Frequency of Coincident Iron Deficiency among Children with B Thalassemia Trait

Anshuman Paria ¹Baishakhi Paria ², Soma Sengupta ³, Goutam Das ⁴
¹(Dept of Neonatology, SSKM hospital, WB)
²(Department of Community Medicine, National Medical College)
³(Dept of Anesthesiology, SSKM hospital, WB)
⁴(Department of Pediatric Medicine, North Bengal Medical College, West Bengal)

Abstract: β Thalassemia Trait(BTT) produces mild ineffective erythropoiesis and associated increased iron absorption from the gut. Based on these reasons it had been suggested that BTT confers an advantage in maintaining iron balance, in which case prevalence of iron deficiency should be lower in those with the trait. This Study was carried out to determine the frequency of coexistence of iron deficiency anaemia among children with β Thalassemia Trait (BTT) aged 1 to 12 yrs. 6 out of the 46 (13%) children with BTT were found to have concomitant iron deficiency (serum ferritin <12 µg/l) in comparison to 5 of the 25(20%) healthy non anemic controls, a difference which is not statistically significant. Microcytosis (MCV<75 fl) was significantly more associated with BTT as compared to control group. The difference between HbA2% of iron replete BTT and iron deficient BTT was not found to be significantly different. Based on these data, the following inferences can be drawn: Iron deficiency is no less common in children with β Thalassemia Trait than in those without. Any advantage conferred by BTT in prevention of Iron deficiency is probably trivial, and so a combined state of BTT and iron deficiency should be searched for in presence of microcytic anaemia . Iron supplements should be given in case of coincident iron deficiency in children with BTT as timely intervention can preclude the psychomotor adverse effects of iron deficient. Microcytosis (MCV<75 fl) can be regarded as a sensitive screening tool for β Thalassemia Trait. The percent elevation of HbA2 in BTT was striking even in presence of iron deficiency and concern about the reliability of HbA2 quantitation in presence of iron deficiency does not appear to be relevant and the test can be used confidently as a discriminant.

I. Introduction

Anaemia is the commonest co-factor of morbidity in many pediatric patients, both in indoor and office practice. Over 30% of world’s population i.e. 1500 million¹ are suffering from anaemia majority, of whom are suffering from microcytic anaemia. Common causes of microcytic anaemia in children include iron deficiency, beta thalassemia, while the rarer causes include lead poisoning, anaemia of chronic disease, and sideroblastic anaemia. Iron deficiency is the more widespread micro nutrient deficiency affecting 1.5 billion people globally.¹ Iron plays a pivotal role in various metabolic processes and its deficiency is the commonest cause of anaemia in the pediatric practice. Affected children become inattentive, disruptive, irritable, and restless. Work capacity and immunity are decreased.

Thalassemia syndromes result from quantitative defects in globin chain synthesis. This heterogenous group of recessively inherited anaemia can be caused by more than 200 different mutations affecting genes of α or β globin clusters. β thalassemia trait affect individuals heterozygous for a mutation that affects β globin synthesis i.e. synthesis of one of the β genes is affected by mutation while the other gene is unaffected. They have mild anaemia with disproportionate reduction in MCV and MCH. High A2 β Thalassemia trait, the commonest form of β Thalassemia trait is electrophoretically characterized by HbA2>3.5%, while HbF varies from 1-5%.³

Serum ferritin gives a sensitive reproducible and quantitative estimation of body iron stores. A low ferritin level is diagnostic of Iron deficiency. Ferritin is also a acute phase reactant, rising in inflammation, malignancy or hepatocellular damage. Ferritin is generally raised in anaemia of chronic diseases.

In clinical practice β thalassemia trait (BTT) must be distinguished from iron deficiency anaemia (IDA). Several indices and formulas have been developed to assist in the diagnosis of BTT (Mentzer index, Shine and Lal index, England and Fraser index)³ ---all of which are subject to error when both conditions coexist.
II. Materials And Methods

This cross sectional observational Study was conducted in Department of Pediatrics in a multispeciality hospital in Kolkata, from March 2009 to August 2011 was carried out to determine the frequency of coexistence of iron deficiency anaemia among children with β Thalassemia Trait (BTT) aged 1 to 12 yrs.

All mildly anaemic children who visited the pediatric OPD with trivial illnesses were screened for presence of microcytic anaemia. Those with microcytic anaemia were examined for the presence of β Thalassemia Trait. Such methodology eventually identified 46 children within the age group of 1-12 years who were carriers of beta thalassemia. These 46β Thalassemia Trait children were compared with 25 randomly selected healthy non anaemic children who were designated as CONTROLS.

Inclusion Criteria: All children with red cell microcytosis, who were subsequently detected to have β Thalassemia Trait.

