ORIGINAL ARTICLE

Assessment of Reticulocyte-haemoglobin Equivalent (Ret-He) as a Diagnostic Indicator for Iron Deficiency Anaemia

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ABSTRACT

Introduction: Iron deficiency anaemia (IDA) is the most common cause of anaemia. The diagnosis of IDA, however, remains a challenge and is a problem worldwide. Serum iron study is commonly used for IDA diagnosis but there are some limitations. This study was conducted to evaluate reticulocyte-haemoglobin equivalent (Ret-He) as a screening tool for IDA diagnosis in adults. **Method:** This is a comparative case control study conducted in Hospital Tengku Ampuan Afzan, Kuantan consisting of adult patients with iron deficiency anaemia and a healthy control group. Haematological parameters (Hb, RBC count, MCV, MCH, RDW) inclusive of Ret-He and serum iron parameters (serum iron, transferrin saturation and serum ferritin) were measured. Correlation between Ret-He with other haematological and serum iron parameters were analysed. **Results:** There were 103 IDA adult patients with majority of them being female (85.4%) with median age of 36 years old. Malay ethnicity (79.6%) contributed to the larger proportion of adult IDA patients. The Ret-He value for patient and control groups were 16.50 ± 4.90 pg and 34.80 ± 1.97 pg, respectively. Ret-He was 89.32% sensitive and 100% specific with 100% positive predictive value (PPV) and 73.11% negative predictive value (NPV) when compared to transferrin saturation. There was significant correlation between Hb, MCH, MCV, RDW and serum iron, transferrin saturation and serum ferritin parameters with Ret-He. **Conclusion:** Ret-He together with a complete blood count, may serve as an alternative to the serum iron parameters for screening of IDA in adults.

Keywords: Iron deficiency anaemia (IDA), Reticulocyte haemoglobin equivalent (Ret-He), Screening, Adults, Diagnostic indicator

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INTRODUCTION

Iron deficiency anaemia (IDA) is a state when iron is inadequate to maintain normal physiological function and tissue demands (1) due to iron loss or inadequate iron absorption (2). IDA is the most common cause of anaemia globally, affecting all populations mainly women and children (1). In Malaysia, 85% of anemia is caused by IDA (3). IDA requires specific investigations and management, therefore it is important to differentiate IDA with other causes of anaemia (2).

WHO (2014) defines anaemia as a state when the capacity of blood to transport oxygen in the body is impaired as a consequence of reduced haemoglobin (Hb) level from normal value (4).The mean Hb level for men and women are 13.0 g/dL and 12.0 g/dL, respectively, while for both pregnant women and preschool children are 11.0 g/dL (5). IDA is classically described as a hypochromic microcytic anaemia. Differential diagnoses include thalassaemia, sideroblastic anaemias, certain anaemia of chronic disease, and lead poisoning (6). IDA is a sequence of events which starts with depleted iron stores, followed by iron-deficient erythropoiesis, iron deficiency anaemia and finally tissue effects of iron deficiency (7,8). It is important to diagnose and manage IDA appropriately as well as to prevent it, as the consequences of IDA may cause major impacts on maternal mortality and birth outcomes, child development and intelligence (9) as well as physical and work performance (5).

Gold standard for IDA diagnosis is bone marrow Perls' stain by assessing presence of stainable iron (7). However, in routine practice, full blood count (FBC) parameters such as haemoglobin (Hb), red blood cell (RBC), mean corpuscular volume (MCV),

mean corpuscular haemoglobin (MCH) and red cell distribution width (RDW) as well as iron profile which includes serum iron, total iron binding capacity (TIBC) / unsaturated iron binding capacity (UIBC), transferrin saturation (Tsat) and serum ferritin are used for screening and diagnosis of IDA. Even though these tests are simple and fast, these tests must be interpreted together and there are many underlying condition that may interfere with the true value (7). Therefore, there have been many studies performed to evaluate, assess and demonstrate other parameters that may be considered to replace these tests.

Ret-He has been studied and evaluated for a few years as a potential screening tool for IDA diagnosis. It is an extended FBC parameter obtainable using automated haematology analyser and is available in many laboratories (10-12). Ret-He measures the iron content available in red cells recently produced by the bone marrow (11) . Ret-He was chosen as it is a more stable assay compared to reticulocyte volume and Hb concentration and is not influenced by infection or inflammation (13-14). Ret-He provides the exact measure of functional iron available for erythropoiesis and can be used to monitor iron treatment and need for iron therapy in renal failure patients (13,15). Ret-He measurement is a reliable parameter when compared to gold standard (1). Currently, there are no consensus in using Ret-He for screening and diagnosis of IDA in our local laboratories. Therefore, this study was conducted to evaluate Ret-He among adults' blood samples of IDA for potential use as a screening tool as well as for rapid diagnosis of IDA.

