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Enhancing diagnostic accuracy for iron deficiency in pregnant women through mean reticulocyte volume

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Yuan Xue, Mingjie Chen, Molong Li were students of Professor Guofang Shu and performed the main experiments. Guofang Shu designed the study and wrote the first draft of the manuscript. Zhi He helped with the laboratory work and participated in the design and discussion of the manuscript. Yuexin Zhang revised the manuscript. All authors read and approved the final manuscript

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ABSTRACT

Background and Objectives: Women are more prone to iron deficiency (ID) anemia when pregnant. The diagnostic use of mean reticulocyte volume (MRV) in identifying ID anemia during pregnancy has not been thoroughly investigated. The objective of study is to evaluate the effectiveness of MRV in diagnosing ID in pregnant women. **Methods and Study Design:** At first, the MRV of 20 healthy female volunteers (healthy group) was measured on specific days throughout one month. Subsequently, clinical data from 724 pregnant women were thoroughly examined. These women were divided into two groups: 282 with ID (research group) and 442 without ID (control group). Parameters such as MRV, reticulocyte hemoglobin equivalent (RHE), red blood cell volume distribution width-standard deviation (RDW-SD), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT), reticulocyte count (RET), MRV/MCV ratio, and serum ferritin (SF) were analyzed and compared. **Results:** The MRV remained consistent over a month for 20 healthy individuals. In addition, there were significant differences in MRV, RHE, RDW-SD, MCV, MCH, MCHC, HCT, RET, and MRV/MCV between the research group and control group. The receiver operating characteristic (ROC) analysis showed that the areas under the curve (AUCs) for these measures were as follows: 0.840, 0.837, 0.676, 0.654, 0.639, 0.602, 0.571, 0.550, and 0.816, respectively. Ultimately, there was a substantial disparity in MRV prior to and following therapy with oral iron treatments. **Conclusions:** In healthy women, MRV remains stable and reliably diagnoses ID, while assessing oral iron treatment effectiveness during pregnancy.

Key Words: pregnancy, iron deficiency, mean reticulocyte volume, reticulocyte hemoglobin equivalent, oral iron treatment

INTRODUCTION

Anemia, which is mostly brought on by a lack of iron, is a significant health concern that affects people all over the world. In China, pregnant women had an incidence of roughly 19.8% of the disease.¹ Several negative consequences are related to iron deficiency anemia (IDA), including premature birth, low birth weight, infection, and neurological impairment in childhood.² These effects are associated with both the mother and the fetus. Therefore, the diagnosis and treatment of iron deficiency (ID) are essential to improve the outcome and quality of life of expectant mothers and the children they are carrying.

Both the diagnosis of ID and the monitoring of the patient's response to treatment are predicated on the availability of highly precise and sensitive tests. These tests can give early and accurate detection, as well as lead to the prevention of iron overload, which is associated with dangers and possible adverse effects.³ Currently, the diagnosis of ID can be made by measuring hematologic parameters of a complete blood count. These parameters include the concentration of hemoglobin (Hb), mean corpuscular volume (MCV), and hematocrit (HCT). Additionally, serum iron measurements such as serum ferritin (SF), transferrin, and transferrin receptor (TfR) can also be used. The gold standard is considered the measurement of SF along with transferrin saturation, values reflecting the body's iron stores.^{4,5} However, when interpreting the results of these tests, it is necessary to take into account the fact that certain hematologic parameters are influenced by the physiological changes that occur during pregnancy. Throughout the early stages of pregnancy, for instance, there is an increase in the mass of red blood cells, whereas throughout the late second and third trimesters, there is a drop in Hb, HCT, and MCV. In addition, inflammation, the generation of liver transport proteins, and hemodilution all have an impact on particular parameters, some of which include serum iron and ferritin.^{6,7} These days, hematological analyzers that make use of flow cytometry cannot only provide information on regular parameters, but also information on the status of iron through the measurement of reticulocyte indices.⁸ It has been observed that the Hb of reticulocyte is a reliable predictor for ID in newborns, children, and adolescents.^{9,10} This is one of the advanced cellular indices. The significance of other indices, such as mean reticulocyte volume (MRV) and MRV/MCV, in the diagnosis of ID has to be further investigated.

