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Studies on the Haematological Aspects of Beta (β) – Thalassemia in Tamilnadu

Shanthi G¹, Balasubramanyam D^{2*}, and Srinivasan R³

¹. Scientist, Micro Therapeutics Research labs Private Ltd, Chennai, India

². Professor, Directorate of Centre for Animal Production Studies, Chennai, India

³. Professor,(Retired),Department of Zoology, Presidency college, Chennai, India.

ABSTRACT

Hematological parameters such as the total Hb, PCV, RBC count, MCV, MCH, WBC, Differential counts and Platelets were analysed for β -thalassemia major, β -thalassemia intermedia and β -thalassemia trait. All the red cell parameters decreased drastically in β -thalassemia major, moderately decreased in β -thalassemia intermedia and minimally decreased in β -thalassemia trait. An elevated level of WBC count was present in β -thalassemia major. The total Hb, PCV, and RBC count revealed a highly significant variation, while for MCV and MCH no significant variation was found among the β -thalassemia major, Intermedia and Trait. Between β -thalassemia trait males and females, males showed a higher significant variation for total Hb, PCV, RBC, MCV and MCH.

Keywords: Haematology; Clinical manifestation; β -thalassemia; Tamil Nadu.

**Corresponding author*

INTRODUCTION

In India, about 50 million individuals are affected by genetic diseases and nearly 1,30,000 genetically affected children are being added every year [1]. Some genetical problems are unique for our country. The multiracial and multi-environmental conditions of the country and its people may be the contributing factors for the incidence of several genetical disorders like haemoglobinopathies, thalassemia, chromosomal disorders, etc.

Thus the congenital malformations and genetic diseases are the important causes for the early mortality and morbidity in India [2]. Thalassemia, which is caused by an autosomal recessive gene, forms the commonest monogenic blood disorders in the world.

Thalassemia comes from Greek word “Thalas-Sea and emia-Blood” as it was originally found in people bordering Mediterranean Sea. The wide spread incidence of thalassemia in the world may be due to mass migration and partial resistance to malarial parasites. Generally thalassemia characterized by microcytic, hypochromic anaemia due to reduced or absence of globin chain synthesis resulted in imbalance in the globin chains. This hereditary blood disorder is caused due to mutations in the globin gene that are responsible for the synthesis of haemoglobin molecule. Imbalanced production of the globin chain leads to change in the red cell morphology, bone marrow precursor cells and reticulocytes. The excess globin chain synthesis causes precipitation, which is common to all thalassemia syndromes [3,4,5]. The haematological parameters such as MCV, Osmotic fragility and RBC count are useful to discriminate the normal individual and thalassemia carriers [6].

MATERIALS AND METHODS

Blood samples were collected from the Institute of Child Health and Hospital, Egmore, Chennai. Two ml of blood was drawn aseptically in a sterilized disposable syringe and transferred to a sterile vacutainer containing Ethylene Diamine Tetra Acetic acid (1.0 mg/ml of blood) as anticoagulant. The samples were kept in the refrigerator at 4°C until the blood samples were processed for study. In this study 35 β -thalassemia patients along with their parents and sibs were examined for haematological variations. The haematological parameters such as total Haemoglobin (Hb), Packed cell volume (PCV), Red Blood Corpuscles (RBC) count and White Blood Corpuscles (WBC) count, Differential counts (DC), Mean corpuscular volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelets count were examined. [7,8]

RESULTS

In β -thalassemia major, the mean value for the total Hb was 4.93 ± 1.49 , PCV was 16.96 ± 5.06 , RBC count was 2.61 ± 0.80 , MCV was 65.47 ± 5.11 , MCH was 19.32 ± 3.59 , MCHC was 29.59 ± 5.51 , WBC count was 29.79 ± 28.02 , neutrophils was 39.28 ± 10.88 , lymphocytes was 57.10 ± 11.20 , monocytes was 2.67 ± 1.73 , basophils was 1.67 ± 0.37 , eosinophils was 2.38 ± 1.46 and platelets was 220.31 ± 113.68 .

The mean values of total Hb, PCV, RBC, MCV, MCH, MCHC, WBC, neutrophils, lymphocytes, monocytes, basophils, eosinophils and platelets for β -thalassemia intermedia were 7.56 ± 0.74 , 26.94 ± 4.37 , 3.78 ± 0.58 , 69.28 ± 5.00 , 20.16 ± 1.57 , 29.22 ± 2.85 , 11.18 ± 1.93 , 32.80 ± 7.98 , 57.2 ± 4.96 , 7.67 ± 6.60 , 0.80 ± 0.75 , 5.75 ± 6.50 and 283.20 ± 118.17 respectively.

