Schistocytes Evaluation in Iron Deficiency: An Assessment Adopted From ICSH Nomenclature Guideline

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Research note

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Abstract

Objective The diagnostic validity of schistocyte count in diagnosing iron deficiency in the microcytic population is critical to evaluate. The purpose of this study is to identify the correlation between schistocyte count and iron parameters, and the performance of schistocyte count in diagnosing iron deficiency in the microcytic population.

Results Out of 805 general check-up participants, 65 subjects consisting of 17 males and 48 females aged 18–56 years had Mean Corpuscular Value (MCV) < 80 fL. Serum ferritin examination showed 25 patients with iron deficiency and the other 40 subjects without iron deficiency. There was a significant difference in the schistocyte count between the two groups ($p < 0,001$). Correlation analysis obtained a significant relationship between schistocyte count and serum ferritin ($r=-0,67$, $p < 0,001$). The Receiver Operating Characteristic (ROC) curve analysis provided Area Under the Curve (AUC) of schistocyte count is 0.827 with Youden Index (YI) 55% for a cut-off of ≥0.75%.

Highlights

- Due to higher cost and complicated equipment to detect iron deficiency anemia, a simple assessment tool for laboratory facility located in rural and remote areas is required.
- Recently, the measurement of schistocytes, the red blood cell fragments, are considered as an alternative.
- Schistocyte have significant correlation with iron parameters, and have good performance in diagnosing iron deficiency in the microcytic population.

Introduction

Anemia is a worldwide public health issue affecting approximately 1.62 billion sufferers or approximately a quarter of the world's population in both developed and developing states. Among many different anemia forms, Iron Deficiency Anemia (IDA) is the most widespread [1]. Anemia can be caused by nutritional deficiencies (iron, folate, vitamin B12, and vitamin A), acute and chronic inflammation, parasitic infections, and inherited or acquired disorders that can affect the synthesis of hemoglobin, red blood cell production, or red blood cell survival [2]. In developing countries, such as Indonesia, iron deficiency is responsible for most cases of anemia [3].

In iron deficiency, the microcytic erythrocytes found is caused by a decrease in the concentration of hemoglobin. While iron composes most of the hemoglobin structure, the erythrocytes formed under iron deficiency conditions will present smaller (microcytic) and pale (hypochromic) in morphology [4]. The most common parameters used for iron status measurements are transferrin saturation and serum ferritin. Unfortunately, the iron biochemical parameters as the gold standard to detect iron deficiency are still relatively costly and not affordable enough to be used in daily practice at peripheral health facilities [5, 6]. Due to this condition, a simple alternative assessment tool for laboratory facilities located in rural

and remote areas is considered necessary. Some parameters of erythrocytes have been widely investigated concerning their relation to iron status [7]. In addition to elliptocytes, ovalocytes, target cells, and hypochromia, which have been known to be the most common cells in iron deficiency anemia, it turns out that fragment cells (schistocytes) have also been reported in iron deficiency.

Schistocyte (from Greek *schistos* for "divided" and *kytos* for "hollow" or "cell") is a fragmented part of an red blood cell (RBC) that can be seen at peripheral blood smear as an irregularly shaped body with two pointed ends. Schistocyte occurs due to obstacles in blood vessels, such as fibrin clots, artificial heart valves, or other blood vessels' obstacles. Schistocyte count is considered normal at the level of < 0.5% [8]. On microscopic examination of peripheral blood smears, RBC fragments represented as schistocytes can occur due to several conditions (Table S1) [9].

In iron deficiency, erythrocytes with poikilocytosis morphology, such as schistocytes, have a shorter life span and membrane stiffness. The iron that builds up hemoglobin is also the primary feature of erythrocyte structure. If the iron levels are decreased, there will be an increase in the surface-volume ratio of erythrocytes. This change causes erythrocytes to break easily when passing through capillaries or turbulence, causing erythrocyte fragments to be formed in the bloodstream [10].

