STUDY OF RED CELL INDICES AND RETICULOCYTE COUNT IN PERSON WITH ANEMIA AT A TERTIARY CARE HOSPITAL OF KATHMANDU

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ABSTRACT

Anemia is not a disease but a clinical feature of some other underlying problems. It is one of the most common public health problems in developing countries. The prevalence of anemia differs according to the age group. A cross sectional observational study was conducted in a tertiary hospital, over a period of six months (January 2018 to June 2018). All the cases of anemia in different age group with hematological investigations (hemoglobin and red blood cell indices) including reticulocyte count during the study period were included. A total of 274 blood samples of persons with anemia were evaluated. The mean age of the patients was 41.4 ± 25.37 years. Anemia was more commonly seen in females (68.2%) than males (31.8%) in our study. The most affected age group for anemia was person ≥ 15 years followed by children < 5 years. The least affected group was children of 12- 14 years. Microcytic anemia (decreased MCV and decreased MCHC) was observed in children of 12- 14 years and macrocytic anemia (increased MCV) was seen in group ≥ 15 years. Red cell distribution width (RDW) was more elevated in children of 12 -14 years and showed that there was significant variation of size of RBC in this group. Reticulocyte count was higher in children < 5 years and lower in children of 12- 14 years. Significant correlation was noted for hematocrit and reticulocyte count. This study revealed that the prevalence of anemia increases with age and was more common in females. Baseline study of red blood cell indices and reticulocyte count will help us in diagnosing the type of anemia and in further management.

KEYWORDS

Anemia, hemoglobin, hematocrit, red cell indices, reticulocyte

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INTRODUCTION

Anemia is a global health problem.[1] It is not a disease but it is the expression of underlying diseases. It is important to know the cause of anemia to treat the patients.[2] Anemia is defined as the decrease in Red blood cell (RBC), hemoglobin (Hb) and hematocrit (Hct) level below the lower extreme of the normal range for the age and sex of the individual.[1-3] According to WHO, the anemia cut offs are different for different age group. The hemoglobin cut offs for anemia is- for children 6- 59 months of age- 11 gm%, children 5-11 years of age- 11.5 gm%, children 12-14 years of age- 12 gm%, non pregnant women (15 years and above)- 12 gm%, pregnant women - 11 gm% and men (15 years and above) - 13 gm%.[4]

The prevalence of anemia is usually high in females when compared to males.[5] Another group of person affected with anemia are children of 5- 14 years.[6] To find out the type of anemia, the laboratory test should include Hb, Hct and RBC indices which include mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW).[3] MCV is the average volume of red cell expressed in femtoliters (fl). MCH is the average content of Hb per red cell, expressed in picograms (pg). MCHC is the average concentration of Hb in a given volume of packed red cells, expressed in grams per deciliter (gm/dl). RDW is the coefficient of variation of red cell volume expressed as RDW-CV (%) and RDW- SD (fl).[3] The study of RBC parameters is an effective way of quantitative assessment of RBC. RBC indices provide important guidelines for the diagnosis, classification and monitoring the treatment of anemia.[7]

Morphological classification of anemia shows three types of anemia. The most common type is microcytic hypochromic anemia which shows reduced MCV (mean corpuscular volume) <80 fl and reduced MCHC (mean corpuscular hemoglobin concentration) <30 gm/dl. Normocytic normochromic anemia has normal MCV (82- 100 fl) and macrocytic anemia has MCV >100 fl and normal MCHC.[2,3] Reticulocytes are the non nucleated immature erythroid cells in the peripheral blood that are in a discrete penultimate phase of maturation.[8,9] The reticulocytes percentage in the peripheral blood is an indication of the rapidity of red cell turnover if the patient is in a steady state.[8,10] A low reticulocyte percentage reflects a marrow unable to compensate for anemia and a high reticulocyte percentage reflects a marrow that is attempting to compensate for red cell destruction or recovering from anemia.[8]

The red cells parameters (MCV, MCH, MCHC and RDW) given by automated haematology cell counter are very relevant for the diagnosis of anemia. Reticulocyte count also plays a major role in giving the clue for diagnosis and also in monitoring the bone marrow recovery. Hence, it is worthwhile to evaluate these parameters in person with anemia.

MATERIALS AND METHODS

This was a cross sectional observational study done in a department of Pathology of a tertiary hospital (Nepal Medical College Teaching Hospital, NMCTH) for a period of six months (January 2018 to June 2018). Ethical approval was taken from Institutional Review Committee (IRC) of NMCTH. A total number of 274 cases of anemia (on the basis of definition as per WHO criteria) along with reticulocyte count during the study period were included. Relevant clinical history of the patients was obtained from the patient's file. Children under 6 months, pregnant females and patients with chronic illness (heart diseases and kidney diseases) were excluded from the study.