In β -thalassemia trait the mean value was 11.26 ± 1.74 for total Hb, 36.95 ± 5.48 for PCV, 5.40 ± 0.58 for RBC count, 68.52 ± 7.75 for MCV, 20.87 ± 2.58 for MCH, 30.62 ± 3.62 for MCHC, 8.48 ± 2.55 for WBC, 59.34 ± 10.62 for neutrophils, 37.45 ± 10.23 for lymphocytes, 2.65 ± 1.49 for monocytes, 1.90 ± 0.94 for basophils, 2.65 ± 1.59 for eosinophils and 274.81 ± 128 for platelets count.

Analysis of variance (ANOVA) for red cell indices showed a highly significant variation for the total Hb, PCV, RBC count and no significant variations for MCV and MCH between β -thalassemia major, intermedia and trait. The Multiple range test, Turkey – HSD test, showed significant variation between the individual mutation and were denoted by various alphabets (Table 1). The red cell indices of male and female β -thalassemia children showed mean values of 5.12 ± 1.44 and 4.71 ± 1.57 for total Hb 17.14 ± 5.00 and 16.74 ± 5.39 for PCV, 2.69 ± 0.86 and 2.51 ± 0.75 for RBC count, 64.58 ± 5.44 and 66.55 ± 4.64 for MCV, 19.62 ± 3.68 and 18.95 ± 3.46 for MCH respectively (Table 3). Student't' test showed no significant variation between male and female β -thalassemia children (Table 3). Mean values of 12.22 ± 1.53 and 10.51 ± 1.52 for total Hb, 40.25 ± 5.61 and 34.38 ± 3.78 for PCV, 5.60 ± 0.58 and 5.25 ± 0.54 for RBC count, 71.97 ± 7.64 and 65.82 ± 6.80 for MCV and 21.92 ± 2.46 and 20.08 ± 2.43 for MCH were observed for male and female of β -thalassemia trait (Table 4). Student't' test showed highly significant variation among total Hb, PCV, MCV and MCH parameters, while the RBC count showed a significant variation between the male, female of β -thalassemia trait (Table 4). In β -thalassemia total Hb was 10.4 g/dl, the PCV was 30.0%, the RBC count was 4.7×10^6 , the MCV was 63.8 fl and the MCH was 22.1 pg (Table 5). The values of total Hb, PCV, RBC count, MCV and MCH for sickle cell trait were 14.5 g/dl, 42.6%, 5.8×10^6 , 73.4 fl and 25.0 pg respectively (Table 5). The values were elevated when compared with mean values of red cell indices of β -thalassemia trait (Table 1, 5).

One of the β -thalassemia trait showed a Hereditary Persistence of Fetal Hemoglobin (HPFH) in which a value of 17.0 g/dl for total Hb, 63.8% for PCV, 7.5×10^6 for RBC count, 85.1 fl for MCV and 22.7 pg for MCH (table 5) was observed. The HPFH observed in one of the sibs was found to have values of 14.7 g/dl, 46.5%, 5.3×10^6 , 87.7 fl and 27.7 pg for total Hb, PCV, RBC count, MCV, and MCH respectively.

The peripheral smears observed for β -thalassemia major and intermedia showed the presence of hypochromic, microcytic, poikilocytic, anisocytic, nucleated red cells (normoblast), fragmented cells, tear drop cells and target cells in varied numbers and a few ovalocytes, spherocytes, elliptocytes were also observed. In most of the β -thalassemia trait the peripheral smear was found to have mild hypochromic, microcytic, poikilocytic, anisocytic and a very few

nucleated red cells. Some tear drop cells and target cells were also observed. In β -thalassemia the peripheral smear showed the presence of sickle cells in addition to other abnormalities of red blood corpuscles such as hypochromic, microcytic, tear drop cells and target cells that characterized β -thalassemia major.

