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RESEARCH ARTICLE

Mean Platelet Volume as a Prognostic Indicator in Sickle Cell Anemia

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ABSTRACT

Aim: To assess the use of mean platelet volume (MPV) as a prognostic indicator of sickle cell disease (SCD).

Materials and methods: Fifty sickle cell patients aged 18 to 60 years, attending the Medicine outpatient department (OPD) and sickle cell clinic, or admitted with an ongoing crisis, were chosen as the study group, and 50 healthy participants were chosen as controls. A pro forma detailing relevant clinical history was obtained, with emphasis on admissions. Then, a blood sample was collected and analyzed for various parameters.

Patients were classified into two categories:

- Category 1: Patients with <3 reported vaso-occlusive crises in the previous year, and
- Category 2: Patients with >3 reported vaso-occlusive crises in the previous year, or admitted to the medicine ward/ intensive care unit (ICU), currently with an ongoing crises.

Results: Mean hemoglobin (Hb) values were found to be statistically lower in sickle cell patients compared with controls, while mean total leukocyte count (TLC) and mean neutrophil % values were higher in sickle cell patients. Mean platelet volume in category 2 patients was higher compared with category 1 patients ($p < 0.001$), and values correlated with the mean neutrophil percentages. Also, MPV, TLC, and neutrophil % values showed a statistically significant drop ($p < 0.001$) 2 weeks after treatment for sickle cell crises.

Conclusion: Thus, MPV can be used as a potential marker to identify patients at a greater risk of developing vaso-occlusive crises in the future, which, in a developing country like India, can be a key step toward affordable, primary prevention of future emergencies.

Keywords: Hydroxyurea, Sickle cell anemia, Vaso-occlusive crises.

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INTRODUCTION

Sickle cell anemia, a disease very prevalent in India, is caused by a mutation in the β-globin gene that changes the sixth amino acid from glutamic acid to valine, forming sickle hemoglobin (HbS), provoking unpredictable episodes of vaso-occlusion and premature red blood cell (RBC) destruction, which manifests as acute pain and tissue ischemia.[1] A key component of vaso-occlusion is increased platelet activation and reactivity, and thus, an increased platelet volume, resulting in an elevated platelet distribution width.[2] Also, higher concentrations of platelet microparticles have been detected in sickle cell anemia patients who reported a greater number of crises.[3] In central India, also known as the sickle cell belt, patients unfortunately develop relatively severe manifestations compared with peers in developed countries, with vaso-occlusive crises and stroke being two of the most major complications.[4-6]

Mean platelet volume has been implicated as a good marker of prognosis in various inflammatory and hemolytic disorders.[7] Also, since an increased production of platelets leads to an increase in the average platelet size, and larger platelets are more reactive, the MPV values can also be used to make inferences about disorders causing platelet destruction.[8]

For patients of sickle cell anemia, hydroxyurea therapy for crises often proves to be prolonged and expensive, which burdens the large majority who come from tribal and poorer socioeconomic classes. Furthermore, not much data are available regarding the use of MPV as a prognostic indicator, especially for a developing country like India, which constitutes a large population of global sickle cell patients. Thus, the purpose of this study would be to assess the role MPV can play in prior identification of patients at a greater risk of crises and, if found to have a positive correlation, propose a revised indication for early institution of hydroxyurea therapy, for better patient outcomes.

Aim

To assess the use of MPV as a prognostic indicator of SCD.

Objective

- To correlate MPV of patients with known histories of complications to control group participants.

- To compare MPV with blood indices including total platelet count (TPC), TLC, and total Hb.
- To assess the feasibility of using MPV as an indicator of prognosis.
- To propose a revised indication for hydroxyurea therapy in SCD patients with higher MPV compared with peers.

MATERIALS AND METHODS

After obtaining due consent from the Institutional Ethics Committee, a prospective cross-sectional study was conducted in the Department of Medicine, and Central Clinical Laboratory, Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha, India, for a period of 2 months, from June 1, 2017 to July 31, 2017.

Study participants were 50 sickle cell patients aged 18 to 60 years, attending the Medicine OPD and sickle cell clinic, and/or admitted to the medicine ward/ICU with a current, ongoing sickle cell crisis, confirmed by qualitative and/or quantitative Hb electrophoresis. A control group of 50 healthy, non-SCD participants was also chosen. Participants were selected based on simple random sampling in order to eliminate any bias.

