Original Article

Diagnostic accuracy of mean corpuscular volume in detecting coexisting iron deficiency in patients of sickle cell disorders: A hospital-based study

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Abstract

Background: Co-existing iron deficiency in patients of sickle cell disease (SCD) and trait may worsen anemia, and adversely affect neuro-cognitive development and growth. Determining a cut-off below which Mean corpuscular Volume (MCV) can predict iron deficiency in SCD patients can preclude use of more expensive test serum ferritin. **Aims:** This study was conducted to determine the diagnostic accuracy of low MCV in detecting iron deficiency compared to serum ferritin levels in patients with SCD. **Methods:** 60 consecutive patients with SS or AS pattern on hemoglobin electrophoresis were enrolled. The index test (MCV) and the reference standard test (serum ferritin) were performed in a blind and independent manner. The measures of diagnostic accuracy were calculated and the precision of the point estimates were expressed by 95% confidence intervals. As MCV is a continuous variable, we also used multi-level likelihood ratios to compute diagnostic accuracy of MCV at several cut-points. **Results:** The sensitivity of low MCV in detecting iron deficiency was 40.0% (95% CI-20.0-63.6), the specificity was 78.4% (95% CI-61.3-89.6) using serum ferritin as a reference standard. The sensitivity and specificity of predicting coexisting iron deficiency at this point was 60.9% (CI-38.6-80.3%) and 75.7% (CI-58.8-88.2%) respectively. **Conclusions:** The low sensitivity (40%) of microcytosis in detecting iron deficiency indicates that many cases will be missed if MCV alone is used to detect co-existing iron deficiency anemia in SCD patients. No single test is good enough to detect co-existing iron deficiency and a combination of tests might be useful.

Keywords: Diagnostic accuracy, iron deficiency anemia, mean corpuscular volume, sickle cell disease

Background

The prevalence of sickle cell disorders in the "sickle belt" of Central India has been reported to be from 2.9 to 5.5%.[1] Iron deficiency anemia is the most common cause of anemia worldwide, which results in microcytic and hypochromic red cells on the peripheral smear. The hemoglobin indices in iron deficiency will demonstrate a low mean corpuscular hemoglobin and mean corpuscular hemoglobin volume. Serum levels of ferritin, iron, and transferrin saturation will be decreased.[2]

In patients with sickle cell disease, conventional laboratory tests for iron deficiency, such as S. ferritin, total ion binding capacity, transferrin saturation, and mean corpuscular volume (MCV), may be abnormal.

The excess iron deposits are unavailable for erythropoiesis and, hence, may not be reflected in S. ferritin levels. However, iron deficiency anemia can coexist in patients with sickle cell anemia (SCA), as they are not immune to environmental factors that precipitate iron deficiency anemia. These factors, especially

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in the tropics, include poor nutrition, parasitic infestations, and various bacterial infections, which can impair iron metabolism.[3] Although estimates of iron deficiency in patients with sickle cell anemia range between 16-67%,[4-6] iron deficiency in sickle anemia is often missed because physicians think that hemolyzed red cells or blood transfusions replenish iron stores in these patients.[7] Further, these patients often present with normocytic normochromic anemia – an unusual feature of iron deficiency. The presence of microcytosis (low MCV) is usually considered indicative of coexisting thalassemia.[8]

Iron deficiency can worsen anemia and may adversely impact neurocognitive development and growth.[9] It can also result in lowering the intracellular hemoglobin concentration and this may ameliorate sickling. Patients of sickle cell anemia with coexisting iron deficiency may benefit from oral iron supplementation.[5]

At present, the absence of stainable iron in marrow is considered to be the reference standard for diagnosing iron deficiency. However, this test is invasive, painful, and was not feasible for research purposes. Serum ferritin concentrations have been proposed as the best biochemical test that correlates with relative total body iron stores.[5] In normal subjects, a serum ferritin value below 12 ng/ml is usually diagnostic of iron deficiency.[10] However, in patients with chronic inflammatory disease or hemolytic anemia, conditions recognized to elevate serum ferritin, a serum ferritin value below 25 ng/ml has been used to predict absent bone marrow iron stores and response to iron therapy.[5,10-12] Serum ferritin values less than 25 ng/ml are 100% sensitive and 100% specific for iron deficiency anemia in sickle cell disease.[7]

Anemia is one of the most encountered ailments in general family medicine practice in family medicine professionals, and iron deficiency anemia is the most common cause, which might sometimes be accompanied by the concurrent presence of sickle cell anemia.

