Section: General Medicine

Original Research Article

DIAGNOSTIC PERFORMANCE OF RETICULOCYTE HEMOGLOBIN EQUIVALENT IN ASSESSING THE IRON STATUS

K. Senthil[1], K. Muralidharan[2], S.Saravana Madhav[3], A. Mimi Janet[4]

[1]Professor, Department of General Medicine, Madurai medical college, Tamilnadu, India
[2]Associate Professor, Department of General Medicine, Madurai medical college, Tamilnadu, India
[3]Assistant Professor, Department of General Medicine, Madurai medical college, Tamilnadu, India
[4]Resident, Department of General Medicine, Madurai medical college, Tamilnadu, India

Received : *01/10/2023*
Received in revised form : *09/11/2023*
Accepted : *23/11/2023*

Keywords:
Reticulocyte haemoglobin, Anaemia, Iron deficiency, MCV, MCH, MCHC.

Corresponding Author:
Dr. A. Mimi Janet,
Email: mimijanet19@gmail.com

DOI: 10.47009/jamp.2023.5.6.194

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (6); 942-946

Abstract

Background: Reticulocyte haemoglobin equivalent (RET-He) is a potential early detection and management marker, providing a snapshot of iron availability, therapy response, and iron-deficient erythropoiesis. The study aimed to investigate RET-He value in various states and to evaluate its diagnostic performance in iron status assessment. **Materials and Methods:** This cross-sectional observational study was conducted on 50 patients admitted under the Department of General Medicine for Anemia evaluation at Madurai Medical College- Government Rajaji Hospital, Madurai, for six months from January 2022 to June 2022. Laboratory investigations included liver function tests, renal function tests, complete blood count, urine examination, red cell indices, reticulocyte haemoglobin equivalent, serum ferritin, and transferrin saturation. **Result:** The mean age of participants is 45.6 years, with 63.6% females and 36.4% males. There is a significant difference in haemoglobin, MCV, MCH, MCHC, Reticulocyte haemoglobin equivalent, Sr. Iron, Sr. Ferritin, Total Saturation, and TIBC between groups, but no difference in RBC, Platelet count, HCT, and RDW. There is a significant positive correlation of moderate strength in Reticulocyte haemoglobin equivalent, Total Saturation, Sr. Iron, RBC, MCV, and MCH between reticulocyte count, but no correlation in Sr. Ferritin. A significant positive correlation of strong strength in Hb, MCHC, and HCT between reticulocyte count. The Peripheral smear in Iron deficiency anaemia patients revealed a Microcytic hypochromic picture in 42.4%, Microcytic hypochromic with anisopoikilocytosis in 42.4%, and Dimorphic picture in 6.1%. **Conclusion:** Reticulocyte Hemoglobin equivalent (RET-He >30 pg) is a potential marker for diagnosing iron deficiency anaemia with excellent diagnostic sensitivity, but serum ferritin evaluation is necessary for coexisting conditions.

INTRODUCTION

Iron deficiency is one of the most prevalent forms of malnutrition. Globally, 50% of anaemia is attributable to iron deficiency and accounts for approximately nearly a million deaths annually worldwide.[1] Early detection is, thus, crucial for proper and timely management to prevent such consequences. Presently, there is still no single best test for diagnosing this condition. Conventional iron biomarkers are also affected by acute-phase responses and influenced by diurnal variation and dietary intake. Due to these diagnostic difficulties, an alternative marker is sought. Reticulocyte haemoglobin equivalent (RET-He) is one of the potential markers. Its measurement is not only rapid but also convenient and cost-effective.[1-3]

Reticulocyte haemoglobin content has been reported as a marker that provides a snapshot of iron availability for erythropoiesis in the bone marrow, an indicator of iron therapy response, and an early marker of iron-deficient erythropoiesis.[2,4] It enables an early detection and indication of ID owing to its short life span (1-2 days) in the circulation compared to mature erythrocytes.[2] RET-He is the first peripheral blood count marker to be abnormal in the presence of iron deficiency. It is also an early indicator of response to iron therapy, of which effect is detected about two days after initiation of the optimal treatment as opposed to ferritin, of which the first response occurs in 1-2 weeks RET-He>30 pg positively confirms the non-iron deficiency anaemia (IDA) state.[2-4] The study aimed to investigate RET-He value in various states and to evaluate its diagnostic performance in iron status assessment.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

MATERIALS AND METHODS

This cross-sectional observational study was conducted on 50 patients admitted under the Department of General Medicine for Anemia evaluation at Madurai Medical College- Government Rajaji Hospital, Madurai, for six months from January 2022 to June 2022. Ethical Committee approval and informed consent were obtained before the study started.

