RESEARCH ARTICLE

Hemoglobin, Ferritin levels and RBC Indices among children entering school and study of their correlation with one another

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Abstract

Objective: To study correlation of various complete blood count variables with one another and to ascertain the values of intercept and slope among those having strong correlation.

Methods: The cross-sectional study was conducted in Marach 2017 in the Rabwah town of Punjab province in Pakistan, and comprised randomly selected one in four children studying under the Nazrat Taleem School System. Serum sample from each subject was used to analyse complete blood count on an automated analyser, and ferritin levels were checked by enzyme-linked immunosorbent assay. SPSS20 was used for data analysis.

Results: There were 299 children with a median age of 67 months. Mean haemoglobin level was 12.09±0.82gm/dl. There was a very strong positive correlation between haemoglobin and haematocrit; mean corpuscular volume; and red cell distribution width; mean corpuscular volume and mean corpuscular haemoglobin; red cell distribution width and mean corpuscular haemoglobin ($p<0.001$).Moderate positive correlation was found between haemoglobin and red blood cell count; haematocrit and red blood cell count; mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration ($p<0.05$). Moderate negative correlation was present between red blood cell count and mean corpuscular volume; red blood cell count and red cell distribution width; red blood cell count and mean corpuscular haemoglobin ($p<0.05$).

Conclusions: There were strong to moderate correlation between various complete blood count variables in the studied population. Ferritin level was the only variable which did not have any correlation with any of the other variables.

Keywords: Children, Haemoglobin, Haematocrit, Ferritin, MCV.

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Introduction

Red blood cell (RBC) indices i.e. mean corpuscular volume (MCV)), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW), and RBC count, are valuable parameters in the morphological classification of different types of anaemias. Various types of anaemia lead to different red cell morphology. Different RBC indices can help the clinicians to ascertain the cause of anaemia.[1]

Anaemia can be classified according to the size of the red cell; normocytic (normal MCV), macrocytic (increased MCV), or microcytic (decreased MCV). Iron deficiency anaemia and thalassemia are microcytic anaemias. Anaemias due to Vitamin B12 and folic acid deficiency are macrocytic, and aplastic and haemolytic anaemias are usually normocytic.[1]

RBC counts are decreased in iron-deficiency, aplastic, haemolytic anaemias, and haemorrhage and chronic renal failure. It is raised in dehydration, stress, cyanotic heart diseases, smoking, polycythaemia vera and renal cell carcinoma.[1]

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Studies have shown that in cases of iron deficiency with anaemia, and in latent iron deficiency, MCH is reduced and is moderately accurate in diagnosing iron deficiency.[2,3] RDW is the parameter which measures variation in the size of RBC. RDW increases with the increase in variation in red cell size called anisocytosis. Elevated RDW is an early clue to deficiencies like iron, folate or vitamin B12. RDW helps to differentiate between megaloblastic anaemias, such as folate or vitamin B12 deficiency anaemia with elevated RDW and other causes of macrocytosis with usually normal RDW.[4,5] Elevated RDW is associated with impaired iron mobilisation, ineffective red cell production and increased red cell destruction.[6,7] Increase in RDW has been found to be associated with markers of inflammation like interleukin-6 (IL-6), C reactive protein (CRP) ,and indicates membrane instability.[8-10] In recent years, RDW has been studied as a marker for increased risk of mortality.[11,12] A study showed that high RDW at the time of admission was associated with increased mortality among children admitted in paediatric intensive care unit (ICU).[13]

Although in recent years RBC indices have been the focus of many studies, few have focussed on studying the correlation of these indices with one another. As these parameters are increasingly being used clinically, there is a need to understand how they correlate with one another.

The current study was planned to study correlation of haemoglobin (Hb), haematocrit (Hct), ferritin levels, RBC counts, MCV, RDW, MCH and MCHC with one another, and to ascertain whether or not MCH can be used to predict the value of MCV, RDW and MCHC.

Subjects and Methods

The cross-sectional study was conducted in March 2017 in the Rabwah town of Punjab province in Pakistan, and comprised randomly selected children studying under the Nazrat Taleem School System (NTSS). Rabwah is a town with a population of around 70,000 where 11 schools provide primary-level education and five of them are owned and operated by NTSS.