In the present study, we aimed to investigate the diagnostic value of MRV for ID in pregnant women. To address this point, we split our goal to three: first, to assess the degree of variability and reproducibility of MRV in the healthy population. Second, we try to ascertain the diagnostic accuracy of several cellular indices among pregnant women and to investigate the association between these indices and the presence or absence of iron. Further, we examine the change in MRV that occurred after oral iron supplementation and evaluated its capacity to serve as an indicator of the effectiveness of the treatment we administered.

MATERIALS AND METHODS

Study design

This retrospective cohort study focused on pregnant women attending Zhongda Hospital between January 11, 2022, and December 30, 2022.

Study population

Participation was open to adult pregnant women without infections, inflammations, connective tissue illnesses, liver diseases, chronic kidney diseases, cancer, or other conditions affecting pregnancy. Those undergoing iron reagent treatment were excluded. Pregnant women with ID were enrolled into Research group, while those without ID were enrolled into Control group. Additionally, we recruited twenty healthy non-pregnant adult female volunteers as healthy group to measure the levels of MRV on days 1, 4, 7, 15, 21, and 30 of September 2022. In addition to being registered on the Chinese Clinical Trial Registry (<http://www.chictr.org.cn>; registration number ChiCTR-TRC-14005084), the study was approved by the ethics committee at Zhongda Hospital Affiliated to Southeast University (No. 2021ZDSYLL335-P01).

Test methods and analyzers

In the case of healthy group, 2 mL of venous blood from each participant will be collected and tested on days 1, 4, 7, 15, 21, and 30 of September 2022. Within two hours, the MRV of every single blood sample was detected three times, and the results were compared to those of day 1.

To obtain rapid venous blood from pregnant women, a vacuum blood tube (BD Biosciences) was utilized. MRV, reticulocyte hemoglobin equivalent (RHE), red blood cell volume distribution width-standard deviation (RDW-SD), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte count (RET), and other indicators of each sample were analyzed with a Mindray BC-6900 Auto Hematology Analyzer, which was adjusted with a suitable calibrator. A Beckman Coulter DXI800 Auto Immunologic Analyzer was utilized to analyze the SF. The threshold for diagnosing ID was determined to be SF levels that were less than 15 $\mu\text{g/L}$.¹¹ The information was received from the Laboratory Information System, and a retrospective analysis was carried out on the data.

Treatment

An oral iron supplement, consisting of ferrous succinate 100 mg tablets, was administered to pregnant women who had been diagnosed with ID in Research group. The recommended daily dose for these women was between 200 and 300 mg, as recommended by the treating physician. Following one week, indices were rediscovered and examined to assess the therapeutic efficacy both before and after the administration of the oral iron treatment.

Statistical analysis

Analysis of statistical data was carried out with the help of the SPSS 18.0 program (IBM Corporation, USA). The continuous data are presented as the mean \pm standard deviation (SD). Statistical analysis was performed using the student's t-test. Paired t-test was used for the comparison of MRV in healthy female volunteers at different days. Receiver operating characteristic curves (ROC) were utilized to conduct an analysis of the areas under the curve (AUCs) for the diagnosis of ID in pregnant women with MRV, RHE, RDW-SD, MCV, MCH, MCHC, HCT, and MRV/MCV. Both the worst-case imputation and the exclusion of these samples were utilized to deal with the problem of missing data. We regarded a two-sided p -value less than 0.05 to be statistically significant.

RESULTS

Baseline characteristics

There were 20 healthy female volunteers who participated in the study, with a mean age of 29.1 ± 6.8 years. Table 1 showed the pregnant women who were a part of both the case group and the control group. A total of 282 women, with a mean age of 28.7 ± 5.7 years, and a mean gestation period of 21.4 ± 3.9 weeks, were included in the research group. On the other hand, the control group consisted of 442 pregnant women, with a mean age of 28.6 ± 5.7 years, and a mean gestation period of 22.3 ± 3.1 weeks. There was no statistical difference existed among research group, control group and healthy group regarding age, and no statistical difference existed between research group and control group regarding gestation period (both $p > 0.05$).

