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# Clinico haematological profile in beta Thalassemia trait

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## ABSTRACT

***The most common causes of microcytic hypochromic anemia in India are β Thalassemia & Iron deficiency anemia (IDA). β-Thalassemia is one of the most common single gene disorders in India with an overall prevalence of 3-4 %. Effective screening of β Thalassemia trait (βTT), decrease its incidence. Hb electrophoresis, serum iron profile & RBC indices are used to differentiate βTT from IDA. HbA2, red cell indices are observed as effective screening tests in βTT. Our main objective is to study the hemoglobin electrophoresis & RBC indices in β-Thalassemia Trait & to differentiate from IDA. A retrospective study of 50 patients (OP & IP) admitted in our KMC & hospital, Manipal was done. Study period was 1year (Aug 2015-Sep 2016). HbA2 level is studied by capillary zone electrophoresis method. Hb & red cell indices (MCV, MCH, RDW) were calculated. From red cell indices formulas –Mentzer (MI-cut off-13), Srivastava (SI-3.3), Shine & Lal (S&L-595), England & Frazer (E&F-1.39) were derived & serum iron profiles (Fe 2+, TIBC, Ferritin) was done to distinguish βTT from IDA. Vitamin B12 & folate assay was done. Sensitivity, specificity & Youden index were also calculated. All 50 pts were diagnosed as β-Thalassemia Trait (HbA2≥3.5. Hb≤8gms/dl, MCV<60fL, MCH5x106/mm3, RDW>16%). Mean age group is 50yrs, M>F.3/50 cases (6%) shows HbF along with HbA2.2/50 (4%) has HbS .1 case shows IDA features with βTT. 49/50 (98%) cases of βTT showed-≤ cut off values of all index (MI,SI, S&LI ,E&FI). Serum iron profile was normal in 24/50 (48%), serum ferritin in 5/50 (10%). vitamin B12 & folate levels seen in 9/50(18%).1 case showed iron profile & index. The MI is the most sensitive (>90%) & specific (>83%). βTT & IDA are the M.C. causes of microcytic anemia. Hemogram parameters and RBC indices have significant role in βTT. HbA2, MCV & MI are the most sensitive tool to detect βTT.***

***Keywords:*** *βTT, electrophoresis, RBC Indices*

## 1. REVIEW OF LITERATURE

### 1.1 Background

Iron Deficiency Anemia (IDA) and Beta (β) thalassemia trait (BTT) are common causes of microcytic hypochromic anemia, apart from sideroblastic anemia and anemia of chronic disease. Both IDA and Thalassemia cause reduced hemoglobin synthesis, however, by distinct mechanisms. IDA occurs due to a derangement in the iron metabolism, which can either be acquired, owing to nutritional deficiencies or due to mutations in genes encoding for the various transport molecules involved in the kinetics of iron metabolism. Evidently, a complete serum iron profile would confirm the diagnosis, along with a complete review of peripheral blood picture, and red blood cell indices.

Thalassemia, however, are a heterogeneous group of inherited disorders, characterized by reduced synthesis of hemoglobin, which results due to mutations causing reduced or complete absence of synthesis, of one or more of the globin chains of the hemoglobin molecule[1]. They are most commonly encountered in individuals of Mediterranean, African and South - east Asian descent, hence, the name 'Mediterranean anemia' has been conferred.

Thalassemia is divided mainly into alpha and β thalassemia types, depending on the type of globin chain affected. Other rare types include delta thalassemia, and combination hemoglobinopathies: combination of HbE/HbS/HbC with β - thalassemia. β - thalassemia is further divided into β – thalassemia major, β – thalassemia minor/trait[4] and β – thalassemia intermedia, depending on the number of β globin genes mutated.

## 2. HISTORICAL BACKGROUND

The initial description of thalassemia as hereditary disorders was given by Thomas Cooley in 1925[6] , in 5 children who presented with anemia, hepatosplenomegaly, discoloration of the skin and classical facies – hence the name – Cooley's anemia. Later, in 1932, George Whipple[7] coined the term 'thalassemia' which originates from the Greek word – '*Thalassa*' which means 'sea' and '*emia*' – meaning blood.

