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Implementation of RBC Scatterogram for diagnosis and to evaluate treatment response in Iron deficiency Anemia.

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Abstract

OBJECTIVE- The aim of this study was to evaluate the significance of RBC Scatterogram using an Automated Hematoanalyzer in the diagnosis of Iron deficiency Anemia (IDA) and to see the treatment response in comparison to other conventional iron parameters.

MATERIAL AND METHODS - A total of 10 adult patients with Iron Deficiency Anemia (Serum Hemoglobin <11g/L and serum ferritin <15ng/mL) were enrolled.

RESULTS –Scatterogram in IDA patients consisted mostly microcytic RBCs which gradually changed to normocytic RBCs with treatment response. RBC Scatterogram showed a significant positive correlation with Hemoglobin, MCV & RDW.

CONCLUSION – Our data demonstrate that RBC Scatterogram is a useful parameter that can be confidently used in the diagnosis of IDA , and also in evaluating its response to treatment.

CONFLICT OF INTEREST – None detected.

(Key words- RBC scatterogram ,hematoanalyzer, Iron deficiency Anemia)

1. INTRODUCTION-

Anemia is an enormous problem worldwide, and consequently the complete blood count (CBC) is the most frequently requested laboratory investigation. The actual value of haemoglobin, expressed in g/dL, is used to define anemia, but does not provide any information about its probable cause. In order to guide clinicians regarding the possible underlying pathology, the assessment of erythropoiesis, which forms an integral part of the CBC, is required.

Iron deficiency (ID) is well recognized as the most common nutritional deficiency [1,2] disorder in the world[3] and the principal cause of global anemia,[4] leading to adverse sequelae, namely, growth retardation, [5,6] neurocognitive deficit,[1] impaired immune system,[3]and increased risk of prematurity and maternal mortality.[7]Early detection is, thus, crucial for proper and timely management to prevent such consequences. Routinely we use low hemoglobin, low MCV/MCH, high RDW in CBC and low Serum ferritin, for diagnosis of iron deficiency anemia and increase HB and increase in serum ferritin look for response to treatment. Problem with serum ferritin is this also an inflammatory marker and hemoglobin does not change significantly immediately. So we proposed a simple method like scatterogram change in RBCto find out early response to iron deficiency anemia to treatment.

2. METHODS-

Aims and Objectives- Single test RBC scatterogram can help in diagnosis & treatment response of iron deficiency anemia.

This study was conducted in MKCG Medical college and Hospital, Brahmapur, Odisha from May 2021 to June 2021

Inclusion criteria : .

The sample consisted of ten diagnosed cases of Iron deficiency anemia, aged from 10 years to 45 years of both sexes who were havingMicrocytic Hypochromic Anaemia, and was confirmed as cases of iron deficiency anemia by biochemical tests (Serum Ferritin, Serum Iron, TIBC). These cases were reviewed and followed up after treatment of Iron Deficiency anaemia for evaluation of response in scatterogram .

Exclusion criteria: Patients having chronic blood loss.

This was a prospective pilot study, performed at MKCG MEDICAL COLLEGE & HOSPITAL. An ethical approval was obtained from the Ethics Committee for Human Research of MKCG Medical college & Hospital, Berhampur University. Blood samples from the study group of population were obtained usingtubes containing ethylenediaminetetraacetic acid (EDTA) for CBC analysis and in regular tubes for biochemical tests. The hematology analysis of the samples was performedusing a Sysmex XN 550N automated hematology analyzer.Reticulocyte counts were generated by Sysmex XN.Biochemical testing (typically included ferritin, serum iron, TIBC) to confirm the iron status was carried out using a turbidometry.

Anemia was defined, following the WHO criteria, by hemoglobin (Hb) level <120 g/L in women (W) or <130 g/L in men. Iron status was defined by serum ferritin as the reference method. Low

serum ferritin (<33.71 pmol/L or 15 ng/mL according to the World Health Organization [WHO]; 1 ng/mL = 2.247 pmol/L) signified Iron Deficiency in both women and men. [8]

3. RESULTS & OBSERVATIONS-

Table 1 *:Biochemical tests of Ten patients (denoted as alphabet A to J) for confirming IDA*

PARAMETRES	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Ferritin (mcg/L)	7	8	9	11	10	12	11	10	13	12
TIBC (mcg/dl)	440	420	410	380	360	356	370	380	386	392
Serum Iron (mcg/dl)	10	11	13	15	14	16	15	14	15	15

Table 2 :*CBC of Ten patients (denoted as alphabet A to J), both at the time of diagnosis of IDA and after treatment.*