MRV changes of volunteers in a month

In healthy female individuals, MRV levels on days 4, 7, 15, 21, and 30 demonstrated significant stability, with no statistical difference compared to the baseline on day 1 ($p > 0.05$; Table 2). This suggests consistent MRV values over the one-month observation period.

Laboratory test results in case group and control group

Table 1 reveals notable disparities in MRV, RHE, RDW-SD, MCV, MCH, MCHC, HCT, RET, and MRV/MCV between the research and control group ($p < 0.01$). The ROC curve shown in Figure 1 demonstrates that the MRV, RHE, and MRV/MCV have high diagnostic effectiveness for ID, with comparative AUC values of 0.840, 0.837, and 0.816 respectively. The ROC curve was analyzed to determine the cutoff value that yields the optimal balance

between sensitivity and specificity. Table 3 presents a summary of the AUCs, confidence intervals for AUCs, sensitivities, and specificities. These values were calculated at the point of maximal efficacy. The MRV demonstrated the highest diagnostic efficacy with a sensitivity of 74.9%, specificity of 80.1%, and a Youden index of 0.550. The RHE had the second highest diagnostic efficacy with a sensitivity of 87.1%, specificity of 66.3%, and a Youden index of 0.534. Furthermore, MRV/MCV exhibited a notable diagnostic efficacy, second only to MRV and RHE (with a sensitivity of 74.4%, specificity of 78.7%, and a Youden index of 0.532).

Monitoring therapeutic effect with MRV and SF

Indices (MRV and SF) were reexamined and assessed to evaluate the therapeutic impact before and following oral iron treatment (Table 4). Following therapy, both MRV and SF levels exhibited a notable rise in all 49 pregnant women diagnosed with iron insufficiency. Moreover, a statistically significant difference was observed between the pre-treatment and post-treatment values ($p < 0.001$).

DISCUSSION

Our investigation revealed that there was no statistically significant alteration in MRV among the healthy participants over one month. This suggests that MRV is a consistent and reliable metric. Nevertheless, the MRV exhibited a notable decrease in pregnant women with ID in comparison to those without ID. Furthermore, there was a considerable improvement in MRV following the administration of oral iron therapy. Additional research is required to ascertain the impact of additional variables on MRV.

Insufficient iron in the human body leads to the utilization of stored iron, resulting in a decrease in ferritin levels. Iron reserve depletion occurs when there is insufficient timely supply of iron, resulting in the production of iron-deficient red blood cells although Hb levels remain normal. Continued negative iron balance can impact the production of Hb and result in the development of IDA.¹²⁻¹⁴ Pregnant women who need more iron cannot fulfill this requirement only through their regular diet, resulting in a significant prevalence of ID and IDA during pregnancy. It is well-established that ID and IDA are linked to higher rates of maternal and fetal health problems, such as low birth weight, preterm birth, and intrauterine growth restriction.¹⁵ Therefore, it is necessary to use tests that are extremely precise and sensitive to identify ID and assess the effectiveness of oral iron treatment, to enhance the final result. The reticulocyte serves as an early and highly responsive marker, as it remains present

in the peripheral blood for a brief duration of 1-2 days. Laboratory tests, such as reticulocyte indices, are highly valuable as they offer direct insights into Hb synthesis in bone marrow progenitors. Reticulocyte characteristics serve as indicators of bone marrow precursors and have proven clinically valuable in identifying disorders such as IDA, sideropenic erythropoiesis, and functional ID. These parameters are now widely recognized as the standard test for diagnosing these clinical illnesses.¹⁶⁻¹⁸ Prior research has suggested that MRV is a promising method for detecting hereditary spherocytosis and is crucial for distinguishing between various forms of hemolytic anemia.^{19,20} Nevertheless, determining whether MRV can serve as a reliable indicator of ID necessitates additional investigation.