 

### 2.1 Epidemiology – Worldwide

Panos [8] (2005), and Riewpaiboo *et al.*[9] (2010) reported Thalassemia to be a major health problem in the world. Three to five lakh children are born with the homozygous state of the disease i.e. Thalassemia major. Lookopoulos *et al.*[10] in 2001, reported it to be of common occurrence in the Mediterranean, Middle east, and southeast Asian countries, like India and China. Wetherall et al. [11](2001) reported that over 60000 to 70000 children get diagnosed with β Thalassemia major in the Mediterranean region out of the 300,000 affected children annually.

### 2.2 Epidemiology - Thalassemia In India

Sukumaran *et al.* [12] (1973), Modell and Petrou *et al.*[13] (1983) in their study, reported a β Thalassemia carrier state of 1-3% in the Indian subcontinent, with few subgroups reporting a frequency of, as high as 17%. Shah *et al.*[14] in 2004, showed a higher prevalence of the disease in question, in the northern and eastern regions of the country, when compared to the southern states.

## 3. CLASSIFICATION

**Table 1: Genetic Classification of β Thalassemia[15]**

| Clinical syndromes | Genotypes | Clinical features |
|---|---|---|
| β thalassemia major | ( $\beta^0/\beta$, $\beta^+/\beta^+$, $\beta^0/\beta^+$) | Severe; transfusion dependent |
| β thalassemia intermedia | ( $\beta^0/\beta^+$, $\beta^+/\beta^+$, $\beta^0/\beta$, $\beta^+/\beta$) | Severe; regular transfusion not required |
| β thalassemia mino | ($\beta^0/\beta$, $\beta^+/\beta$) | Asymptomatic with mild or no anemia with mild red cell abnormalities |

### 3.1 Inheritence Of Beta Thalassemia Trait

β Thalassemia trait patients are asymptomatic and usually present with a mild microcytic hypochromic anemia. They follow the Mendelian pattern of inheritance and are most commonly autosomal recessive.[2]

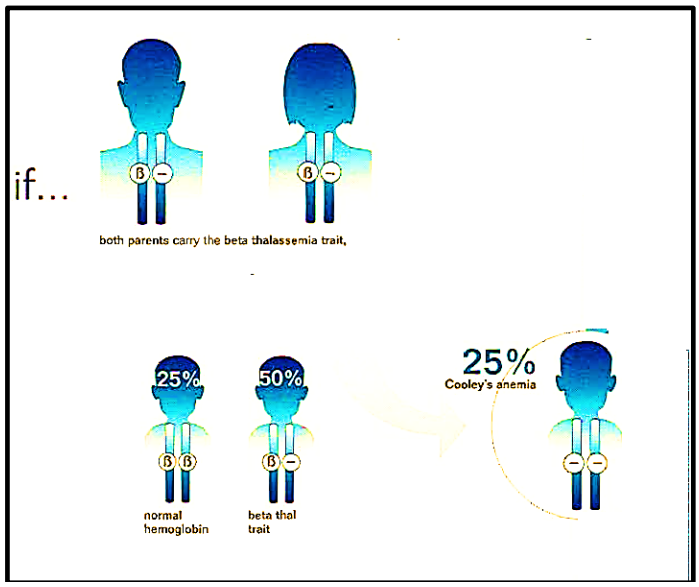


**Fig. 1: Inheritance Model showing the Pattern of Inheritance in β Thalassemia, when both parents are carriers.[5]**

### 3.2 Etiology

More than 1000 mutations are seen in β-thalassemia and related disorders. Predominantly, they are point mutations involving the beta globin chain. Deletion and frameshift mutations are reported to be rare in BTT. Verma I. C. *et al.*[17] (1997) studied the regional distributions of beta-thalassemia mutations in India. They characterized the various mutations in 1,050 carriers of beta-thalassemia trait. By using amplification refractory mutation system technique, 98.2% of subjects were identified; 91.8% of subjects had 5 common mutations, which are listed below:

a) IVSI-5 (G>C),
b) 619-bp deletion
c) IVSI-1 (G>T)
d) codon 8/9 (-G) and
e) codon 41/42 (-TCCT).

