DIAGNOSTIC ACCURACY OF RETICULOCYTE HAEMOGLOBIN EQUIVALENT (RET-HE) IN DETECTING IRON DEFICIENCY ANAEMIA KEEPING SERUM FERRITIN AS GOLD STANDARD

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ABSTRACT

Objective: To determine the diagnostic accuracy of Ret-He in detecting IDA, keep ingserum ferritin as gold standard

Study Design: Cross sectional study.

Place and Duration of Study: Department of Haematology, AFIP Rawalpindi, from Jan to Jul 2017.

Methodology: Study involved 165 female patients who presented with anemia (hemoglobin <11g/dl). Ret-He was determined having a level of <27.8 pg/L as diagnostic of IDA. Diagnosis of IDA was confirmed on serum ferritin level <11 ng/ml taking it as gold standard. Accordingly, diagnostic accuracy of Ret-He was determined considering low serum ferritin level as gold standard.

Results: The mean age of the patients was 27.52 ± 13.57 years. Mean hemoglobin level was 9.17 ± 1.59 g/dl while mean serum ferritin level was 15.20 ± 12.89 ng/ml. Ret-He ranged from 11.0 pg/L to 39.4 pg/L with a mean of 23.34 ± 8.21 pg/L. IDA on the basis of serum ferritin level was diagnosed in 75 (45.5%) patients while Ret-He labeled IDA in 85 (51.5%) patients. When cross-tabulated, there were 70 TP, 15 FP, 5 FN and 75 TN cases which yielded 93.33% sensitivity, 83.33% specificity, 87.88% accuracy, 82.35% PPV and 93.75% NPV for Ret-He in diagnosing IDA keeping serum ferritin as gold standard. Thus the optimal cut-off value of Ret-He was found to be <27.6 pg/L which gave 93.33% sensitivity and 83.33% specificity in detecting IDA keeping serum ferritin as gold standard.

Conclusion: Ret-He was found as fast and reliable technique to diagnose IDA in female population. However, further studies are required for confirmation of its efficacy.

Keywords: Diagnostic Accuracy, Iron deficiency anemia, Reticulocyte Haemoglobin Equivalent (Ret-He), Serum ferritin.

INTRODUCTION

Iron Deficiency Anaemia (IDA) is one of the most common types of anaemia globally particularly in developing countries[1]. Iron deficiency anaemia leads to sever morbidity, reduce school performance, and has long-term effect on psychomotor and mental development[2]. Cut off values defined by WHO for children under 5 years of age and expecting women is 11g/dl while for non-pregnant women and men are 12 and 13g/dl respectively. Africa and South Asia arethemost susceptible regions showing highest prevalence (40%) in all age group except male[3]. IDA results from inadequate dietary intake and blood loss due to worms infestation in developing countries; while vegetarian diet, chronic blood loss or malabsorption are the common causes in developed countries[4]. Iron deficiency assessment can be performed by diagnostic tests for Haemoglobin concentration (Hb), Red cell distribution width (RDW), zinc protoporphyrin, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), serum transferrin receptor (Tfr), Serum ferritin as well as bone marrow biopsy[5].

However, these tests have their own limitations. MCV, MCH, RDW assesspresence of iron in mature red cell, therefore are slow and insensitive as early indicators. On the one hand, investigations for Zinc protoporphyrin and Tfr

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are expensive while on the other, bone marrow biopsy isitselfan invasive procedure[6]. With regards to Serum ferritin, it is an acute phase reactantand thus unreliable in presence of acute or chronic inflammation[7]. Therefore, a need arises for a diagnostic test, which is readily available, cost effective and accurate for diagnosing IDA and eliminate the need for iron studies. Reticulocyte haemoglobin content (Ret-He) can be one such sensitive early stage indicator for iron deficiency prior to anaemia, which is a direct measurement of iron content in reticulocytes[8]. Abundant nucleic acid remaining in immature erythrocytes are stained with fluorescent dye and its measurement is based on principle of flow cytometery. Ret-He cut-off point of 27.8 pg/L had sensitivity of 43.8%, specificity 85.3%, Positive Predictive Value (PPV) 58.3% and Negative Predictive Value (NPV) 76.3% with Odds ratio (OR) 4.5 (95%CI 1.1 to 17.7)[9].

