



Correlation of newer RBC indices (Micro RBC % and Macro RBC %) and iron profile in iron deficiency anaemia patients

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Abstract

Introduction: In India nutritional anemia, mainly iron deficiency anemia still continues to be the most prevalent anemia and is commonly seen in women in reproductive age group in economically backward sections. Iron profile to diagnose iron deficiency anemia is an expensive investigation and hemogram is the only investigation done for most cases. This test was done to assess if newer indices such as micro and macro RBC percentage can provide additional information regarding the severity of iron deficiency anemia.

Objectives: The objective was to correlate newer RBC indices such as micro and macro RBC % with iron profile in cases of microcytic hypochromic anemia.

Methodology: Study was conducted at R L Jalappa Hospital and Research Centre, a rural tertiary and academic teaching hospital attached to Sri Devaraj Urs Medical College with prospective study of one year between August 2018 to July 2019. A total of 156 samples were analysed for complete blood count, iron profile and peripheral smear examination. The data was entered in excel sheet and analysed using SPSS 22 software.

Results: The newer RBC index namely micro RBC percentage showed strong correlation with statistical significance using ANOVA test when compared with parameters such as serum iron, total iron binding capacity, serum transferrin, serum transferrin saturation and unsaturated iron binding capacity. Macro RBC percentage showed statistical significance with all parameters of iron profile except total iron binding capacity.

Conclusion: In resource limited settings, micro and macro RBC percentage provide valuable information regarding the severity of iron deficiency anemia

DOI Number: 10.48047/nq.2022.20.19.nq99477

NeuroQuantology 2022;20(19):5125-5138

Introduction

In India nutritional anaemia, predominantly iron deficiency anemia still

continues to be most common cause of anemia with its incidence being highest in adolescent girls, ranging from 67- 69% as per



the National Nutrition Monitoring Bureau¹⁻² Survey conducted. Anemia hence would also lead to reduced productivity of the population in the already economically backward³ countries where it is prevalent. In most cases all microcytic hypochromic anemias are treated as iron deficiency without confirmatory investigations such as total iron binding capacity, serum ferritin or bone marrow Perl's stain. As tests cost higher and the cost of treatment being cheaper at times, this may bring us conclusion that all microcytic hypochromic anemias are iron deficiency anemia. Hence other causes having adequate to increased iron stores must be identified as those patients will not benefit from the iron therapy given prophylactically.

Although newer RBC indices such as Macro and Micro RBC percentage are available with every complete blood count in most newer analysers, the utility of those tests is not made proper use by neither clinicians, nor studied by researchers completely. Only few studies have been done on these newer RBC parameters have been done in evaluating their utility in chronic kidney disease and iron deficiency anemia and⁴⁻⁶ variable results were found.

Many studies have been done using individual and combination of RBC indices to differentiate between the different causes of⁷⁻¹¹ microcytic hypochromic anemia. Few studies have shown that Micro RBC percentage is useful in identifying the etiology of microcytic anemia and mainly differentiate between iron deficiency anemia, thalassemia¹²⁻¹⁵ and chronic kidney disease. The Macro and Micro RBC percentage parameters are taken from either ends of a RBC histogram and detect the percentage of microcytes and macrocytes which can be used to narrow down the causes of anemia. This study aims to identify the utility of these already available newer parameters with specific tests to detect iron insufficiency and deficiency such as serum iron, transferrin, transferrin saturation and total iron binding capacity.

Hence it will help distinguish iron

deficiency anemia from the other anemias which present as microcytic hypochromic anemia but with normal to increased iron stores such as anemia of chronic disease or thalassemia. This will be useful in developing countries as may provide valuable information regarding the cause before starting on iron therapy routinely. **Objectives**

To study the correlation between newer RBC indices (Micro RBC % and Macro RBC %) and iron profile in iron deficiency anemia patients.

Methodology

Laboratory investigations were conducted at R L Jalappa Hospital and Research Centre, a rural tertiary and academic teaching hospital attached to Sri Devaraj Urs Medical College with prospective study period of one year and between August 2018 to July 2019.

50 healthy individuals with normal hemoglobin and RBC indices was taken as controls to obtain a reference range for the newer RBC parameters such as Macro and Micro RBC percentage. Patients included in the study are cases of microcytic hypochromic anemia with serum iron profile. Exclusion criteria included if a) they were already on treatment with iron / vitamin supplements b) cases without iron profile

Samples were collected in EDTA vacutainer and processed within 4 hours in a Sysmex XN 550 5 part differential hematology analyser for complete blood counts (which includes the newer RBC parameters). Peripheral smear slides were prepared from the same sample and stained with Leishman Stain following standard protocol following which peripheral smear will be interpreted. Blood was also be collected for serum iron profile in vacutainer without any anticoagulant and allowed to clot and the serum analysed for serum iron profile composed of transferrin, serum iron, transferrin saturation, total iron binding capacity and unsaturated iron binding capacity. Data was collected for patients and controls included in the study. Data includes demographics of the patient, clinical diagnosis, complete blood count including routine RBC indices (mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration and red cell distribution width), newer RBC indices



(micro and macro RBC percentage, iron profile and peripheral smear report. Routine RBC indices include.

