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ORIGINAL ARTICLE

RED CELL DISTRIBUTION WIDTH AS A SCREENING TOOL IN CLASSIFYING MICROCYTIC HYPOCHROMIC ANEMIAS

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ABSTRACT: Prevalence of anemia in India is a well known fact. The National Family Health Survey-3 (NFHS-3) data suggests that anemia is widely prevalent among all age groups and is particularly high among the most vulnerable – nearly 58 per cent among pregnant women, 50 per cent among non-pregnant and non-lactating women, 56 per cent among adolescent girls (15–19 years), 30 per cent among adolescent boys and around 80 per cent among children under 3 years of age. Major cause of which is iron deficiency anemia followed closely by thalassemia syndrome with the latter having a prevalence of 10.4%. Present study was conducted to find out whether a small parameter called RDW(Red Cell Distribution Width) could be helpful in differentiating between two major causes of anemia i.e. Iron deficiency anemia & thalassemias. This study was conducted on 660 patients having anemia. Out of 660 patients, 287 patients had microcytic hypochromic anemia. 101 patients could not be classified into any of the above. In remaining 186 cases, RDW as a test showed a sensitivity of 82.27% in classifying microcytic hypochromic anemia as iron deficiency anemia. Thus, by this study we tried to showcase the utility of RDW as a simple, economical and a screening tool in evaluation of microcytic hypochromic anemia.

KEYWORDS: RDW, IDA, thalassemia, MCV, microcytic hypochromic anemia.

INTRODUCTION AND AIMS: Complete blood counts have become the backbone to almost all clinical diagnostic elucidation. RDW as a parameter is calculated by virtually all the modern hematology analyzers available. Current study was carried out to elucidate the role of red cell distribution width (RDW) in subtyping the different causes of microcytic hypochromic anemia and check its potential in segregating iron deficiency anemia from thalassemia syndromes.

Anemia is a public health problem that affects populations in both rich and poor countries and is widely prevalent amongst all the ages. Although the primary cause is iron deficiency, it is seldom present in isolation. India is among the countries with high prevalence of anemia in the world.[1,2] The present study basically tried to investigate whether in context of huge burden of anemia in our country, can we simplify our approach towards detecting the same with stress on use of red cell distribution width (RDW) as a primary means by which the same may be envisioned.

Three factors are responsible for the pathogenesis of IDA: a) Impaired Hemoglobin synthesis- as the serum iron falls & transferring saturation achieves a critical value of <15%, the iron is so depleted that Hb production is impaired resulting in microcytosis first followed by hypochromia of red cells. b) Impaired cellular proliferation - This is reflected in the marrow erythroid hyperplasia which is not proportionate to the degree of anemia. c) Diminished iron containing proteins - Many of the enzymes like cytochrome c, cytochrome oxidase and myoglobulin are reduced leading to the epithelial changes seen in IDA.

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IDA progresses through three stages before manifesting clinically as iron deficiency anemia. The first stage, also called prelatent iron deficiency or iron depletion, represents a reduction in iron stores without reduced serum iron levels. This stage is usually detected by a low serum ferritin measurement. Latent iron deficiency is said to exist when iron stores are exhausted but the blood hemoglobin level remains higher than the lower limit of normal. The mean corpuscular volume usually remains within normal limits, but a few microcytes may be detected on a blood smear. Finally, in the third stage, the blood hemoglobin concentration falls below the lower limit of normal and iron deficiency anemia is apparent.[3]

Further, a case of iron deficiency anemia is evaluated by the following investigations:- S. ferritin, S. iron, S. transferrin saturation, S. transferrin receptor assay, red cell indices, bone marrow iron evaluation & erythrocyte protoporphyrin. Treatment of a diagnosed case of iron deficiency anemia is by either oral iron preparations or parenteral therapy.[4]

The thalassemia syndromes are a heterogeneous autosomal recessive group of disorders caused by inherited mutations that decrease the synthesis of adult hemoglobin HbA[4].