### 3.2 Beta Thalassemia Trait Associated With Other Syndromes

In rare cases of BTT, the defect does not lie in the beta globin chain alone. When associated with other syndromes, a molecular defect is identified in the gene encoding the transcription factor - TFIIH (Beta thalassemia trait associated with tricothiodystrophy), or X-linked factor GATA-1 (Thrombocytopenia with Thalassemia)[16].

### 3.3 Pathophysiology

The gene for beta globin chain is located on chromosome number 11[4]. A point mutation, results in reduced amount beta globin chain synthesis but it does not impair normal functioning of hemoglobin since there is a normal allele. This person however carries the genetic trait that may be transmitted but will not experience any significant health problems.[5]

 

## 4. CLINICAL FEATURES

Urbinati Nathan *et al.*[18] in 2006, stated that β-Thalassemia trait patients are generally asymptomatic. Agarwal MB and Mehta BC[16] studied 143 symptomatic and 171 asymptomatic BTT patients over 11 years. Besides anemia, jaundice, abdominal pain and hepatosplenomegaly were seen in 20 – 25% of the cases. Premawardhena *et al.*[26] evaluated 217 cases of BTT and documented anemia in 22 (10.14%) patients. Hepatomegaly was seen in 16 (7.3%) cases. They also noted an increased susceptibility of BTT patients to infections.

**Table 2. Clinical symptoms and signs in BTT**

| S no. | | Anemia | Jaundice | Hepatomegaly | Splenomegaly |
|---|---|---|---|---|---|
| 1 | Mazza U. *et a.l*[22] , n= 254 | 118 (46%) | - | 25 (10%) | 48 (19%) |
| 2 | Gardikas c. *et al.*[23] n= 260 | 123 (47.3%) | - | - | - |
| 3 | Premavardhena *et al* .[26], Srilanka n =217 | 22(10.14%) | 3(1.3%) | 16(7.3%) | 5(2.3%) |

### 4.1 Hematological parameters in BTT

Way back in 1975, an expert group on 'Abnormal Hemoglobin and Thalassemia', and the International Council for Standardization in Hematology (ICSH), recommended two sets of investigations for diagnosing these disorders. It incorporates correlation of clinical presentation and the hematologic parameters which are as follows[27]

a) Hemoglobin
b) Packed cell volume or Hematocrit
c) RBC indices - includes (MCV, MCH, MCHC and RDW).
d) Hemoglobin electrophoresis at alkaline or acid pH and quantification of Hb $A_2$ and Hb F. However, the present method of choice for the same is cation-exchange HPLC and capillary zone electrophoresis.
e) Discriminant factors are used to differentiate BTT from IDA

## 5. SAMPLE COLLECTION

Laboratory investigations are performed on Ethylene diamine tetra acetic acid (EDTA) anticoagulated venous blood samples. CBC should be done within 4 hours of collection. If the Hb electrophoresis test is delayed, it should be stored at 4° C, and should be preferably tested within one week of collection, owing to eventual denaturation of Hb.

### 5.1 Complete Blood Count (CBC)

Hemoglobin, total RBC count, and red blood cell indices, are the key components of CBC, to aid in the diagnosis of BTT.

### 5.2 MCV Mean Corpscular Volume

It is the average **volume** of red cells. It can be directly measured by automated hematology analyzers. In normal adult individuals, MCV ranges from 80-100fl; in BTT it can have a wide range between 56.3 – 87.3 fl as reported by Rund *et al*.[28]

### 5.3 RDW – Red cell distribution width

It is a measure of the degree of variation in red cell size. IDA is characterized by a significant increase in RDW; in contrast, BTT produces an uniform microcytic picture and therefore a normal RDW. RDW acts as adjunct to diagnosis but not as a lone indicator.