Exclusion criteria considered were:

- Patients having sickle cell trait, sickle cell-β thalassemia, or sickle cell HbC disease.
- Patients on treatment including glucocorticoids, anticoagulants, or antiplatelet drugs, or presenting with febrile illness.

Written consent of the participant was obtained first, having explained in detail about the purpose, methodology, and implications of the study.

Next, a pro forma detailing relevant clinical history was obtained, with emphasis on number, frequency, duration, and severity of Emergency Department admissions brought on by vaso-occlusive crisis.

This was then followed by a meticulous clinical examination to assess the presence of any infection, pallor, jaundice, or related signs of sickle cell anemia.

Collection of Blood Sample

Skin over the median cubital vein was first disinfected by applying surgical spirit over the cubital fossa, along with application of a tourniquet proximal to the fossa. Then, using a sterile standard venipuncture needle, blood was collected in a potassium ethylenediaminetetraacetic acid (EDTA) bulb for sampling and complete blood count, and analyzed within 15 minutes of collection.

Calculation of MPV

The blood collected was placed in a siliconed, graduated centrifuge tube containing 0.3 mL of 45% disodium EDTA, and mixed. Then, the sample was spun at 900 rotations per minute (r.p.m.) for 15 minutes, and supernatant plasma was separated; 20 mm^3 of platelet-rich plasma obtained was then diluted in 100 mL of 0.85% saline giving a dilution ratio of 1/5,000.

ELECTRONIC COUNTING

Based on the principle of detection of electrolyte displacement, the MPV was then obtained from the 1/5,000 dilution using a Coulter counter (Beckman Coulter's Z Series: Z1 counter, manufactured by Beckman Coulter, Germany) with a 50 orifice, aperture current setting 7, and threshold 25.

Correlation of MPV to Frequency of Vaso-occlusive Crisis

Sickle cell patients were classified into two categories:

- Category 1: Patients with <3 reported vaso-occlusive crises in the previous year, and
- Category 2: Patients with >3 reported vaso-occlusive crises in the previous year, or admitted to the medicine ward/ICU, currently with an ongoing crisis.

Values of MPV of all sickle cell patients were then compared with those of the control group, and values of category 1 patients with patients of category 2, in correlation with their clinical history of past/present crises.

Correlation of MPV with Other Blood Indices

Rise in blood indices, such as TPC, TLC, and Hb, is associated with a greater number of complications and crises in SCD. Thus, the MPV values of category 2 patients with >3 crises were compared with these indices first, followed by MPV values of category 1 patients with <3 crises, which were hypothesized to be comparatively lesser.

Assessment of MPV as an Indicator of Prognosis

Values of MPV demonstrating a linear relationship with the number of vaso-occlusive crises in the previous year would confirm the feasibility of its use as a marker for early identification of patients at greater risk.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences software, and p-values <0.05 were regarded as statistically significant for all comparisons; t-test was used to compare different groups and a one-way analysis of variance test was used to assess intergroup differences.

RESULTS

Fifty sickle cell patients aged 18 to 60 years, attending the medicine OPD and sickle cell clinic, and/or admitted to

Table 1: Reasons cited for visit to sickle cell OPD

Reason cited	*No. of patients (n = 50)*	*Percentage*
Periodic follow-up	48	96
Bone pain and body ache	27	54
Fever	14	28
Symptoms and correction of anemia	11	22
Cough	8	16

Table 3: Comparison of category 1 and 2 patients

Parameter	*Category 1 (n = 21)*	*Category 2 (n = 29)*	*p-value*
Hb% (gm/dL)	9.2 ± 1.7	7.9 ± 2.1	0.63
TLC ($\times10^9$/L)	7.3 ± 1.1	9.9 ± 1.8	0.27
Neutrophil %	54.1 ± 5.3	63.2 ± 5.6	0.39
TPC ($\times10^9$/L)	308 ± 45	286 ± 43	0.82
MPV (fL)	8.2 ± 0.5	9.8 ± 1.1	**<0.001**

Bold value indicates statistical significance

the medicine ward/ICU with a current, ongoing sickle cell crisis, and a control group of 50 healthy, non-SCD participants were selected by simple random sampling. The study was conducted over a period of 2 months from June 1, 2017 to July 31, 2017.

Reasons of sickle cell patients for visit to the OPD and sickle cell clinic were documented. The five most common reasons cited are shown in Table 1.

Hematological parameters of both groups were obtained, and quantitative data have been represented as mean ± standard deviation; p-values <0.05 were accepted as significant for all variables. Comparison of parameters of the study population and the control group is listed in Table 2.

