Original Article

Screening of Thalassemia Major or Intermedia on Routine Complete Blood Count in An Outpatient Setting

Screening of Thalassemia

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ABSTRACT

Objective: To find out the relationship between Red cell distribution width (RDW-CV) and level of fetal hemoglobin on HPLC.

Study Design: Prospective, observational cross-sectional study

Place and Duration of Study: This study was conducted at the Institute of Hematology, Baqai Medical University, Karachi from September 2018 till March 2019 or a period of six months.

Materials and Methods: Patients were seen in Baqai Institute of hematology and their blood samples were sent to Hematology laboratory of Dow university Ojha complex, Karachi. A total of 394 consecutive blood samples aged 8 months to 5 years were analyzed for Hb. HPLC. Complete blood count was done immediately on fully automatic hematology analyzer while Hb. HPLC was done the same day on automatic Adam's Arkray HPLC analyzer.

Results: On the basis of RDW-CV, two groups were created as Group 1 & 2 having RDW-CV less than 30.9 and more than 30.9 respectively. Mean RDW and Hb-F levels for group 1(n = 248) and 2 (n = 146) were 18.5, 1.32 % and 35.62 and 65 % respectively.

Conclusion: Very high RDW-CV on routine CBC has significant relation to high values of Hb-F which is characteristic laboratory feature of the disease.

Key Words: Thalassemia, Anemia, (RDW) Red cell distribution width, failure to thrive, Hb-HPLC, Globin chains, anisocytosis

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INTRODUCTION

Hemoglobin (Hb) found in red blood cells is vital for oxygen transport in body. In a normal individual about seven different types of hemoglobins are synthesized different stages of life [(1).] Four of them are transient (Hb Gower 1&2 and Hb. Portland 1&2), seen only during the embryonic period while fetal Hb (Hb-F) is predominant Hb in fetal life and makes a major proportion at birth. In normal children and adults Hb-A, comprises a major portion (96-97%) with small amounts of Hb-A2 (2-3.3%) and Hb-F (0.2-1.0 %)[1].

Each molecule of Hb i.e. A, A2 and F consists of two alpha globin chains paired respectively with two beta, delta and gamma globin chains attached to one heme molecule. Alpha chain production is controlled by two alpha genes on Chromosome 16 while non- alpha chains (beta, gamma and delta) are synthesized by beta, gamma and delta genes present on Chromosome 11[2]. All these globin genes show an autosomal recessive transmission pattern[3]. At birth production of gamma chains and so Hb-F gradually start decreasing and by the age of six months reaches a very low level. At the same time the production of beta globin chains and so Hb-A, start increasing and by the age of twelve months constitutes almost all the Hb in the circulation[1]. In beta thalassemia major (BTM) there is a mutation in beta gene causing reduced or absent synthesis of beta globin chains while alpha genes are produced normally creating an imbalance between the two globin chains with a relative excess of alpha chains causing an excess of unpaired alpha chains resulting in precipitation of unpaired alpha chains in the red blood cells producing hemolysis of these cells inside the bone marrow cavity causing ineffective erythropoiesis culminating in severe anemia[4]. Beta thalassemia major / intermedia (BTM/I) are hereditary disorders of hemoglobin synthesis with a gene carrier rate of 5-7 % in Pakistan[5].

In the majority of patients, it presents before completing the first year of life when Hb-F production continues with a limited capacity as it tries to compensate for the absent or decreased production of Hb-A. The net result is severe isolated anemia with

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variation is size and shape of red cells (aniso-poikilocytosis) along with hypochromic microcytic red cells in the peripheral blood. There is little or no Hb-A with a relative increased proportion of Hb-F on a background of severe anemia. This is due to ineffective & dyserythropoietic erythropoiesis by the bone[6]. Bone marrow expands to compensate this anemia but again is ineffective. This results in various complications of disease like expansion of bone marrow cavity at the expense of thinning of bone cortex and bony deformities, extramedullary erythropoiesis resulting in Hepatosplenomegaly. The child suffers from failure to thrive unless treated[7].