Determination of MCV is a routine hematologic procedure that requires no extra effort as it is performed using the automated cell counter. Low MCV is seen with iron deficiency anemia, thalassemia, sideroblastic anemia, and anemias of chronic disease. Determining a cut-off below which MCV can predict iron deficiency in patients with sickle cell disorders can preclude the use of a more expensive and additional investigation, i.e., serum ferritin. This study was carried out to determine the diagnostic accuracy of low MCV in detecting iron deficiency compared to serum ferritin levels in patients with sickle cell anemia and trait.

Material and Methods

Study setting

This study was carried out in a tertiary care teaching hospital in rural Central India. This study was approved by the Institutional Ethics Committee.

Study design

This is a diagnostic hospital-based study where we evaluated the diagnostic accuracy of MCV compared to serum ferritin levels in the diagnosis of iron deficiency in sickle cell patients.

Study population

Sixty consecutive patients (incident as well as prevalent) from the Medicine and Obstetrics/Gynecology departments detected with an SS or AS pattern on hemoglobin electrophoresis were enrolled in this study. Patients diagnosed as SS or AS on hemoglobin electrophoresis were traced using the Hospital Information System to the wards. Informed consent was taken from patients before including them in the study.

Subjects

Inclusion criteria

Cases of sickle cell disease included 60 consecutive patients who had been detected with either SS or AS pattern on hemoglobin electrophoresis.

Exclusion criteria

Patients who had received recent blood transfusions or oral iron supplementation in the previous 2 weeks were excluded from this study. Patients who did not volunteer to be part of the study or did not give consent were excluded.

Data collection methods

The following methods were used to determine hematologic parameters for each case: (a) Hemoglobin electrophoresis was performed using cellulose acetate paper at pH 6.5.; (b) Values for hemoglobin, complete blood counts and red cell indices (MCV, MCH, MCHC) were obtained using an automated cell counter (ACT diff 2, Beckman-Coulter); and (c) Reticulocyte counts were performed using supravital stains with standard methods.

All hematologic parameters including complete blood counts, red cell indices, hemoglobin electrophoresis, and reticulocyte count were obtained from Pathology records. The patients were traced and were personally interviewed by the investigators in the wards. After taking informed consent, the investigators recorded evidence of fever, inflammation, leukocytosis, and raised C Reactive Protein (CRP) if present. They also verified if the patients had been recently transfused with blood or received oral iron supplementation. A 2 ml blood sample was collected in a plain bulb by venipuncture. The serum was separated from the sample.

Using an ELISA-based commercial kit, serum ferritin was performed on all these samples. The test was an indirect solid-phase enzyme immunoassay for the quantitative measurement of serum ferritin.

All patients received the index test (MCV) and the reference standard test (serum ferritin) in a blind and independent manner. Serum ferritin was used as the reference standard. We used a cut-point of 25 ng/ml to diagnose iron deficiency anemia.

Statistical analysis

All data were entered in Microsoft Excel spreadsheets. R software studio Version 4.1.2 was used to analyze data. The measures of diagnostic accuracy (sensitivity, specificity, positive predictive value, negative predictive value, positive and negative likelihood ratios) were computed, and the precision of the point estimates was expressed by 95% confidence intervals. Because MCV is a continuous variable, we also used multi-level likelihood ratios to compute the diagnostic accuracy of MCV at several cut points.