MCV <76 fl, MCH <26 pg and RBC count > 5 x $10^6$ /µL are observed as effective screening parameters for BTT. Various discriminant factors by using CBC indices have been derived mathematically to differentiate IDA from BTT[29]:

- Mentzer index(MI)
- Green and King index(GKI)
- Red cell distribution width index (RDWI)
- England and Fraser index (EFI)
- Srivastava index (SI)

**Table 3. Comparison of RBC parameters in BTT**

| | n | | Mean value | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Hb (gm%) | Hct (%) | RBC x $10^6$/µL | MCV (fl) | MCH (pg) | MCHC (gm/dL) | RDW (%) |
| Rahim *et al*.[31] | | | | | | | | | |
| <10yrs | 59 | BTT | 10 | - | 5.7 | 58.3 | 18.6 | 32.6 | 11.8 |
| >10yrs | 94 | | 11.6 | - | 5.9 | 61.4 | 19.4 | 36.1 | 15.1 |
| <10 yrs | 114 | IDA | 10.12 | - | 4.45 | 69.7 | 22.12 | 31.14 | 14.20 |
| >10 yrs | 56 | | 10.25 | - | 4.48 | 71.64 | 22.16 | 30.23 | 15.60 |
| Januaria *et al*.[32] | 30 | BTT | 10.9 | 34.6 | 5.4 | 63.8 | 20.4 | 31.8 | 16 |
| | 155 | IDA | 10.0 | 32.2 | 4.4 | 73 | 22.7 | 31.2 | 17.9 |

 

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Premavardhini *et al.*[26] | 217<br>96 | BTT<br>IDA | 10.9<br>11.7 | -<br>- | -<br>- | <80<br><80 | <27<br><27 | ND<br>ND | ND<br>ND |
| Raiz Ahmed Quazi *et al.*[33] | 26<br>- | BTT<br>IDA | -<br>- | -<br>- | 5.1<br>- | 72.8<br>- | 19.9<br>- | 31.2<br>- | 13.9<br>- |
| Zahid Ullal *et al.*[35] | 230<br>570 | BTT<br>IDA | 10.7<br>9.25 | -<br>- | 6.3<br>4.0 | 67.2<br>55.3 | 19<br>21.1 | -<br>- | 14.2<br>20.2 |
| Trivedi Dhara *et al.*[34] | 135<br>81 | BTT<br>IDA | 10.4<br>9.34 | 37.2<br>34.1 | 5.6<br>4.34 | 63.1<br>70.6 | 18.8<br>21.56 | 29.6<br>29.8 | 17.1<br>17.9 |
| Elahe *et al.*[36] | 151<br>- | BTT<br>IDA | 11.7<br>- | 37.4<br>- | 5.69<br>- | 66.2<br>- | 20.7<br>- | 31.2<br>- | -<br>- |
| Yi-Ping Wang *et al.*[37] | 65<br>- | BTT | M-12.7<br>F-11.7 | - | 5.8 | 66.1 | - | - | 15.5 |
| | | IDA | - | - | - | - | - | - | - |
| Nongnuch Sirachainan *et al.*[38] | 91<br>40 | BTT<br>IDA | 12.6<br>11.2 | 38.8<br>35.0 | 5.2<br>4.9 | 75.3<br>71.6 | 24.5<br>23.0 | 32.2<br>31.6 | 14.8<br>16.1 |

M – Male; F – Female

Zahid Ullal *et al.*[35] studied 800 cases of microcytic hypochromic anemia with 230 BTT cases and 570 patients with IDA. The mean Hb, RBC count, Hb $A_2$ and MCV were higher and RDW was lower when compared to patients with IDA. Trivedi Dhara *et al.*[34] also did a similar study and obtained similar result except that the RDW in the two groups were comparable (17.1 in BTT and 17.9 in IDA). Elahe *et al.*[36] studied 504 cases of microcytic hypochromic anemia and documented higher Hb and MCV in the non BTT group compared to patients with BTT.