Statistical analysis:

Data was analysed by using statistical tool SPSS 22 version. Categorical data is represented in the form of Frequencies and proportions. Test of significance was done using ANOVA analysis.. p value <0.05 considered statistically significant.

Results

50 patients who had normal hemogram were taken to define the normal range for

percentage of micro and macro RBCs as no defined percentage is available. The normal range for micro RBC was 2.3 +/- 1.5 % and for macro RBC was 4.3 +/- 0.5%.

The demographic data of the patients show that microcytic hypochromic anaemia was more common in females(55%) and between the age group of 61-70 year. In men it was noted to be more common 51-60 years. In reproductive age group between 20-50 years the cases were seen in women predominantly (64%)

Demography of patients with microcytic hypochromic anemia

Age Group (in years)	Male (No. of cases)	Male (%)	Female (No. of cases)	Female (%)	Total
<10	8	11.59	7	8.05	15
11-20	2	2.90	8	9.20	10
21-30	3	4.35	11	12.64	14
31-40	4	5.80	13	14.94	17
41-50	15	21.74	14	16.09	29
51-60	17	24.64	16	18.39	33
61-70	10	14.49	17	19.54	27
>70	10	14.49	1	1.15	11
Total	69	100.00	87	100.00	156

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Table 4 : Age and Sex Distribution of cases

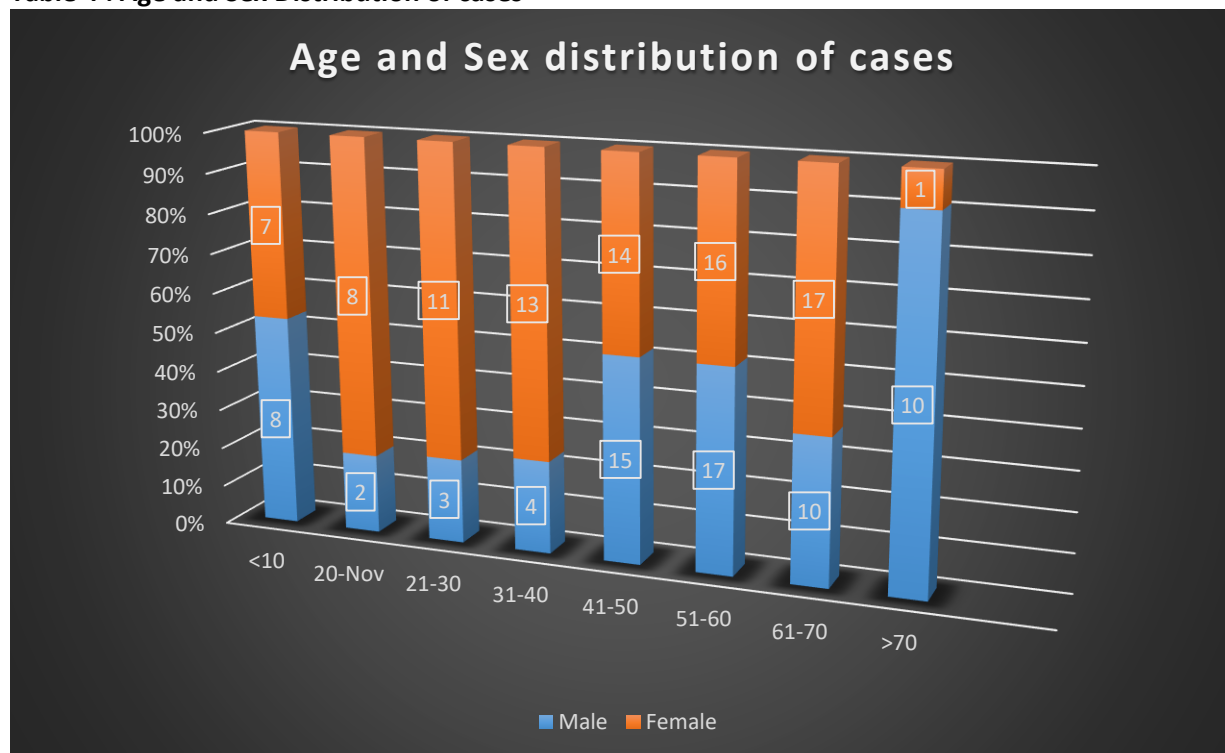


Fig 4: Age and Sex Distribution of cases



Total Iron (ug/dl)	No. of cases	Micro RBC	Macro RBC	Hb	MCV	MCH	MCHC
<20	65	43.7	1.9	8.4	64.5	18.9	28.7
20-50	54	17.3	4	8	72.9	25.8	31.9
50-170	23	13	5.9	7.9	73.7	27.3	32.6
>170	14	36.4	4	6.9	68.3	21.9	29.8
p value		<0.0001	0.0002				

Table 5: Correlation of Serum Total iron with RBC indices.