Results

Our study included 14 males and 46 females. Maximum cases (36.6%) were in the age group between 20 and 29 years. No child below the age of 12 years was included in this study. Of the 60 cases, 48 (80%) cases had AS pattern and 12 cases (20%) had SS pattern on hemoglobin electrophoresis.

The criteria for anemia were as follows: (a) Adult males: hemoglobin below 13 gm/dl and (b) Adult females: hemoglobin below 12 gm/dl. All patients except one female were anemic according to the normal reference values for anemia.

The mean hemoglobin levels were 8.5 ± 2.1 gm/dl. The mean hemoglobin levels of adult males with sickle cell disease were 8.2 ± 2.3 gm/dl, while the mean hemoglobin levels in adult females with sickle cell disease were 8.5 ± 2.0. There was no significant difference between the mean hemoglobin levels in males and females ($P = 0.67$). The mean hemoglobin levels in AS patients were 8.7 ± 2.1 gm/dl compared to 7.6 ± 1.7 gm/dl in SS patients and this difference was not statistically significant ($P = 0.13$).

Table 1 shows the distribution of cases according to MCV. Normal MCV values in adult were taken as 76-96 fl.[13] Eighteen (30%) of the total study group showed microcytosis, while MCV was raised in five (8.3%) patients. The mean MCV values of the study group were 79.9 ± 11.9 fl. Mean MCV in adult males was 82.0 ± 10.1 fl while it was 79.2 ± 12.5 fl in adult females. The difference in MCV between males and females was not statistically significant (i = 0.46). The mean MCV was 77.1 ± 10.7 fl in patients with AS pattern compared to 91.1 ± 10.2 fl in SS patients. The mean MCV was significantly higher in patients with SS pattern ($P = 0.00015$).

Table 2 depicts the distribution of cases according to serum ferritin levels. We used a cut-point of 25 ng/ml to diagnose iron deficiency anemia. Thus, the diagnosis of iron deficiency anemia patients of sickle cell disease was established based on the following criteria: adult male (>12 years): Hb <13 g/dl and serum ferritin <25 ng/ml; and adult female (>12 years): Hb <12 g/dl and serum ferritin <25 ng/ml.

Table 1: Distribution of cases according to mean corpuscular volume

MCV (in fl)	Adult males	Adult females	Total
Low MCV<76 fl	3	15	18 (30%)
Normal MCV 76-96 fl	10	27	37 (62%)
Raised MCV >96 fl	1	4	5 (8%)
Total	14	46	60 (100%)

Table 2: Distribution of cases according to serum ferritin levels

Serum ferritin level (in ng/ml)	Adult males	Adult females	Total
≤25 ng/ml	5	18	23 (38%)
>25 ng/ml	9	28	37 (62%)
Total	14	46	60 (100%)

Table 3: Iron deficiency anemia in patients of sickle cell disease according to hemoglobin electrophoresis

Iron deficiency anemia	AS	SS	Total
Present	21	2	23 (38%)
Absent	27	10	37 (62%)
Total	48	12	60 (100%)

Table 4: Diagnostic accuracy of low MCV in detecting iron deficiency anemia (IDA) compared to low serum ferritin

	IDA present	IDA absent	Total
Low MCV	10	8	18 (30%)
Normal MCV	13	29	42 (70%)
Total	23	37	60 (100%)

Using these criteria, Table 3 shows the distribution of iron deficiency anemia in patients of sickle cell disorders. Twenty-three of the 60 (38.3%) cases of sickle cell disorders were iron deficient. Twenty-one of the 48 (43.7%) patients of AS type and 2 of the 12 (16.6%) patients with SS pattern were iron deficient.

We compared the diagnostic accuracy of low MCV (less than 76 fl) in detecting iron deficiency using low serum ferritin levels (less than 25 ng/ml) as reference standard, as depicted in Table 4.

The sensitivity of low MCV in detecting iron deficiency was 40.0% (CI-20.0-63.6), and the specificity was 78.4% (CI-61.3-89.6). The positive predictive value and the negative predictive value of this test were 50.0 (CI-25.5-74.5) and 70.7 (CI-54.3-83.4), respectively.