According to researchers' knowledge, studies on RBC fragment as an alternative parameter to detect iron deficiency in Indonesia are still limited. This study aims to determine the correlation between schistocyte count and iron parameters and schistocyte count performance in diagnosing iron deficiency in the microcytic population.

Materials And Methods

This research is a cross-sectional observational study with a population consisting of general check-up participants. Participants underwent complete blood tests using ADVIA 2120i® (Siemens, Tarrytown, NY, USA). Inclusion criteria included all male and female screening participants who had microcytic erythrocyte morphology (MCV < 80 fL). Subjects were excluded if they were pregnant. Serum ferritin was examined in subjects with Mean Corpuscular Value (MCV) < 80 fL using Cobas 6000® analyzer series (*Roche Diagnostics Corporation, Indianapolis, USA*). Schistocyte was counted in 1000 RBC per subject with the International Council for Standardization in Haematology (ICSH) 2015 guideline morphology criteria. Schistocyte count was done with good reliability results between two examiners (r = 0.944, p = 0.016). The relationship between variables was determined using the Spearman correlation test. The schistocyte count was further compared to the Ferritin level using Receiver Operating Characteristic (ROC) curve to diagnose iron deficiency.

Data analysis used in this study was Statistical Package for the Social Sciences (SPSS) (*version 25; IBM Corporation, Armonk, New York, USA*). The normality test for hematology parameter, RBC fragment, and iron status was the Kolmogorov-Smirnov test, followed by the mean difference test using the Mann-Whitney U test due to abnormal data distribution. The relationship between variables was determined using the Spearman correlation test. The schistocyte count was further compared to the Ferritin level

using ROC curve to diagnose iron deficiency. The schistocyte count ability as a deficiency marker is illustrated by the Area Under Curve (AUC) area, where the most optimal sensitivity and specificity cut-off is determined by the Youden Index (YI) analysis.

Results

Of the 805 general check-up participants, 65 subjects had MCV < 80 fL. This microcytic subjects consisted of 17 males and 48 females with an age range of 18–56 years. All of the microcytic subjects were then examined for their iron status in the form of serum ferritin levels. Figure 1 shows the peripheral blood smear results in both microcytic subjects with iron deficiency, which has fragmented red blood cells, and the ones without iron deficiency, which has no fragmented cells. The erythrocyte index results from the complete blood count and iron status of all microcytic subjects are shown in Table 1.

Table 1
The characteristic of a hematology profile and schistocyte count

Parameter	Iron Deficiency (n = 25)	Non-Iron Deficiency (n = 40)	*p*-value
Haemoglobin (g/dL)	10.48 ± 1.43	13.19 ± 1.65	0.000[a*]
Hematocrit (%)	34.98 ± 3.20	41.94 ± 4.61	0.000[a*]
Erythrocyte count (x10^6 cells/μL)	4.96 ± 0.41	5.78 ± 0.62	0.000[a*]
MCV (fL)	73.5 (50–79)	74.7 (58–80)	0.237[b*]
MCH (pg)	21.90 (13–26)	23.8 (17–25)	0.018[b*]
MCHC (%)	29.83 ± 1.84	31.4 ± 1.20	0.000[a*]
Schistocyte	13.00 (2–45)	4.00 (0–37)	0.000[b*]

[a] Student T-test; [b] Mann Whitney-U test; [*] Statistically significant ($p < 0.05$)

The subjects were then grouped into iron deficiency (ID) and non-iron deficiency (non-ID) based on serum ferritin levels. In this study, 25 ID subjects and 40 non-ID subjects were obtained.

There was a significant difference in the schistocyte count between the two groups ($p < 0.05$). Spearman correlation test in *Figure S2* shows a correlation between schistocyte count and serum ferritin level (r=-0,67, $p < 0,001$), as well as between schistocyte count and transferrin saturation (r=-0.58, $p < 0.001$).