**Table 4: Discriminant factors with formulae**[29]

| INDEX | FORMULA | βTT | IDA |
|---|---|---|---|
| Mentzer Index (MI) | (MCV/RBC) x 100 | ≤ 13 | >13 |
| Green And King Index (GKI) | $MCV^2$ x RDW/Hb x100 | ≤ 65 | >65 |
| Red Cell Distribution Width Index (RDWI) | MCV x RDW/RBC | ≤220 | >220 |
| England And Fraser Index (EFI) | MCV-(5xHb)-RBC-K where K=3.4 | ≤ 0 | >0 |
| Srivastava Index (SI) | (MCH/RBC) x 100 | ≤ 3.8 | >3.8 |

**Table 5. Sensitivity and specificity of discriminant factors**

| Indices From Other Study | | MI | | GKI | | EFI | | RDWI | | SI | | Shine and Lal index | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SEN (%) | SPE (%) | SEN (%) | SPE (%) | SEN (%) | SPE (%) | SEN (%) | SPE (%) | SEN (%) | SPE (%) | SEN (%) | SPE (%) |
| Margreet Schoorl *et al.* [43] | BTT (n=34) | 48 | 95 | 64 | 97 | 51 | 96 | - | - | - | - | - | - |
| | IDA (n=142) | 94 | 47 | 97 | 56 | 97 | 45 | - | - | - | - | - | - |
| Ebrahim *et al.*[44]* | | 72 | 82 | - | - | 67 | 90 | - | - | 64 | 44 | 64 | 44 |
| Sahli *et al.*[44]** | | 77 | 100 | - | - | 44 | 96 | - | - | 88 | 100 | 86 | 88 |
| Nikitha *et al.*[44] n=185 BTT | | 85.41 | 81.75 | - | - | 40 | 95.63 | - | - | 78.38 | 78.58 | - | - |
| Rahim *et al.*[31] | BTT (n=114) | 93 | 85 | 83 | 85 | 71 | 92 | 97 | 72 | 81 | 92 | - | - |
| | IDA (n=59) | 85 | 93 | 85 | 83 | 92 | 71 | 72 | 97 | 92 | 81 | - | - |
| Trivedi Dhara *et al.*[34] | BTT (n= 135) | 83 | 80 | 72.5 | 70 | 79 | 90 | - | - | 92 | 47 | - | - |
| | IDA (n= 81) | 80 | 83 | 70.3 | 72 | 90 | 79 | - | - | 47 | 92 | | |
| Aziz Batebi *et al.*[39] n= 444-BTT | | 86.3 | 85.4 | - | - | 87.2 | 62.9 | - | - | - | - | 83.1 | 90.6 |
| TA Santhi *et al.*[45], n =10 | | 93.3 | 53 | - | - | 84.7 | 90.2 | 94.1 | 87.8 | - | - | 63.8 | 94.1 |

 

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mahendra Singh *et al.*[46] n =;7/100-BTT | 71.7 | 57.1 | - | - | 57.1 | 100 | 87.3 | 90.5 | - | - | 85.7 | 2.6 |
| Kunal *et al.*[47] n=124/316-BTT | 58.97 | 95.65 | - | - | 52.5 | 86.9 | 78.2 | 73.1 | - | - | 94.9 | 56.5 |

*Ebrahim et al - MI-<13, SLI-<1004, SI-<4.1, EFI-<6.5; **Sahli et al. - MI-<12.5, SLI-<1083, SI-<3.7, EFI-<5.3

Margreet Schoorl *et al.* [43] studied the sensitivity, specificity of 34 BTT and 142 IDA cases and concluded that the formulae are useful to discriminate the two groups and detect patients who need to undergo further testing. Eight hundred and forty eight cases of microcytic hypochromic anemia were studied by Nikita Tripathi *et al.*[44] with 185 cases of BTT. They found Mentzer index to be the best formula with a sensitivity of 85.41% and specificity of 81.75%. Trivedi Dhara *et al.*[34] studied 135 cases of BTT and 81 cases of IDA and showed that RDW-SD with a sensitivity of 91.11% and specificity of 93.82% is superior to RDW-CV which had a sensitivity of 20.7% and specificity of 76.29 in patients with BTT. They therefore recommend RDW-SD as the best discriminant factor to differentiate between BTT and IDA.

## 6. HEMOGLOBIN ELECTROPHORESIS

### 6.1 Historical Background

Stern *et al*.[32] in (1945), used electrophoresis to investigate human red cell proteins. Initially, Hb A and Hb F were the primary Hb bands which were named. Later, Kunkel *et al*.[48] in 1955**,** first recognized the increased level of Hb $A_2$ in BTT patients.