PARAMETRES	**A**		**B**		**C**		**D**		**E**		**F**		**G**		**H**		**I**		**J**	
HB	4.1	5.7	5.5	7.7	6.1	8.7	9.7	11.1	8.8	10.1	9.1	10.9	8.5	10.8	7.8	9.6	8.2	10.2	8.0	9.9
RBC	2.51	2.69	2.90	3.13	3.55	3.61	4.28	3.76	3.96	4.25	3.91	4.53	3.65	4.35	3.75	4.29	3.95	4.25	3.5	4.21
HCT	14.8	16.7	18.7	24.7	22.7	29.3	29.9	33.4	25.5	32.8	27.5	34.5	25.1	33.6	23.8	31.8	25.6	32.5	24.1	32.5
MCV	58.9	62.1	64.4	78.9	63.9	81.4	69.7	88.8	64.4	77.2	70.3	76.2	68.8	77.2	63.4	74.2	64.8	76.5	68.9	77.2
MCH	**16.3**	21.2	18.9	24.6	17.2	24.2	22.6	29.5	22.2	23.8	23.3	24.1	23.3	24.9	20.8	22.4	20.8	24.0	22.9	23.5
MCHC	**27.8**	36.3	29.4	31.2	26.9	29.7	32.4	33.2	34.5	30.8	33.1	31.6	33.9	32.1	32.8	30.2	32.0	31.3	33.2	30.4
RDW-CV	**22.6**	17.8	19.1	16.7	20.2	17.5	18.4	14.2	18.5	**15.2**	17.9	15.1	19.1	14.8	20.4	14.6	19.3	16.6	18.8	15.5
RETIC	**0.17**	0.16	0.03	0.02	0.07	0.04	0.05	0.05	0.03	**0.06**	0.06	0.04	0.07	0.05	0.09	0.06	0.06	0.05	0.07	0.06
RETIC %	7.1	6.20	1.19	0.65	2.18	1.31	1.28	1.38	1.01	**1.50**	1.71	1.02	2.1	1.19	2.50	1.60	1.63	1.41	2.1	1.6

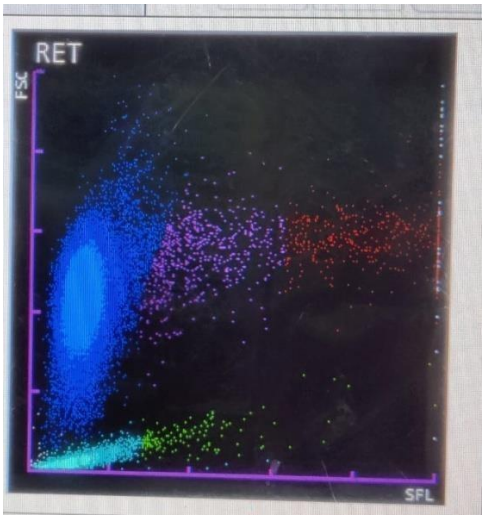
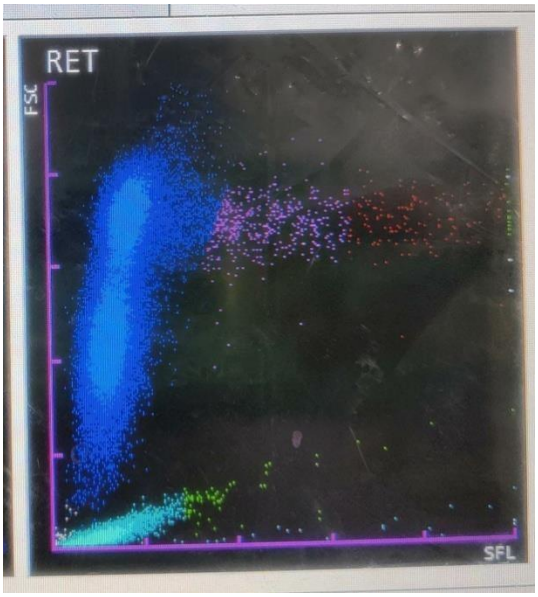


Figure 1 RBC Scatterogram. : At the time of diagnosis of IDA



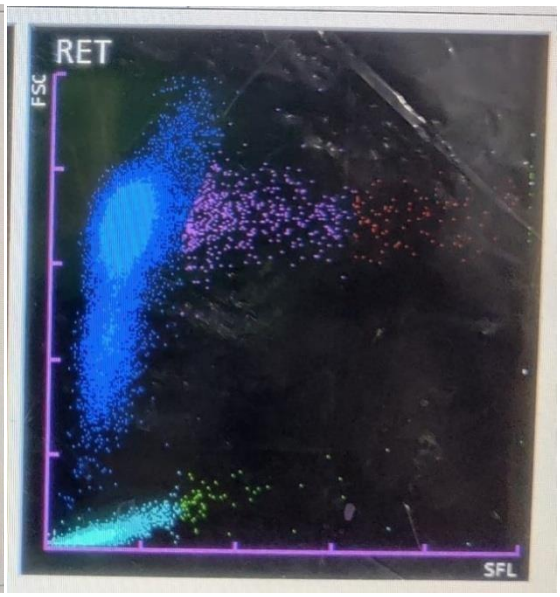


Figure 2:RBC scatterogram After 2 week of treatment response , Figure 3: RBC scatterogramAfter 1 month of treatment response.