Receiver operating characteristic (ROC) curve analysis was performed to analyze the usefulness and optimal cut-off of MCV to predict iron deficiency among patients suffering from

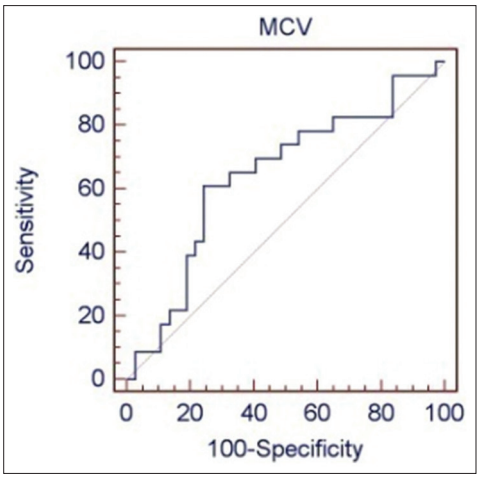

Figure 1: Receiver operating characteristic (ROC) curve of MCV for predicting iron deficiency anemia

sickle cell anemia, as depicted in Figure 1. The area under the curve of MCV for predicting iron deficiency anemia based on the criteria stated earlier was 0.642 (CI: 0.507 – 0.761). The optimal cut-off where the sum of the sensitivity and specificity was highest was at an MCV of 77.6 fl. The sensitivity and specificity of predicting coexisting iron deficiency at this point were 60.9% (CI: 38.6-80.3%) and 75.7% (CI: 58.8-88.2%), respectively. The likelihood ratio for a low MCV and high MCV was 2.5 and 0.52, respectively. Normally, the cut-off for low MCV is considered 76 fl. At this cut-off of MCV, the sensitivity and specificity were 43.5% and 78.4%, respectively.

Discussion

As MCV is routinely performed for all patients of sickle cell anemia, a cut-off at which it can also determine Iron deficiency anemia and help the family physicians and their patients by decreasing the cost of investigations by avoiding additional much costlier investigation serum ferritin. Evidence in this regard can help family physicians make rational decisions.

Of the 60 cases in our study, 48 (80%) cases had AS pattern and 12 cases (20%) had SS pattern on hemoglobin electrophoresis. The numbers of sickle cell heterozygotes are higher than the homozygotes in the general population, and hence, the distribution was similar in this hospital-based study as well.

The mean hemoglobin levels in the study population were 8.5 ± 2.1 gm/dl. There was no significant difference between the mean hemoglobin levels in males (8.2 ± 2.3 gm/dl) and females (8.5 ± 2.0). The mean hemoglobin levels in AS patients were 8.7 ± 2.1 gm/dl compared to 7.6 ± 1.7 gm/dl in SS patients, and this difference was not statistically significant (P = 0.13). Our findings are similar to those of Mohanty *et al.*[6] whose study population is geographically adjacent to the population that we have studied. They found mean hemoglobin levels of 9.9 ± 2.2 gm/dl in AS patients and 8.7 ± 2.5 gm/dl in SS patients as against 9.9 ± 2.0 gm/dl in AA subjects. Similarly, in a study conducted by Panigrahi *et al.*[13] in a geographically adjacent population, they observed similar findings with mean hemoglobin levels of 8.2 ± 2.6 gm/dl in SS patients. They did not report the values for AS patients.

The mean MCV was 77.1 ± 10.7 fl in our patients with AS pattern compared to 91.1 ± 10.2 fl in SS patients. The mean MCV was significantly higher in patients with SS pattern (P = 0.00015). Vichinsky *et al.*[5] found the mean MCV in transfused HbSS patients to be 90 fl (range: 80-108 fl). They found mean MCV in non-transfused patients to be 82 fl (range: 61-95 fl). Our findings in HbSS patients are similar to those of transfused patients, as they may have received transfusions in the past. Mohanty *et al.*[6] found a mean MCV of 63.5 ± 8.9 fl in AS patients, 72.5 ± 8.4 fl in SS patients as against 66.4 ± 8.5 fl in AA subjects in a community-based study. Panigrahi *et al.*[13] reported that MCV was 73.59 ± 8.92 in patients with SS pattern. It was reported to be 84.95 ± 11.10 in a study conducted by Sani *et al.*[14] Our values of MCV seem to be higher than those of Mohanty *et al.*'s,[6] Panigrahi *et al.*'s,[13] and Sani *et al.*'s[14] study probably because ours is a hospital-based study, and increased reticulocyte counts may have elevated the MCV.