Based on the AUC analysis, we discovered that MCV, MCH, MCHC, RDW-SD, HCT, and RET exhibited low accuracy in diagnosing ID in pregnant women. On the other hand, MRV, RHE, and MRV/MCV had similar AUC values and demonstrated excellent diagnostic performance for ID in pregnant women. The MRV and MCV are valid indicators of bone marrow hematological function. They have been utilized to forecast the reticulocyte response in individuals who have received autologous and allogeneic bone marrow transplantation (BMT).²¹

In our study, we found that the most accurate cut-off value for diagnosing ID was 100.05 fl when using MRV as a measure. At this cut-off value, the diagnostic sensitivity was 74.9% and the diagnostic specificity was 80.1%. Additionally, we observed that at a lower cut-off value of MRV, specifically 91.35 fl, the diagnostic sensitivity increased to 100%. This means that ID could be quickly ruled out in 6.49% (47 out of 724) of pregnant women. Conversely, at a higher cut-off value of MRV, specifically 112.25 fl, the diagnostic specificity increased to 100%. This means that ID could be quickly ruled out in 2.2% (16 out of 724) of pregnant women. The optimal threshold for diagnosing ID using RHE was found to be 29.85 pg. At this threshold, the diagnostic sensitivity was 87.1% and the specificity was 66.3%. When the threshold was lowered to 27.25 pg, the sensitivity increased to 100%, allowing for quick exclusion of ID in 6.21% of pregnant women. Conversely, when the threshold was raised to 33.75 pg, the specificity increased to 100%, allowing for quick exclusion of ID in 1.8% of pregnant women.

MRV demonstrated a good level of sensitivity and specificity in diagnosing ID during pregnancy, with the advantages of being minimally invasive, cost-effective, and fast.²² Concurrently, MRV can precisely indicate the condition of reticulocytes in the body and demonstrated a noteworthy reaction following oral iron treatment. Hence, MRV holds

significant potential for the clinical identification of ID and assessment of treatment in pregnant women.

There are several constraints associated with this study. Initially, it is important to note that this investigation is a retrospective cohort study conducted at a single site. This design has the potential to introduce bias in the selection of cases. Furthermore, the findings of this study have not undergone validation in a separate group of individuals. A prospective study, particularly one conducted across multiple centers, is necessary to further examine the clinical importance of utilizing MRV in pregnant women.

Indices (MRV and SF) were reexamined and assessed to evaluate the therapeutic impact prior to and following oral iron treatment (Table 4). Following therapy, both MRV and SF levels exhibited a significant rise in all 49 pregnant women diagnosed with iron insufficiency. Moreover, a statistically significant difference was observed between the pre-and post-treatment values ($p < 0.001$).

Conclusion

The results of our study demonstrated that MRV, a parameter used to measure blood flow, is highly stable in healthy women. Furthermore, it serves as a reliable indicator for detecting ID and monitoring the effectiveness of oral iron treatment in pregnant women.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest.

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Table 1. Comparison of baseline indicators: research group vs. control group

Group	Cases	SF ($\mu\text{g/L}$)	MRV (fL)	RHE (pg)	MCV (fl)	RDW-SD	RET %	HCT (%)	MCH (%)	MCHC (%)	MRV/MCV
Research	282	8.2 \pm 2.1	97.1 \pm 6.3	29.0 \pm 1.9	90.2 \pm 4.0	43.4 \pm 2.7	2.0 \pm 0.5	0.4 \pm 0.1	30.3 \pm 1.6	335.2 \pm 6.5	1.1 \pm 0.3
Control	442	27.2 \pm 19.1	104.3 \pm 4.3	31.3 \pm 1.3	92.3 \pm 3.6	45.0 \pm 2.9	2.0 \pm 0.5	0.4 \pm 0.1	31.2 \pm 1.3	337.4 \pm 5.8	1.1 \pm 0.5
<i>t</i> value		20.734	16.971	16.829	7.367	7.356	2.799	3.659	7.807	4.726	15.570
<i>p</i> value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.005