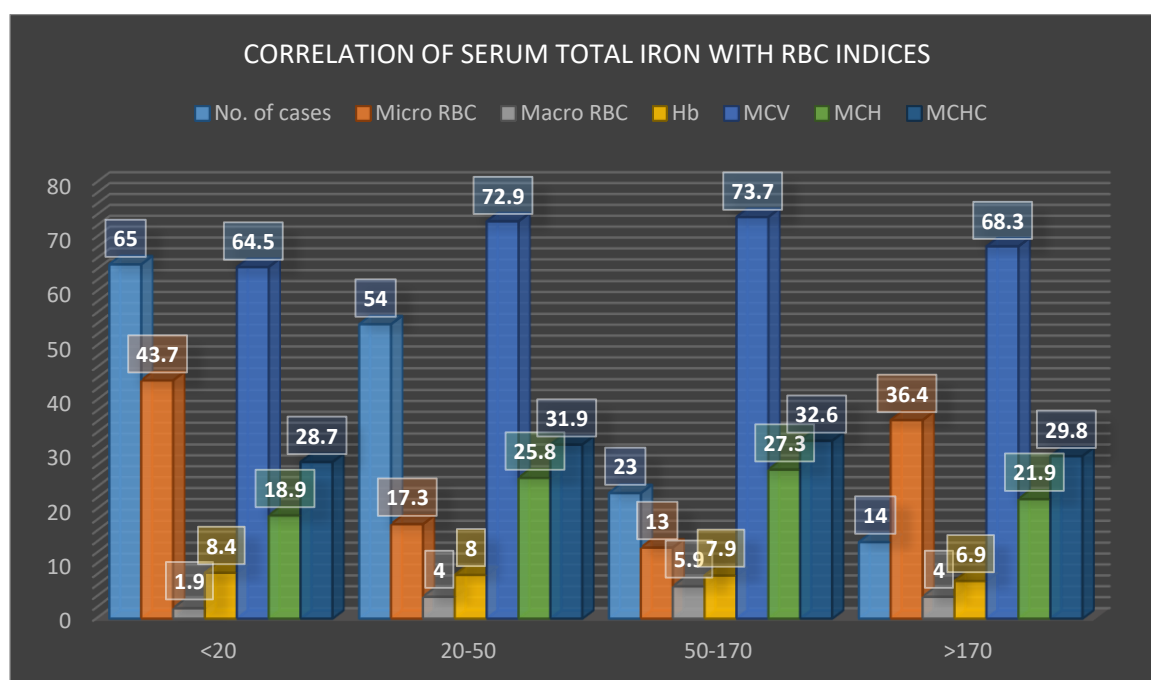


Fig 5: Correlation of Serum Total iron with RBC indices.

The results show both micro and Macro RBC % showing a correlation with total iron values with p value being significant on ANOVA test. As expected, as the total iron levels reduce the percentage of micro RBCs increased.

TIBC (mcg/dl)	No. of cases	Micro RBC	Macro RBC	Hb	MCV	MCH	MCHC
<260	41	17.8	3.9	7.5	72.8	26.1	32.4
260-500	81	29.4	3.7	7.5	69.1	22.9	30.5
>500	34	43.4	2.2	10	64.5	18.7	28.1
		0.0001	0.10				