At present, the absence of stainable iron in marrow is considered to be the reference standard for diagnosing iron deficiency. However, this test is invasive, painful, and was not feasible for research purposes. Serum ferritin concentrations have been proposed as the best biochemical test that correlates with relative total body iron stores.[5] In normal subjects, a serum ferritin value below 12 ng/ml is usually diagnostic of iron deficiency.[9,10] However, in patients with hemolytic anemia, conditions recognized to elevate serum ferritin, a serum ferritin value below 25 ng/ml has been used to predict absent bone marrow iron stores and response to irontherapy.[5,7,9] Serum ferritin values less than 25 ng/ml are 100% sensitive and 100% specific for iron deficiency anemia in sickle cell disease.[7]

We used a cut-off point of 25 ng/ml to diagnose iron deficiency anemia. Serum ferritin was low (<25 ng/ml) in 23 of the 60 cases (38.3%). The serum ferritin levels in the study ranged between 2-510 ng/ml (mean = 68.7 ± 100.5 ng/ml). The mean ferritin levels in adult males were 81.2 ± 110.8 ng/ml while they were 64.9 ± 98.1 ng/ml in adult females. This difference was not statistically significant (P = 0.39). The average serum ferritin levels in patients with AS type was 66.7 ± 107. 0 ng/ml and the average serum ferritin levels in patients with SS type was 76.8 ± 71.9 ng/ml. This difference was not statistically significant (P = 0.09). Authors have reported raised varying serum ferritin levels for patients with sickle cell disease. Hussain *et al.*[15] reported raised serum ferritin values in patients with sickle cell anemia compared to controls. While Mohanty P *et al.*[16] reported serum ferritin levels ranging from 12-2093 ng/mL; they found low serum ferritin levels only in 20 out of 208 subjects (10%) and reported that most patients had high serum ferritin levels. They reported statistically significant difference between high serum ferritin levels in patients with SS pattern compared to controls. The

serum ferritin concentration in unselected patients with sickle cell anemia in the steady state is normal or only modestly elevated according to studies conducted by Rao KR *et al.*,[17] Oluboyede OA *et al.*,[18] Vichinsky *et al.*,[5] Russo-Mancuso *et al.*,[19] O'Brien,[7] and Serjeant GR *et al.*[20] In three studies undertaken by Vichinsky *et al.*,[5] Davies *et al.*,[21] and O'Brien,[7] the samples were stated to have been taken during the steady state. Raised ferritin concentrations were not found in un-transfused patients, and in transfused subjects, the concentrations were lower than would be expected in similarly transfused thalassemia patients. Sani *et al.*[14] reported that patients with SS type had mean serum ferritin levels of 589.33 ± 427.61 ng/mL and in controls were 184.53 ± 119.74 ng/ml. The wide range of values in both AS and SS patients reflects that serum ferritin values may be normal, increased or decreased in these patients. Since our study population was hospital-based, samples were not always collected in a steady state as patients could have been in crises. This could have elevated the serum ferritin levels falsely. However, despite this feature, we found low ferritin levels in 38.3% of our patients.