After approval from the ethics review committees of Fazl-e-Omar Hospital, Rabwah, and NTSS, the sample size was calculated using the online calculator of the University of California, San Francisco, United States. Pearson correlation coefficient between serum ferritin and RDW was taken as 0.420,$\beta = 0.20$ and $p = 0.50$.[14]

One in four children getting admission in the preparatory (Prep) class (first year of school) was selected by computer randomisation. Children having fever or any other signs or symptoms of infectious disease or inflammation and those receiving iron therapy were excluded. After taking informed consent from the parents of each child, 5ml blood was drawn for full complete blood count(CBC) and ferritin levels. CBC was checked using Medonic M20 analyser, and ferritin levels were checked by enzyme-linked imunosorbent assay (ELISA) (Statfox200). Names, Hb, Hct, MCV, RDW, RBCcount, MCH, MCHC and ferritin levels were noted on a pre-designed proforma.

SPSS 20 was used for data analysis. Shapiro-Wilk test showed that Hb and Hct levels had normal distribution and ferritin levels, MCV, RDW, RBCcount, MCH and MCHC had non-normal distribution. Median values and interquartile ranges (IQRs) were used to ascertain central tendency and spread of the continuous variables with non–normal distribution. Mean values and standard deviations were used to ascertain central tendency and spread of continuous variables with normal distribution. Outliers were marked by using box plots of SPSS and their values were changed by mean values. Spearman test was used to ascertain correlation among ferritin, Hb, Hct, MCV, RDW, MCH and MCHC levels as well as RBC counts.Value of Spearman coefficient (rr) 0-0.19 was taken as very weak, 0.20-0.39 as weak, 0.40-0.59 as moderate, 0.60-0.79 as strong, and 0.80-1.0 as very strong correlation.

Simple linear regression was done to check the predictive value of MCH to predict the values of dependant variables MCV, RDW and MCHC. If the X-axis was the independent and Y-axis the dependant variable, the point where the line crossed the Y-axis or X-axis was taken as the 'intercept', while the 'slope' was defined as the steepness of the line. The values of intercept and slope were obtained by simple linear regression. The intercept and slope defined the linear relationship between the two variables, and was used to estimate the rate of change in one variable with unit change in the other variable .If intercept was 'a' and slope was 'x', the value of independent variable was 'b', the estimated value of dependant variable(y) was found using the equation$y=a+bx$.[15]

$P<0.05$ was taken as significant . Hb<11.5 gm/dl was defined as anaemia, and ferritin level<12ng/dl was categorised as iron deficiency.[16]

Results

Of the 299 children, 166 (55.6%) were girls and 133 (44.6%) were boys, with an overall median age of 67 months (IQR: 6months). Of the total, 63(21.1%) children were anaemic and 270 (90.3%) had low ferritin levels (Table-1).

The correlation coefficients and their significance were worked out and only ferritin level was found to have no moderate, strong or very strong correlation with any other variable (Table-2; Figures 1-2).

Simple linear regression was done between MCH, as an independent variable, and MCV, RDW and MCHC, as dependant variables. MCHC was a better predictor for MCV compared to RDW and MCHC (Table-3).

Table-1: Mean/Medianlevelsofdifferentvariablesinmale,female,andallsubjects.

Variable	Male Mean±SD	Female Mean±SD	All Mean±SD
Haemoglobin gm/dl	12.11±0.77	12.08±0.87	12.09±0.82
Haematocrit %	35.67±2.16	35.67±2.14	35.67±2.28
	Median (IQR)	Median (IQR)	Median (IQR)
Ferritin ng/ml	4.40(2.85-7.30)	5.20(3.10-7.40)	4.90(3-7.3)
RBCcount 1012/l	4.65(4.39-4.94)	4.60(4.32-4.86)	4.65(4.37-4.88)
MCV fl	77.40(74.10-79.70)	78.35(75.35-80.95)	77.80(74.80-80.40)
RDW fl	55.70(53.35-58.10)	56.w10(53.37-58.92)	55.90(53.40-58.30)
MCH pg	26.40(25.20-27.30)	26.70(25.40-27.70)	26.50(25.20-27.40)
MCHC g/dl	34.10(33.70-34.40)	33.90(33.40-34.40)	34.00(33.50-34.40)