Key point to be remembered is that the hemoglobin chains synthesized are of normal structure. According to the chain whose synthesis is impaired, the thalassemias are called α-, β-, γ-, δ-, δβ-, or ∈γδβ-thalassemias. Another point to be made is that it is not the lack of the affected globin chain but the accumulation of the unaffected ones that cause hemolysis (in α-Thalassemia) & ineffective hematopoiesis (primarily in β-thalassemia)[4, 5]

In India, it is found throughout the country with highest incidence noted in certain communities such as sindhis, Punjabis, Bengalis, gujaratis and lohanas.[6] In India, combined carrier rate of β-Thalassemia, Hemoglobin E & sickle cell disease is 3.9%. In a multicity study, prevalence of 2.7%, 5.5% & 10.4% were found in school children of Mumbai, Delhi & Calcutta respectively.[4, 7] In β^{+} thalassemia, β- globin chains are present but reduced in quantity because molecular defects have resulted in production of unstable or decreased amounts of m-RNA. In β^{0}-thalassemia, β -chain synthesis is absent.

A further consequence of the red blood cell membrane damage is the loss of the normal asymmetric distribution and increased surface exposure of the procoagulant, negatively charged phospholipids phosphatidylserine and phosphatidylethanolamine. The anionic phospholipids increase thrombin generation, which leads to activation of platelets and endothelial cells. Activated monocytes and granulocytes, found in patients with thalassemia, could contribute to the endothelial damage and the hypercoagulable state[3]

Cases of β-thalassemia major, present within first year. At birth, baby is asymptomatic because HbF level is high but after 3months, HbF production wanes & β-chains are not produced resulting in anaemia, failure to thrive, intermittent infections and poor feeding. Untransfused or poorly transfused children succumb to infections & cachexia usually within 4-5 years of age. In regularly transfused patients, iron overload can result in further complications and hence the need for iron chelation15. Other then the reduced hemoglobin & red cell indices & few with increased red cell counts, a typical case of thalassemia presents with microcytic hypochromic anemia on peripheral smear along with presence of target cells, basophilic stippling, howell jowell bodies and nucleated red blood cells. Bone marrow is markedly hypercellular with erythroid hyperplasia usually of normoblastic type & M:E ratio reversal to 1:1 to 1:2. Features of dyserythropoiesis are seen. Myelopoiesis & megakaryopoiesis are normal.[3, 4, 5]

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To evaluate such a case, other than a peripheral smear, iron profile, hemoglobin electrophoresis at alkaline/ acidic pH & confirmatory investigation like high performance liquid chromatography can be done.[5] To detect fetuses carrying point mutations for thalassemia, antenatal chorionic villous sampling or cord blood HPLC can be undertaken.[7, 8]

MATERIALS & METHODS: Our study was conducted on the indoor patients that were admitted on routine basis in our New Civil Hospital, Surat in the period extending from September2011 – November 2013. Inclusion criteria that is used in this study are:- a)All anemic patients. b) All patients with Mean Cell Volume (MCV) <80fl. Exclusion criteria that were kept in mind while performing the study include:- a) Patients having history of recent blood transfusions. b) All patients with known chronic diseases.

Random patients were selected from the requests sent by the different clinicians, their personal details noted upon & the fresh blood sampled in EDTA bulbs of the same were used to perform the routine hematological investigations as:- a) Complete Blood Counts including measuring Hemoglobin, red blood cell counts, hematocrit, red cell indices including Mean Corpuscular Volume (MCV), Mean Hemoglobin Concentration(MHC), Mean Cell Hemoglobin Concentration(MCHC) and lastly, red cell distribution width(RDW) using Sysmex KX21i, 3- part cell counter with standard calibration. b) Blood smears of the same were made & stained using giemsa stain to evaluate the peripheral cell morphology.

On evaluation of the above mentioned tasks, using WHO defined criteria for anemia, males with Hb< 13 g% & females with Hb <12g% were selected for the study.

These patients were further checked for their Red Cell Indices especially MCV and all those with MCV values <80fl were further evaluated using peripheral smear morphology. All cases of macrocytic anemia, post hemorrhagic samples & others were hence taken out from the specific study group at this stage.

Now, significant causes of microcytic hypochromic cases were further followed up by getting requisite investigations done in cohort with the concerned clinicians, sickle cell laboratory & Surat Raktdan Kendra Centre, Surat. Iron profile in the form of serum ferritin was done as and when feasible keeping in mind the financial constraints of the patient. High Performance Liquid Chromatography (HPLC) for detecting thalassemias & other hemoglobinopathies was employed using HPLC machine by BIORAD.