### 6.2 Principle

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].

### 6.3 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].

**Table 6: Hb $A_2$ levels in pregnant and non-pregnant women (Zhan hui Ou *et al.*[50] )**

| S. no | Hb $A_2$ study | Pregnant women with BTT n =55 | Non-pregnant women with BTT n =85 | Pregnant women without BTT n =96 | Non -Pregnant without BTT n =114 |
|---|---|---|---|---|---|
| 1. | Hb $A_2$ (Mean) | 4.93 | 4.89 | 2.59 | 2.50 |
| 2. | Hb $A_2$ (>3.5%) | n = 55 | n = 85 | None | none |
| 3. | Hb $A_2$ (3.0-3.5%) | None | None | n=9 | n=6 |

Zhan hui Ou et al.[50] studied the Hb $A_2$ levels in both pregnant and non-pregnant women using a cut off value of 3.5%. They noted that pregnancy did not change the Hb $A_2$ level significantly and along with CBC parameters can be used as an appropriate method to diagnose BTT.

**Table 7: Hb A, Hb $A_2$ and Hb F values in BTT**

| S no. | Other Studies | Hb A % | Hb $A_2$ % | Hb F % |
|---|---|---|---|---|
| 1. | Leela Pant *et al*.[51] n= 216 | 89 | 5.2 | 1.08 |
| 2. | A Mosca *et al*.[52], n=31 At birth | - | 0.5 | 73.8 |
| | n=12 ; 3months | - | 3.2 | 27 |
| | n=10 ;6months | - | 4.8 | 8.2 |
| | n=14; 9-10months | - | 5.1 | 4.4 |
| | n=8 ; 1 yr | - | 4.8 | 4.1 |
| 3. | Hafiza Alauddin *et al*.[53] n=218;BTT | - | 5.23 (CE) 5.14 (HPLC) | - |
| 4. | Promil Jain *et al*.[54] n=36 | 82.2 | 6.5 | 0.7 |
| 5. | Trefor N Higgins *et al*.[55], n=91 | - | 5.01(CE) 5.50 (HPLC) | - |
| 6. | Santosh B Bhokare *et al*.[56], n= 33 | 84.5 | 5.27 | 1.2 |

*CE- Capillary electrophoresis, HPLC- High performance liquid chromatography*

 

Leela Pant et al.[51], studied a total of 4600 cases who were screened for Thalassemia by HPLC method, of which 290 cases were detected with abnormal hemoglobin. With a cut off of >3.5% as the diagnostic criteria for BTT, they detected 216 cases (74.48%). They discuss the problem of establishing the cut off for Hb $A_2$ when the value is in the borderline range of 3.5 to 4%. They have quoted different authors who have used different range for borderline cases, such as 3 to 4% by Rangan *et al* and Colah *et al*. Rangan *et al.*[82]evaluated patients with Hb $A_2$ levels of 3.4 to 3.9% and found mutations in 8 out of 25 cases (32%). Trefor N Higgins *et al*.[55] compared the Hb $A_2$ value in different Hb variants and found the highest Hb $A_2$ levels of 5.01% by CZE method in BTT as against 3.06% in Hb S, 2.76% in Hb D Punjab, 3.65% in Hb E and 2.91% in Hb C.

**Table 8. SENSITIVITY, SPECIFICITY, PPV, NPV of Hb $A_2$ by HPLC (Noura M Kablan *et al.*[63])**

| S no. | Study Groups | Cut Off Hb A2 | SEN (%) | SPE (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|
| 1. | MCHC Anemia (n= 20) | >2.2 | 100 | 21.43 | 47.6 | 100 |
| 2. | Beta Thal Intermedia (n=12) | >3.2 | 33.33 | 92.86 | 66.7 | 76.5 |
| 3. | BTT (n=52) | >3.6 | 84.62 | 100 | 100 | 77.8 |

*MCHC- Microcytic hypochromic*

Noura M Kablan et al. [63], studied a total of 112 cases in three different groups; 20 cases were patients with microcytic hypochromic anemia, 12 cases belonged to beta thalassemia intermedia and 52 cases of BTT. There were 52 cases of BTT with the cut off >3.6 and showed 100% specificity. The cut off values were determined by ROC curve in the three groups separately. They found Hb $A_2$ level between 3.5 – 7% in patients with BTT.

