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Significance of Haemoglobin Electrophoresis in Detecting Abnormal Hb variants

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1. Introduction

The most common causes of microcytic hypo chromic anemia are iron deficiency anemia (IDA) and beta thalassemia trait (BTT). Thalassemia is the group of genetic blood disorders. It is characterized by reduced synthesis of normal hemoglobin due to absence or decreased synthesis of one or more globin chains. Thalassemia is divided into alpha and beta thalassemia. Beta thalassemia is also known as Mediterranean anemia. Beta-Thalassemia is a hereditary disorder characterized by the absent or decreased synthesis of β- chain , results in the imbalance between α and β chain. Beta thalassemia is further divided into beta thalassemia major, minor/trait and intermediate. Thalassemia major is also called "Cooley's anemia". Thalassemia minor is also known as "beta-thalassemia trait", carrier /heterozygous thalassemia.[4] In beta thalassemia trait (BTT) one of two globin genes is abnormal. Patients with thalassemia trait act as a carrier of the disease. Thalassemia trait also combines with variant hemoglobin (HbE, HbS) to produce the other disorders like HbE Beta thalassemia, sickle cell beta thalassemia.[5] Differential diagnosis is important for prognosis and treatment of the disease. Iron deficiency anemia is detected by serum iron profile. Beta thalassemia trait is detected by Hb electrophoresis method with HbA_2level (>3.5%).

2. Materials and Methods

Study design - Prospective and Retrospective.

Study Method

Inclusion criteria

1) All patients (213 numbers) irrespective of the age and gender diagnosed as Beta Thalassemia Trait, with increased Hb A_2 by capillary zone electrophoresis method.

Data Collection

- The clinical details of the patients were collected from the medical records department of hospital
- Hemoglobin electrophoresis was done using Capillary Zone Electrophoresis (CZE) method.

Principle of Hb Electrophoresis

Electrophoresis is a test, based on the movement of electrically charged molecules under an applied electric field. When the proteins on the membrane are exposed to a charge gradient, they get separated from each other[28]. Hb electrophoresis can be done on filter paper, cellulose acetate membrane, starch gel, or citrate agar gel. They are performed on lysed RBCs as no bands are formed in the presence of plasma proteins. Capillary zone electrophoresis (CZE) is another high resolution semi-automated method (Sebia, Evry, France) employed in the detection of abnormal hemoglobins. The system is equipped with 8 capillaries in parallel, allowing multiple and simultaneous analyses. Each capillary can be used at least 3000 times[48]. In this, electrophoresis is carried out in a capillary tube, by permitting higher voltages; the main advantage is a lesser amount of sample required for each run. Capillary iso-electric focusing (cIEF) has several advantages over High performance liquid chromatography (HPLC) method and gel electrophoresis method: decreased manual labor, lower cost, and detection of even minor hemoglobin components accurately[48].

Significance of Hb A_2

Hemoglobin A_2, is composed of two alpha and two delta chains. The level of Hb A_2 increases in patients with BTT (≥3.5). The main limitation of diagnosing BTT by gel electrophoresis is that Hb C, E and O Arab move in the same region as A_2. Capillary electrophoresis simplifies Hb A_2 detection, as Hb A_2 is seen in a different zone and separates it from other hemoglobins[49].

3. Results

The clinico-hematological profile of 213 patients with BTT were studied in our hospital with HbA2 level more than 3.2%. The results are depicted as mean, median and range for various variants of Hb. The result is as follows.

Type of Hb (n=213)	Median	Mean	Range	Biological Reference Range
Hb A (%)	94.3	94.08	88.5-96.1	94.8-97.8
Hb A_2 (%)	5.10	5.11	3.8-6.7	2.2-3.5
Hb F (%)	0.80	1.22	0.2-6.4	≤2

The overall median of HbA was 94.3 % and the range was 88.5 to 96.1%. The overall median of HbA_2 was 5.10 % and ranged from 3.8 to 6.7 %.

The median HbF was found to be 0.80% with the range from 0.2 to 6.4%. There were 11 cases with HbF >3%. Six of these patients were antenatal women with HbF ranging from 3.1 to 6.2%, three were female patients aged 24, 26 and 30 years and two cases were children aged 1year and 2years, with aHb F of 6.1 and 6.4 respectively.