The diagnosis of iron deficiency was established with reduced serum ferritin levels. In cases where, bone marrow examination was done, in addition to above, bone marrow iron stores were used as criteria to diagnose iron status of patients. Cut off values for low hemoglobin has been mentioned earlier. Cut off values for high red cell distribution width (RDW) was defined as a value more than 16%. S. ferritin <15 µ/l was considered as cut-off ruling in favor of iron deficiency anemia.

ETHICS: Samples collected for the study were original samples derived from the patients.
STASTISTICS: Chi - square test was employed.

DISCUSSION: Our study comprised of intake of 660 cases of anemia as defined by the cut –offs set by WHO & evaluating them on the basis of mean cell volume (MCV) to further categorize them into microcytic anemia & after a slew of tests performed to come to a diagnosis, detecting whether red cell

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distribution width holds its ground as far as differentiating the two most common causes of microcytic hypochromic anemia are concerned.

Out of 660 cases screened for anemia, 287 belonged to the microcytic variety & our investigations were directed in their favor only to come to a possible diagnosis of the same (Fig. 1). Around 76% cases were later on diagnosed safely as those of iron deficiency anemia followed by approximately 45 cases of thalessemia syndromes including β-thalassemia trait, β-thalassemia major & other major hemglobinopathies (Fig. 2)

For this purpose we employed, iron profile, basically serum ferritin, bone marrow iron store levels for ruling in favor of iron deficiency anemia & high performance liquid chromatography, wherever possible to rule out thalassemia or sickle positive cases.

Many cases of anemia that were initially part of the study due to low hemoglobin, were excluded on the basis of higher than prescribed values of MCV due to varied causes like, macrocytosis due to any cause, etc. Further it was seen that there was conflicting data in cases of sickle cell positive cases. Many of them had MCV lower than 80 fl but since the literature we referred & things known to us keep them in the category of a normocytic anemia, hence they were not taken in the specific study group. There was a dropout of 101 patients that could not be diagnosed due to other reasons (Table 5).

A β-thalassemia trait was usually suspected with increased RBC values but this finding was not very conclusive. Red cell distribution widths of all the microcytic cases was deeply studied & our findings have been summarized (Tables 1, 2, 3, 4). In all, Red Cell Distribution Width(RDW) as a test showed a sensitivity of 83% & 82.27% in classifying microcytic hypochromic anemia & iron deficiency anemia respectively (fig. 3 & 4) (Tables 1 & 2).

Thus, the present study goes in favor of previous study by Flynn et al.[9, 10, 11] While the same had a sensitivity of 46.67% in truly diagnosing beta thalassemia trait with significant p-values. Another point to ponder upon is that usually thalassemia syndromes exhibit not as great an elevation in RDW as seen in cases of iron deficiency anemia (Fig. 3 & 5) (Table 3).

Red cell distribution width has provided an automated method of detecting the heterogeneity of distribution of RBCs in a particular case. This measure of heterogeneity was documented by Bessman et al[12] to divide microcytic anemia into two categories. Those with elevated RDW included iron deficiency anemia, δβ –thalassemia, HbH disease & those with normal RDW as comprised of heterozygous thalassemia & anemia of chronic disease. Differentiating the two most important causes in question is indeed every clinician's headache.

Now we must remember that bone marrow iron studies are invasive as a procedure & serum ferritin is an expensive tool in work up of a case of iron deficiency. Further, high performance liquid chromatography though very sensitive, but does have a cost factor of its own plus the unavailability of the machine at many large centers too. Here comes the importance of such a routine tool in red cell distribution width that is calculated for each and every patient and can be easily interpreted in cohort with red blood cell parameters and a good knowledge of peripheral smear to come to a likely diagnosis.

CONCLUSION: Our study does show the significance of red cell distribution width as an initial screening tool in every case of microcytic hypochromic anemia. Since there is a huge prevalence of iron deficiency anemia & thalassemia in our population, an approach can be adopted in which the

complete blood counts, peripheral smear morphology & RDW can be used to provide the first level of screening, then therapeutic trial of iron can be given. RDW can be monitored to see the outcome of the therapy. If the patient does not respond, further iron studies & hemoglobin electrophoresis can be advised.

Our study does show to some extent in good light the likely hood of red cell distribution width as a initial screening tool, if not diagnostic, at least as far as iron deficiency cases are concerned.

