DIAGNOSTIC SIGNIFICANCE OF HEMATOLOGICAL PARAMETERS IN DIAGNOSIS OF, β–THALASSEMIA TRAIT AND MICROCYTIC ANEMIA

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ABSTRACT:*The main objective of this study is to evaluate the extent to which hematological markers can differentiate between β – thalassemia trait and microcytic anemia. It is a retrospective study, conducted in a tertiary care hospital. Study included 46 patients of β – thalassemia trait and 41 patients of microcytic anemia. Blood sample of these patients were collected for analysis of hematological parameters and extent of hemoglobinization.*

In all 87 patients, all hematological parameters (hemoglobin, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, platelet count, red cell distribution width), parameters for hemoglobinization (HbA1, HbA2 and HbF) and discriminating factor ($\frac{Mean\ corpuscular\ volume\ (MCV)}{Red\ blood\ cell\ count\ (RBC)}$) *were measured. Among all parameters red blood cell count (p = 0.004), platelet count (p = 0.002) and discriminating factor (p = 0.04) showed statistically significant results, while hemoglobin levels (p = 0.819), mean corpuscular volume (p = 0.343), mean corpuscular hemoglobin concentration (p = 0.154), white blood cell count (p = 0.192), lymphocyte counts (p = 0.301), neutrophils (p = 0.177), mixed white blood cells (p = 0.184), red cell distribution width (p = 0.502), HbA1 (p = 0.31), HbA2 (p = 0.224) and HbF (p = 0.627) did not show any statistically significant results. Different hematological parameters shows variety in the cases of β – thalassemia trait and iron deficiency and play important role in differentiation of these two conditions as clinical picture of these diseases resemble each other.*

Keywods: β – thalassemia trait, Microcytic anemia, Hematological parameters, Discriminating factors.

INTRODUCTION

Thalassemia is most common inherited disorder present worldwide with Pakistan lying in its belt [1]. Thalassemia are group of hereditary blood disorders characterized by anomalies in the synthesis of α – or β – chains of hemoglobin called α – thalassemia or β – thalassemia respectively. [2]. β-thalassemia trait is heterozygous state which may present with chronic anemia along with splenomegaly and skeletal changes to an almost symptomless state. Clinically these patients may present with features of iron deficiency anemia and iron supplementation will lead to iron overload. [3]. Iron deficiency anemia is most common micronutrient deficiency. Severe iron deficiency may lead to serious neurological impairments [4]. Microcytic anemia in case of thalassemia results from impaired globin chain synthesis and decreased hemoglobin synthesis. On the basis of classic hematological parameters, subjects with microcytic anemia are discriminated from subjects with anemia owing to thalassemia. As state of iron deficiency proceeds, the mean corpuscular volume, mean cell hemoglobin and red blood cell count tend to decline, but results in both microcytic anemia overlap [5-7].Usually, hemoglobin electrophoresis is essential for definite diagnosis of β – thalassemia trait cases. Normally HbA_2 is less than 3.2% but in β – thalassemia trait it is more than 3.5%. In areas where modern equipments for diagnosis are not available, simple morphological characteristics can distinguish between patients with microcytic anemia and β – thalassemia trait [8].

METHODS AND MATERIALS

It is a retrospective study comprising of total 87 patients, out of which 46 were diagnosed as β – thalassemia trait and remaining 41 had hypochromic microcytic anemia. In thalassemia trait group 16 (34.48%) were male and 30 (65.21%) were female. In microcytic anemic group, 12 (29.26%) were male while 29 (70.73%) were female (Table 1). Patients with different ages were included. Age of patients in thalassemia trait group were ranged from 1 year to 31 years with mean age of 16.45 years, while patients in microcytic anemia group were ranged from 1 to 50 years with mean age of 16.39 years (Table 2). All hematological parameters were measured using Sysmex automated blood analyzer X – 100. Discriminating factor (DF) was calculated using formula:

$$DF = \frac{Mean\ corpuscular\ volume\ (MCV)}{Red\ blood\ cell\ count\ (RBC)})$$

Concentration of hemoglobin was measured using hemoglobin electrophoresis technique. Data was collected and analyzed using SPSS version 20.0. Mean, range, standard deviation, standard error, coefficient of variation and level of significant were analyzed by applying t – test. A p value of <0.05 was considered statistically significant.