Inclusion Criteria

Age >18 years, anaemia, Hb <12 – females, <13 in males were included.

Exclusion Criteria

Patients on iron supplements, Hemoglobinopathies, and Malignancy were excluded.

Laboratory investigations included liver function tests, renal function tests, complete blood count, urine examination, red cell indices, reticulocyte haemoglobin equivalent, serum ferritin, and transferrin saturation.

Statistical Analysis

The data were entered in an MS Excel sheet and analysed using SPSS version 16. Continuous data with normal distribution was expressed as mean with standard deviation. Categorical data were expressed as frequency and percentage. Fisher's exact test was used to compare the frequency between the groups. An unpaired 't' test was used to compare the mean values between the two groups. Pearson's correlation test measured the variables' direction and degree of association. ROC was constructed to predict the cut-off value for various variables to predict iron deficiency anaemia status. P<0.05 was considered statistically significant.

RESULTS

The mean (SD) age is 45.6 years, and 63.6% are females and 36.4% are males. There is no significant difference in age, gender, and Peripheral smear between groups [Table 1].

There is a significant difference in haemoglobin, MCV, MCH, and MCHC between groups, but no difference in RBC, Platelet count, HCT, and RDW.

There is a significant difference between groups in Reticulocyte haemoglobin equivalent, Sr. Iron, Sr. Ferritin, Total Saturation, and TIBC [Table 2].

There is a significant positive correlation of moderate strength in Reticulocyte haemoglobin equivalent, Total Saturation, Sr. Iron, RBC, MCV, and MCH between reticulocyte count, but no correlation in Sr. Ferritin.

A significant negative correlation of moderate strength in TIBC and RDW between reticulocyte count. A significant positive correlation of strong strength in Hb, MCHC, and HCT between reticulocyte count [Table 3].

Correlation of reticulocyte-Hb equivalent (%) showed a significant positive correlation of moderate strength in Reticulocyte count, Sr. Iron, Sr. Ferritin, Hb, MCV, MCH, MCHC, and HCT. There is a significant positive correlation of weak strength in total saturation and no correlation in RBC. A significant negative correlation of moderate strength exists in TIBC and RDW [Table 4].

The total saturation percentage showed the highest area under the curve (AUC), with an AUC of 0.957 and a p-value of < 0.0001, indicating a strong association with IDA. The serum iron showed the second highest AUC, which had an AUC of 0.845 and a p-value of < 0.0001, also showing a significant relationship with IDA. The other parameters with significant AUCs and p values were reticulocyte haemoglobin equivalent percentage, TIBC, and reticulocyte count. Serum ferritin showed an insignificant AUC of 0.633 and a p-value of 0.127, suggesting that it was not a good predictor of IDA [Table 5].

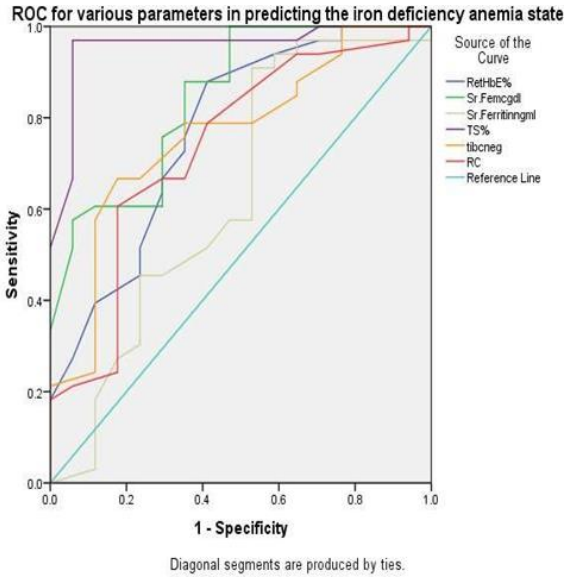

Figure 1: ROC for various parameters in predicting the iron deficiency anaemia state