HCT: hematocrit; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; MRV: mean reticulocyte volume; RDW-SD: red blood cell volume distribution width-standard deviation; RET: reticulocyte; RHE: reticulocyte hemoglobin equivalent; SF: serum ferritin
p < 0.01 is considered significant

Table 2. MRV over different days (n=20)

	D 1	D 4	D 7	D 15	D 21	D 30
Mean	101.0	101.1	101.1	101.2	101.2	101.1
SD	5.0	5.0	5.0	5.0	5.0	5.1
<i>t</i> value		0.5	1.6	1.5	1.5	1.2
<i>p</i> value		0.593	0.117	0.155	0.155	0.23

MRV: mean reticulocyte volume; D: day; SD: Standard deviation
p > 0.05 is considered significant

Table 3. AUC of ROC curve (95%) for diagnosing iron deficiency in pregnant women

Parameter	AUC \pm SE	CI (95%)	Sensitivity	Specificity	FNR	FPR	Youden index
MRV	0.840 \pm 0.016	0.810 - 0.871	0.749	0.801	0.251	0.199	0.550
RHE	0.837 \pm 0.016	0.806 - 0.868	0.871	0.663	0.129	0.337	0.534
MCV	0.654 \pm 0.021	0.598 - 0.680	0.563	0.645	0.437	0.355	0.209
MRV/MCV	0.816 \pm 0.017	0.782 - 0.850	0.744	0.787	0.256	0.213	0.532
RDW-SD	0.676 \pm 0.021	0.635 - 0.716	0.568	0.695	0.432	0.305	0.263
RET %	0.550 \pm 0.022	0.507 - 0.592	0.462	0.635	0.538	0.365	0.096
HCT	0.571 \pm 0.022	0.529 - 0.614	0.439	0.677	0.561	0.323	0.116
MCH	0.639 \pm 0.021	0.613 - 0.694	0.590	0.635	0.410	0.365	0.225
MCHC	0.602 \pm 0.022	0.559 - 0.644	0.656	0.518	0.344	0.482	0.174

AUC: area under the curve; CI: confidence intervals; FNR: false-negative rate; FPR: false-positive rate; MRV: mean reticulocyte volume; RHE: reticulocyte hemoglobin equivalent; RDW-SD: red blood cell volume distribution width-standard deviation; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; HCT: hematocrit; RET: reticulocyte; ROC: receiver operating characteristic

Table 4. Comparison of SF and MRV before and after oral iron supplement in pregnant women (n=49)

	SF		MRV	
	Mean	Standard deviation	Mean	Standard deviation
Before	7.716	2.0527	95.776	6.3973
After	19.873	13.7169	103.114	4.3447
<i>p</i> value	< 0.001		< 0.001	
<i>t</i> value	6.39		9.89	

MRV: mean reticulocyte volume; SF: serum ferritin
 $p < 0.001$ is considered significant.