When classified based on "No. of crises in previous year," sickle cell patients were divided into two categories: category 1: patients with <3 reported vaso-occlusive crises in the previous year, which comprised 21 patients (42%), and category 2: patients with >3 reported vaso-occlusive crises in the previous year and/or admitted to the ward/ICU with a current, ongoing sickle cell crisis, which comprised of 29 patients (58%). When subclassified, out of the 29 category 2 patients, 7 were currently admitted for an ongoing sickle cell crisis, receiving appropriate treatment, which were the focus of pre- and posttreatment

Table 2: Comparison of the study group and the control population

Parameters	*Study group (n = 50)*	*Control population (n = 50)*	*p-value*
Age	34 ± 14.82	35 ± 16.27	0.29
Sex (M:F)	18:32	23:27	0.81
Hb% (gm/dL)	8.6 ± 2.4	12.2 ± 1.5	**<0.001**
TLC ($\times10^9$/L)	9.1 ± 1.7	7.5 ± 1.2	0.38
Neutrophil %	58 ± 7.1	52 ± 4.7	0.67
TPC ($\times10^9$/L)	296 ± 44	302 ± 56	0.21
MPV (fL)	9.2 ± 1.7	8.9 ± 1.4	0.08

Bold values indicate statistical significance

comparison of hematological parameters taken at the time of admission, 5 days after initiation of treatment, and 2 weeks after initiation of treatment, results of which are depicted in Table 4.

Mean hemoglobin (gm/dL) obtained in category 1 patients was 9.2 ± 1.7, while in category 2 patients, it was 7.9 ± 2.1. Mean TLC ($\times10^9$/L) obtained in category 1 patients was 7.3 ± 1.1, while in category 2 patients, it was 9.9 ± 1.8. Mean neutrophil % obtained in category 1 patients was 54.1 ± 5.3, while in category 2 patients, it was 63.2 ± 5.6. Mean TPV ($\times10^9$/L) obtained in category 1 patients was 308 ± 45, while in category 2 patients, it was 286 ± 43. Mean MPV (fL) obtained in category 1 patients was 8.2 ± 0.5, while in category 2 patients, it was 9.8 ± 1.1.

Comparing parameters of category 1 and category 2 sickle cell patients are shown in Table 3.

A subgroup of seven patients from category 2, having experienced >3 sickle cell crises in the past year, and currently admitted with ongoing sickle cell crises, was the prime area of focus, and their hematological parameters were regularly monitored following ward or, in cases deemed necessary, ICU admission. Following hydroxyurea therapy combined with other appropriate treatment, the five parameters were monitored at 5 days posttreatment to assess if there was any immediate change seen, and 2 weeks posttreatment. Parameter values obtained at 5 days posttreatment and 2 weeks posttreatment are listed in Table 4.

While there was no significant change in hematological parameters noted 5 days after initiating treatment, TLC with neutrophil % and MPV showed a statistically significant decrease ($p < 0.001$) 2 weeks posttreatment.

Table 4: Comparison of hematological parameters at admission, 5 days posttreatment, and 2 weeks posttreatment

Parameters	*At admission*	*5 days posttreatment*	*p-value*	*2 weeks posttreatment*	*p-value*
Hb% (gm/dL)	7.4 ± 1.8	7.8 ± 1.6	0.53	8.5 ± 1.3	0.28
TLC ($\times10^9$/L)	10.3 ± 1.5	9.5 ± 1.4	0.12	8.9 ± 1.2	**<0.001**
Neutrophil %	64.7 ± 4.1	63.8 ± 3.7	0.37	59.2 ± 2.8	**<0.001**
TPC ($\times10^9$/L)	261 ± 39	257 ± 46	0.79	274 ± 38	0.62
MPV (fL)	10.6 ± 0.8	10.4 ± 0.7	0.21	9.7 ± 0.5	**<0.001**

Bold values indicate statistical significance

DISCUSSION

Sickle cell anemia is a disease that is unfortunately quite prevalent among rural and tribal communities of India today, with estimates reporting the prevalence of heterozygotes varying from 1 to 40%,[9] and often manifests as crises, infections, acute chest syndromes, limb pain, or weakness.[10] A variation of the normal variety of adult hemoglobin (HbA), HbS, occurs due to mutation in the hemoglobin, beta gene that causes substitution of valine for glutamic acid at the 6th position of the β-globin subunit of the hemoglobin molecule. The term "sickle cell disease" refers to any condition in which the production of HbS leads to pathophysiological systemic consequences.[11] Among rural communities, risk factors for sickle cell crises have been found to include Mahar caste ($p = 0.007$), noncompliance ($p = 0.000$), and protein energy malnutrition ($p = 0.0015$), criteria that are quite prevalent across a majority of rural communities of India.[12]