Table 1: Comparison of age, gender and peripheral smear between groups

Parameter		No iron deficiency anemia (N=17)	Iron deficiency anemia (N=33)	P-value
Age in years		45.3 20.5	45.6 16.7	0.962 (NS)
Gender	Female	13 (76.5%)	21 (63.6%)	0.524 (NS)
	Male	4 (23.5%)	12 (36.4%)	
Peripheral smear	MCHC	5 (29.4%)	14 (42.4%)	0.175 (NS)
	MCHC-APC	5 (29.4%)	14 (42.4%)	
	Dimorphic	2 (11.8%)	2 (6.1%)	
	MCNC	0	1 (3%)	
	NCNC	5 (29.4%)	2 (6.1%)	

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Table 2: Comparison of various parameters for iron deficiency anaemia status

	Mean ± SD		P-value
	No iron deficiency anemia (N=17)	Iron deficiency anemia (N=33)	
Hb (g/dL)	8.8 ± 1.8	7.7 ± 1.7	0.03*
RBC (million/cc)	3.4 ± 0.6	3.3 ± 0.7	0.541 (NS)
Platelet count (L/cc)	3.2 ± 1.5	3.9 ± 1.1	0.057 (NS)
HCT (%)	28.2 ± 4.9	25.5 ± 5.1	0.999 (NS)
MCV (fL)	78.1 ± 11.2	71.2 ± 9.1	0.022*
MCH (pg)	25.9 ± 4.2	23.5 ± 4.1	0.049*
MCHC (%)	29.3 ± 3.2	26.6 ± 4.2	0.026*
RDW (%)	14.6 ± 2.1	15.5 ± 1.8	0.127 (NS)
Reticulocyte count (%)	1.8 ± 0.36	1.48 ± 0.36	<0.0001*
Reticulocyte haemoglobin equivalent (%)	28.5 ± 5.3	23.3 ± 4.4	0.001*
Sr. Iron (mcg/dL)	43.7 ± 13.8	27.1 ± 7.9	<0.0001*
Sr. Ferritin (ng/ml)	42 ± 27.2	27.9 ± 17.3	0.03*
Total Saturation %	21.3 ± 7.2	11.5 ± 3.3	<0.0001*
TIBC (mcg/dL)	393.9 ± 40.5	438.1 ± 38.3	<0.0001*

Table 3: Correlation of reticulocyte count for various parameters

Correlation of reticulocyte count (%) with	Pearson's 'r'	P value	Inference
Reticulocyte hemoglobin equivalent	0.511	<0.0001*	Significant positive correlation of moderate strength
TIBC (mcg/dL)	-0.448	0.001*	Significant negative correlation of moderate strength
Total saturation (%)	0.501	<0.0001*	Significant positive correlation of moderate strength
Sr. Iron (mcg/dL)	0.401	0.004*	Significant positive correlation of moderate strength
Sr. Ferritin (ng/ml)	0.207	0.149 (NS)	No correlation
RDW %	-0.541	<0.0001*	Significant negative correlation of moderate strength
Hb (g/dL)	0.748	<0.0001*	Significant positive correlation of strong strength
RBC (million/cc)	0.459	0.001*	Significant positive correlation of moderate strength
MCV (fL)	0.521	<0.0001*	Significant positive correlation of moderate strength
MCH (pg)	0.519	<0.0001*	Significant positive correlation of moderate strength
MCHC (%)	0.606	<0.0001*	Significant positive correlation of strong strength
HCT (%)	0.613	<0.0001*	Significant positive correlation of strong strength

Table 4: Correlation of reticulocyte – Hb equivalent for various parameters

Correlation of reticulocyte-Hb equivalent (%) with	Pearson's 'r'	P value	Inference
Reticulocyte Count (%)	0.511	<0.0001*	Significant positive correlation of moderate strength
TIBC (mcg/dL)	-0.404	0.004*	Significant negative correlation of moderate strength
Total saturation (%)	0.368	0.009*	Significant positive correlation of weak strength
Sr. Iron (mcg/dL)	0.513	<0.0001*	Significant positive correlation of moderate strength
Sr. Ferritin (ng/ml)	0.41	0.003*	Significant positive correlation of moderate strength
RDW %	-0.471	0.001*	Significant negative correlation of moderate strength
Hb (g/dL)	0.576	<0.0001*	Significant positive correlation of moderate strength
RBC (million/cc)	0.211	0.141 (NS)	No correlation
MCV (fL)	0.667	<0.0001*	Significant positive correlation of moderate strength
MCH (pg)	0.587	<0.0001*	Significant positive correlation of moderate strength
MCHC (%)	0.557	<0.0001*	Significant positive correlation of moderate strength
HCT (%)	0.503	<0.0001*	Significant positive correlation of moderate strength