Table 6 – Correlation of Serum Total Iron Binding Capacity with RBC Indices



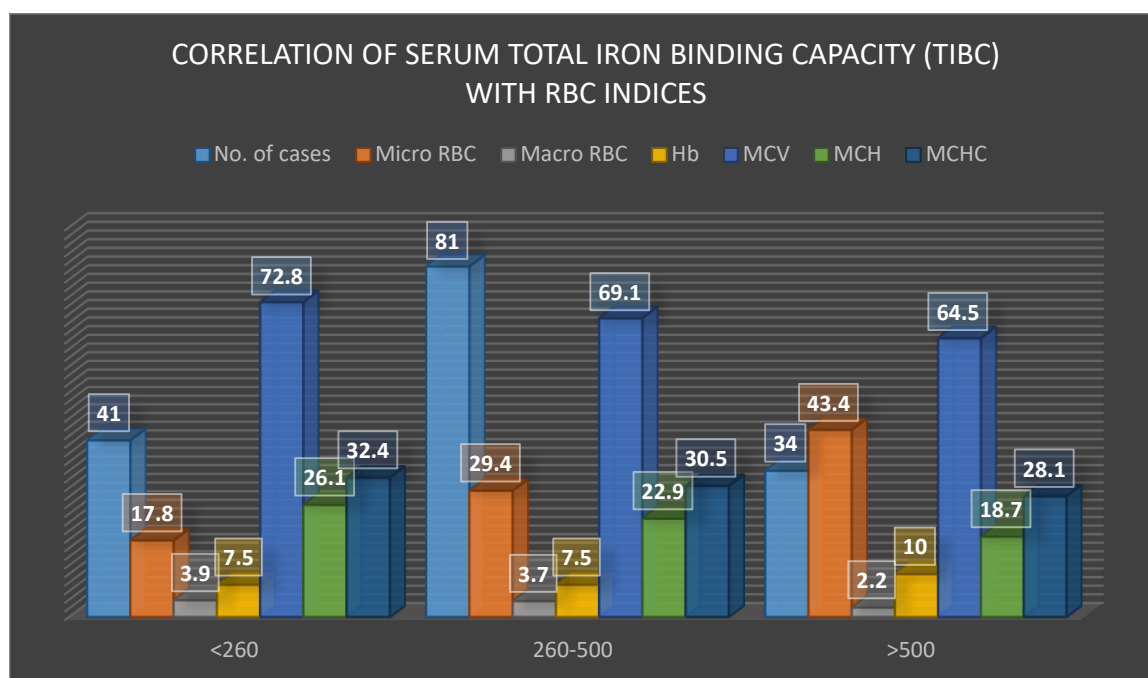


Fig 6 : Correlation of Serum Total Iron Binding Capacity with RBC Indices

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The results show micro RBC % showing a correlation with total iron binding capacity values with p value being significant on ANOVA test. As expected, as total iron binding capacity increases, the percentage of micro RBCs increased

Sr	Transferrin (mg/dl)	No. of cases	Micro RBC	Macro RBC	Hb	MCV	MCH	MCHC
< 175		37	17.3	3.7	7.7	73.2	26.3	32.7
175-320		70	26.2	4.1	7.6	69.9	23.8	31
>320		49	43	2.3	7	64.9	18.8	28.1
p value			0.03	0.01				

Table 7 : Correlation of Serum Transferrin with RBC Indices



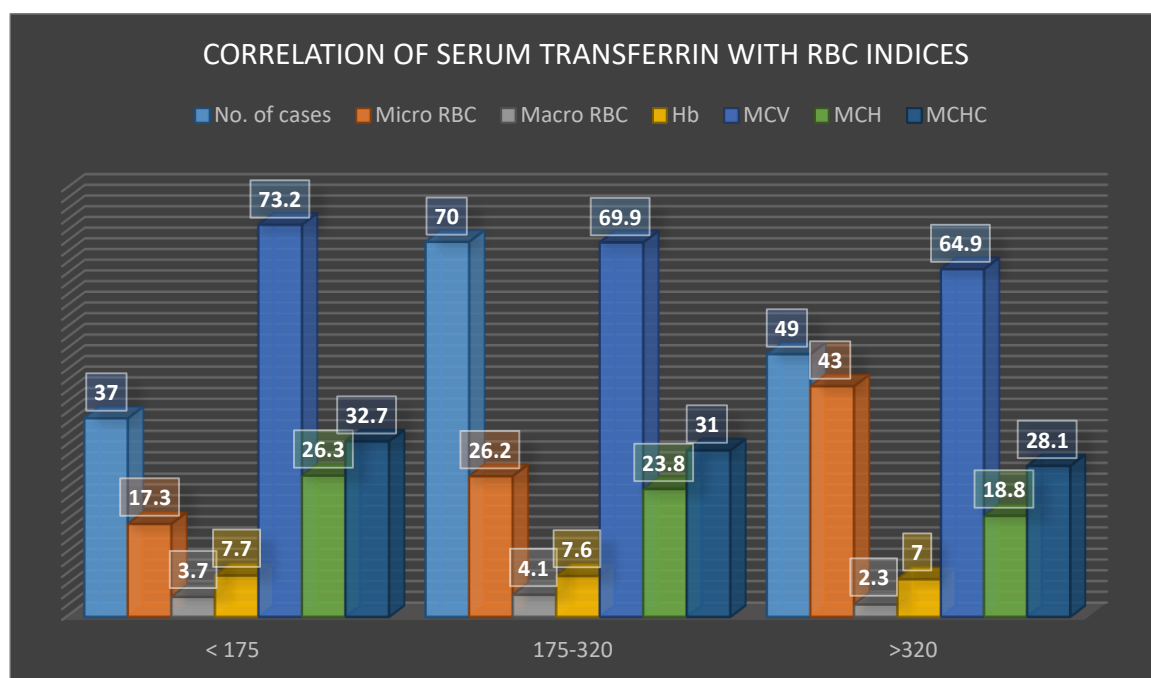


Fig 7: Correlation of Serum Transferrin with RBC Indices

The results show both micro and macro RBC % showing a correlation with serum transferrin values with p value being significant on ANOVA test. As expected, as serum transferrin increases, the percentage of micro RBCs increased.

