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Evaluation of anaemia in children using minimum haematological parameters and correlation of red cell distribution width with mean corpuscular volume for prediction of early stages of nutritional anaemia

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Abstract
We had discussed and classified childhood anaemia using minimum haematological parameters. Six months cross sectional study was done on 3033 children (under 15 years). Complete blood count (CBC) parameters were analyzed, with correlation of red cell distribution width (RDW) and mean corpuscular volume (MCV). 46.6% children were anaemic. Male: female ratio was 1.3:1. Anaemia burden was highest among children of age 11-15 years (n=560, 18.4%) followed by 0-5 years (n=500, 16.4%) with predominance of normocytic anaemia with high RDW (n=672, 47.4%) followed by microcytic anaemia with high RDW (n=463, 32.7%), indicating predominance of early nutritional anaemia in adolescents and frank iron deficiency anaemia in preschool children respectively. Maximum children had moderate (n=803, 56%), followed by mild (n=343, 24.2%) and severe anaemia (n=269, 19%). Of 381, 104 (27.3%) children with anaemia and thrombocytopenia, had high MCV, due to megaloblastic anaemia. Early nutritional anaemia represents tip of iceberg. RDW and MCV can predict early anaemia in limited resource settings. Earliest detection can definitely reverse condition.
Keywords: *anaemia, children, iron deficiency, mean corpuscular volume (MCV), megaloblastic, nutritional, red cell distribution width (RDW).*

Introduction

Anaemia is serious global public health problem often associated with increased risk of morbidity & mortality, especially in young children, adolescent girls and pregnant women. In 2013, global burden of anaemia affected 27% of world's population or 1.93 billion people. In developing countries, burden accounts for 89% of all anaemia related disability. Women of reproductive age group & preschool children seems to be particularly affected. In more than 60% cases, iron deficiency anaemia is the prime cause.[1] India being the largest hub of anaemia in world, accounts for approximately 147.9 million cases followed by China with 75.8 million & Nigeria 24.7 million cases.[2] In 2011, worldwide 43% of under-five children were anaemic, and prevalence in developing South Asia & East Africa were 58% & 55%, respectively.[3,4] In our study, we will analyze and classify anaemia in children, so as to address and prevent the dire consequences, which can otherwise be avoidable.

Materials and Methods

This cross sectional study was undertaken at Department of Pathology of our Government medical college and associated tertiary hospital on total 3033 children between zero to fifteen years of age, who visited our department for complete blood count (CBC) during January 2018 to June 2018. Before beginning this research, approval was obtained from the ethical committee of our institute.

Inclusion Criteria: All children between zero to fifteen years of age who visited our hospital's outpatient paediatric clinic and pathology department requiring CBC investigation.

Exclusion Criteria: Children suffering from

- Haemoglobinopathies
- Pancytopenia
- Chronic disorders like liver disorders, immunodeficiency syndromes
- Cancer
- Shock or severe blood loss
- or those who were on treatment for anaemia or seriously ill patients
- or those who were consuming multivitamins and/ or mineral supplements regularly

Children were sampled for CBC analysis by following method: Under all aseptic precaution, two milliliters (ml) venous blood was collected in EDTA vials, after obtaining informed consent from the parents of children who were included in the study. As generally preferred for children, winged steel needle, 23 or 23 gauge with an extension tube (a butterfly) was used. CBC analysis was performed in Automated LH 750 Haematology Analyzer. The values obtained by automated analyzers were regularly monitored by running controls daily, ensuring accuracy of the results obtained. Finally various parameters of CBC such as haemoglobin (Hb), Red blood cell count, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red cell distribution width (RDW), total white blood cells and platelet counts were recorded for every patient. The values thus obtained were plotted on Microsoft Office Excel spreadsheets for further analysis. Colour coding for normal, low and high values were decided and desired filters were applied for data extraction using Excel spreadsheets. We preferred standard World Health Organization (WHO) criteria to diagnose anaemia age and sex wise. Accordingly anaemia was defined as Hb concentration, less than (<) 11 gm/dl for children between 6-59 months of age, <11.5 gm/dl for 5-11 years and <12 gm/dl for 12-14 years. For various other haematological parameters, standard normal reference range were set, as defined for specific age and sex, and then our data was analyzed accordingly.