Table 5: ROC curve characteristics of various parameters for predicting iron deficiency anaemia

Parameter	Area under curve	P value
Reticulocyte-Hb equivalent (%)	0.771	0.002*
Sr. Iron (mcg/dL)	0.845	<0.0001*
Sr. Ferritin (ng/ml)	0.633	0.127 (NS)
Total Saturation %	0.957	<0.0001*
TIBC (mcg/dL)	0.764	0.002*
Reticulocyte Count (%)	0.737	0.006*

Table 6: Validity indexes of various measurements at their cut-off values for predicting iron deficiency anaemia status

Measurement	Critical limit	Sensitivity	Specificity
Reticulocyte-Hb equivalent (%)	28.5	88%	60%
Sr. Iron (mcg/dL)	50	100%	42%
Sr. Ferritin (ng/ml)	25	51%	60%
Total Saturation %	15	97%	83%
TIBC (mcg/dL)	425	57%	89%
Reticulocyte Count (%)	1.75	67%	65%

ISSN (O): 2687-5365; ISSN (P): 2753-6556

The Peripheral smear in Iron deficiency anaemia patients in our study revealed a Microcytic hypochromic picture in 42.4%, Microcytic hypochromic with anisopoikilocytosis in 42.4%, and Dimorphic picture in 6.1%. The mean reticulocyte count was 1.6% with a p-value <0.0001, which is statistically significant in our study. A statistically significant association exists between Reticulocyte Hemoglobin equivalent and Iron deficiency anaemia. Hence, according to the study results, the Reticulocyte Hemoglobin equivalent has 88% sensitivity in diagnosing Iron deficiency anaemia.

DISCUSSION

Iron deficiency is a worldwide health issue that significantly challenges developing nations. Simple iron supplementation may address anaemia and reduced iron storage, but the diagnostic indicators may take weeks or months to change. Better indicators that detect the effects of iron therapy in a shorter amount of time are required due to the large global prevalence of iron deficiency and IDA. Reticulocyte has a short lifespan before it turn out to be mature. Instead of waiting for the treatment course to end, which might take up to three months, using this brief window to assess the impact of iron therapy would make it easier to decide whether to study the treatment option.[1-4]

Thus, in this study, we have determined RET-He value in various states and evaluated its diagnostic performance in iron status assessment. Our study shows that the mean age is 45.6 years, with 63.6% females and 36.4% males. We have observed a significant difference between groups in haemoglobin, MCV, MCH, MCHC, Reticulocyte haemoglobin equivalent, Sr. Iron, Sr. Ferritin, Total Saturation, and TIBC. There is a significant positive correlation of moderate strength in Reticulocyte haemoglobin equivalent, Total Saturation, Sr. Iron, RBC, MCV, and MCH between reticulocyte count, but no correlation in Sr. Ferritin. A significant negative correlation between moderate strength in TIBC and RDW in reticulocyte count, whereas a significant positive correlation of strong strength in Hb, MCHC, and HCT between reticulocyte count.

Numerous investigations have been conducted to explore the clinical use of RET-He. The findings reported that RET-He is a useful diagnostic tool for iron-restricted erythropoiesis,[4] ID,[5-8] and IDA. [9,10] In several articles, Reticulocyte Hb measurement is clinically valuable in diagnosing iron-deficient conditions. Previous studies used CHr in adults,[11] pediatric/adolescent iron deficiency,[7,13,14] and renal failure anaemia.[14,15] Whereas some studies have used the RET-He parameter, which has revealed a rational correlation with CHr.[16] Moreover, our study reported that the Peripheral smear in iron deficiency anaemia patients revealed a Microcytic hypochromic picture in 42.4%, a Microcytic hypochromic with anisopoikilocytosis in 42.4%, and a Dimorphic picture in 6.1%. Our study's mean reticulocyte count was statistically significant (1.6% with a p-value <0.0001).