MATERIALS AND METHODS

Ethical approval

The study was conducted at Department of Pathology, Hospital Tengku Ampuan Afzan, Kuantan. Ethical clearance to conduct the study was obtained from Medical Research and Ethics Committee, Ministry of Health Malaysia [NMRR-16-2486-33677 (IIR)] and Universiti Putra Malaysia's Research Ethics Committee [UPM/TNCPI/RMC/JKEUPM/1.4.18.2 (JKEUPM)].

Study design and sampling

This was a comparative cross-sectional study of two groups; adult IDA patients versus healthy control individuals in Hospital Tengku Ampuan Afzan (HTAA), Kuantan. The inclusion criteria of the patients' group were Malaysian citizen, aged between 15 to 60 years old and the results of both FBC and iron study must be available concurrently or not more than three days apart. For the control group, inclusion criteria include healthy Malaysian citizen, aged between 15 to 60 years old and the results of both FBC and iron study must be available concurrently or not more than three days apart.

Laboratory Investigations

Patients' Group: All information regarding patient's age, gender, ethnicity and other related investigation results such as iron study (serum iron, TIBC and serum ferritin) and FBC (Hb, MCV, MCH, RDW and Ret-He) results were obtained from the Record Unit, Haematology Unit, Pathology Department, Hospital Tengku Ampuan Afzan, Kuantan.

Control Group: Healthy individuals were randomly selected among the medical students or hospital staff in HTAA, Kuantan, Pahang to get the median and reference range for normal value of Ret-He. All respondents were screened for anaemia at a cut off level of 12 g/dL for women and 13 g/dL for men. Only respondents with normal Hb, MCV and MCH were eligible for further iron study (serum iron, TIBC / UIBC, Tsat and serum ferritin). Respondents with normal serum iron, Tsat and serum ferritin as well as normal level of Hb, MCV and MCH were included to get the median value for Ret-He.

About 6 mL of blood was taken and collected into one EDTA tube for FBC including Ret-He and two lithium heparin tubes for biochemical iron markers (serum iron, TIBC / UIBC and serum ferritin), 2 mL in each tube.

Sysmex XN-3000 series (Sysmex,Japan) automated hematology analyser was used in this study for FBC and Ret-He measurement. Haemoglobin concentration was measured using sodium lauryl sulphate (SLS)-haemoglobin method. RBC, HCT and RDW were measured by RBC channel using sheath flow direct current detection method. Both MCV and MCH were calculated manually by analyser. Reticulocyte parameter was measured via RET channel by principle of flow cytometry method using semiconductor laser. The laser counted and classified the cells by irradiating cells at 633nm laser beam.

For biochemical iron markers i.e. serum iron and TIBC/ UIBC were analysed using ARCHITECT c4000 (Abbott Core Laboratory, USA) and these parameters were measured using a direct colorimetric method. As for serum iron, it was released from transferrin at pH4.8 and reduced to ferrous state which then form a stable colored complex with Ferene-S*. For UIBC, sample was added to an alkaline buffer containing known amount of iron to saturate available binding sites on transferrin. Free unbound iron was reduced to ferrous state and complexed with Ferene- S*. UIBC was determined by subtracting the quantity of unbound iron from the total added quantity and TIBC was the sum of serum iron and UIBC. A two-site sandwich immunoassay using direct chemiluminometric technology on ADVIA Centaur CP (Siemens,USA) was used for measurement of serum ferritin.

HTAA Pathology laboratory is accredited under Laboratory Accreditation Scheme MS ISO 15189. Standard operating procedures were applied during measurement of blood samples mentioned.

Statistical analysis

SPSS Statistic Version 22 was used for statistical analysis. Descriptive analysis was used to determine demographic characteristics (age, gender and ethnicity) among adults' retrospective data of IDA in HTAA, Kuantan. The median value of Ret-He for both patients' and control groups were determined by analytical statistical test (Wilcoxon Signed Rank test). Subsequently, Mann Whitney test was used to compare the median value of Ret-He between patients' group and control group among adults' blood samples. Correlations of Ret-He value with other haematological and biochemical parameters were determined by Spearman correlation test. Sensitivity and specificity with positive predictive value (PPV) and negative predictive value (NPV) were calculated by comparing Ret-He to Tsat.

RESULTS

There were 347 cases of iron deficiency anaemia (IDA) available, however only 103 data were included in this study as per the inclusion criteria. For control group, 30 out of 60 respondents consented and met the inclusion criteria.

