2 hrs. In our study the samples were analysed after 2 hrs of storage in room temperature. The only study with a lower MPV in cases compared to controls is Tohji et al[11], who did not specify the time after venepuncture at which samples were analysed or temperature at which it was stored. In the Indian study by Shah et al blood sample was obtained from the patient within 48 hrs. of onset of symptoms. Samples were collected in EDTA bottles and kept in room temperature and processed after 6 hrs. In the current study the platelet count is showing a slightly lower trend in the cases with an average of 2.56 ± 0.58 (1.43-4.40) when compared to the controls in which the average was 2.69 ± 0.83 (1.60-5.40). This trend is however not statistically significant. This pattern has been seen in all the other case control studies which included O'Malley et al, Butterworth et al[12], Tohji et al and Shah et al.

The platelet mass being a product of platelet count and mean platelet volume is almost a constant and there is no statistical correlation of platelet mass to thrombotic stroke in this study. This has not been looked upon in other western studies.

We have further demonstrated by multiple logistic regression analysis that MPV with p value of 0.004 and an adjusted OR of 2.8, is one of the most important risk factors associated with stroke along with metabolic syndrome.

It has been suggested that MPV and platelet count are under independent hormonal control, although control of platelet production remains obscure. Some have suggested a role for interleukin-6, interleukin-3, thrombopoietin, and colony-stimulating factors. It is, however, generally accepted that platelet volume and count are determined at thrombopoiesis.[8] and as in ischemic heart disease, these findings may implicate primary changes occurring at the bone marrow (megakaryocyte) level. Moreover, an increase in megakaryocyte size and ploidy (DNA content) coincides

with an increase in MPV. This suggests the possibility that activation of megakaryocytes, as heralded by an increase in MPV, is a feature of thrombotic stroke.

CONCLUSION

1. This study has shown an elevation of MPV in acute phase of thrombotic stroke. With this relationship and adjusting for other significant variables in multivariate regression analysis, it can be stated that an increases in MPV is independently associated with stroke. The observations here suggest a role for larger platelets in the genesis of cerebral thrombosis.

2. Platelet mass was found to be more or less a constant. i.e this study has shown not only an elevation of MPV but a slight reduction of platelet count in acute phase of thrombotic stroke though no statistical correlation was established.

3. This study did not find a statistically significant correlation between clinical severity of stroke and mean platelet volume.

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