SD: Standard deviation; IQR: Interquartile range. RBC: Red blood cell; MCV: Mean corpuscular volume; RDW: Red cell distribution width; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration

Discussion

Iron deficiency was found in >90% of the subjects in the current study. Ferritin levels had weak positive correlation with MCV and MCH, and very weak positive correlation

Table-2: Spearman Correlation Coefficients between Ferritin, haemoglobin, haematocrit, and RBC Indices.

Spearman'srho	Haemoglobin	Haematocrit	Ferritin	RBCcount	MCV	RDWa	MCH	MCHCa
Haemoglobin (g/dl)Correlation Coefficient	1	0.955	0.170	0.424	0.331	0.341	0.369	0.321
p-value	-	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001
Haematocrit %Correlation Coefficient	0.955	1	0.118	0.535	0.252	0.297	0.227	0.051
p-value	<0.001	-	0.041	<0.001	<0.001	<0.001	<0.001	0.367
Ferritin Correlation Coefficient ng/dl	0.170	0.118	1	-0.120	0.215	0.151	0.229	0.197
p-value	0.003	0.041	-	0.037	<0.001	0.009	<0.001	<0.001
RBC count Correlation Coefficient 10^{12}/dl	0.424	0.535	-0.120	1	-0.581	-0.477	-0.587	-0.282
p-value	<0.001	<0.001	0.037	-	<0.001	<0.001	<0.001	<0.001
MCV Correlation Coefficient Fl	0.331	0.252	0.215	-0.581	1	0.909	0.953	0.330
p-value	<0.001	<0.001	<0.001	<0.001	-	<0.001	<0.001	<0.001
RDW Correlation Coefficient Fl	0.343	0.297	0.151	-0.477	0.909	1	0.839	0.207
p-value	<0.001	<0.001	<0.009	<0.001	<0.001	-	<0.001	<0.001
MCH Correlation Coefficient Pg	0.369	0.277	0.299	-0.587	0.953	0.839	1	0.560
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	<0.001
MCHC Correlation Coefficient g/dl	0.312	0.052	0.197	-0.282	0.330	0.207	0.560	1
p-value	<0.001	0.367	0.001	<0.001	<0.001	<0.001	<0.001	-

RBC: Red blood cell; MCV: Mean corpuscular volume; RDW: Red cell distribution width; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration.

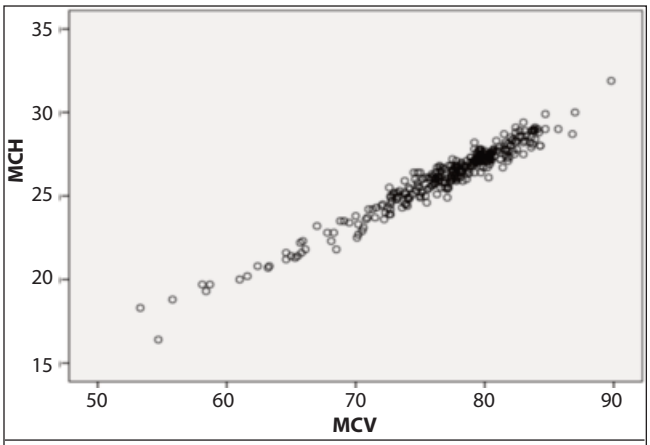

Figure-1: Scatter plot between MCV and MCH.

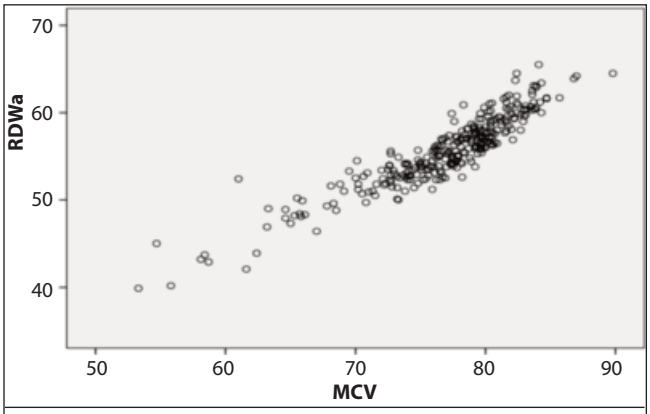

Figure-2: Scatter plot between MCV and RDWa.

with Hb, hematocrit, RDW and MCHC levels. Ferritin levels also had very weak negative correlation with RBCcount.