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Unsaturated iron binding capacity (mcg/dl)	No. of cases	Micro RBC (%)	Macro RBC (%)	Hb (%)	MCV (%)	MCH (%)	MCHC (%)
< 190	33	14.6	4.7	7.5	73.6	26.7	32.4
190-350	56	22.2	4.2	8.2	71.7	24.8	31.7
> 350	67	42.7	2.2	8.2	64.6	19.2	28.5
P value		<0.0001	0.01				

Table 8 : Correlation of Serum Unsaturated iron binding capacity with RBC Indices



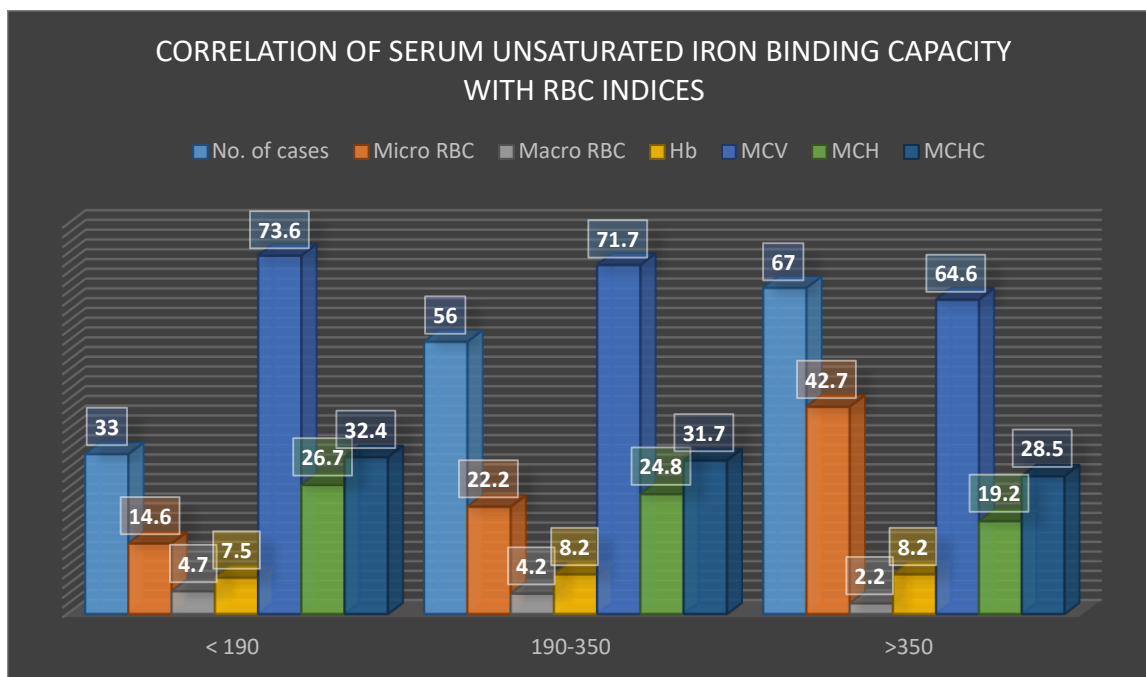
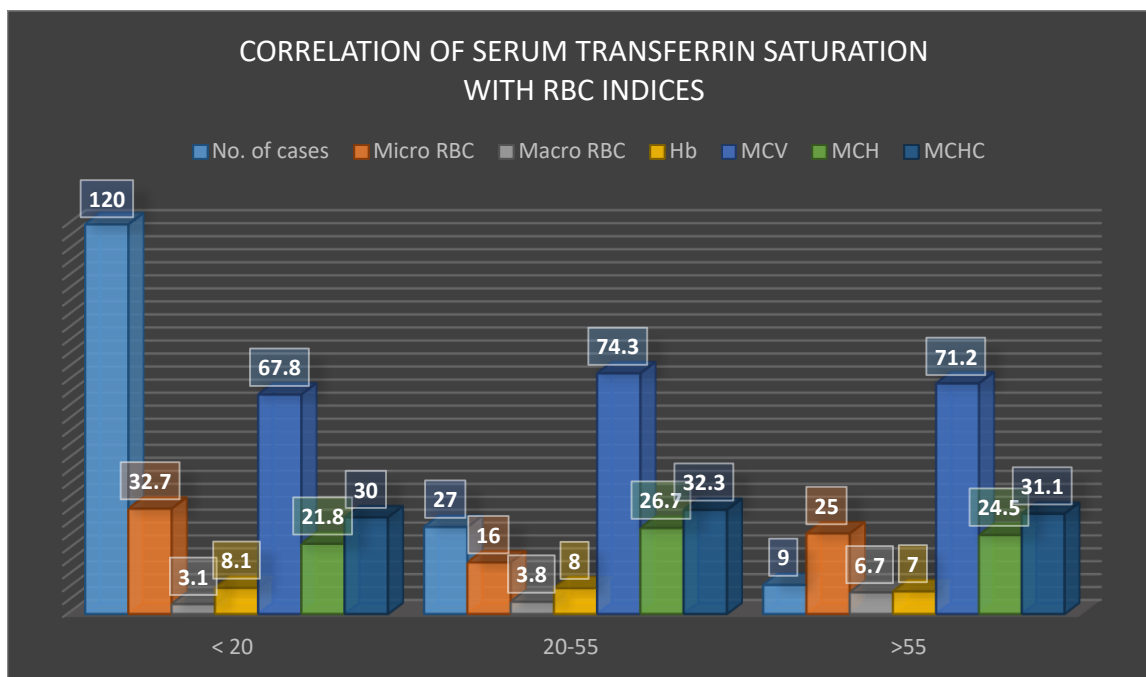