In the severe forms of BTM/I, the Hb level ranges between 2-8 g/dl. Mean corpuscular volume (MCV) and mean corpuscular Hb (MCH) are significantly low, but, unlike thalassemia trait, thalassemia major is associated with a markedly elevated red cell distribution width (RDW), reflecting the extreme anisocytosis. The white blood count is usually elevated and this is due, in part to miscounting circulating nucleated red blood cells as leukocytes. Platelet count is usually normal, unless the spleen is markedly enlarged. Hb electrophoresis usually reveals an elevated Hb F fraction, which is distributed heterogeneously in the RBCs of patients with β thalassemia[8]. The disease is diagnosed by Hb electrophoresis or HPLC (high performance liquid chromatography), which is expensive and not easily available in rural areas. If left untreated, the patient dies within few months due to severe failure to thrive and various disease complications. In order to save the life of child, regular blood transfusion is started to maintain the hemoglobin level at adequate level followed by iron chelation therapy due to anticipated iron overload[9]. This treatment continues lifelong or until cure is achieved by allogenic bone marrow transplant. Another treatment which has gained popularity nowadays is the use of Hydroxyurea in these patients. Hydroxyurea (HU), is a ribonucleotide reductase inhibitor, acts by increasing Hb-F production and partially correcting α and non-α globin chains imbalance, thus ameliorating the hemolytic symptoms of these patients[10]. is a chemotherapeutic agent and has been used since a long time in the treatment of various malignancies especially in the treatment of chronic myelocytic leukemia before the introduction of tyrosine kinase inhibitors. This drug is beneficial in certain types of mutations of the disease and the blood requirement of the patient decreases significantly. It is said that hydroxyurea initiates the formation of Hb-F by stimulating its gene and so the level of hemoglobin don't fall to very low levels requiring blood transfusion. But these modalities do not offer cure. Up till now allogenic bone marrow or stem cell transplantation is the only means of acquiring a cure is HLA matched sibling is present but is highly expensive, available only in specialized centers and has high risk of morbidities and mortalities. The outcome of stem cell depends upon the age of patient and the number of blood transfusion he has received. The older the age and more the number of transfusions may lower a better outcome of this disease. So, it is important to diagnose it an early stage so that the patient may have received lesser transfusions. Major proportion of Pakistan is underdeveloped and many people living under poverty line along with scarcity of medical and diagnostic facilities, these patients remain undiagnosed for quite some time and are therefore more prone to develop various complications of the disease. This mounts the importance of early diagnosis and treatment of the disease by a screening investigation which should be easily available with a low cost. CBC is the first diagnostic investigation done for any disease and most of the time is the prime investigation that a physician requests for the patient. In our observation RDW parameter in a routine CBC closely parallels with anisocytosis of red cells in peripheral blood. In cases of severe anisocytosis, this parameter reaches very high values which are usually not seen in simple clinical conditions. This variation in size of red cells is called anisocytosis.

RDW value is directly proportional to the degree of anisocytosis[11]. RDW can be reported statistically as coefficient of variation (CV), the term which will be used in this article or standard deviation (SD)[12]. RDW-SD is expressed in femtolitres & actually measures the width of the RBC size distribution histogram and is measured by calculating the width (in fL) at the 20% height level of the RBC size distribution histogram (Image1) thus making it independent from MCV while RDW-CV (expressed in %) is calculated from a formula which is SD & and MCV: RDW-CV (%) = 1 SD of RBC volume/MCV x 100%, as RDW-CV and is mathematically derived from MCV, it is therefore affected by the average RBC size (MCV). The reference range for RDW-SD is 39-46 fL while for RDW is 11.6-14.6%. Reference ranges may vary depending on the individual laboratory and patient's age[13].

Aim of the study was to use this inexpensive routine CBC parameter for the screening of the beta thalassemia major or Intermedia, so that definite diagnostic investigations and treatment can be initiated early. In this study we plan to correlate high value of RDW-CV on routine CBC with the Hb-F OR fetal hemoglobin concentration on Hb. HPLC.

MATERIALS AND METHODS

This prospective descriptive study was conducted in Institute of hematology, Baqai medical college 16th September 2018 till 16th March 2019 for a period of six months. Inclusion criteria were age limit from 8 months to five years of age of both genders irrespective of their transfusion status. Sample size was calculated from

online RaoSoft Sample size calculator by adjusting the margin of error (d) at 5%, confidence level at 95%, with response distribution at 50%. The recommended sample size was 377 but we took it as 394. Samples were received from all over Karachi and interior of Sindh province. About 2 ml of whole blood sample was taken from a good peripheral vein in EDTA purple top with a vacutainer and CBC was performed within three hours of sample collection on Cell Dyne Sapphire automatic hematology analyzer which incorporates MAPSS (Multi angle polarized scatter separation technology). Quality control is our daily routine three times a day and checked on Levy Jennings chart and accepted only if it did not have any violation of Westgard rules. Remaining sample was run for Hb. HPLC fully automatic Adam's Arkray analyzer the same day which has the ability to run analysis any time and even individually.

A Total of 394 samples were analyzed for CBC and Hb. HPLC of patients less than five years of age. The demographic data along with results were entered in the computer. The results were computed by SPSS version 20 and mean, median, standard deviation was obtained. The data was analyzed forming two groups of RDW-CV i.e. 30.9% or above as group one (GP I) and 30.8% or below as group 2 (GP II) 30.9 and subjected to student t test in order to obtain P value. A p value of less than 0.05 was considered significant.