While the current study focussed on children starting first year of school, other studies have evaluated correlation of ferritin levels with RBC indices in different groups of population. A study showed that among pregnant women of second and third trimester ferritin levels had moderate negative correlation with RDW, and a weak positive correlation with RBC count. No correlation was seen between ferritin levels and MCV, Hb, MCH and MCHC.14 On the other hand, another study showed that serum ferritin of pregnant women had weak positive correlation with Hb, MCV and MCH.[17] Different studies have tried to ascertain the appropriateness of different RBC indices to predict the status of iron stores. As none of the above-mentioned indices had strong correlation with ferritin levels, researchers studied the correlation of the combined cell index(CCI) with ferritin levels. This index is calculated by the formula: RDW×104×MCV-1×MCH-1. CCI had a weak negative correlation with ferritin levels in males, and moderate negative correlation in females.[16,18]

Table-3: Results of Simple Regression Analysis, while taking MCH as independent variable and MVC, RDWa and MCHC (dependent variables).

	MCV	RDWa	MCHC
Adjusted R Square	0.91	0.72	0.35
Intercept	13.91	10.95	29.98
Slope	2.41	1.71	0.91
p-value	<0.001	<0.001	<0.001

MCV: Mean corpuscular volume; RDW: Red cell distribution width; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration.

Our study showed that MCV, RDW, and MCH had a very strong correlation with each other. Another study showed that MCV and MCH had a very strong correlation, but there

was moderate negative correlation between MCV and RDW[19].The same study[19] also showed moderate positive correlation between Hb and RBC count, as was the case in the current study. The earlier study[19] included only children with iron deficiency anaemia, while majority of the children in the current study had normal Hb levels.

We found very strong correlation between RDW and MCV. Studies should be conducted to find out the reason behind this very strong correlation between these two indices. It is known that iron deficiency leads to low MCV and high RDW.[20]

As majority of the children included in the current study had iron deficiency, it would have been expected to find negative correlation between MCV and RDW. A study[20] explored the utility of RDW in the diagnosis of iron deficiency among children with microcytic hypochromic anaemia, and showed that RDW was significantly higher among cases of iron deficiency anaemia compared to those without it. Detailed analysis showed that among the cases of iron deficiency anaemia, RDW increased significantly with the increase in the severity of anaemia. Among the cases of mild anaemia, there was little difference between mean RDW of iron deficiency and non-iron deficiency groups.[21]As in our study, most of the children did not have anaemia,[19] and this can explain ferritin's very weak positive correlation with RDW.

Our study showed moderate positive correlation between RBC count and Hb and Hct, and there was negative moderate correlation between RBC count and MCV, MCH and RDW. This means that when increased amount of Hb is being formed, it leads to formation of increased number of RBCs of smaller size and with less amount of Hb present in each cell. A similar study showed strong positive correlation between RBC counts and Hb, and very strong positive correlation between RBC count and Hct.[19]

One study showed that among adult Pakistani males, there was significant positive correlation between RBC count and Hb, but correlation between RBC count and Hct was non-significant.[22]

In the current study, MCHC had a very weak positive correlation with ferritin levels weak positive correlation with Hb, MCV and RDW, and moderate positive correlation with MCH. A study showed that mean cell Hb, MCH and MCHC were only moderately accurate in diagnosing empty iron stores in children and young adults, and normal values of these tests do not exclude empty iron stores in anaemic patients.[2]

Conclusions

Other than Hct and Hb which had strong correlation with each other, RBC indices MCV, RDW and MCH had a very strong correlation with one another. Ferritin level was the only variable which did not have any correlation with any other variable.

Disclaimer: None.

Conflict of Interest: None.

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