In the ROC curve analysis of schistocyte count with serum ferritin as the gold standard, an AUC of 0.827 was obtained (Fig. 2). From the YI analysis, a 55% value was obtained, while the cut-off for schistocyte count was 0.75 with a sensitivity of 80% and specificity of 75%. At the cut-off, schistocyte count had a Positive Predictive Value (PPV) of 61%, Negative Predictive Value (NPV) of 86%, Positive Likelihood Ratio of 2.67, and Negative Likelihood Ratio of 0.286.

Discussion

According to the World Health Organization (WHO), anemia affects 2 billion people worldwide and is mostly due to iron deficiency anemia [11]. Schistocyte is associated with iron deficiency because iron-deficient erythrocytes are fragile. The formation occurs most often due to mechanical damage to erythrocytes, usually due to turbulent blood flow, contact with roughened vascular surfaces, or shearing by intravascular fibrin strands [12]. ICSH Working Group recommended the automated counting of RBC fragments, such as schistocytes, as a useful routine screening tool in the laboratory [13].

Based on the subjects' characteristics, females were likely to have microcytic red blood cells than males, with an age range of 18–56 years old. In other studies, it is found that hypochromic microcytic anemia is more common in premenopausal females because they lose blood with each menstrual cycle. After the female population, pre-school aged children suffer the most from anemia because of a lack of iron in their primary diet. The male population is usually resistant to anemia due to circulating testosterone levels. Among 65 microcytic subjects, 26% of them were males, a slightly higher number than the percentage of adult males who are globally afflicted with anemia, which is 12.7% [14, 15].

There was a significant difference in the schistocyte count between iron deficiency subjects compared to non-iron deficiency subjects ($p < 0.05$). Microcytic subjects with iron deficiency had fragmented red blood cells, while the ones without it had none. A study on direct red cell membrane deformability measurements indicated that iron deficiency might increase membrane rigidity, causing reduced RBC deformability, which produced fragmented cells [10]. Schistocyte, a fragmented red cell, usually occur in cytoskeletal RBC abnormalities, such as acquired and inherited RBC disorders in association with marked anisopoikilocytosis [13]. Another study also stated that RBC fragments are most numerous in diseases with marked anisopoikilocytosis, such as iron deficiency anemia [16].

Reduced hemoglobin levels with low MCV may suggest iron deficiency anemia, indicating red cell morphology and other ancillary investigation for iron deficiency. Further tests such as serum ferritin and total iron-binding capacity (TIBC) helps to differentiate microcytic anemia. For instance, low serum ferritin is expected in iron deficiency [17, 18]. This statement was confirmed in our study, where there was a statistically significant inverse relationship between schistocyte count and serum ferritin levels in the correlation analysis of this study ($r=-0.67$; $p < 0.001$), indicating an association of higher schistocyte count with lower serum ferritin levels leading to the direction of iron deficiency.

The AUC obtained from the ROC curve analysis (0.827) is said to have sufficient diagnostic ability, where the minimum AUC is equal to, or more than 0.7. This indicates that schistocyte count has a sufficient ability to mark iron deficiency in the microcytic population. A schistocyte count and grading are recommended and may be valuable when schistocytes are the dominant feature for the diagnosis and follow-up of anemia [19]. Schistocyte is termed few or 1 + if the number is < 1%; moderate or 2 + if 1–2%; and many or 3 + if > 2% [20]. Meanwhile, in another reference, schistocyte is termed occasional if it is less 1%; 1 + if 1–3%; 2 + if 3–6%; and 4 + if it is more than 12% [19].

Any positive smear findings represent relevant clinical information that must be described in complete blood counts as per local consensus indicating the morphological changes and cell percentages relevant for the diagnosis and monitoring of patients [21].

Conclusion

Schistocyte count has a significant correlation with iron parameters and can be used as a marker for iron deficiency in the microcytic population. Health facilities that do not have access to iron parameters examination can perform a schistocyte count.

Limitations

This study only used 65 subjects as a preliminary study. Future work can use more subjects to obtain more convincing results.

