Research Article

Significance of Red Blood Cell Indices in Beta-Thalassaemia Trait

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Abstract

Background: Thalassemia is among the most common genetic disorders worldwide. Detection of hypochromic and microcytosis by the measurement of red blood cell indices is a preliminary step.
Objectives: To enlighten the importance of analysis of Red Blood Cell indices as a proactive step before ordering Hb electrophoresis.
Patients and Methods: Retrospective analysis of Complete Blood Count (CBC) and Hemoglobin (Hb) electrophoresis of 378 patients who were sent to the hematology laboratory of Al-Karama Teaching Hospital in the period from 1st of January 2009 to 31st of December 2011. For those patients, CBC and Hb electrophoresis results were reviewed. Patient data were tabulated and processed using SPSS software. P value of less than 0.05 was considered as significant.
Results: Of the 378 patients referred to hematology laboratory in Al-Karama Teaching Hospital in Wasit Governorate, 199 (52.6%) were females. Male to female ratio was 0.8:1. Pallor was the predominant cause of referral for Hb electrophoresis (187 patients, 49.5%).The mean age was (21.5 ± 15.22 years) with a range of (1-73 years). The Hb level was ranging from 4.5g/dl to 18.0 g/dl with a mean of (11.1 ± 2.7 g/d). Red cell volume level was ranging from 49.1 to 103.0 (fL), the mean was (73.4 ± 11.4 fL). Red cells distribution width (RDW) level showed the minimal reading of (25.6 fL) to a maximum one of (89.0 fL) with a mean of (43.60 ± 8.0 fL). About fifty eight percent (218 cases) were having normal Hb electrophoresis results. Thalassemia trait was detected in 37.3% of referred cases (141 cases). P value was less than (0.05) in the measurements of mean of both normal and thalassemia trait patients in regard to Mean cell volume (MCV), Red blood cells (RBC) count, Mean cell hemoglobin (MCH) and Mean cell hemoglobin concentration (MCHC) (0.0001 for MCV, RBCs, RDW and MCH, while 0.042 for MCHC). The mean Hb level for both normal and thalassemia trait cases were almost similar (both were 11.2 g/dl).
Conclusion: Illogical ordering of Hb electrophoresis might lead to overloading of diagnostic services and over expenditure.

Key words: **RBC indices, Thalassemia trait.**

INTRODUCTION

Thalassemia is a hereditary anemia resulting from defects in hemoglobin production. [1] Thalassemia is the name of a group of genetic blood disorders characterized by anemia due to enhanced red blood cell destruction. Hemoglobin, the oxygen-carrying component of the red blood cells consists of two different proteins, an alpha and a beta. If the body doesn't produce enough of either of these two proteins, the red blood cells become defective and cannot carry sufficient oxygen. The resulting anemia is usually severe with several health problems like enlarged spleen, bone deformities, fatigue and requires regular life-long transfusion, therapy and medical supervision. [2] Accordingly, lifelong care is required, [3] and financial expenditures for proper treatment are substantial. [4] Thalassemia is among the most common genetic disorders worldwide; 4.83 percent of the world's population carry globin variants, including 1.67 percent of the population who are

heterozygous for α-thalassemia and β- thalassemia. [5] β-thalassemia is caused by any of more than 200 point mutations and, rarely, by deletions. [1] Thalassemia is clinically heterogeneous because various genetic lesions variably impair globin-chain synthesis. However, genotypic variability at known loci is often insufficient to explain the disparate phenotypes of individual patients with the same genotype. [6] Researchers have paid much attention to differentiating thalassemia minor from the other types of hypochromic and microcytic anemia, because other types of hypochromic anemia are not transferred as a hereditary disorder. A definitive differential diagnosis between thalassemia minor and iron deficiency anemia (IDA) is based on the results of hemoglobin electrophoresis, serum iron levels, and ferritin calculation. [7] Detection of hypochromic and microcytosis by the measurement of red blood cell (RBC) indices is a preliminary step. The Coulter counter is used for the rapid assessment and measurement of RBC indices. [8] The investigation for thalassemia in a patient may be made for clinical or genetic reasons. Initial investigations usually include the complete blood count (CBC) together with evaluation of iron status by ferritin analysis and the quantification of hemoglobin's HbA_2 and HbF. In patients who are iron replete, the presence of a raised HbA_2 with thalassemic indices in the CBC is diagnostic of thalassemia. Although confirmation by DNA studies may be performed, this is unnecessary in the majority of cases. The diagnosis of α thalassemia is made by exclusion (patient is iron replete with thalassemia indices and a normal HbA_2 level) or by a positive hemoglobin H preparation. [9]

The present study is aimed to enlighten the importance of analysis of red blood cell indices as a proactive step before ordering Hb electrophoresis so as to perpetuate and, cost effectively, implement the use of such readings as screening method for thalassemia.