From total of 103 IDA patients, 88 (85.4%) were female which contributed to most IDA cases in HTAA, Kuantan within this study period and only 15 (14.6%) patients were male (Table I). With regards to ethnicity, 82 (79.6%) patients were Malay, followed by 14 (13.6%) patients from Indian ethnicity, 5 (4.9%) patients from Chinese ethnicity and 2 (1.9%) patients from others ethnicity (Table I). The median age for IDA was 36-year-old with interquartile range (IQR) of 22.

Haematological and biochemical parameters for patients and control group are shown in Table I. The findings were reported based on normality distribution of each parameter concerned. In this study, the median for Ret-He was 16.50 pg with IQR of 4.90 and 34.80 pg with IQR of 1.97 for IDA group and control group respectively (Table I).

The findings from this study suggested that the Ret-He range for IDA (patient group) was 11.60 to 21.40 pg (16.50 ± 4.90 pg) and for control group was between 32.83 to 36.77 pg (34.80 ± 1.97 pg). This study highlighted that, Ret-He value between these two groups has clearly separated the patients and control groups as shown in Fig.1.

The correlation between Ret-He and other hematological and biochemical parameters of iron status were analysed by Spearman correlation test. The results are presented in Table II.

This study highlighted that there was a significant correlation (p = 0.01) between haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and red cell distribution width (RDW) with Ret-He. It showed excellent positive correlation (rho= 0.83 and rho= 0.85), respectively, between MCV and MCH with Ret-He and good positive

Table I : Demographic, haematological &biochemical parameters for patients and control group

Demographic, n(%)		Parameter (unit)	Mean(SD)*/Median(IQR)#	Mean(SD)*/Median(IQR)#
			Patients	Control
Patients	**Control**			
Female : 88 (85.4)	18 (60.0)	Hb (g/dL)	6.85 (1.70)*	13.99 (1.42)*
Male : 15 (14.6)	12 (40.0)	RBC (X 10^9/dL)	3.89 (0.74)#	4.90 (0.84)#
		MCV (pg)	62.50 (0.74)#	84.95 (2.48)#
		MCH(fL)	17.40 (4.30)#	28.55 (1.04)*
Malay : 82 (79.6)		RDW (%)	19.97 (2.68)*	12.91 (0.62)*
Indian : 14 (13.6)				
Chinese: 5 (4.9)		Serum iron (µmol/L)	2.60 (1.50)#	17.05 (9.60)#
Others : 2 (1.9)		TIBC (µmol/L)	73.70 (14.30)#	58.53 (6.62)*
		Transferrin saturation	3.27 (2.10)#	27.88 (19.42)#
		Serum ferritin(µg/L)	5.80 (4.30)#	40.85 (59.73)#
Age : 36yr (22)#		Ret He (pg)	16.50 (4.90)#	34.80 (1.97)#

Notes:

*Mean (SD) is used when the data is normally distributed.

#Median (IQR) is used when the data is not normally distributed.

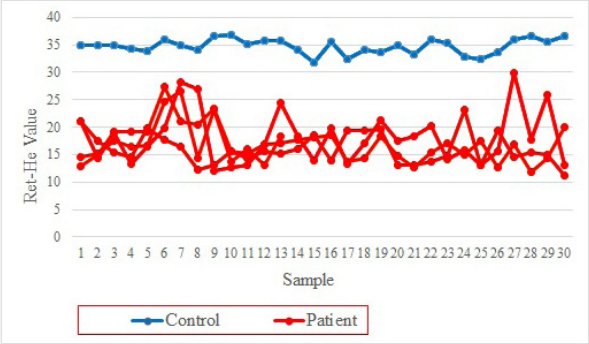

Fig. 1: Comparison of Ret-He value between patients and control group
For Fig.1, each dot represents a Ret-He value either belonged to a patient or control. Hence, there is clear separation of Ret-He value between patients and control group. Patients=103. Control, n=30

Table II : Correlation between Ret-He with haematological & biochemical iron status parameters among adults' blood samples of IDA in HTAA, Kuantan

Variables	Ret-He	
	Rho	p
Hb (g/dL)	0.69	0.01
RBC(X 10^9/dL)	0.16	0.12
MCV(pg)	0.83	0.01
MCH(fL)	0.85	0.01
RDW(%)	-0.59	0.01
Serum iron(µmol/L)	0.50	0.01
TIBC(µmol/L)	-0.01	0.90
Transferrin saturation	0.42	0.01
Serum ferritin(µg/L)	0.62	0.01

Table III : Ret-He and and Transferrin saturation (Tsat) in iron deficiency anaemia (IDA) vs control group. Ret-He as diagnostic test

Ret-He	Tsat <16%	Tsat >16%	Total
Positive	89.32% (92/103)	0 (0/0)	92
Negative	10.67 % (11/103)	100% (30/30)	41
Total	103	30	133

Note: The positive predictive value (PPV) is 100% whilst negative predictive value (NPV) is 73.11% when Ret- He is used as diagnostic test. Ret-He range for IDA in this study is 11.6-21.4pg (16.50±4.90)pg

correlation (rho= 0.69) between Hb and Ret-He. RDW showed inverse correlation (rho= -0.59) with Ret-He. However, there was no correlation (p= 0.12) found between red blood cells (RBC) and Ret-He.