Ethylene diamine tetra acetic acid (EDTA) anticoagulated blood sample of in patient and OPD patient was run in Sysmex 5 parts hematology automatic cell counter- XS- 500i. Investigations like Hb, Hct, RBC indices (MCV, MCH, MCHC and RDW-CV) were obtained. The slides for reticulocyte count were prepared by mixing the blood sample with equal amount of dye (New methylene blue).The mixture was then incubated at 37°c for 15 minutes and the film was prepared on the slide. After drying the slide, reticulocytes were counted under the oil immersion.

The value of Hb, Hct, RBC indices and reticulocyte count was obtained and entered in MS Excel 2003. Analysis was done using SPSS (Statistical Package for Social Sciences) version 16.

Age wise distribution of Hb, Hct, MCV, MCH, MCHC, RDW and reticulocyte count was found out. Mean of each variables were compared with different age group. ANOVA test was applied. The level of significance (∞) was set at 5% and p value < 0.05 was considered significant.

RESULTS

There were 975 samples of Complete blood count (CBC) and out of this 274 cases had anemia during the study period. The prevalence of anemia in our hospital during the study period was 28.1%. The age of the person with anemia ranged from 6 months to 93 years with the mean age of 41.40 ± 25.37. Anemia was more commonly seen in female (68.2%) than male (31.8%). (Fig. 1)
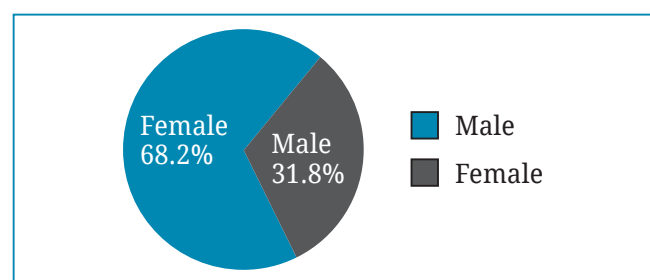

Fig. 1: Sex wise distribution of anemia

Anemia was most commonly seen in the age group ≥ 15 years (82.2 %) followed by age group, children < 5 years (14.6%). (Figure 2)
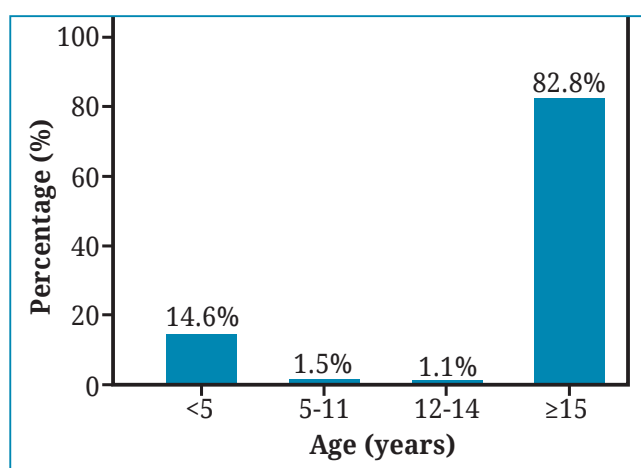

Fig. 2: Age wise distribution

Females of age group ≥ 15 years were found to be affected more with anemia (59.5%) followed by male (31.8%) in the same age group (Table-1).

Table-1: Age and sex wise distribution of anemia			
Age (years)	**Female** n **(%)**	**Male** n **(%)**	**Total** n **(%)**
< 5	19 (16.9)	21 (7.7%)	40 (14.6%)
5-11	2 (0.7%)	2 (0.7%)	4 (1.5%)
12-14	3 (1.1%)	0 (0%)	3 (1.1%)
≥ 15	163 (59.5%)	64 (23.4%)	227 (82.8%)
Total	187 (68.2%)	87 (31.8%)	274 (100%)

The mean Hct value was 29.39 ± 7.80. The mean Hct was significantly different among the age groups. Mean Hct level was lowest in children of 12-14 years and high in children < 5 years (Table-2).

Table-2: Agewise comparison of Hct levels			
Age (years)	**Mean ± SD**	**Sample Mean ± SD**	**P value**
< 5	32.93 ± 7.47		
5-11	30.72 ± 6.24	29.39 ± 7.80	0.018
12- 14	27.30 ± 4.15		
≥ 15	28.78 ± 7.77		

Comparison of mean MCV values in different age group was shown in Table-3. Low MCV (65.33 ± 5.46) was seen in children of age group 12- 14 years and high MCV was seen in age group, ≥ 15 years. This showed that microcytic hypochromic anemia was common in growing children and macrocytic anemia was common in adult patients.   Similarly, MCH was also very much decreased in children of 12- 14 years (Table-4). Decreased MCH is suggestive of hypochromic anemia.