Results

A total of 3033 children were studied, of which 1415 (46.6%) had anaemia. Among anaemic patients, 798 (56%) were males and 617 (43.6%) were females. Male: female ratio was 1.3:1. Thus, number of males affected with anaemia were more as compared to females [Table 1].

Table 1: Age and sex wise distribution of Haemoglobin (Hb) values in children

Age	Normal Hb	Low Hb			High Hb
		Total	Males	Females	
0-5 years	43	500	292	208	357
6-10 years	324	355	204	151	143
11-15 years	511	560	302	258	240
Total	**878**	**1415**	**798**	**617**	**740**

Higher RDW (>14.5) values were noticed almost equally in males (n=720, 90.2%) & females (n=554, 89.7%) Most children were found to be affected by moderate anaemia (n=803, 56%) in our study, followed by mild (n=343, 24.2%) and severe anaemia (n=269, 19%). In age group 0-5 years, total 500 (16.4%) children were anaemic. Microcytic anaemia with high RDW predominated followed by macrocytic anaemia with high RDW [Table 2].

Table 2: Age wise distribution of morphological subtypes of anaemia with RDW correlation

MCV	Age wise distribution (in years)	RDW	
		High	Normal
Microcytic	0-5	191	1
	6-10	147	6
	11-15	125	3
	Total	**463 (32.7%)**	**10**
Normocytic	0-5	130	16
	6-10	158	12
	11-15	384	19
	Total	**672 (47.4%)**	**47**
Macrocytic	0-5	155	7
	6-10	32	0
	11-15	27	2
	Total	**214 (15.12%)**	**9**

Total 149 (30%) anaemic children also had associated thrombocytopenia, of which 81 had high MCV, possibly due to megaloblastic anaemia [Table 3]

Table 3: Age wise distribution of thrombocytopenia and macrocytic anaemia

Age	Total number of anemic children with thrombocytopenia (bicytopenia)	Total number of anemic children with thrombocytopenia & high MCV
0-5 years	149	81
6-10 years	98	10
11-15 years	134	13
Total	**381**	**104 (27.3%)**

In age group 6-10 years, total 355 (11.7%) children were anaemic while in 11-15 years, 560 (18.4%) children were anaemic. Thus maximum anaemic children were in age range 11-15 years followed by 0-5 years and minimum were in age group 6-10 years [Table 1]. In age group 6-10 years and 11-15 years, normocytic anaemia with high RDW predominated [Table 2]. 98 (27.6%) anaemic children between 6-10 years and 134 (24%) between 11-15 years had associated thrombocytopenia, out of which, 10 and 13 children between 6-10 years and 11-15 years respectively, had high MCV suggesting megaloblastic anaemia as etiology. Thus, 104 out of 381 (27.3%) children with anaemia and thrombocytopenia, also had high MCV, probably due to megaloblastic anaemia. [Table 3].

Discussion

Anaemia is classified on basis of etiology (nutritional, hemolytic etc), morphology (normocytic, microcytic or macrocytic) or erythropoietic response (hypoproliferative, ineffective). Red cell indices play an important role in morphological typing of anaemia[5]. As different anaemia has different and characteristic impact on size of red cells, hence just by analyzing size of red cells either with help of electronic counters or by analyzing peripheral smears, we can easily classify anaemia. It is very necessary to classify anaemia so as to introduce the specific treatment in patient's management regime.

Morphological classification of anaemia is based on the size of red cells which in turn is reflected by Mean Corpuscular Volume (MCV). Thus morphologically three types of anaemia are: normocytic (normal MCV), microcytic (decreased MCV) and macrocytic (increased MCV). Respective Mean Corpuscular Hemoglobin (MCH) values closely parallels the value of MCV. Microcytic hypochromic anaemia may either be due to really low Mean Corpuscular Hemoglobin Concentration (MCHC) or due to optical properties of thin microcytes which make them appear hypochromic on smears while on electronic counters may create discrepancy as microcytic normochromic picture due to normal MCHC. Addition of one more parameter Red Cell Distribution Width (RDW) helps to further typify various anaemia [Table 4][5].