Further, correlation studies and ROC analysis were used to examine the efficacy of Ret-He as a marker for iron deficiency and IDA compared to other iron markers and serum ferritin, the gold standard non-invasive marker. A statistically significant association exists between Reticulocyte Hemoglobin equivalent and IDA. Hence, according to the study results, the Reticulocyte Hemoglobin equivalent has 88% sensitivity in diagnosing iron deficiency anaemia.

Almashjary et al. showed a significantly positive correlation of Ret-He with ferritin and TS and a significant negative correlation with TIBC.[17] Also, other studies conducted by Toki et al. and Uçar et al. demonstrated the same findings.[6,18] Further, Almashjary et al. suggested that as a diagnostic marker, Ret-He indorses advanced sensitivity and specificity with a cut-off of <21.55 and <28.25 pg for iron deficiency anaemia and iron deficiency, respectively. This further suggested that Ret-He is a highly sensitive marker that may quickly reflect iron incorporation and is valid for identifying and diagnosing various stages of iron shortage.[17]

The findings of Almashjary et al,[17] were greater than those of Toki et al. and Uçar et al. With a sensitivity of 49.1% and a specificity of <90%, Uçar et al. identified iron insufficiency using a Ret-He cut-off value of <25.4 pg.[6,18] Toki et al. diagnosed ID with a sensitivity of 68% and a specificity of >90% using a Ret-He cut-off value of 28.5 pg. The specificity to identify ID fell back to 81%, but the sensitivity rose to 92% at a higher cut-off (30.9 pg).[6]

The collective findings of this study should encourage the medical community to use Ret-He as a possible marker for high-validity detection and diagnosis of various stages of iron deficiency, particularly in endemic areas of IDA. Furthermore, it supports Ret-He as a very sensitive and useful marker that can quickly reflect the bone marrow response to iron treatment. The use of this marker in several erythropoietic diseases, including hemoglobinopathies, anaemia of chronic inflammation, anaemia of infection, and sideroblastic anaemia, will require further research to determine reference ranges and appropriate cut-offs. Most contemporary haematology analysers use Ret-He, a straightforward and integrated marker that doesn't need an extra tube. It is necessary to address the drawbacks of RET-He, not the possible benefits. While prior research suggests that RET-He may be able to predict early response to iron therapy, the restoration of iron stores is the ultimate goal of treatment monitoring or follow-up, for which ferritin measurement is essential.

CONCLUSION

Reticulocyte haemoglobin equivalent is a quick, practical, and affordable measurement method. Our

ISSN (O): 2687-5365; ISSN (P): 2753-6556

findings further support the idea that RET-He >30 pg is a possible marker with great diagnostic sensitivity for ruling out iron deficiency anaemia. Serum ferritin testing is still required for the diagnosis and other factors when iron deficiency anaemia coexists with other non-ID illnesses, as is frequently the case in actual clinical practice.

Limitations

This study has some limitations. Although serum ferritin is an acute-phase protein, its levels rise in the presence of chronic inflammation, and serum ferritin levels are still frequently used to assess iron status. Thus, when assessing RET-He, inflammation should be taken into account. Our main shortcoming is that inflammation has not been taken into account. Second, we cannot extrapolate the results to the entire Indian population due to the small sample size. Since the sample size is small, we haven't determined the cut-offs for RET-He.