Table-3: Agewise comparison of MCV values			
Age (years)	**Mean ± SD**	**Sample Mean ± SD**	**P value**
< 5	80.34 ± 12.73		
5-11	80.70 ± 5.65	108.60 ± 4.74	0.975
12- 14	65.33 ± 5.46		
≥ 15	114.64 ± 5.21		

Table-4: Agewise distribution of MCH values			
Age	**Mean ± SD**	**Sample Mean ± SD**	**P value**
< 5	25.57 ± 5.19		
5-11	25.77 ± 3.67	24.79 ± 5.26	0.185
12- 14	18.90 ± 2.00		
≥ 15	24.71 ± 5.28		

MCHC decreases when MCV is decreased as it is a measure of the average concentration of Hb inside a single RBC. MCHC was decreased in all age group except in group ≥ 15 years. The lowest value of MCHC was seen in children of 12- 14 years who showed low MCV (Table-5).

Table-5: Agewise distribution of MCHC values			
Age	**Mean ± SD**	**Sample Mean ± SD**	**P value**
<5	31.34 ±1.76		
5-11	30.40 ± 2.36	32.64 ±24.94	0.971
12- 14	28.96 ±2.31		
≥ 15	32.96 ±27.39		

RDW was increased in all age group. (Table-6) Increased RDW values indicate greater variation in size of the RBCs. The variation was more in case of children of 12- 14 years which showed the presence of microcytic hypochromic anemia in this age group.

Table-6: Agewise distribution of RDW values			
Age	**Mean ± SD**	**Sample Mean ± SD**	**P value**
<5	16.18 ± 2.61		
5-11	15.07 ± 1.37	16.47 ± 3.31	0.755
12- 14	17.03 ± 1.95		
≥ 15	16.54 ± 3.45		

Reticulocyte count was slightly reduced in children of 12 – 14 years. However in other age group it was within the normal range (Table-7).

Table-7: Agewise distribution of Reticulocyte			
Age	**Mean ± SD**	**Sample Mean ± SD**	**P value**
<5	2.59 ±2.16		
5-11	0.60 ± 0.40	1.41 ± 1.45	0.000
12- 14	0.40 ± 0.30		
≥ 15	1.23 ± 1.20		

When different variables are compared with male and female, Hct and MCH was significant. (Table-8)

Table-8: Sexwise comparison with Hct, MCV, MCH, MCHC, RDW and Reticulocyte

	Sex	(n)	Mean	Std. Deviation	P value
Hb	Male	87	8.700	2.6005	0.134
	Female	187	10.187	9.0383	
Hct	Male	87	27.651	7.7366	0.011
	Female	187	30.212	7.7158	
MCV	Male	87	83.295	13.4339	0.548
	Female	187	120.374	574.8973	
MCH	Male	87	25.879	5.6968	0.020
	Female	187	24.290	4.9817	
MCHC	Male	87	34.299	32.7216	0.455
	Female	187	31.874	20.4070	
RDW	Male	87	17.003	3.8797	0.072
	Female	187	16.229	2.9937	
Retics	Male	87	1.585	1.6605	0.196
	Female	187	1.340	1.3541	

DISCUSSION

Anemia affects both developing and developed countries and is more common in children and females of reproductive age group. It is such an important issue affecting a large population worldwide.[2] Blood cell index gives us quantitative assessment of RBCs. Advent of automated hematology cell counter has not only improved accuracy and precision but has also reduced subjective errors.[1]

The prevalence of anemia is an important health indicator.[4] The prevalence of anemia ranges from 30% to 98% in different studies from different regions.[5] Prevalence of anemia in Nepal was 36.2% in the year 2006 which was shown by WHO Global database on anemia[11]. In our study, anemia was seen in 28.1% cases among 975 CBC samples run during the study period. Similar finding was seen in a study done in eastern part of Nepal by Sinha *et al*[12] where the prevalence of anemia was 25.6%. Another study done in Nepal showed that the prevalence of anemia among school going children ranged from 31.6% to 45.3%.[13] Prevalence of anemia in different part of India is different ranging from 8.7% to 53.2%.[3,5,14] Bangladesh also gives similar prevalence rate of anemia which ranges from 43% to 49%.[15]

The mean age of the person with anemia was 41.40 ± 25.37 with minimum age of 6 months and maximum age of 93 years in our study. There was more number of cases in age group ≥ 15 years (82.8%). Anemia was more commonly seen in female (68.2%) than male (31.8%). A study done in different parts of India showed similar finding with more number of cases of anemia in adult age group where females were affected more.[6,7,14] Similar finding was seen in a study done in Bangladesh.[15] These findings were not in agreement with study done in Nepal, where more number of males had anemia.[16]