List Of Abbreviations

AUC - Area Under the Curve

ICSH - International Council for Standardization in Haematology

ID - Iron Deficiency

IDA - Iron Deficiency Anemia

MCV - Mean Corpuscular Value

Non-ID - non-Iron Deficiency

NPV - Negative Predictive Value

PPV - Positive Predictive Value

ROC - Receiver Operating Characteristic

RBC - Red Blood Cell

SPSS - Statistical Package for the Social Sciences

TIBC - Total Iron-Binding Capacity

WHO - World Health Organization

YI - Youden Index

Declarations

Ethics approval and consent to participate

This study has received ethical approval from the FK-KMK UGM Ethics Committee (No: KE/FK/0552/EC/2019). All examinations were carried out at the Clinical Pathology Laboratory, FK-KMK UGM, and Clinical Laboratory Unit, RSUP Dr. Sardjito. The written informed consent for this study was obtained.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

TR: provided concept of the study, data analysis, drafted the manuscript and journal submission; GM: selected research participants and data collection; DP: drafted the manuscript; US: helped in data analysis.

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Figures

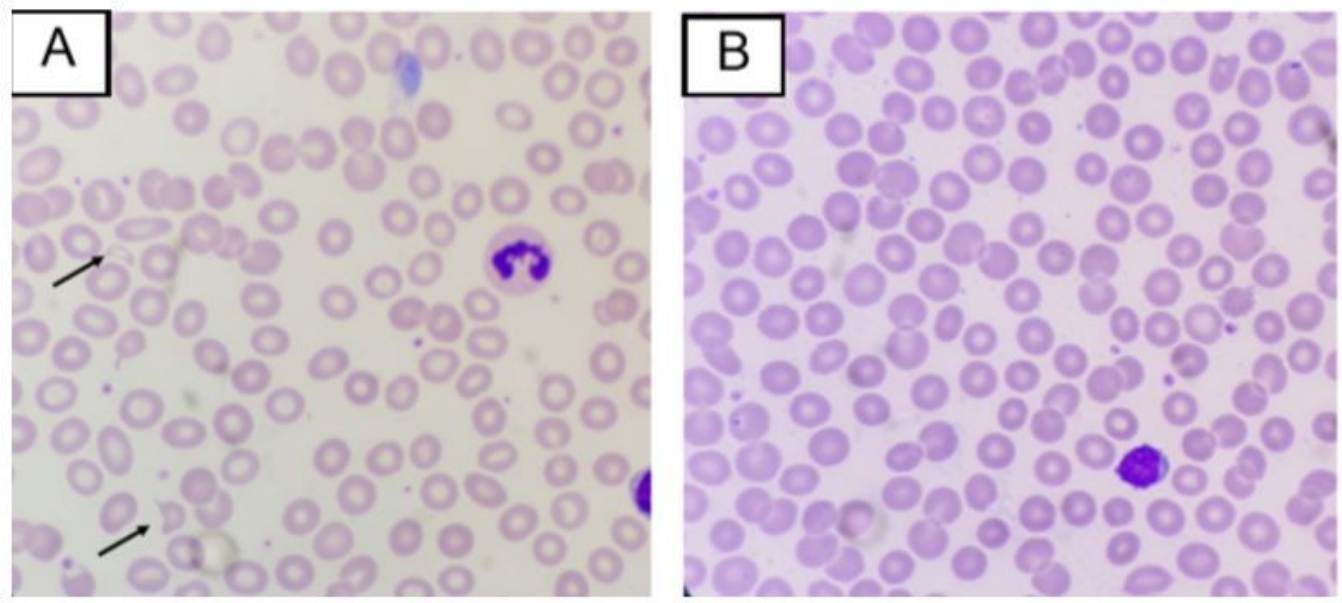

Figure 1

Blood picture of schistocyte in iron deficiency subject A. Iron deficiency subject. Schistocyte as a fragment and triangular found in the smear B. Microcytic non-iron deficiency subject. No fragmented cells were found in the smear.