In figure 1, At the time of diagnosis of Iron Deficiency Anemia (IDA) , the RBC Scatterogram is showing mostly the microcytic population of RBCs with marked anisocytosis. There is also presence of immature reticulocyte response due to underlying anemia.

After diagnosis of IDA the patient is being treated withhematinics and is followed up with CBC after 2 weeks and 1 month duration to look for the treatment response. After 2 weeks of treatment, the RBC scatterogram, in *figure 2* is showing two distinct population of RBCs consisting of both normocytic and microcytic type. The newly formed RBCs are now of normocytic type with normal hemoglobin content. The reticulocytes are now mostly mature

reticulocytes and no more immature reticulocytes are seen in the peripheral blood. The microcytic RBCs are the old RBCs which are present in the patient's blood as lifespan of RBCs are 120 days. After 1 month of treatment, RBC scatterogram in *figure3* , is showing further increase in normocytic population of RBCs with normal reticulocyte response with a small microcytic population which constitutes the old RBCs.

4. DISCUSSION-

The iron parameters that were assessed together with complete blood count to diagnose IDA include serum Fe level, serum ferritin level, TIBC, and TSAT.Automated analysis permits assessment of erythropoiesis at three levels :-

a) Mature erythrocytes

The traditional red blood cell (RBC) indices, namely Mean Cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC), have formed the cornerstone of categorisinganaemias into broad classes in order to direct further investigations as to the probable underlying cause. One of the biggest challenges is to monitor response to treatment, or more often to elucidate why there is a lack of response to conventional therapy. The major limitation of relying on the traditional red cell parameters to assess response is the fact that they only give an indication of average red cell status over the past 120 days, which is the life span of red blood cells.

b) Reticulocytes

The reticulocyte count, expressed either as a percentage or absolute count, is an extremely valuable parameter that is unfortunately significantly under-utilised. It provides an indication of erythropoietic activity with respect to the absolute number of cells generated over the past one to two days .

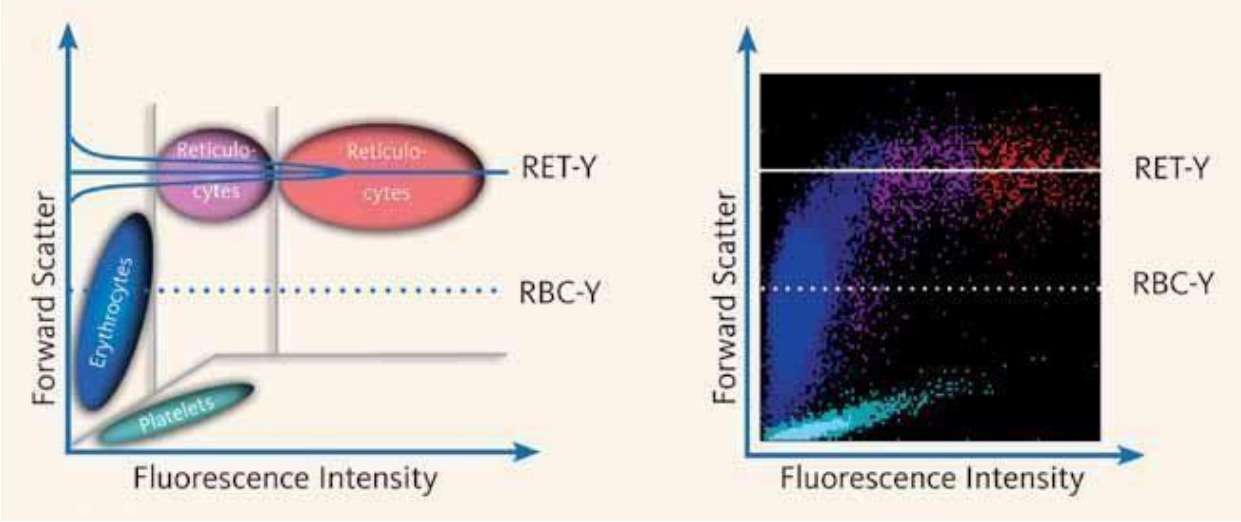


Figure *Schematic depiction of a Sysmex X-class analyser reticulocyte scattergram (left) and an actual scattergram (right) showing how the parameters Ret-He and RBC-He are obtained.*

In the reticulocyte scattergram, forward scatter, a measure of individual cell size, on the y axis is plotted against fluorescence intensity, a measure of RNA content, on the x axis. The Ret-Y and RBC-Y represent the mean forward scatter of the reticulocyte and mature red blood cell clusters respectively.