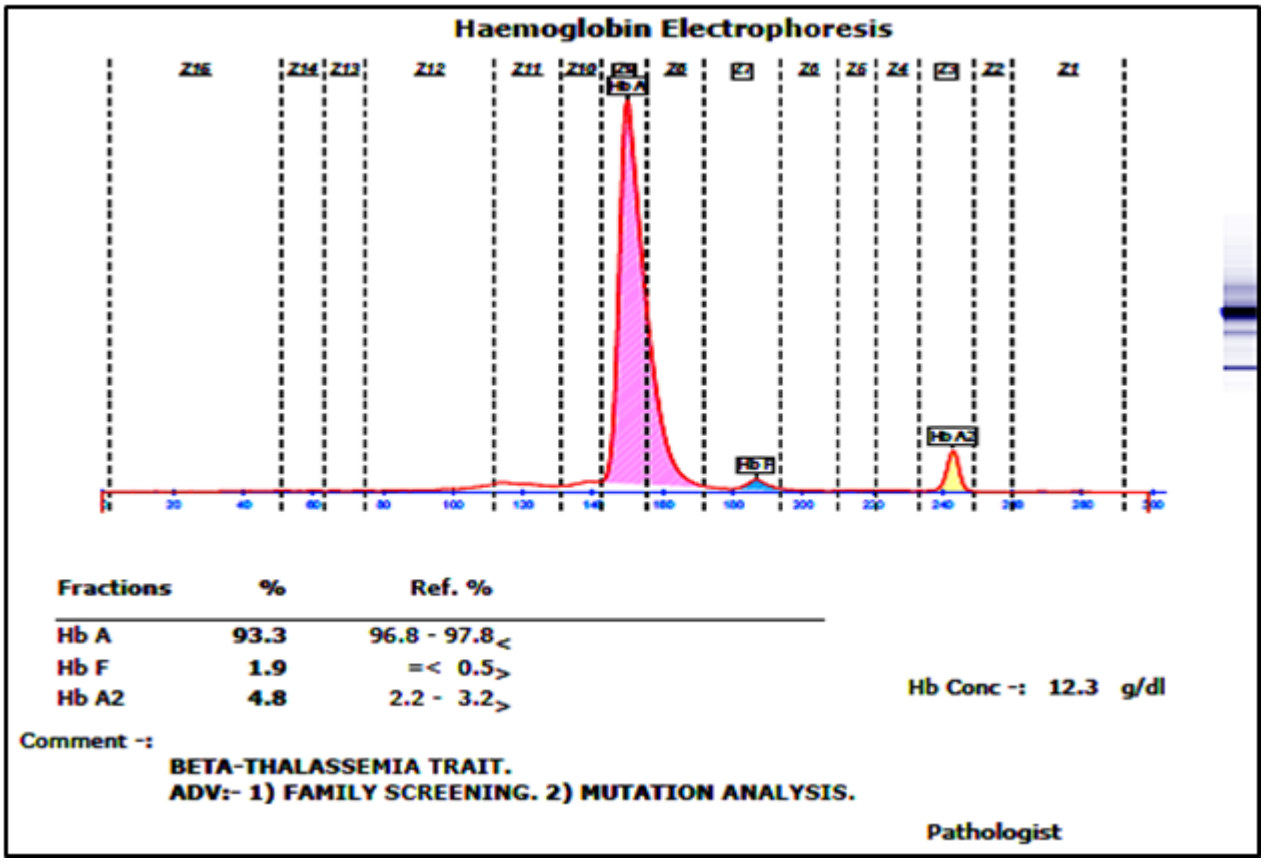

Figure 1: Beta Thalassemia Trait
Hb A:-<96.8; Hb F:-> 0.5; Hb A_2 :- >3.2

4. Summary

The clinico-hematological profile of 213 cases of Beta thalassemia trait was studied in our hospital in the Department of Pathology, Srinivasan Medical College Trichy.

The summary of results are as follows:

1) Epidemiology:-
The patients studied were in the age group from 6 months to 75 years, with median age of 28 years. Out of 213 cases (42.7%) cases were seen in the age group of 21 to 30 years and only 3.4% of cases were seen in the age group of more than 61 years. A female predominance was seen with a male: female ratio of 1:1.77.

2) Presenting Complaints:-
Majority of cases were asymptomatic (54.5%). But symptoms of anemia was seen in (82 out of 213) cases (38.5%). Bleeding was seen in 4 patients, which contribute to the degree of anemia with the hemoglobin range from 8.2 to 11.7 gm% in theses cases.

3) Physical Examination:-
Majority of cases had no organomegaly (88.2%).

4) Treatment History:-
As majority of cases were asymptomatic, most of them had not received any treatment. However blood transfusion and hematinic supplements were given to women in the reproductive age groups.

5) Hematological Profile:-
From the CBC parameters, the overall median of Hb was 9.7 gm%, with the range from 4.4 to 14.7gm%. the lowest value of Hb 4.4 gm% was seen in diabetic patients who got amputated. The overall median of RBC was $5.03x10^6$ /µL , with the range from 2.44 to $7.52x10^6$ /µL. The median Hb and RBC values meets the criteria for BTT. ESR was estimated in only 133 cases , the median value was 9mm/hr with range from 1 to 110mm/hr. The highest value of 110mm/hr was seen in case associated with chronic renal failure,were the renal function test are abnormal and auto-immune serology was positive for pANCA.

The overall median MCV in the present study was found to be 64.30fl with the range from 50.4 to 99.7fl. The median MCH in the present study was 19.6 pg with the minimum value of 15.4pg and maximum of 79.3pg. The median MCHC was 30.70 gm% with the minimum value of 27.2 gm% and 35.4gm%.The median RDW was 16.5 % with the minimum value of 13.9 % and maximum of 41.1 %. The overall median values of CBC indices alse meets the criteria for diagnosing BTT .

Retculocyte count was estimated in only 53 cases out of 213, of which the median reticulocyte % was 1.82% with the range from 0.36 to 7.05 %. The case with maximum value 7.05% was associated with hemolytic blood picture.

6) Hemoglobin Electrophoresis:-
The overall median Hb A was 94.3 % and range was 88.5% to 96.1%. The median Hb A_2 was 5.10% and range was 3.8

% to 6.7%. The median Hb A_2 meets the diagnostic criteria for detecting BTT and is considered as the gold standard.

5. Conclusion

Thus to conclude from the datas of various laboratory parameters which includes Hb, RBC, RBC indices (MCV, MCH, MCHC, RDW) for 213 of Beta thalassemia trait cases, the median values meet the criteria for BTT. Hb A_2 estimation is considered as gold standard for diagnosing BTT cases with the value more than 3.5 .