The objective of study ws to assess iron deficiency, as part of automated complete blood picture since low level of Ret-He was linked tohigher risk of development of IDA. Ret-He has high specificity, which may be a suitable diagnostic tool for early detection of IDA.

METHODOLOGY

This cross-sectional study was conducted in the department of Haematology, AFIP Rawalpindi from January to July 2017. The study involved 165 females who presented with anemia (hemoglobin <11g/dl). Ret-He was determined having a level of <27.8 pg/L as diagnostic of IDA[9]. Diagnosis of IDA was confirmed on serum ferritin level <11 ng/ml taking it as gold standard. Accordingly, diagnostic accuracy of Ret-He was determined considering low serum ferritin level as gold standard[9].

Sample was calculated by non-probability consecutive sampling technique with the help of World Health Organization sample size calculator. While keeping Confidence level at 95%, absolute precision at 10%, sensitivity 43.8%, specificity 85.3% and prevalence 40%, sample size was calculated as 165[9].

The study was conducted after approval by Ethical Committee. The patients selected for study were from those subjects, referred for screening of anemia to the Department of Hematology, AFIP. All subjects fulfilling the inclusion criteria were explained in detail about the study. Informed consent was taken. Patient'particulars were endorsed on a Performa by trainee researcher under supervision.

Three ml blood was taken from each patient fulfilling inclusion criteria. 2ml blood was poured in EDTA tube for measuring Hb, Hct and reticulocytes haemoglobin equivalent using Sysmex XE 5000. Remaining blood was poured in gel tube for serum ferritin estimation by competitive immunoassay method using machine Immulite 2000. Subsequently, demographic and diagnostic data of all the patients including age, gender and Hb values were recorded and entered onapre-prepared Performa.

All the relevant data was analyzed through SPSS version 17.0. Numerical variables were presented by mean ± SD. Categorical variables; was presented by frequency and percentage. A 2x2 contingency table was created to calculate sensitivity, specificity and diagnostic accuracy of Ret-He in identifying iron deficiency anaemia keeping serum ferritin as gold standard. Post stratification diagnostic accuracy was re-calculated. ROC and likelihood ratio was also been calculated.

RESULTS

The patients' age ranged between 2 to 60 years with a mean of 27.52 ± 13.57 years. Majority 91 (55.2%) of the patients were aged between 19-40 years. Hemoglobin level varied from 5.0 g/dl to 10.9 g/dl with a mean of 9.17 ± 1.59 g/dl. Level of Serum ferritin ranged from 1.7 ng/ml to 50.0 ng/ml with a mean of 15.20 ± 12.89 ng/ml while that of Ret-He values fluctuated between 11.0 pg/L to 39.4 pg/L with a mean of 23.34 ± 8.21 pg/L (table-I).

IDA was diagnosed in 75 (45.5%) patients on the basis of low serum ferritin level while Ret-He labeled 85 (51.5%) patients for IDA (table-II).

Cross-tabulation of IDA on Ret-He with that of serum ferritin, there were 70 true positive, 15 false positive, 5 false negative and 75 true negative cases yielding 93.33% sensitivity, 83.33% specificity, 87.88% accuracy, 82.35% positive predictive value and 93.75% negative predictive value for Ret-He in detecting iron deficiency anaemia keeping serum ferritin as gold standard (table-III).

Table-I: Baseline characteristics of study sample (n=165).

Characteristics	Mean Values
Age (years)	27.52 ± 13.57
≤18 years	42 (25.5%)
19-40 years	91 (55.2%)
41-60 years	32 (19.3%)
Hemoglobin (g/dl)	9.17 ± 1.59
Serum Ferritin (ng/ml)	15.20 ± 12.89
Ret-He (pg/L)	23.34 ± 8.21

Table-II: Frequency of iron deficiency anemia (n=165).