RESULTS

As seen in table 1, the total numbers of cases were 394. All were below five years of age. Females were n 184 (46.7%) while Males were n 210 (53%). Comparison of Age, Hemoglobin, RDW-CV and Hb. F value of two groups is shown in table 1. Group I (n 248, 62%, M:F of 130:118) had median age of 2.2 years while group II (n146, 37%, M:F 80:66) had 12 years. Mean Hb levels and Hb-F % in group I and II were 8.14gm/dl, 5.12 gm/dl, 1.03 and 65 % respectively while for RDW-CV it was 18.5 and 35.62. When RDW & Hb-F % of group I & II were compared by t test, the value obtained was highly significant (less than 0.05).

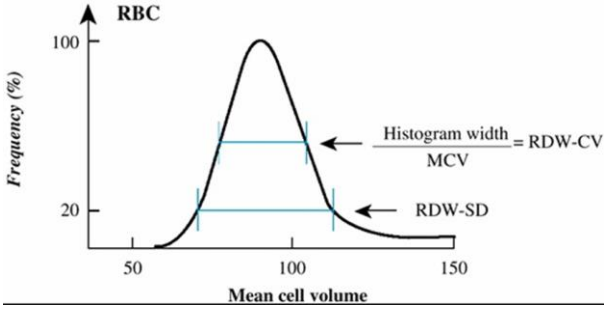

Figure No.1: Co-relation of RDW measurement with MCV

Table 1: Comparison of Age, RDW & Hemoglobin & Hb. F values among both groups

Total n394: Group 1 n248 (M 130 F 118), Group 11 n 146 (M 80 F 66)								
Parameter	Age years		RDW CV%		Hb gm/dl		Hb-F %	
Groups	Group 1	Group 2	Group 1	Group 2	Group1	Group 2	Group 1	Group 2
Minimum	1	0.8	11.4	31	2.5	1.82	0	15.3
Maximum	5	5	30.9	41.8	10.6	8.4	14	95
Mean	2.6	1.51	18.5	35.62	8.14	5.12	1.03	65
Median	2.2	1	16.9	35.6	8.7	5.26	0.8	72.3
SD	2.02	1.10	4.9	3.002	1.48	1.48	1.48	17.6

DISCUSSION

As far as our knowledge is concerned, we have not come across any study till date which has related Hb-F levels with RDW in beta thalassemia major/intermedia. The normal value or RDW-CV is less than 14.5 and is raised at the most from 14 to 29 in other cases like iron deficiency but values above this are usually seen in few conditions like beta thalassemia syndromes and rare disorders of congenital dyserythropoietic anemias.[1]. The results of our study regarding relation of anisocytosis with RDW-CV correlates very well with the study of T. Jameel et al, In which they tried to differentiate BMT with iron deficiency anemia on the basis of anisocytosis by utilizing RDW-CV on a sample size of 620 patients having hypochromic microcytic conditions by performing Serum ferritin and Hb electrophoresis The patients with iron deficiency had an increased RDW-CV then those of Beta thalassemia minor diagnosed by an elevated Hb-A2[14].

Our finding of very high RDW-CV in beta thalassemia major were also found in a study in Turkey in which the RDW-CV was significantly higher in Delta beta thalassemia than iron deficiency or other hypochromic anemias study showed significant elevation of RDW-CV which was not seen in individuals without Beta thalassemia major.[15] Our study was also independent of the transfusion status of the patient and it was seen that transfusion had a minor significant effect on the other group with high RDW-CV. This shows that regular transfusions to maintain peak and trough Hb levels to 12 and 8 gms/dl had no significant effect on RDW-CV

value. This may suggest that bone marrow is still active in these patients producing the abnormal red cells[16]. Our explanation for the unusual rise of RDW-CV in beta thalassemia major is due to anisopoikiloctyosis (combination of anisocytosis and abnormal shapes of red cells). Our finding and explanation are encouraged by a review of Needs T et al in a review from Stat Pearls Publishing in 2018 stating that due to increased anisopoikiloctyosis the RDW is raised as compared to other hypochromic anemias like beta thalassemia trait, iron deficiency anemia or a mixture of both[17].

CONCLUSION

RDW-CV is an important routine parameter on routine CBC without any extra cost. It can be used in an outpatient setting for the screening of beta thalassemia major / intermedia. This study may be helpful in patients who are not yet diagnosed but had received blood transfusions.

Author's Contribution:

Concept & Design of Study:	Nadeem Nusrat
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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