Table. No: 1 Red Cell Indices between β -thalassemia Major, Intermedia and Trait

| Red Cell Indices | Beta -Thalassemia (Mean \pm S.D.) | | | F-Value | P- Value |
|-------------------------|-------------------------------------|-------------------------------|-------------------------------|---------|----------|
| | Major (n=29) | Intermedia (n=5) | Trait (n=57) | | |
| Hb g/dl | 4.93 \pm 1.49 ^a | 7.56 \pm 0.74 ^b | 11.26 \pm 1.74 ^c | 147.33 | 0.000** |
| PCV % | 16.96 \pm 5.06 ^a | 26.94 \pm 4.37 ^b | 36.95 \pm 5.48 ^c | 137.34 | 0.000** |
| RBC $\times 10^6/\mu$ l | 2.61 \pm 0.80 ^a | 3.78 \pm 0.58 ^b | 5.40 \pm 0.58 ^c | 175.37 | 0.000** |
| MCV fl | 65.47 \pm 5.11 | 69.28 \pm 5.00 | 68.52 \pm 7.75 | 2.04 | 0.136 |
| MCH pg | 19.32 \pm 3.59 | 20.16 \pm 1.57 | 20.87 \pm 2.58 | 2.73 | 0.071 |

** -Denotes significant at 1% level.

^{abc} -Denotes significant at 5% level between individual β -thalassemia types of mutations

Table. No: 2 Haematological Variations between B-Thalassemia Major, Intermedia and Trait

| Haematological Parameters | Beta -Thalassemia (Mean \pm S.D.) | | |
|-------------------------------|-------------------------------------|---------------------|---------------------|
| | Major (n=29) | Intermedia (n=5) | Trait (n=57) |
| WBC $\times 10^3/\mu$ l | 29.79 \pm 28.02 | 11.18 \pm 1.93 | 8.48 \pm 2.55 |
| N% | 39.28 \pm 10.88 | 32.80 \pm 7.98 | 59.34 \pm 10.62 |
| L% | 57.10 \pm 11.20 | 57.20 \pm 4.96 | 37.45 \pm 10.23 |
| M% | 2.67 \pm 1.73 | 7.67 \pm 6.60 | 2.65 \pm 1.49 |
| B% | 1.67 \pm 0.37 | 0.80 \pm 0.75 | 1.90 \pm 0.94 |
| E% | 2.38 \pm 1.46 | 5.75 \pm 6.50 | 2.65 \pm 1.59 |
| Platelets $\times 10^3/\mu$ l | 220.31 \pm 113.68 | 283.20 \pm 118.17 | 274.81 \pm 128.37 |
| MCHC g/dl | 29.59 \pm 5.51 | 29.22 \pm 2.85 | 30.62 \pm 3.62 |

Table. No: 3 Comparison of Red Cell Indices between Males and Females of B-Thalassemia Children

| Red Cell Indices | Beta -Thalassemia Children (Mean \pm S.D.) | | t- Value | P- Value |
|-------------------------|--|------------------|----------|----------|
| | Male (n=21) | Female (n=13) | | |
| Hb g/dl | 5.12 \pm 1.44 | 4.71 \pm 1.57 | 0.73 | 0.469 |
| PCV % | 17.14 \pm 5.00 | 16.74 \pm 5.39 | 0.21 | 0.834 |
| RBC $\times 10^6/\mu$ l | 2.69 \pm 0.86 | 2.51 \pm 0.75 | 0.59 | 0.558 |
| MCV fl | 64.58 \pm 5.44 | 66.55 \pm 4.64 | 1.04 | 0.310 |
| MCH pg | 19.62 \pm 3.78 | 18.95 \pm 3.46 | 0.49 | 0.629 |

Table. No: 4 Comparison of Red Cell Indices between Males and Females of B-Thalassemia Trait

| Red Cell Indices | Beta -Thalassemia Trait (Mean ± S.D.) | | t- Value | P- Value |
|------------------------|---------------------------------------|---------------|----------|----------|
| | Male (n=25) | Female (n=32) | | |
| Hb g/dl | 12.22 ± 1.53 | 10.51 ± 1.52 | 4.23 | 0.000** |
| PCV % | 40.25 ± 5.61 | 34.38 ± 3.78 | 4.71 | 0.000** |
| RBC×10 ⁶ µl | 5.60 ± 0.58 | 5.25 ± 0.54 | 2.37 | 0.021* |
| MCV fl | 71.97 ± 7.64 | 65.82 ± 6.80 | 3.21 | 0.002** |
| MCH pg | 21.92 ± 2.46 | 20.08 ± 2.43 | 2.82 | 0.007** |

** - Denotes significant at 1% level.

* - Denotes significant at 5% level.