Hematocrit (Hct) is the volume percentage of RBC in blood. The level of Hct was compared among different age groups and found out that it was statistically significant. Rairikar *et al*[1] also showed that there was a positive correlation between Hb and RBC indices whereas negative correlation was seen between Hb and RDW. In another study done among children of different age groups, Khadiwada *et al* compared the level of Hb among different age groups and found that the study was significant.[13]

The classification of anemia is based on the alteration in the red cell morphology and it points to the particular cause. Morphological characteristics like normocytic, microcytic or macrocytic provides etiological clues.[3] In our study, low MCV (65.33 ± 5.46) was seen in children of age group 12- 14 years and high MCV (114.64 ± 5.46) was seen in age group, ≥ 15 years. This showed microcytic picture in children and macrocytic in person ≥ 15 years. Hence, it was seen that macrocytic anemia was the predominant type of anemia in our study. This finding was in contrast to the studies done by Jadhav *et al*[2] Qureshi *et al*[3] and Kerdany *et al*[17] in which microcytic anemia was the predominant type. Several studies had shown that microcytic hypochromic anemia was common and was suggestive of iron deficiency as the etiology.[5-8,18-20] Studies done in Nepal shows that iron deficiency is very common and it is most commonly seen in females as well as children.[12,19,21]

Macrocytic anemia was also quite common among older adults and comprises of almost 14% of all anemia cases.[20] Macrocytosis is the most commonly associated with vitamin B12, folic acid deficiency followed by alcoholism and liver disease. Study had also showed that alcohol and folic acid plays a secondary role in macrocytosis.[22] Though alcohol consumption is very common among patients who visit our hospital, due to limitation in our study regarding the alcohol intake history we could not conclude the cause of macrocytosis in the group ≥ 15.

In our study low MCH and low MCHC was very common in children of 12- 14 years. MCH is usually decreased in hypochromic anemia. Sex wise comparison of MCH in our study showed that it was statistically significant while other variables like Hct MCV, MCHC and RDW were not significant. Similarly low MCHC was seen in those with low MCV. MCHC was low in all groups except in group ≥ 15 years. This was the group where MCV level was high too. Contrast to our study, other studies showed that MCH and MCHC was decreased in most of the cases with anemia.[1,2,7] The MCH and MCHC was not statistically significant among the different groups in our study.

RDW is a parameter that measures variation in red blood cell size and RDW- CV is expressed in percentage. In our study, RDW was elevated in all the age groups with more elevation in children between 12- 14 years. This was the group with

decreased MCV, MCH and MCHC which was suggestive of microcytic hypochromic anemia. Associations of RDW with other variables were not found statistically significant. Similar finding was revealed in a study done by Jadhav *et al*.[7] Sultana *et al*[23] found that higher level of RDW was significant than MCV, MCH and MCHC in cases of microcytic hypochromic (iron deficiency anemia). In our study, the total number of cases of microcytic anemia was less as it was seen in children of 12- 14 years and there were fewer cases in this group. Choudhary *et al*[24] also showed that most of the cases of anemia showed abnormal RDW. There was increase in RDW level with microcytic anemia. Since iron deficiency anemia is the most common type of anemia RDW can be used as an effective tool for the diagnosis. RDW determination had high sensitivity and specificity for early diagnosis of microcytic (iron deficiency) anemia. RDW is usually not done as a part of CBC in developing countries. The use of RDW can be promoted for early detection and starting treatment of iron deficiency anemia.[24]

Reticulocyte count compared to different groups was statistically significant in our study. It was normal in all groups except in children of 12- 14 years where the reticulocyte count is low. Different range of reticuolyte count was seen in anemia according to the severity in a study done by Rairikar *et al*.[1] Studies had shown that the reticulocyte counts were usually increased in microcytic hypochromic (iron deficiency anemia).[2,5,9] The reticulocyte count indicate different aspects of erythropoietic control, balance of production of red cells in relation to their rate of destruction.[10] The reticulocyte is also increased in the recovery from a iron deficiency anemia or recovery of erythropoeisis after bone marrow suppression.[8,25]

Prevalence of anemia increases with age and in different age group, the prevalence of various types of anemia is different. Hematological parameters guiding the type of anemia are different in different age group. It gives us the idea about the morphological classification of anemia. In younger age group, the most common is microcytic hypochromic anemia and in adult it is macrocytic. RBC indices are very simple tests which can be easily obtained from automated hematology cell counter. They are very helpful in the classification of anemia and for planning the further management.

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