Fig 8 : Correlation of Serum Unsaturated iron binding capacity with RBC Indices

The results show both micro and macro RBC % showing a correlation with Serum Unsaturated iron binding capacity values with p value being significant on ANOVA test. As expected, as Serum Unsaturated iron binding capacity increases, the percentage of micro RBCs increased.

Transferrin saturation (%)	No. of cases	Micro RBC	Macro RBC	Hb	MCV	MCH	MCHC
< 20	120	32.7	3.1	8.1	67.8	21.8	30
20-55	27	16	3.8	8	74.3	26.7	32.3
>55	9	25	6.7	7	71.2	24.5	31.1
p value		<0.0001	<0.0001				

Table 9: Correlation of Serum transferrin saturation with RBC Indices

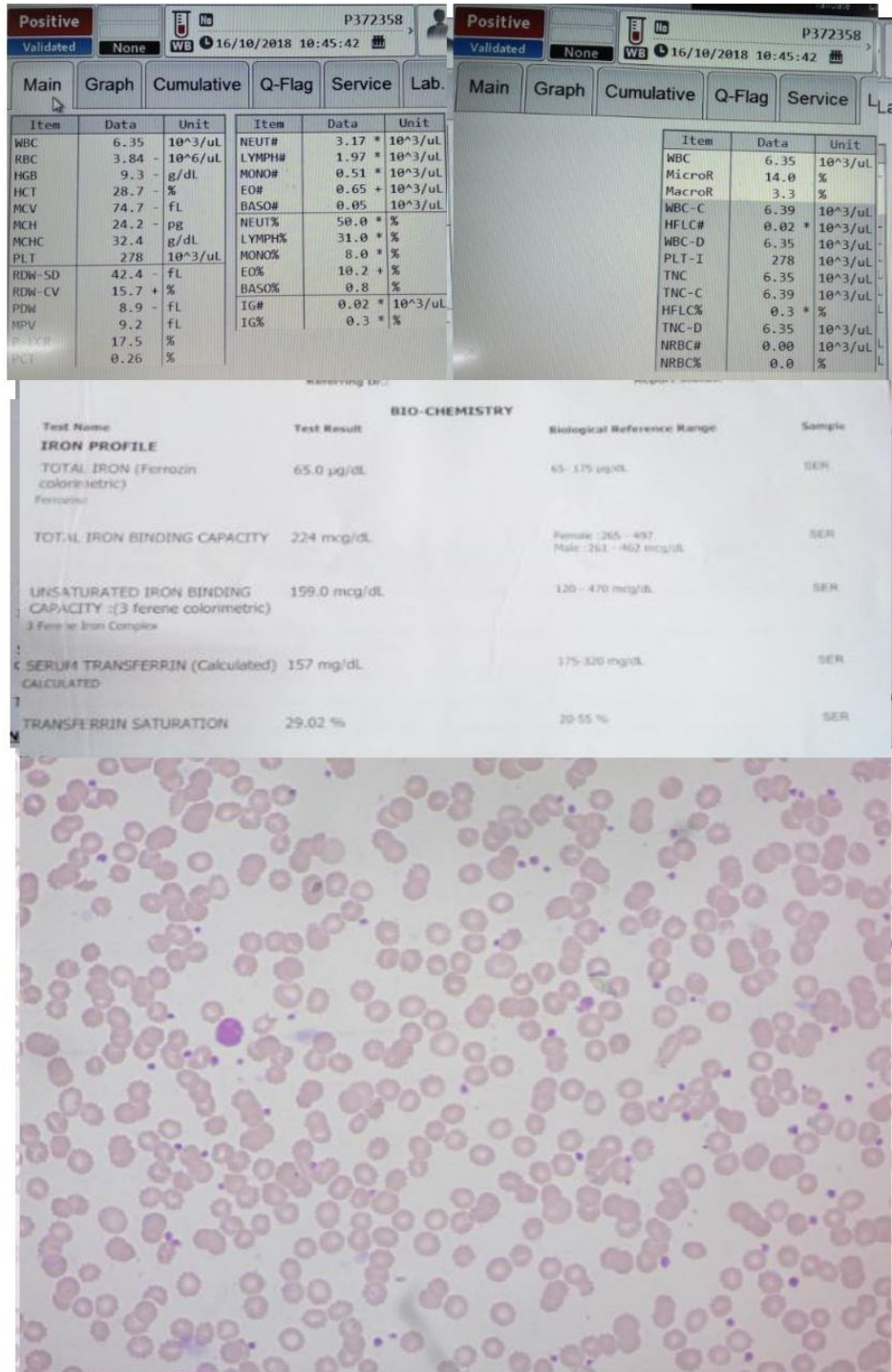


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Fig 9: Correlation of Serum transferrin saturation with RBC Indices

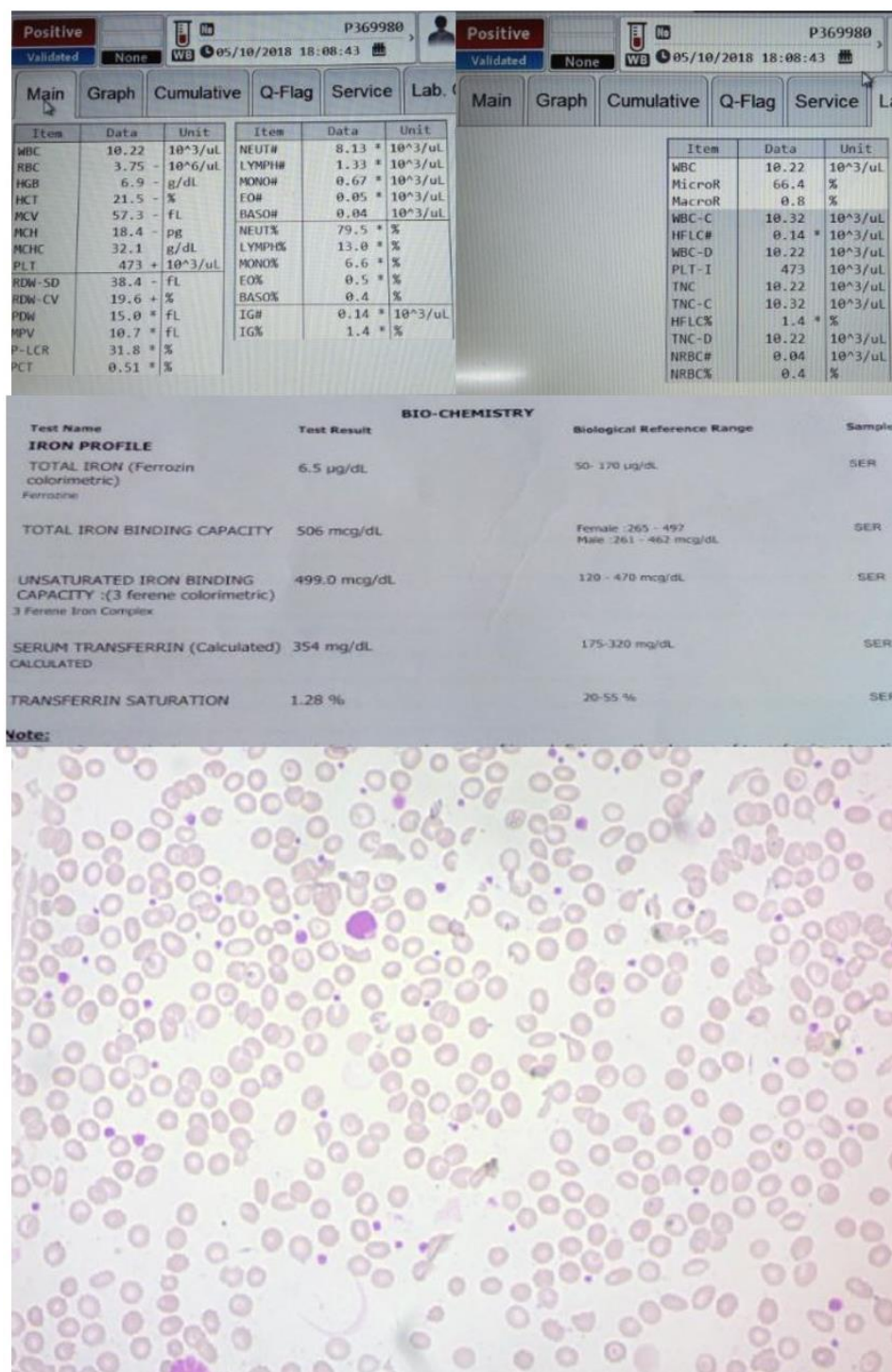
The results show both micro and macro RBC % showing a correlation with serum transferrin saturation values with p value being significant on ANOVA test. As expected, as serum transferrin decreases, the percentage of micro RBCs increased.