Table. No: 5 Red Cell Indices of Sβ-Thalassemia, Sickle Cell Trait, B-Thalassemia Trait With HPFH and a Normal with HPFH

| Beta Thalassemia | No.of Cases | Hb g/dl | PCV % | RBC ×10 ⁶ µl | MCV fl | MCH pg |
|-------------------------------|-------------|---------|-------|-------------------------|--------|--------|
| Sβ | 1 | 10.4 | 30.0 | 4.7 | 63.8 | 22.1 |
| Sickle cell trait | 1 | 14.5 | 42.6 | 5.8 | 73.4 | 25.0 |
| β-Thalassemia Trait with HPFH | 1 | 17.0 | 63.8 | 7.5 | 85.1 | 22.7 |
| HPFH | 1 | 14.7 | 46.5 | 5.3 | 87.7 | 27.7 |

DISCUSSION

The decreased levels of total Hb, PCV, RBC count, MCV, and MCH observed for β-thalassemia are due to an imbalance in the haemoglobin synthesis leading to microcytic and hypochromic anaemia [9]. All the red cells parameters decreased drastically in β-thalassemia major, moderately decreased in β-thalassemia intermedia and minimally decreased in β-thalassemia trait [10,11,12]. The mean PCV for β-thalassemia major and trait presented here, was similar to the reported mean of 20.0% and 35% for β-thalassemia major in Indian population [13] and β-thalassemia trait [14]. The RBC count values reflect the expected trends depending upon the severity of the type of β-thalassemia [15,16,17]. As expected the MCV values were low in β-thalassemia major [18]. In β-thalassemia intermedia the MCV, MCHC depends upon the number of gene and type of mutation. β-thalassemia trait was characterized by low MCV values [19,20]. Decreased MCV in β-thalassemia trait is due to combined effect of the increased red cell production and the mild reduction of haemoglobin formation [17]. As β-thalassemia results from defective haemoglobin synthesis, the WBC count, the differential counts and the platelets count in the affected individual remains unaffected normally. An elevated WBC count has been recorded in β-thalassemia major. An elevated WBC count in β-thalassemia major is due to the presence of immature red cells [21]. However a normal WBC count for β-thalassemia major was observed [12]. The variations observed in the differential counts in this study among β-thalassemia major cases were attributed to the frequent infections whereas in traits it appears normal. Generally a decreased platelets count is with

hypersplenism and increased count following splenectomy [8]. The platelets mean value obtained in the present study corresponds to the reported mean value of 255.0 ± 9.0 [22]. The MCHC are generally not considered for β -thalassemia types as each cell for its volume was not deficient for haemoglobin concentration [23,24]. In the present study decreased levels of MCV and MCH was observed for all β -thalassemia types and supports the report of [10]. A comparison of red blood indices such as total Hb, PCV, RBC count, MCV and MCH between male and female β -thalassemia major had shown that the male registered a higher mean values than female, except for MCV. But the differences were not significant between the sexes [25]. In the present investigation between β -thalassemia trait males and females, males showed a higher significant variation for total Hb, PCV, RBC, MCV and MCH [26]. The total Hb value obtained for $S\beta$ -thalassemia in the study reflects [27]. A higher red cell indices values for the $S\beta$ -thalassemia than those affected only with β -thalassemia is due to the fact that in $S\beta$ -thalassemia, one of the β -gene causes a quantitative defect while the other β -globin gene produces a qualitative defect in the production of β -globin chains, but in the case of β -thalassemia both the β -globin genes produce quantitative defect in β -globin chain production. Correspondingly the sickle cell trait leads to an elevated haematological value than β -thalassemia trait [28, 29]. Observed a normal haematological profile for a compound heterozygous form of β -thalassemia trait with HBFH and a normal individual with HPFH. The peripheral blood smears of β -thalassemia major and intermedia with the striking abnormalities of red blood cells were similar as picturesied earlier by [18, 22,30]. The peripheral smear of β -thalassemia trait also showed red cell abnormalities of hypochromia, microcytosis, poikilocytosis, target cells and teardrop cells [31]. Silvestroni and Bianco [32, 33] presented the occurrence of hypochromia, microcytosis, target cells, teardrop cells with sickle cells in $S\beta$ -thalassemia. All the abnormalities in red blood cells showed the ineffective erythropoiesis and hemolysis.

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