Table 4: RDW correlation with MCV and interpretation[5]

MCV	**High RDW**	**Normal RDW**
Decreased MCV (Microcytic)	Iron deficiency or Red cell fragmentation	heterozygous thalassemia/ anaemia of chronic disease
Normal MCV (Normocytic)	Early iron deficiency/ Early megaloblastic/ Sideroblastic/ Myelophthisis/ combined deficiency/ Sickle cell anemia/ Immune hemolysis/ secondary to chemotherapy	Poor iron utilization/ Acute blood loss/ Acute hemolysis/ Enzyme defects (e.g., G6PD deficiency)/ Liver disease
Increased MCV (Macrocytic)	Megaloblastic/ Sideroblastic/ Myelodysplastic/ Secondary to chemotherapy	Liver disease/ Aplastic anemia/ Myelodysplastic syndrome

RDW depicts degree of anisocytosis & increases proportionately with the severity of iron deficiency anaemia. It has been reported that in early iron deficiency anaemia, RDW is the first parameter to be deranged, following reduction in iron stores. It's reported sensitivity is 90-100% and specificity is 50-70%. In our study, 720 out of total 798 males i.e. 90.2% and 554 out of 617 females i.e. 89.7% had higher RDW which is almost similar to the results obtained in the study done by Khatri et al[6] who have reported raised RDW in 95% males and 97% females. But the point that needs to be emphasized here is that no electronic analyzer can replace the role of pathologist analyzing the peripheral smear evaluating various other parameters such as red cell membrane abnormalities, nucleated red blood cells, inclusions, atypical cells including blasts and various haemoparasites

In our study, total number of anaemic patients in age group 0-5 years were 500 (16.4%), 6-10 years were 355 (11.7%) and 11-15 years were 560 (18.4%) as compared to India's Annual Health Survey report 2014, which has reported anaemia prevalence in Chhattisgarh, as 63.8% in age group 6-59 months, 78.5% in 6-9 years and 71.5% in 10-17 years. This survey, similar to our study, has also indicated higher prevalence of anaemia in adolescent children as compared to under fives. However, in our study, limited sample size reflected less anaemia load as compared to Annual health survey report 2014, that might have represented actual scenario in a better way due to analysis on much larger sample population than that of ours. In our study, total 343 (24.2%) children had mild anaemia, 803 (56%) had moderate and 269 (19%) had severe anaemia. This was similar to the results obtained in the study done by Ncogo et al[7] which reported 24%, 67% and 9% children with mild, moderate and severe anaemia, respectively.

In age group 0-5 years, anaemic children with low MCV and high RDW were 191 which constituted the most common derangement noted in this population. Low MCV and high RDW is indicative of either iron deficiency anaemia or red cell fragmentation [Table 4]. According to the study done by Bessman et al[8] in 2350 subjects, the two most important causes of abnormal red cell fragmentation are due to malignancy with ongoing cytotoxic therapy and severe iron deficiency anaemia. Other causes being disseminated intravascular coagulation, microangiopathic hemolytic anaemia and severe burns. As we had already excluded severely ill and cancer patients from our study, it can be assured that iron deficiency anaemia remains the paramount cause of anaemia in this tender age group. High MCV reflects macrocytic anaemia, the causes of which include megaloblastic anaemia, sideroblastic anaemia, aplastic anaemia, liver disease, myelodysplastic disorders, secondary to chemotherapy. Total 162 children under five years had high MCV, of which 155 had high MCV and high RDW. Predominant cause of such combination includes megaloblastic anaemia, sideroblastic anaemia or aplastic anaemia [Table 4]. Further, of these 155 children with macrocytic anaemia, 81 also had thrombocytopenia which indicates megaloblastic anaemia, thus constituting 52.2% of all macrocytic anaemia and total 16.2% of all anaemia under five years of age. Total 130 children had normal MCV and high RDW, the most significant cause of this combination includes early iron and early megaloblastic anaemia [Table 4]. Thus, in our study, nutritional anaemia was the most common type of anaemia observed, affecting children under five years of age. However, for definite diagnosis of iron deficiency anaemia or megaloblastic anaemia, iron studies or vitamin B12 and red cell folate assays respectively, are must. In a government tertiary care center, still the majority of patients belong to ignorant, less educated and low socio economic strata. These patients are generally reluctant to make health of their children a priority, unless they develop serious ailment. They not only deprive them of primary care but also regular medical follow up. In our study, we thus failed to motivate such patients for special investigations.