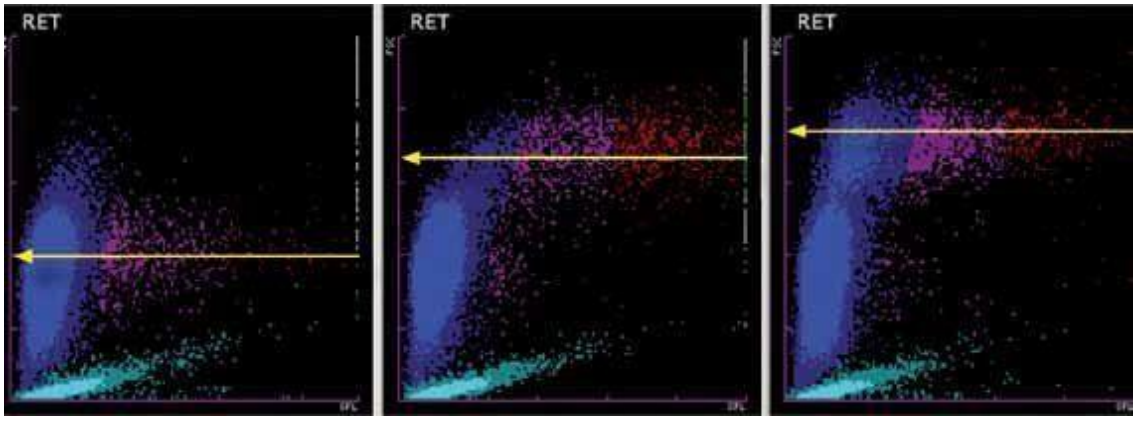


Figure *The blue cluster represents mature RBCs, whereas the red and purple clusters represent very young and maturing reticulocytes respectively.*

Note the mature RBC cluster with normal haemoglobin content in panel

Reticulocyte indices can be classified into three groups, according to the fluorescence intensity that reflects the maturity of reticulocytes (LFR: normal values between 81.0% and 96.4 %; MFR: between 1.1% and 15.2 %; HFR: between 0.03% and 3.95% of the total reticulocytes)[9,10]

The reticulocyte count is one of the most common hematological tests to classify and monitor the treatment of different types of anemia, as well as to determine if the bone marrow is functional [11]

An increase of immature reticulocytes in the blood of individuals with iron deficiency anemia represents a response to anemia, as long as the medullary tissue and the indispensable factors for erythropoiesis are preserved. Anemic hypoxia stimulates the release of erythropoietin in the bone marrow, increasing cell proliferation and differentiation. If the reticulocyte concentration increases in the medulla, its maturation will be completed in the blood. [9,12]

The reticulocyte indices analyzed in the study are related to the amount of RNA contained in reticulocytes and, consequently, to their degree of immaturity. In more severe anemia, the maturation time of reticulocytes in the medulla decreases, and an increased number of immature reticulocytes are released into the peripheral blood. These reticulocytes will remain more than 48 hours in the peripheral blood until they turn into red blood cells. Therefore,the immature reticulocyte count in the peripheral blood will be higher. [13,14]

5. CONCLUSION-

Aim of our present study is to investigate the role of RBC scatterogram in diagnosing and monitoring the treatment response in Iron Deficiency Anemia. The hemoglobin &Mean corpuscular volume (MCV) donot respond immediately after treatment of IDA. Serum ferritin in addition to being an inflammatory marker, it also doesnot increase soon after treatment of IDA. So to find out the immediate response of treatment in IDA patients, we did the RBC Scatterogram Study in adults with Iron Deficiency anemia. The RBC scatterogram is often the neglected part of an automated analyzer that if well interpreted, has a good potential to provide diagnostically relevant information about Iron Deficiency Anemia and its response to the treatment only by a single, simple & economical test. Despite the limitation derived from the small number of patients and the prospective nature of the study, according to the data of literature, our data confirms that by doing a RBCscatterogram analysis we can diagnose & evaluate the treatment response for Iron deficiency anemia without any other biochemical tests.

6. CONFLICT OF INTEREST STATEMENT-

The authors of this paper have no conflict of interest , including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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