As stated earlier, the diagnosis of iron deficiency anemia in patients of sickle cell disease was established based on certain criteria. Using these criteria, 23 of the 60 (38.3%) cases of sickle cell disorders were found to be iron deficient. The reported estimates of iron deficiency in patients with sickle cell anemia range between 16-67%.[4-7] Vichinsky *et al.*[5] detected iron deficiency anemia in 9% of their patients. Lanzkowsky P *et al.*[22] reported a 21% incidence of nutritional iron deficiency in black children living in a low socio-economic environment. Okeahialam and Ob[23] reported iron deficiency anemia in 31% of sickle cell anemia subjects. King *et al.*[24] found that 8.5% of HbSS and HbSC subjects had iron deficiency anemia. Kassim *et al.*[25] in a study from Yemen reported that overall, 13.3% of patients had iron deficiency, while iron deficiency was present in 20.5% of the non-transfused group among the study subjects. Das *et al.*[4] in a hospital-based study in Orissa found a 23% incidence of iron deficiency anemia in sickle cell anemia patients. Mohanty *et al.*[6] found iron deficiency anemia in 28.1% of 1329 sickle cell patients (AS and SS). Our study shows that iron deficiency in Indian patients with sickle cell disease is much more common than is usually suspected.

No single test is sensitive and specific enough to assess the state of iron balance in sickle cell anemia. The difficulty in assessing the state of iron balance in sickle cell anemia has been demonstrated by several authors. Transferrin saturation, MCV, free erythrocyte protoporphyrin, and marrow iron stores have all been shown to have drawbacks in studies conducted by Peterson *et al.*,[26] Vichinsky *et al.*,[5] Davies *et al.*,[27] and Rao *et al.*[15] We acknowledge that our reference standard, serum ferritin, was not sensitive enough as the results are known to vary in a number of situations including inflammation, crises, and blood transfusion. In the absence of a gold standard, this was the best possible test available to diagnose iron deficiency. Measurements of ferritin to assess the state of this balance are only useful if performed in the steady state patient. As this was a hospital-based study, it was not possible to make sample collections always in the steady state, and this could have affected our results. The findings of this study must be viewed in this context.

We compared the diagnostic accuracy of low MCV (less than 76 fl) in detecting iron deficiency using low serum ferritin levels (less than 25 ng/ml) as reference standard. The sensitivity of low MCV in detecting iron deficiency was 40.0% (95% CI-20.0-63.6), and the specificity was 78.4% (95% CI-61.3-89.6). The positive predictive value and the negative predictive value of this test were 50.0 (95% CI-25.5-74.5) and 70.7 (95% CI-54.3-83.4), respectively. Vichinsky *et al.*[5] stated that age-dependent MCV values were a useful screening test for iron deficiency. Alquazi *et al.*[28] reported that MCV had a sensitivity of 73.6% and a specificity of 69% in detecting iron deficiency. However, our findings do not concur with those of Vichinsky *et al.*[5] and Alquazi *et al.*[28] Microcytosis was not found to be a sensitive test to detect coexisting iron deficiency in patients with sickle cell disorders.

We found that the optimal cut-off on ROC curves, where the sum of the sensitivity and specificity was highest, was at an MCV of 77.6 fl. The sensitivity and specificity of predicting coexisting iron deficiency at this point were 60.9% (CI: 38.6-80.3%) and 75.7% (CI: 58.8-88.2%), respectively. This cut-off is very close to the conventionally used cut-off for low MCV, i.e., 76 fl.

The major limitation of our study is that as it is a hospital-based study, the study findings cannot be generalized to other community settings. Our study has several strengths as our patients represent the core rural Indian population; by including consecutive outpatients and inpatients, we tried to avoid selection bias. We performed blind and independent comparisons between the index test (MCV) and the reference standard test (serum ferritin). As MCV is a continuous variable, we also used multi-level likelihood ratios to compute the diagnostic accuracy of MCV at several cut points.

The low sensitivity (40%) of microcytosis in detecting iron deficiency indicates that many cases will be missed if MCV alone is used to detect coexisting iron deficiency anemia in sickle cell patients. However, the specificity of this test is relatively better (78.4%). We believe and conclude that no one test is good enough to detect coexisting iron deficiency, and a combination of tests might be useful.

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Conflicts of interest

There are no conflicts of interest.

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