RESULTS

In patients with thalassemia trait hematological parameters showed a wide range of variety (Table 3). Hemoglobin (Mean = 10.18 gm/dl ± 0.25 SE, SD = 0.173), red blood cell count (Mean = 5.59 X 10^{12}/L ± 0.86 SE, SD = 0.589), mean corpuscular volume (Mean = 65.56 fl ± 1.9 SE, SD = 7.43), mean corpuscular hemoglobin (Mean = 19.63 pg ±0.56 SE, SD = 3.81), mean corpuscular hemoglobin concentration (Mean = 29.31 gm/dl ± 0.32 SE, SD = 2.17), white blood cell count (Mean = 8.59 X 10^9/L ± 0.31 SE, SD = 2.16), platelets (Mean = 345.58 10^9/L ± 17.33 SE, SD = 117.56), lymphocyte count (Mean = 33.65% ± 1.64 SE, SD = 11.14), neutrophil

count (53.63% ± 2.01 SE, SD = 13.67), mixed white blood cells (Mean = 12.95% ± 0.76 SE, SD = 5.21), red cell distribution width (Mean = 19.05% ± 0.41 SE, SD = 2.8), discriminating factor (Mean = 11.69 ± 0.32 SE, SD = 2.21), HbA_1 (Mean = 95.03% ± 0.02 SE, SD = 0.135), HbA_2 (Mean = 3.84% ± 0.25 SE, SD = 0.17) and HbF (Mean = 1.11% ± 0.25 SE, SD = 1.73). There were different ranged of same parameters in patients having hypochromic microcytic anemia (Table 4). Hemoglobin (Mean = 8.48 gm/dl ± 0.38 SE, SD = 2.45), red blood cell count (Mean = 3.73 X 10^{12}/L ± 0.1 SE, SD = 0.657)), mean corpuscular volume (Mean = 66.43 fl ± 0.86 SE, SD = 5.55), mean corpuscular hemoglobin (Mean = 20.84 pg ± 0.78 SE, SD = 5.01), mean corpuscular hemoglobin concentration (Mean = 28.88 gm/dl ± 0.49 SE, SD = 3.19), white blood cell count (Mean = 8.46 X 10^9/L ± 0.33 SE, SD = 2.13), platelets (Mean = 307.46 10^9/L ± 20.2 SE, SD = 129.98), lymphocyte count (Mean = 41.08% ± 2.45 SE, SD = 15.72), neutrophil count (Mean = 49.82% ± 2.5 SE, SD = 16.01), mixed white blood cells (Mean = 9.34% ± 0.63 SE, SD = 4.07), red cell distribution width (Mean = 19.38% ± 0.85 SE, SD = 5.5), discriminating factor (Mean = 18.45 ± 0.7 SE, SD = 4.54), HbA_1 (Mean = 96.79% ± 0.12 SE, SD = 0.799), HbA_2 (Mean 1.93= % ± 0.11 SE, SD = 0.741) and HbF (Mean = 1.25% ± 0.37 SE, SD = 0.24). Individual hematological parameters in both groups were also compared (Table 5). In comparison, hemoglobin (coefficient of variation = - 0.161, p = 0.819), red blood cell count (coefficient of variation = -0.175, p = 0.004), mean corpuscular volume (coefficient of variation = 6.65, p = 0.343), mean corpuscular hemoglobin concentration (coefficient of variation = - 1.118, p = 0.154), white blood cell count (coefficient of variation = 0.946, p = 0.192), platelets (coefficient of variation = 7291.71, p = 0.002), lymphocyte count (coefficient of variation = - 29.39, p = 0.301), neutrophil count (coefficient of variation = -40.3, p = 0.177), mixed white blood cells (coefficient of variation = - 4.16, p = 0.184), red cell distribution width (coefficient of variation = - 1.74, p = 0.502), discriminating factor (coefficient of variation = - 3.21, p = 0.04), HbA1 (coefficient of variation = - 0.004, p = 0.31), HbA2 (coefficient of variation = - - 0.24, p = 0.224) and HbF (coefficient of variation = 0.003, p = 0.627).

Table 1: Gender distribution among groups of β – thalassemia trait and microcytic anemia

Group	No. of cases	Male	Female
Thalassemia trait	46	16	30
Microcytic anemia	41	12	29

Table 2: Age distribution among groups of β – thalassemia trait and microcytic anemia

Group	No. of cases	Range	Mean
Thalassemia trait	46	1 – 31 years	16.54 years
Microcytic anemia	41	1 – 50 years	16.39 years

Table 3: Descriptive analysis of hematological parameters in patients with β – thalassemia trait

Parameter	Range	Mean	Standard Deviation
HbA1	94.70 – 95.40	95.03 ± 0.02 SE	.13577
HbA2	3.60 – 4.20	3.8478 ± 0.25 SE	.17093
HbF	0.80 – 1.60	1.1130± 0.25 se	.17334
Hb	6.10 – 12. 80	10.1824± 0.25 SE	1.73559
RBC	2.55 – 4.36	5.5943 ± 0.86 SE	.58936
MCV	51 – 86.20	64.5630 ± 1.9 SE	7.43913
MCH	12.40 – 29.20	19.6391 ± 0.56 SE	3.81194
MCHC	23.30 – 32.40	29.3174 ± 0.32 SE	2.17882
WBC	4.50 – 13.60	8.5928 ± 0.31 SE	2.16612
Platelets	181 – 623	345.5870 ± 17.33 SE	117.56938
Lymphocytes	17 – 60	33.6522 ± 1.64 SE	11.14594
Neutrophils	25 – 78	53.6304 ± 2.01 SE	13.67619
Mixed	2 – 30	12.9565 ± 0.76 SE	5.21518
RDW	14.30 – 27.80	19.0500 ± 0.41 SE	2.80038
DF	8.84 – 19.77	11.6993 ± 0.32 SE	2.21573