PATIENTS AND METHODS:

Retrospective analysis of complete blood count (CBC) and hemoglobin (Hb) electrophoresis for 378 patients who were sent to the hematology laboratory of Al-Karama Teaching Hospital in Wasit Governorate during the period from 1st of January 2009 to 31st of December 2011. These investigations were ordered by different physicians in both private and governmental sectors and from their own point of view. For those patients, CBC and Hb electrophoresis results were reviewed. A chart review was performed to determine age, gender, cause of referral and results of the investigations. Patients' data were tabulated and processed using SPSS software. [10] Qualitative data were expressed as frequency. Quantative data were expressed as mean and standard deviation (SD). One sample t test was used for P value. P value of less than 0.05 was considered as significant.

RESULTS

Of the 378 patients referred to hematology laboratory in Al-Karama Teaching Hospital, 199 (52.6%) were females. Male to female ratio was 0.8:1 as shown in table (1).

Table (2) describes the frequencies of referral causes. Pallor was the predominant cause of referral for Hb electrophoresis (187 patients, 49.5%), followed by positive family history of thalassemia in 185 (48.9%) patients. The descriptive data of the patients are shown in table (3); the mean age was 21.5 ± 15.2 years with a range of (1-73 years). The Hb level was ranging from 4.5 g/dl to 18.0 g/dl with a mean of 11.1 ± 2.7 g/d. Mean Cell Volume (MCV) level was ranging from 49.1 to 103.0 (fL), the mean was 73.4 ± 11.4 (fL). Red cells distribution width (RDW) level showed the minimal reading of 25.6 to a maximum one of 89.0 (fL). The mean RDW level was 43.60 ± 8.0 (fL).Strikingly, about fifty eight percent (218 cases) were having normal Hb electrophoresis results. Thalassemia trait was detected in 37.3% of referred cases (141 cases), while others types of hemoglobinopathy as Thalassemia intermedia, high Hb H and thalassemia major were reported in (1.9%, 1.3 &1.1%) respectively as shown in table (4).

Table (5) shows the differences and statistical significance of blood indices in relation to Hb electrophoresis results. P value was less than (0.05) in the measurements of mean of both normal and thalassemia trait patients in regard to MCV, RBCs number, MCH and MCHC (0.0001 for MCV, RBCs, RDW and MCH, and 0.042 for MCHC). The mean Hb level for both normal and thalassemia trait cases were almost similar (both were 11.2 g/dl).

Table No 1: Gender distribution of (378) patients referred for Hb electrophoresis.

sex	Frequency	percent
female	199	52.6
male	179	47.4
Total	378	100.0

Table No 2: Causes of referral for Hb electrophoresis test.

	Frequency	Percent
Pallor	187	49.5
Family history	185	48.9
Splenomegaly	4	1.1
Others*	2	0.6
Total	378	100.0

***One patients with history of jaundice and another as checkup post bone marrow transplantation because of persistent pallor.**

Table No.3: Descriptive data for all patients sent for Hb electrophoresis.

Items	No.	Minimum	Maximum	Mean	SD
Age in years	378	1.0	73.0	21.504	15.2277
Hb level (g/)	378	4.5	18.0	11.1	2.7
RBC count $\times 10^{12}$	378	2.06	7.50	5.0292	0.95098
Hct level (L/L)*	378	0.15	0.54	0.3605	0.07031
MCV(fL)**	378	49.1	103.0	73.454	11.4615
MCH level(pg)***	378	11.0	35.3	22.401	5.0858
MCHC level(g/dl)****	378	21.0	39.8	33.2	3.04
RDW level (fL)*****	353******	25.6	89.0	43.603	8.0812

*** Haematocrit level. ** Mean cell Volume. *** Mean cell Hemoglobin. **** Mean cell Hemoglobin concentration. ***** Red Cell Distribution Width. ******Missing data by the coulter counter.**

Table No 4: Hb electrophoresis results.