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Fig 10 Indices, Iron Profile and Peripheral smear in early iron deficiency anemia



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Fig 11 Indices, Iron Profile and Peripheral smear in Iron deficiency anemia

Discussion

As anaemia is common in most developing countries, we have always been looking for cost effective alternatives of biochemical tests to diagnose nutritional anaemia in the form of measured and calculated RBC Indices. Macro RBC and micro

RBC are newer indices measured by sysmex automated haematology analysers and they are counted in the RBC channel by using sheath flow direct current method using red cell distribution width.

As most studies have shown, in India anaemia is slightly more prevalent in women



than man and similar results have been seen in our study too with 55% of the cases being women. The age group with most number of cases with anaemia is also similar to other studies and being the 4th to 6th decade which accounts for majority of the cases in our study too.⁵⁶⁻⁵⁷

Several studies have been done to detect the best biochemical tests and most studies either suggest one or a combination of tests of iron profile in diagnosis of iron deficiency anaemia and to rule out other causes of microcytic hypo chromic anaemia such as thalassemia and anaemia of chronic disease.⁵⁸⁻⁵⁹

In this study we have hence correlated the micro and macro RBC percentage along with previous well known indices with iron profile. As expected micro RBC percentage have significant correlation with all parameters of iron profile.

In iron deficiency anemia, the serum iron and serum transferrin saturation along with RBC parameters such as MCV, MCH and MCHC reduces whereas serum total iron binding capacity and serum transferrin saturation increases. With lower MCV, not only the size of microcytes reduces but also the number of microcytes reduces with the severity of iron deficiency in anaemia

This is evident with the findings in the current study with mean microcyte RBC percentage being increased in cases of low serum iron, serum transferrin saturation and high total iron binding capacity and serum transferrin levels.

The values also correlated with mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. As this study didn't workup all cases of microcytic hypo chromic anaemia with electrophoresis, other causes of microcytic hypo chromic anaemia cannot be ruled out using these newer RBC indices.

The present study also identified normal reference level of micro RBC percentage to be less than 4% and cases with severe iron deficiency anaemia with serum iron values less than 20ug/dl, serum total iron binding capacity more than 500mcg/dl, serum

transferrin saturation less than 20% and serum transferrin levels more than 320mg/dl had a mean micro RBC percentage of more than 40% and even in early iron deficiency anemia the mean micro RBC percentage was more than 15%. With these findings it can be suggested that micro RBC% not only suggests the severity of anaemia but can also be used in detection of early iron deficiency anaemia. The micro RBC percentage had an inverse relation with macro RBC percentage and the values appeared lower in severe iron deficiency anaemia rather than early iron deficiency anaemia patients.

As this research parameter is available only in few sysmex analysers there are no studies which have tried to identify its utility in diagnosing anemia or identifying the severity of anemia. However as the principle is based on pulse height detection and impedance, we are sure that it will be soon incorporated into several analysers making it a useful addition to existing RBC indices.

Conclusion:

In conclusion, RBC indices have always been essential in morphological classification of anemia and have provided valuable information hinting the probable cause of anemia along with peripheral smear examination. In resource poor settings and in rural population where tests such as iron profile and peripheral smear examination are not available, newer indices such as micro RBC and macro RBC % act as a complementary investigation to the hemogram indices in providing valuable information regarding the severity of iron deficiency.

Hence we suggest incorporation of newer RBC indices such as percentage of micro and macro RBC to routine hemogram along with regular RBC indices which can help the clinician to decide the treatment protocol and intensity in a case of iron deficiency anaemia.

These newer indices also aid in locations where pathologists aren't there in providing information regarding the percentage of microcytes and macrocytes which may aid in differentiating iron deficiency anaemia alone from combination of iron and B12 deficiency patients which may



not be identified by routine RBC indices currently provided in the hemogram.

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