REFERENCES

1. Stoltzfus RJ. Iron deficiency: Global prevalence and consequences. Food Nutr Bull 2003;24:S99–103. https://doi.org/10.1177/15648265030244s206.
2. Chinudomwong P, Binyasing A, Trongsakul R, Paisooksantivatana K. Diagnostic performance of reticulocyte hemoglobin equivalent in assessing the iron status. J Clin Lab Anal. 2020;34:e23225. https://doi.org/10.1002/jcla.23225.
3. Auerbach M, Staffa SJ, Brugnara C. Using reticulocyte hemoglobin equivalent as a marker for iron deficiency and responsiveness to iron therapy. Mayo Clin Proc 2021;96:1510–9. https://doi.org/10.1016/j.mayocp.2020.10.042.
4. Franck S, Linssen J, Messinger M, Thomas L. Potential utility of ret-Y in the diagnosis of iron-restricted erythropoiesis. Clin Chem 2004;50:1240–2. https://doi.org/10.1373/clinchem.2004.030254.
5. Poffenroth C, Mabbett C, Kendrick C. The reticulocyte haemoglobin equivalent (RET-He) and laboratory screening for iron deficiency. New Zealand J. Medical Lab. Sci. 2017;71:120. https://doi.org/10.3316/informit.210936625649545.
6. Toki Y, Ikuta K, Kawahara Y, Niizeki N, Kon M, Enomoto M, et al. Reticulocyte hemoglobin equivalent as a potential marker for diagnosis of iron deficiency. Int J Hematol 2017;106:116–25. https://doi.org/10.1007/s12185-017-2212-6.
7. Brugnara C, Zurakowski D, DiCanzio J, Boyd T, Platt O. Reticulocyte hemoglobin content to diagnose iron deficiency in children. JAMA. 1999;281(23):2225-2230. https://doi.org/10.1001/jama.281.23.2225.
8. Jarc E, Preložnik Zupan I, Buturović Ponikvar J, Snoj N, Podgornik H. Primerjava eritrocitnih in retikulocitnih parametrov pri ugotavljanju pomanjkanja železa. Slov Med J 2017;86:19–27. https://doi.org/10.6016/zdravvestn.2403.
9. Wollmann M, Gerzson BMC, Schwert V, Figuera RW, de Oliveira Ritzel G. Reticulocyte maturity indices in iron deficiency anemia. Rev Bras Hematol Hemoter 2014;36:25–8. https://doi.org/10.5581/1516-8484.20140009.
10. Sanyoto A, Suega K, Adnyana L, Bakta IM. Diagnostic test equivalent hemoglobin reticulocyte in iron deficiency anemia. Indones Biomed J 2017;9:143. https://doi.org/10.18585/inabj.v9i3.267.
11. Mast AE, Blinder MA, Lu Q, Flax S, Dietzen DJ. Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. Blood 2002;99:1489–91. https://doi.org/10.1182/blood.v99.4.1489.
12. Ullrich C, Wu A, Armsby C, Rieber S, Wingerter S, Brugnara C et al. Screening healthy infants for iron deficiency using reticulocyte hemoglobin content. JAMA. 2005;294:924-930. https://doi.org/10.1001/jama.294.8.924.
13. Stoffman N, Brugnara C, Woods ER. An algorithm using reticulocyte hemoglobin content (CHr) measurement in screening adolescents for iron deficiency. J Adolesc Health 2005;36:529. https://doi.org/10.1016/j.jadohealth.2004.09.011.
14. Fishbane S, Galgano C, Langley RC Jr, Canfield W, Maesaka JK. Reticulocyte hemoglobin content in the evaluation of iron status of hemodialysis patients. Kidney Int 1997;52:217–22. https://doi.org/10.1038/ki.1997.323.
15. Tessitore N, Solero GP, Lippi G, Bassi A, Faccini GB, Bedogna V, et al. The role of iron status markers in predicting response to intravenous iron in haemodialysis patients on maintenance erythropoietin. Nephrol Dial Transplant 2001;16:1416–23. https://doi.org/10.1093/ndt/16.7.1416.
16. Brugnara C, Schiller B, Moran J. Reticulocyte hemoglobin equivalent (Ret He) and assessment of iron-deficient states. Clin Lab Haematol 2006;28:303–8. https://doi.org/10.1111/j.1365-2257.2006.00812.x.
17. Almashjary MN, Barefah AS, Bahashwan S, Ashankyty I, ElFayoumi R, Alzahrani M, et al. Reticulocyte hemoglobin-equivalent potentially detects, diagnoses and discriminates between stages of iron deficiency with high sensitivity and specificity. J Clin Med 2022;11:5675. https://doi.org/10.3390/jcm11195675.
18. Uçar MA, Falay M, Dağdas S, Ceran F, Urlu SM, Özet G. The importance of RET-He in the diagnosis of iron deficiency and iron deficiency anemia and the evaluation of response to oral iron therapy. J Med Biochem 2019;38:496–502. https://doi.org/10.2478/jomb-2018-0052.

ISSN (O): 2687-5365; ISSN (P): 2753-6556