Table-4. Descriptive analysis of hematological parameters in patients with microcytic anemia

Parameter	Range	Mean	Standard Deviation
HbA1	95 – 98.1	96.7951 ± 0.12 SE	.79967
HbA2	1 – 3.40	1.9317 ± 0.11 SE	.74143
HbF	0.90 – 1.60	1.2585 ± 0.37 SE	.24081
Hb	3.60 – 12.20	8.4976 ± 0.38 SE	2.45229

RBC	1.89 – 4.76	3.7359 ± 0.1 SE	.65774
MCV	49.70 – 73.90	66.4341 ± 0.86 SE	5.55984
MCH	11.80 – 30.50	20.8488 ± 0.78 SE	5.01279
MCHC	22.20 – 34.40	28.8805 ± 0.49 SE	3.19564
WBC	4.60 – 14	8.4656 ± 0.33 SE	2.13499
Platelets	181 - 815	307.4634 ± 20.2 SE	129.98271
Lymphocytes	18 – 86	41.0488 ± 2.45 SE	15.72093
Neutrophils	10 – 74	49.8293 ± 2.5 SE	16.01390
Mixed	2 – 20	9.3415 ± 0.63 SE	4.07805
RDW	13.90 – 40.60	19.3878 ± 0.85 SE	5.50124
DF	12.27 – 36.35	18.4584 ± 0.7 SE	4.54494

Table 5: Comparison between hematological indices among patients with β – thalassemia trait and iron deficiency

Parameter	Thalassemia trait group	Microcyic anemia group	Coefficient of variation	P value
HbA1	95.03 ± 0.02 SE	96.7951 ± 0.12 SE	-0.004	0.31
HbA2	3.8478 ± 0.25 SE	1.9317 ± 0.11 SE	-0.24	0.224
HbF	1.1130± 0.25 SE	1.2585 ± 0.37 SE	0.003	0.627
Hb	10.1824± 0.25 SE	8.4976 ± 0.38 SE	-0.161	0.819
RBC	5.5943 ± 0.86 SE	3.7359 ± 0.1 SE	-0.175	0.004
MCV	64.5630 ± 1.9 SE	66.4341 ± 0.86 SE	6.658	0.343
MCH	19.6391 ± 0.56 SE	20.8488 ± 0.78 SE	-0.86	0.376
MCHC	29.3174 ± 0.32 SE	28.8805 ± 0.49 SE	-1.118	0.154
WBC	8.5928 ± 0.31 SE	8.4656 ± 0.33 SE	0.946	0.192
Platelets	345.5870 ± 17.33 SE	307.4634 ± 20.2 SE	7291.718	0.002
Lymphocytes	33.6522 ± 1.64 SE	41.0488 ± 2.45 SE	-29.359	0.301
Neutrophils	53.6304 ± 2.01 SE	49.8293 ± 2.5 SE	-42.302	0.177
Mixed	12.9565 ± 0.76 SE	9.3415 ± 0.63 SE	-4.616	0.184
RDW	19.0500 ± 0.41 SE	19.3878 ± 0.85 SE	-1.744	0.502
DF	11.6993 ± 0.32 SE	18.4584 ± 0.7 SE	-3.213	0.04

(P value < 0.05 was considered statistical significant).

DISCUSSION

Among all parameters correlated, red blood cell count, platelet count and discriminating factor showed statistically significant results. Although other parameters showed a wide range of variety, but did not show any statistically significant results. Hemoglobin was much lower in microcytic anemia group due to prominent deficiency of iron. Mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration was lower than normal reference ranges, but were not significant to differentiate between these two groups. Red cell distribution width is an indicator of aniosycytosis but was increased in both groups, so not significant in differentiation. Differentiation in two types of anemia is very essential [9]. Different studies have been performed in context to compare different parameters. Ferrara M. *et al* performed cohort study on 458 patients, showing a wide variety of values among hematological parameters [10]. Matos JF et al performed different study and used various indexes to different between two anemias [11]. Another study done by Nalbantoglu B. et al used different formulas to discriminate between two groups [12]. Nessa and others performed a different study to discriminate using different indexes between hematological indices [13].

CONCLUSION

Clinical picture of both these common anemia is quite similar, while treatment modalities are quite different from each other. Iron deficiency anemia is diagnosed by using more sensitive and specific methods like serum ferritin concentration, serum iron concentration, total iron binding capacity, serum transferrin receptor assay, etc. Evaluation of β – thalassemia trait is done by confirmatory polymerase chain reaction (PCR assay). As these technologies are very expensive and not widely available, we can differentiate between these two types of anemia on the basis of hematological markers. Different indices play important role in discriminating thalassemia trait and iron deficiency anemia.

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