In India, sickle cell trait most occurs commonly in rural communities of central India (southeast Gujarat, Maharashtra, Madhya Pradesh, Chhattisgarh, and western Orissa) with another small focus in the south of the country (northern Tamil Nadu and Kerala), and trait frequencies as high as 40% have been documented in some communities. In these areas, the deoxyribonucleic acid configuration of the β-globin locus differs from that of the African genotype, suggesting that this is a fourth independent HbS mutation, which is known as the Asian haplotype. This haplotype is often related to high levels of fetal hemoglobin and frequent occurrence of α-thalassemia, both of which tend to comparatively decrease the tendency of sickling and improve the clinical course.[13] Sickle cell disease found in rural communities of India is predominantly of the SS variety.[14]

With regard to the treatment of sickle cell crises, studies undertaken, particularly in India, have shown that fixed low-dose hydroxyurea was sufficient for a clinical and hematological improvement, with hemoglobin levels increasing in most cases, resulting in a significant decrease in the number of blood transfusions required in the future.[15,16]

In the present study, the aim was to assess if MPV could be used as a suitable tool to predict future risk of vaso-occlusive crises in a sickle cell anemia patient, and its role as a criterion for early institution of hydroxyurea therapy, along with its use as a prognostic indicator. Earlier studies using MPV as criteria had included multisystem disorders, like diabetes mellitus,[17] hypertension,[18] and cardiovascular disorders like ischemic heart disease[19,20] with their associated complications, which showed that MPV values were raised in scenarios of increased inflammatory and thrombotic activity.

Criteria in the present study were similarly compared. As data from Table 2 indicate, mean Hb values were statistically lower in sickle cell patients compared with control population, while mean TLC and mean neutrophil % values were comparatively higher in sickle cell patients. This correlates with the chronic erythrolysis occurring in sickle cell anemia patients due to increased sickling of RBCs, along with an increasing frequency of ongoing infection, or inflammatory process, as the majority of sickle cell patients presented to the OPD with chief complaints of recurrent infections over the past 2 weeks and/or body pain.

While comparing the mean MPV between the study group and control population showed no significant difference, data from Table 3 show that the mean MPV in category 2 patients was statistically higher compared with category 1 patients ($p < 0.001$). This in turn again points toward an increased frequency of inflammatory processes and, thus, severity of underlying sickling in category 2 patients who already have a history of recurrent infections, and vaso-occlusive crises, most specifically in 4 patients out of 29 (13.8%) who required blood transfusions in addition to routine therapy, in the past year. As Table 3 also indicates, mean MPVs correlated with the mean neutrophil percentages, both of which were comparatively higher in category 2 patients compared with category 1 patients, which are associated with active, ongoing infection and inflammation in the body.

As indicated in Table 4, while there was no significant change within the first 5 days posttreatment, MPV, TLC, and neutrophil % values showed a statistically significant drop ($p < 0.001$) 2 weeks after institution of treatment for sickle cell crises, which could be linked with increased RBC oxygenation capacity, decreased infection, and decreased thromboinflammatory activity, associated with an overall improvement in the health of the sickle cell patient.

With regard to treatment guidelines for sickle cell crises, the current criteria for initiation of hydroxyurea therapy include:

- Patients experiencing repeated episodes of acute chest syndrome or
- Patients presenting with more than three crises per year requiring hospitalization.

Since the criteria do not include a hematological parameter, MPV may be proposed as an additional recommendation, since even patients with subclinical, unapparent SCD, but discernibly high MPV values can benefit with early institution of hydroxyurea therapy, to decrease long-term risk of development of sickle cell crises.

CONCLUSION

As the present study indicates, MPV can be used as a potential marker to identify patients at a greater risk of

developing crises in the near future, and if implemented sooner, treatment with hydroxyurea can be instituted early on, resulting in a decrease of morbidity and mortality due to complications in SCD. Also, in a predominantly developing country like ours, aside from greatly benefiting patients from areas like interior Maharashtra, which is a prominent sickle cell belt of India, it would mean lesser health care costs for relatives as well as hospitals, and would thus be a key step toward affordable, primary prevention of future emergencies.

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