**Table 9. Serum iron profiles in BTT**

| S no. | | Serum iron (µgm/dL) | Serum ferritin (ng/mL) | Serum TIBC (µgm/dL ) |
|---|---|---|---|---|
| 1. | M.Reza Keramati *et al.*[58]<br>n=150<br>M – 64; F -86 | 97.5 | 146 | 344 |
| 2. | Sherchand O *et al.*[59] , n = 8 | 82 | - | 261 |
| 3. | IF Estevao *et al.*[60] Brazil;<br>F -18-40 yrs, (n=10)<br>>40 yrs(n =18) | -<br>- | 53.5<br>127 | -<br>- |
| | M -18-40 yrs (n =6)<br>>40 yrs (n = 8) | -<br>- | 154<br>474 | -<br>- |
| 4. | Sarika Verma *et al.*[61]<br>New Delhi.<br>n = 30 (BTT with IDA) pre treatment | 47.1 | 6.8 | 502 |
| | Post treatment | 70 | 25.9 | 383 |

M.Reza Keramati *et al.*[58] studied serum iron profile in 150 cases of BTT out of total 291 cases which includes, IDA, alpha thalassemia, normal case and concomitant BTT and IDA. The serum iron profile in BTT on comparision with IDA cases showed a significant increase in the serum iron level (97.5µgm/dL) in contrast to IDA cases with serum iron (32µgm/dL). The serum ferritn in BTT was higher (140ng/mL) in contrast with IDA were the levels are significantly much lower.

**Table 10: Study of Serum iron and vitamin B12 ,folate (Yi Ping Wang *et al.*[37] )**

| S no. | BTT, n =65 | Serum iron (µgm/dL) | TIBC(µgm/dL) | Vitamin B12 (pg/mL) | Folate (ng/mL) |
|---|---|---|---|---|---|
| 1. | M- n=(14)<br>F – n=(51)<br>Control-130 M :n= (28),<br>F :n=(102) | 98.9<br>96.8<br>99.4<br>96.2 | 301.2<br>307 | 654.9<br>655.4 | 13.6<br>13.6 |
| **S no.** | | **Mean age** | **Hb (gm%)** | **Iron deficiency seen in** | **Vitamin B12 deficiency seen in** |
| 1. | BTT<br>n =(183), Asma et al. [66]<br>M(47), F(136) | F-48yrs<br>(74.4%) | F<br>(10.4gm%)<br>M<br>( 11.4 gm%) | F-<br>(n=32, 17.5%)<br>M-<br>(n=3,1.6%) | F-<br>n =(33), 28%.<br>M-<br>n =(12), 30%. |

Yi Ping Wang et al.[37] ,studied serum iron profile and vitamin B12 and folate levels in 65 cases of BTT, of which 14 were male and 51 were female. He also used healthy individuals as control groups (n=130) of which 28 were male and 102 were female. The serum iron profile was found to be higher in both test and control group. The vitamin B12/ folate assay was also found to be higher in both test and control groups. The mean serum iron, vitamin B12 and folate levels are found to be similar in both the test and the control groups. The author stated that BTT had no effect on the GI disturbances and presence of parietal cell autoantibodies.

 

Asma et al.[66] , studied in 183 cases of BTT, of which 47 were male and 136 were female. Out of 183 cases, 32 were female associated with IDA and 3 were male. Serum vitamin B12 was studied in 159 cases, of which 45 had serum vitamin B12 level <179pg/mL, of which 33 were female and 12 were male. The author studied 28.7% of vitamin B12 deficient cases and 10.3% of folate deficient cases.