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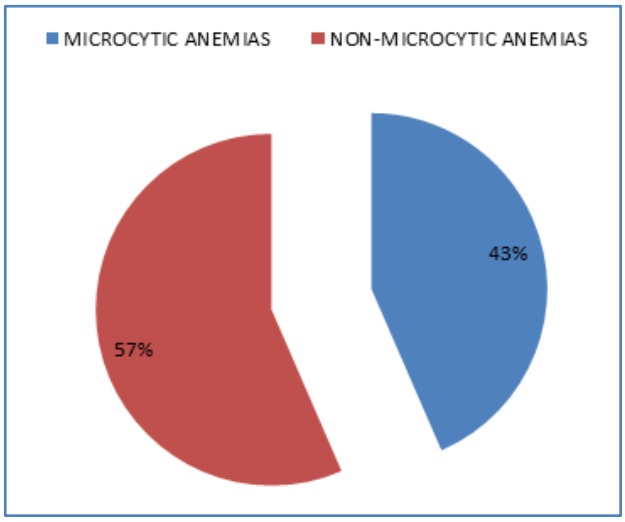

FIG. 1: SHOWING DISTRIBUTION OF ANEMIA ON THE BASIS OF MCV (MEAN CORPUSCULAR VOLUME)

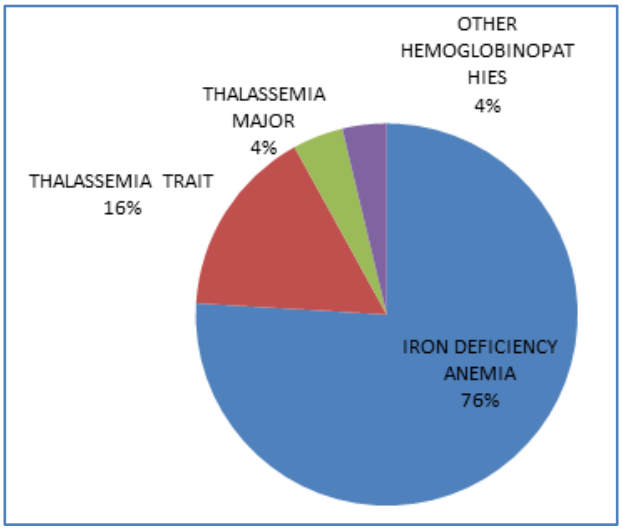

FIG. 2: DISTRIBUTION OF CAUSES OF MICROCYTIC ANEMIA

TYPE	INCREASED RDW	NORMAL RDW
IDA	116	25
THALASSEMIA TRAIT	14	16
THALASSEMIA MAJOR	5	3
OTHER HEMOGLOBINOPATHIES	6	1
Chi-square test value-17.2; p-value(2-tail)-0.00003		

TABLE 1: SHOWING DISTRIBUTION OF RDW IN CASES OF MICROCYTIC ANEMIA

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RDW	IDA	NON-IDA
INCREASED	116	25
NORMAL	25	20
Chi-square test- 13.27; p-value(2-tail)-0.00026		

TABLE 2: SHOWING DISTRIBUTION OF RDW IN CASES OF IDA

RDW	THALASSEMIA TRAIT	NON-THALASSEMIA TRAIT
INCREASED	14	127
NORMAL	16	29
Chi-square test-16.56;p-value(2-tail)- 0.000047		

TABLE 3: SHOWING DISTRIBUTION OF RDW AMONG THALASSEMIA CASES

RDW	DECREASED MCV	NORMAL MCV
INCREASED	293	118
NORMAL	60	162
Chi-square test-114.5;p-value(2-tail)- 0.0000001		

TABLE 4: SHOWING EFFECTIVENESS OF RDW AS A SCREENING TOOL FOR MICROCYTIC ANEMIAS

MICROCYTIC ANEMIA	NO. OF CASES
IRON DEFICIENCY ANEMIA	141
THALASSEMIA TRAIT	30
THALASSEMIA MAJOR	8
OTHER HEMOGLOBINOPATHIES	7
UNDIAGNOSED DUE TO OTHER REASONS	101

TABLE 5: DISTRIBUTION OF CAUSES OF MICROCYTIC ANEMIAS

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