Results	Frequency	per cent
Normal	218	57.7
Thalassemia trait	141	37.3
Thalassemia intermedia	7	1.9
High Hb H	5	1.3
Thalassemia major	4	1.1
High Hb F and abnormal curve	1	0.3
Sickle cell trait	1	0.3
Sickle thalassemia	1	0.3
Total	378	100.0

Table No.5: Correlation between blood indices according to type of Hemoglobin electrophoresis

Item	Thalassemia patients/ Mean (SD)	Normal persons/ Mean (SD)	P value
MCV (fL)	65.3 (2.4)	79.2 (5.3)	0.0001
RBC $\times 10^{12}$	5.7 (0.58)	4.6 (0.79)	0.0001
Hct (L/L)	0.37 (0.04)	0.36 (0.07)	0.123
Hb (g/dl)	11.2 (1.8)	11.2 (2.9)	0.991
RDW (fL)	40 (5.8)	45.1 (7.2)	0.0001
MCHC (g/dl)	29.9 (2.0)	30.5 (3.4)	0.042
MCH (pg)	19.4 (2.4)	24.4 (5.3)	0.0001

DISCUSSION

The analysis of the data including Hemoglobin electrophoresis and CBC results from the haematology laboratory in Al-Karama teaching hospital during the period from January, 1st 2009 to December 31st 2011 showed a slight plurality of females over males.

Pallor was the major cause behind ordering hemoglobin electrophoresis for patients (visiting the hospital for different reasons). Pallor is a major complaint in clinical practice that may face clinicians. On the other hand, the hemoglobin electrophoresis test in Iraq is not available all the times on governmental bases and is expensive if done in private laboratories. That is why the clinician must be skilfully oriented, having a high index of suspicion when interpreting primary CBC results, which should be sent before Hb electrophoresis. Family history lies second as a cause for referral which might explain why patients with normal hemoglobin (maximum Hb recorded in this study was 18 gm/dl) were sent for Hb electrophoresis. The individual with Thalassemia minor are usually asymptomatic and, unless diagnosed by lab testing, they may be unaware of the carrier status. The basic tenet in the overall managements of β-thalassemia, as for many autosomal recessive disease, is the prevention of its homozygous form by detection and

education of carriers. [11] The red cell parameters that can be used include hemoglobin, RBC count, MCH, MCV and RDW. All those parameters are important to give the clinician clues to select who is the suspected case that should be sent for Hb electrophoresis or other advanced investigations which may not be available all the time. If these facts were neglected, misusing investigation ordering may result. For (278) suspected cases of thalassemia, more than half of them were with normal Hb electrophoresis results. By analysis of blood indices for both normal Hb electrophoresis and thalassemia trait Hb electrophoresis groups separately, we can find statistical difference between the two groups regarding blood incidence parameters. The most striking and consistent finding in suspected thalassemia trait group is combination of relatively high RBC count with a slightly low to normal hemoglobin and a disproportionate reduction of MCV and MCH in relation to hemoglobin, which is similar to what is mentioned in Maj GS study. [12] The MCV mean was (65.3 ± 2.4 fL) for thalassemia trait group which is lower than MCV in persons with normal Hb electrophoresis (79.2 ± 5.3 fL). The RBC count mean in patients with thalassemia minor was (5.7×10^{12}) which was higher than normal Hb electrophoresis group mean (4.6×10^{12}). These differences between these two parameter is statistically significant (p value 0.0001) for both, which is identical to what reported in Zahid study. [13] Hb level as well as Hct levels were nearly equal for both groups with no significant statistical difference (p value 0.9, 0.1 respectively) which is compatible to what written in Maj GS study. [12] RDW value is another important parameter which will help clinician to differentiate between thalassemia patients and other causes of anemia, RDW level is normal in patients with thalassemia trait and elevated in patients suspected to have iron deficiency anemia. [14] The RDW mean in thalassemia trait group was normal (40 ± 5.8 fL), but it was high is non thalassemia trait group (45 fL), which carries considerable statistical difference (p value 0.0001). Therefore, iron deficiency anemia should be expected when we face high RDW reading. MCHC and MCH means were (29.9 ± 2.0 g/dl), (19.4 ± 2.4 pg) respectively in group of thalassemia trait. This is lower than what is found in non-thalassemia trait group (30.5 g/dl, 24.4 pg) for each. This difference is statistically significant for MCHC (p value 0.04) and highly significant for MCH (p value 0.0001), which were compatible to facts mentioned in Aziz study. [8]

Conclusion: Rational, cost effective ordering of Hb electrophoresis is highly required in clinical practice settings. The red cell indices are extremely useful in screening for thalassemia and are usually sufficient to suspect the diagnosis. These can be useful to efficiently screen the individual who may require more investigations and therefore, to reduce sending cases with non-suspicious primary RBC indices for further Hb electrophoresis. Illogical ordering of Hb electrophoresis might lead to overloading of diagnostic services and over expenditure. One simple way to avoid over ordering of this investigation is to remove it from the standard forms or to ask for explicit justification for ordering them.

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