All iron status biochemical parameters showed significant correlation (p= 0.01) with Ret-He except for total iron binding capacity (TIBC) parameter. Serum ferritin had a good positive correlation (rho= 0.62), while serum iron and Tsat only had fair positive correlation (rho= 0.50 and 0.42, respectively) with Ret-He. However, from this analysis, there was no correlation (p= 0.90) found between TIBC and Ret-He.

The sensitivity and specificity together with positive and negative predictive value of Ret-He compared to Tsat was shown in Table III. Ret-He was 89.32% sensitive and showed 100% PPV when compared to Tsat in this study.

DISCUSSION

In developing countries, iron deficiency anaemia (IDA) contributes to the most frequent cause of anaemia (5). Several previous studies found that demographic characteristics are associated with factors which contribute to IDA development. Proportion of IDA cases are greater in female gender (3,5,16-18), equally seen in our study. The female gender has a higher risk of developing IDA than the male gender especially during the reproductive age due to various reasons for example menstrual bleeding, pregnancy as well as blood loss during delivery and nutritional factor (18-21).

As for ethnic distribution of IDA, Indian ethnicity contributed to the majority of anaemia cases in Malaysia (18,21,23) mainly due to vegetarianism, popular in their culture (21,24). However, in this study, the Malay ethnicity contributed to the greater proportion of IDA as the majority of the respondents were Malays, which represent the population distribution in Kuantan (25).
IDA was noted to develop around 40 years of age especially in female (20) similarly seen in this study and study by Torino et al. in 2015 which described median age of IDA was 42 years old (17).

Variation of Ret-He values for control group were seen from 30.70 pg to 39.20 pg depending on population normality distribution (14,10,17,26,27). From our study, we found that Ret-He values for control group was 32.83 to 36.77 pg which is similar to another previous local study (27). Hence, a total of 30 respondents in this study was used as reference value of Ret-He indicator for control group corresponded to the CLSI guideline which required minimum of 20 individuals to get a reference value (28).

Ret-He value in our patients' group (11.60 to 21.40 pg) was dissimilar to a previous study by Torino et al. in 2015 (16.90 to 32.60 pg) which overlapped with the control group's Ret-He values (17). However, there was no local study available for comparison. IDA group was able to be differentiated from the control group using our Ret-He value as there was no overlapping results between these two groups in comparison with other parameters such as RBC and TIBC.

In this present study, the correlation of Ret-He with haematological and biochemical parameters in IDA were investigated and correlated. Results showed a significant positive correlation between Ret-He value with Hb, MCV, MCH, serum iron, transferrin saturation and serum ferritin and a significant negative correlation

between Ret-He with RDW. Our findings are partly in concordance with Ageeli et al., in 2013, who reported that Ret-He can be used to diagnose IDA (14).

Ret-He also showed good sensitivity and excellent specificity with good PPV against Tsat as parameter to diagnose IDA. Tsat < 16% is commonly used to diagnose IDA in general and is usually measured together with serum ferritin (29). This would help to diagnose IDA when other comorbidities are present as it is more practical to test for serum ferritin and Tsat at the same time to avoid repeated blood withdrawals and laboratory visits (29). Tsat also is recognized as most efficient alternative to ferritin in most circumstances (30), hence it was used as a comparator in our study. In addition to that, Ret-He parameter was available in most automated haematology analyser in our local laboratories which has been used daily for FBC. It can be done within a short period of time depending on the turnaround time (TAT) in each laboratory.

Apart from that, in the era where most laboratories faced financial constraint, Ret-He test may help as the cost is cheaper compared to iron profile. A set of iron profile requires different reagent and machine to run for different test such as serum iron, TIBC and serum ferritin. On top of that, when different test is performed, the more laboratory staffs are needed to run the tests and it is also time consuming.

CONCLUSION

Assessment on Ret-He showed that it is comparable with haematological and biochemical parameters that have been traditionally used in screening and diagnosis IDA in adults. On top of that, Ret-He has a reference interval value in determining IDA in adults. Therefore, Ret-He can be used for screening IDA among adults population in HTAA, Kuantan, Pahang. However, since the study used retrospective data and patients' underlying diagnoses were absent, thus it is recommended to consider these factors in future study to validate the findings using larger sample and prospective study design.

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