Criteria	Iron Deficiency Anemia	Frequency	Percentage (%)
Serum Ferritin (<11 ng/ml)	Positive	75	45.5
	Negative	90	54.5
	Total	165	100
Ret-He (<27.8 pg/L)	Positive	85	51.5
	Negative	80	48.5

Table-III: Diagnostic performance of Ret-He in iron deficiency anemia (n=165).

Ret-He	Serum Ferritin		Total
	<11 ng/ml (IDA)	>11 g/ml	
<27.8 pg/L (IDA)	70*	15***	85
>27.8 pg/L	5**	75****	80
Total	75	90	165

*True Positive = 70, ***False Positive = 15, **False Negative = 5, ****True Negative = 75

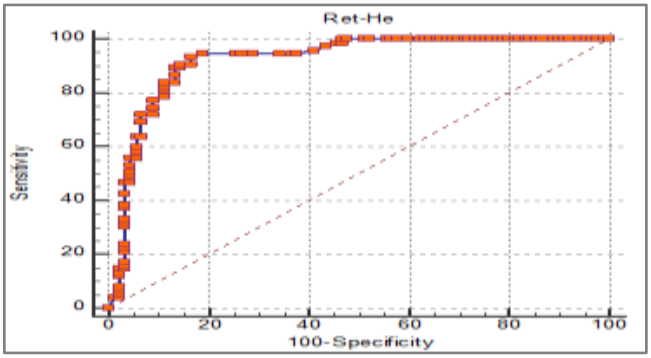
Table-IV: Diagnostic statistics of the study.

Statistic	Value
Sensitivity	93.33%
Specificity	83.33%
Accuracy	87.88%
Positive Likelihood Ratio	5.60
Negative Likelihood Ratio	0.08
Disease prevalence	45.45%
Positive Predictive Value	82.35%
Negative Predictive Value	93.75%

When an ROC curve was plotted, Ret-He (<27.8 pg/L) had an area under curve of 0.922 (Significance level P<0.0001 and 95% Confidence interval = 0.870 to 0.958) (fig-1). The optimal cut-off value of Ret-He was found to be <27.6 pg/L which gave 93.33% sensitivity and 83.33% specificity in detecting IDA, keeping serum ferritin as gold standard (fig-2).

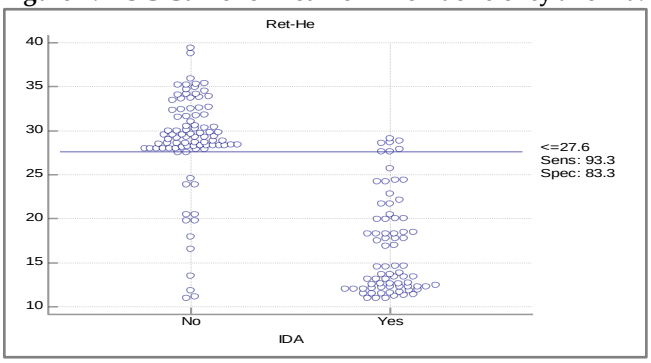
DISCUSSION

Iron Deficiency Anaemia (IDA) is one of the most common type of dietary anemia world over especially in developing countries[1]. Recent published literature has concluded reticulocyte haemoglobin equivalent (Ret-He) to be a good predictor of iron deficiency anemia.



Area under the ROC curve (AUC) = 0.922, 95% Confidence interval = 0.870 to 0.958, Significance level *p* (Area=0.5) <0.0001

Figure-1: ROC Curve for Ret-He in iron deficiency anemia.



Youden index J = 0.7667, Associated criterion ≤27.6, Sensitivity = 93.33%, Specificity = 83.33%

Figure-2: Plot diagram for Ret-He and optimal diagnostic cut-off.