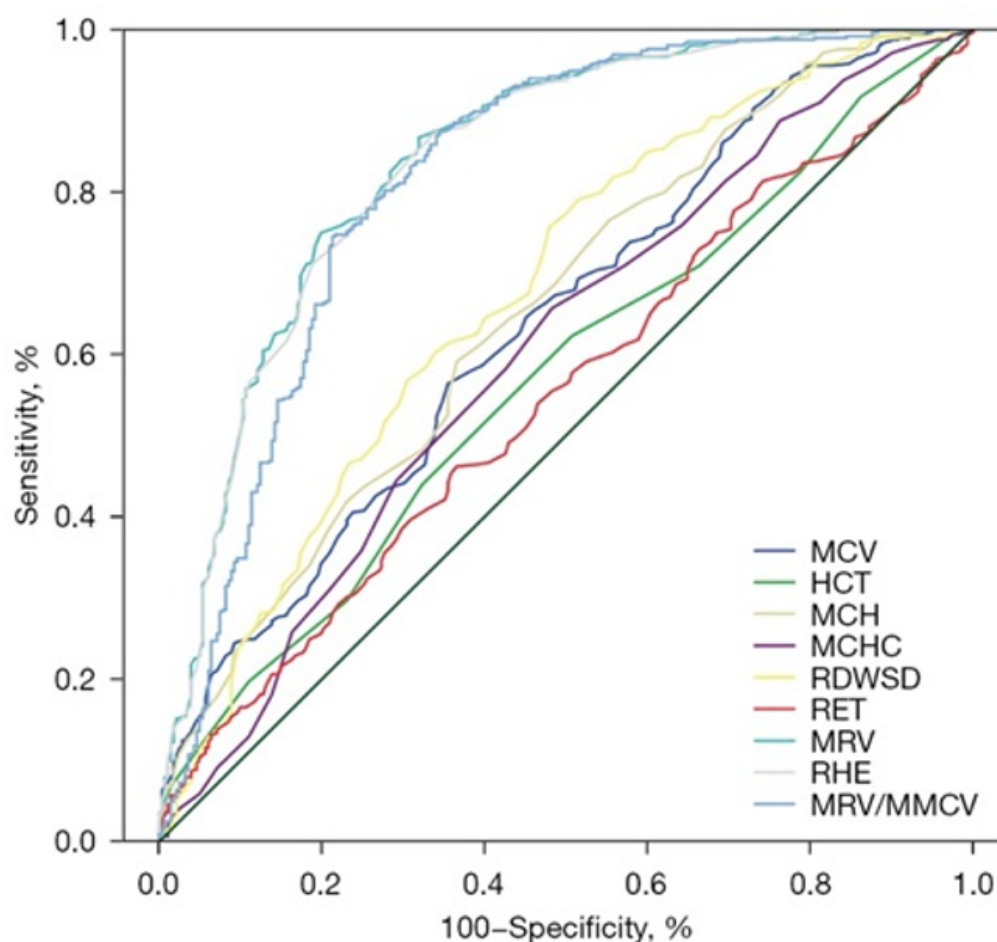


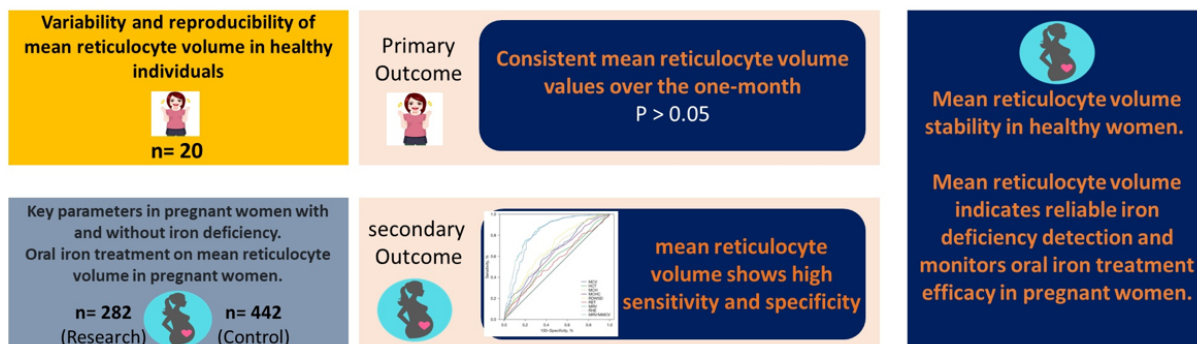
Figure 1. Receiver operating characteristic (ROC) curves of each indices in the diagnosis of iron deficiencies in pregnant women (area under the curve: MRV [0.840] > RHE [0.837] > MRV/MCV [0.816] > RDW-SD, [0.676] > MCV [0.654] > MCH [0.639] > MCHC, [0.602] > HCT [0.571] > RET [0.550]). AUC: area under the curve; HCT: hematocrit; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; MRV: mean reticulocyte volume; RDW-SD: red blood cell volume distribution width-standard deviation; RET: reticulocyte; RHE: reticulocyte hemoglobin equivalent; SF: serum ferritin.

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Retrospective cohort study

Evaluation of the effectiveness of mean reticulocyte volume in diagnosing iron deficiency in pregnant women



Graphical abstract.