According to Strauss et al[9], nutritional status particularly in children of 0-5 years is considered as one of the most important index of child survival and household's living standard. The most important cause of iron deficiency anaemia is increased iron demand due to rapid growth[3], particularly, in age group four to six months, when all the reserved iron stores in infants have depleted and the child completely becomes dependent on dietary iron supply[10]. Another significant factors are less education, low socio economic status, and weak mothers due to inadequate dietary intake. Due to lack of nutritional education, mothers introduce nutritionally poor foods early, and avoid internationally recommended guideline to exclusive breast feed up to six months of age [11,12], thus leading to iron deficiency anaemia[4]. Iron deficiency anaemia affects cognitive and motor development and increased susceptibility to infections. So to prevent this, it is necessary to introduce iron fortified cereals and meat products rich in vitamin B12 in the community[4]. According to WHO guidelines, at home, MNP, a single-dose powder packet containing iron, zinc, vitamin A, other vitamins and minerals, can be used to sprinkle on any semisolid food so as to increase essential nutrient's content in infant's diet without changing usual dietary habits[13] This along with regular supplementation of iron folic acid tablets/ syrup to children can entirely change the scenario of entire world. The main concern here is that despite the initiative taken by the government to provide Iron folic acid (IFA) tablets/ syrup to children (aged 6-35 months), it's been evident by Annual health Survey report 2011-12, which state that only 38.7% of the total population of children in Chhattisgarh, between age 6-35 months, actually received IFA supplementation. This indicates severe deficit in both ends, i.e. receiving and dispensing, and that needs to be addressed on urgent basis.

In age group 6-15 years, we found normocytic anaemia with high RDW predominated indicating early iron deficiency/ early megaloblastic/ sideroblastic/ myelophthisis/ combined deficiency/ sickle cell anemia/ immune hemolysis or secondary to chemotherapy [Table 4]. Amongst all these, only nutritional anaemia again could have been the most common cause, as the children suffering from rest of the serious ailments, cancer and haemoglobinopathies were already excluded from our study. The important point to note here is that in children aged 6-15 years, due to predominance of normocytic anaemia with high RDW, we can deduce that such children were suffering from early stages of anaemia, hence frank microcytosis or macrocytosis couldn't manifest. However, in age group 6-10 years, of 32 anaemic children with high MCV, 10 also had thrombocytopenia; while in age group 10-15 years, of 27 anaemic children with high MCV, 13 had associated thrombocytopenia, suggesting frank megaloblastic anaemia as etiology. Overall, in age group 0-15 years, out of total 214 children with macrocytic anaemia, majority i.e. 104 (48.5%) suffered from megaloblastic anaemia associated with thrombocytopenia. Lack of access to necessary micronutrients in dietary habits[3] and lack of hygiene and parasitic infestation(14) can definitely be blamed to cause nutritional anaemias in young children. Infection due to intestinal helminths and *plasmodium* causing malaria are the most common, preventable, infectious causes of anaemia in children[15,16], especially in endemic countries like India. Regular distribution of healthy nutritious supplements, regular de-worming treatment of children and exhaustive malaria control measures should be endorsed nationwide[17]. By implementing these measures and paying little attention to children of age group 6-15 years, better is the possibility that anaemia load and associated morbidities can be drastically reduced.

Overall in our study most common morphological type of anaemia in children was normocytic with high RDW followed by microcytic anaemia with high RDW [Table 2]. High RDW values thus can classify anaemia and diagnose early nutritional anaemia in a population with limited resources. Also this research indicates that, early stages of iron and megaloblastic anaemia were much more common, and hence remain undiagnosed, than established anaemias [Table 2, 4][5] This imply that these early anaemia cases represent just a tip of iceberg of anaemia in our community. Thus we need exhaustive workup to screen all children such that earliest detection can reverse the condition simply by implementing healthy food supplementation and other measures as already discussed.

Conclusions

Hence, we can deduce that by correlating RDW with MCV, early stages of nutritional anaemia can be predicted. This simple correlation can be utilized for anaemia screening, especially in limited resource settings or remote areas with no/ limited access to special investigations. This study also reflects the need of nutritional status upliftment of such children along with an urgent necessity to secure house hold socio-economic conditions such that in a developing nation like India, and particularly in a state which is also known as "Rice bowl of India", no child or any person remains deprive of proper balanced nutrition.

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