### 6.4 Significance of serum ferritin

Ferritin is a high molecular weight protein which contains 20% iron. It is mainly found in hepatocytes and reticulo-endothelial cells. It is a storage form of serum iron. Low levels of serum ferritin are seen in iron deficiency anemia and increased levels are seen in acute or chronic inflammatory conditions, as ferritin is an acute phase reactant. It is used as screening test to distinguish iron deficiency anemia from BTT patients. The level is usually normal (45-340ng/mL) or increased in thalassemia trait conditions.[66]

Edwards *et al*.[67] (1981), Fargion *et a*l. [68](1982) noticed high levels of serum ferritin in patients with beta-thalassemia trait, and even in those patients without any h/o transfusions. Fargion *et al*.[69](1985) suggested the modulating effect of beta-globin gene mutation on iron metabolism. Camaschella [70] (2005), stated that individuals with serum ferritin levels of >300ngm/mL (males) and >200ngm/mL(females), although asymptomatic, should be investigated for iron overload. The hemochromatosis gene (HFE) mutation plays an important role in determining the serum ferritin and transferrin saturation levels, as stated by Lynas *et al*.[71] (1997).

### 6.5 Molecular Genetic Analysis

PCR-based procedures, most commonly reverse dot-blot analysis or primer specific amplification are used to detect the mutations in beta globin chain gene. Reverse transcriptase-PCR(RT-PCR), sequencing of genomic DNA are some nucleic acid based methods employed in the diagnosis of thalassemia trait.[72] Sylvie *et al*.[81] (2008) stated, molecular testing can be done at any age.

## 7. PITFALLS

a) Coinheritance of alpha-thalassemia- These individuals have normal RBC indices but Hb $A_2$ levels are high. It is essential to perform Hb electrophoresis for diagnostic purposes.
b) Co-inheritance of delta thalassemia – These patients have reduced to normal, or sometimes, increased Hb $A_2$ levels, which is seen in beta-thalassemia carrier state. Double heterozygosity of delta and beta thalassemia can be distinguished from alpha-thalassemia by globin chain synthesis or globin gene analysis.[4]
c) Silent mutation, is a very mild mutation seen in residual beta chains, with normal RBC indices and normal Hb $A_2$. They are known as atypical carriers ($\beta^{++}$ mutation).
d) Other causes of beta-thalassemia trait with normal Hb $A_2$ include, associated iron deficiency cases, folic acid deficiency cases, heterozygosity for liver diseases and HbH disease.

## 8. SCREENING TESTS FOR INDIVIDUAL GROUPS

### 8.1 Prenatal Testing

Kan *et al*.[73] (1974), and, Wong *et al*.[74] (1978) introduced the application prenatal tests for the early detection of fetal anomalies and genetic aberrations. The tests can be invasive or non-invasive. Altay and Babak *et al*.[76] (1995) studied several molecular methods using Chorionic villus sampling (CVS), amniocentesis and cordocentesis for prenatal diagnostic tests. Thakur *et a*l.[77](2000) reported the importance of prenatal diagnosis for thalassemia. In India, Aditi *et al*. [(79)] (2004) studied the profile of beta-thalassemia in eastern India and its prenatal diagnosis.[7]

## 9. AMNIOCENTESIS

Fetal cells in amniotic fluid are aspirated and analyzed by molecular testing, to detect the presence of mutations in the fetus.

### 9.1 Cordocentesis

Under ultrasound guidance, about 2-3 ml of fetal blood is aspirated and subjected to gel electrophoresis and other molecular testing, if required[79].

### 9.2 Chorionic Villus sampling (CVS)

This test is performed around 10-11 weeks of gestation under ultrasound guidance, and then subjected to molecular testing.[80]

## 10. TREATMENT

### 10.1 Blood Transfusion

Blood transfusion with packed red blood cells forms the mainstay of treatment. Approximately, 150-200ml/kg, packed cell transfusion is administered to children with BTT. A pre and post transfusion Hb levels, along with a regular check of iron balance, helps in monitoring response to therapy and impending complications, like iron overload.[57]

## 11. PROGNOSIS

The prognosis of BTT is fair, with a chronic protracted clinical course. In patients with an additional Gilbert mutation, there is an increased risk of cholelithiasis.[65]

 