In this study, the mean age of the patients was 27.52 ± 13.57 years. A similar mean age of 26.07 ± 5.04 years was reported by Anjum *et al* among pregnant females presenting with anemia at Allied Hospital, Faisalabad[10]. Taseer *et al* (2011)

also observed similar mean age of 28.28 ± 5.20 years among anemic females in Karachi[11]. A similar mean age of 27.01 ± 5.97 years was also reported by Alene *et al* among Ethiopian females with anemia[12].

In this study, the mean hemoglobin level was 9.17 ± 1.59 g/dl while the mean Ret-He was 23.34 ± 8.21 pg/L. Torino *et al* reported similar mean hemoglobin of 10.1 ± 1.65 g/dl among Brazilian patients with anemia[13] while Dalimunthe *et al* observed it to be 8.60 ± 1.07 g/dl among anemic patients in Indonesia[14]. Our findings are also in line with that of Mehta *et al* who reported mean Ret-He level of 19.19 ± 4.02 pg/L in Indian anemic patients[15] while Rungngu *et al* observed it to be 25.84 ± 4.87 pg/L in Indonesian children with IDA[16].

We also observed that 45.5% of anemic patients are suffering from Iron deficiency anemia. In this study our results are near to similar to that of Ansari *et al* who reported 44.5% of anemic females presenting at Jinnah Postgraduate Medical Centre Karachi, had iron deficiency anemia[17]. Naz *et al* observed comparatively higher frequency of 54.3% for iron deficiency anemia in anemic expecting females presenting at the same hospital[18]. Rungngu *et al* described similar data of 40.0% for iron deficiency anemia in Indonesia[16].

Taking a cut-off value of 27.8 pg/L, we observed 93.33% sensitivity, 83.33% specificity and 87.88% accuracy for Ret-He in identifying iron deficiency anaemia, keeping serum ferritin as gold standard with an area under curve of 0.922 (95% Confidence interval = 0.870 to 0.958, Significance level $p<0.0001$). The optimal cut-off value of Ret-He was found to be <27.6 pg/L which gave 93.33% sensitivity and 83.33% specificity in detecting iron deficiency anaemia. Our results are similar to those of Brugnara *et al* who reported the sensitivity and specificity of Ret-He (≤27.2 pg/L) around 93.3% and 83.2% respectively[19]. Deng *et al* using a same cut-off value of 27.8 pg/L reported the sensitivity and specificity at 88.0% and 90.0% respectively[20]. Mehta *et al* observed 22.4 pg/L as the best cut off value of Ret-He for predicting iron deficiency anemia with sensitivity and specificity around 98.88% and 84.21% respectively[15]. Mateos *et al* also reported Ret-He to have a sensitivity of 90.7% and specificity of 80.1% for IDA among children[21]. Another study reported similar diagnostic results for Ret-He with cut off value at 26 pg/L, giving 83% sensitivity and 75% specificity for IDA[22]. Fishbane *et al* reported a sensitivity level of 100% and specificity at 80% for Ret-He (26pg/L) to diagnose iron deficiency[23]. Lorenz *et al* concluded an optimal cut off value of 29 pg/L for Ret-He (yielding 85% sensitivity and 73% specificity) for IDA with AUC-ROC of 0.92[24].

The present study, has found that Ret-He was 93.33% sensitive and 83.33% specific in detecting iron deficiency anaemia with an area under curve of 0.922. In the light of this evidence, it can be supported that Ret-He should be a preferred choice for diagnostic workup of patients suspected of anemia, in future practice.

The scope of this study was restricted to female patients with focus on those in child bearing age (19-40 years). It was also an established fact that majority of such Pakistani females suffer from iron deficiency anemia[10'11'17'18] which may be an aggravating factor in itself. Limitation of our study was that Bone marrow biopsy was not done in these cases to prove them false positive or negative. There was a need to have further studies in other age groups involving both male and female genders with stratification to establish the diagnostic perfor-mance of Ret-He with more confidence. Such a study is highly recommended in future research.

CONCLUSION

Ret-He was found as fast and reliable technique to diagnose IDA in female population. However, further studies are required for confirmation of its efficacy and usefulness.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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