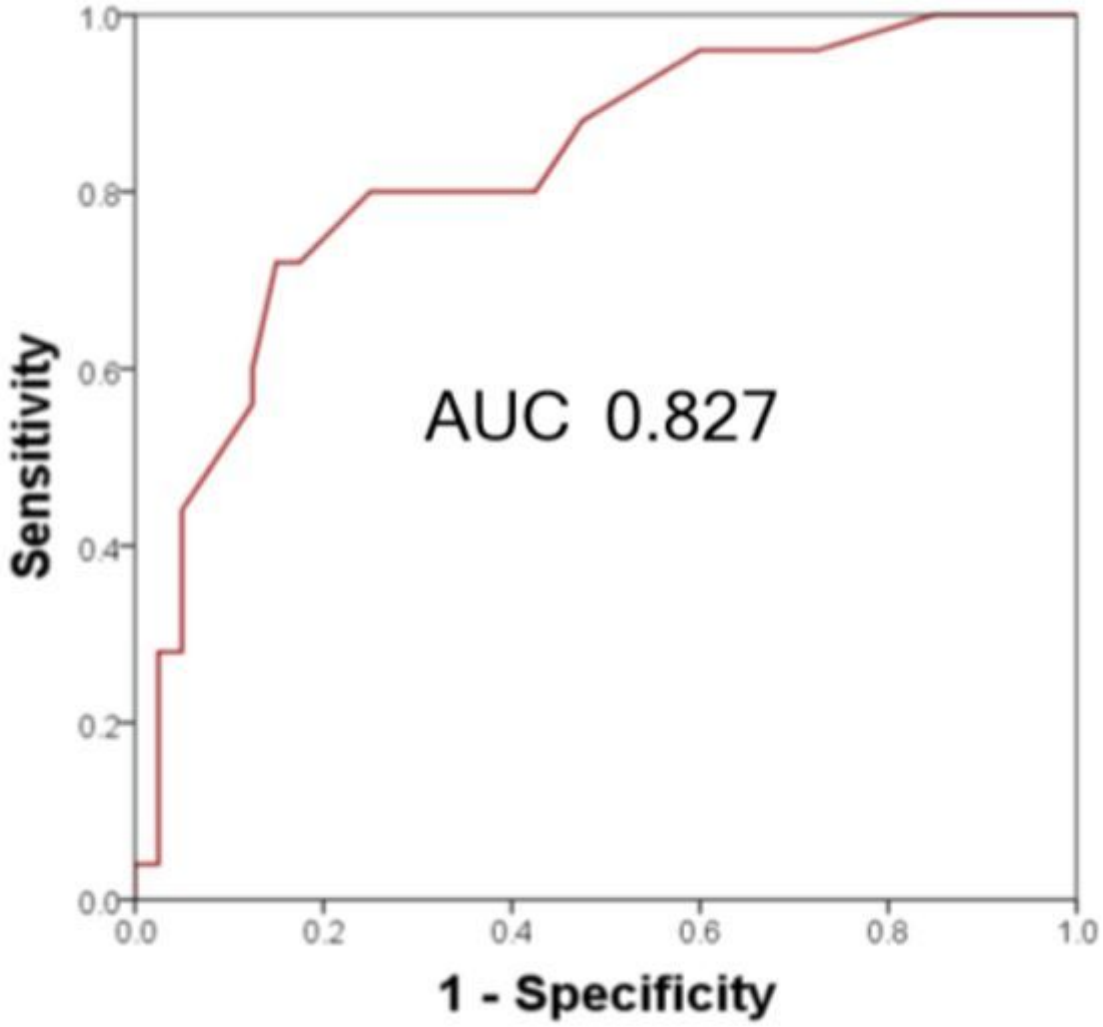

Figure 2

ROC curve and diagnostic performance of schistocyte

Supplementary Files

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