Exclusion Criteria: Children with anaemia suffering from long standing systemic diseases like JIA, SLE, Bronchiectasis, chronic Osteomyelitis, Chronic liver disease etc. Children who have taken iron supplements in last 6 months.

Methodology: All children (1-12 yrs) visiting pediatric out patient department with trivial illnesses were screened clinically for pallor. Detailed enquiry and physical examination, with help of questionnaires, were carried out to exclude those children who are suffering from anaemia of chronic disease. Venous blood samples were drawn from all remaining children to elicit hemoglobin(Hb%) and mean corpuscular volume (MCV).

On subsequent visit, those 1-6 yr old with Hb%<11 gm% and 6-12 yr olds with Hb%<12% were diagnosed as anemic, as per WHO criteria. MCV values of less than 75 fl were considered as microcytic. Venous blood samples were drawn from all such children with microcytic anaemia to perform Hb electrophoresis/High Performance Liquid Chromatography (HPLC). Same samples were used for estimation of plasma ferritin levels.

Children with HbA2% >3.5% were considered to have β Thalassemia Trait. A child was considered to have coincidental iron deficiency if serum ferritin level was <12 microgram/litre.

Healthy non anaemic children (1-12 yrs) were selected as controls. A child will be considered to have iron deficiency if plasma ferritin <12 microgram / litre. Data thus obtained will be analysed by standard statistical methods.

Investigations: Hemoglobin electrophoresis was done by agar gel electrophoresis at alkaline Ph. HPLC was done by Variant Hemoglobin Testing System (Biorad laboratories, Hercules, CA).Serum Ferritin was done by ELISA technique, using kit supplied by Omega Diagnostics. Hemoglobin% and MCV were tested with Sysmex automated electronic cell counter.

Ethical Issue and Consent: A written informed consent was obtained from the parents or the guardians of the children regarding their willingness to participate in the study and to undergo the investigations. The study was approved by the ethical committee of the institution.

III. Results And Analysis

46 β Thalassemia Trait children were chosen as subjects of this study. 22 of these children were boys, the rest were girls. These beta thalassemia carriers were identified on the basis of microcytic anaemia and HbA2 >3.5% on the basis of HPLC/Haemoglobin electrophoresis.

25 age and sex matched healthy non anemic controls were chosen for comparison. Control group comprised of 11 boys and 14 girls. Healthy controls were chosen on basis of absence of ill health, absence of anaemia, and absence of history suggestive of intake of iron supplements in the past. All children of carrier (BTT) group and control group belonged to 1 to 12 yr age group.

Age and sex wise distribution of BTT children and healthy carriers are depicted in details in Table I. Table III shows that there is no statistically significant age difference between the BTT (β Thalassemia Trait with mean ± S.D = 6.04 ± 2.98) group and Healthy non anemic control group (mean ± S.D = 5.89 ± 3.11).

Student’s T Test revealed a statistically non significant p value of 0.84 when both groups were compared on age structure. Table II shows comparison between β Thalassemia Trait and healthy control group. When BTT (n=40, trait without iron deficiency) and BTT+I.D. (n=6, β Thalassemia Trait with iron deficiency) were compared, significant differences were obtained regarding MCV and FERRITIN levels. Table II further reveals that differences regarding Hb% and HbA2% were found to be statistically insignificant while comparing children from BTT group with those from BTT+I.D. group. Comparing BTT (trait without iron deficiency) with healthy non anaemic controls (n=25), values (mean ± s.d.) of MCV, Hb%, HbA2% of both groups were found to be statistically significantly different from each other (p<0.0001). Comparison of ferritin values of the two
groups yielded a p value of 0.0036. Statistically significant and highly different p values were returned when all 4 parameters (MCV, Ferritin, HbA2%, Hb%) of BTT+I.D. (β Thalassemia Trait with iron deficiency) were compared with normal non anemic healthy children (n=25)

**Mean Corpuscular Volume (MCV):** MCV of β Thalassemia Trait (BTT)(63.56±3.34 pg, n=46) was found to be statistically highly and significantly different (t statistic =19.016)(p value=0.0000) when compared with MCV of healthy carriers (80.4±3.98, n=25), data being analysed by Student’s T Test. [Table III]

**Iron Status:** Of the 46 β Thalassemia Trait (BTT) children, 6 (13%) were iron deficient, while 5 out of 25 (20%) healthy non anemic controls were iron deficient. This difference was not found to be statistically significant (p=0.66). When iron deficiency was compared between β Thalassemia Trait children and healthy controls in 1-5 yr, 5-9 yr, 9-12 yr age groups, FISHER’S EXACT TEST returned statistically insignificant p values of 0.39, 0.74, and 0.60 respectively.

Similarly when ferritin levels of BTT children (28.6±16.1μg/dl) and healthy controls (22.3±8.4μg/dl) were compared with help of T TEST, insignificant p value of 0.07 was obtained.

### IV. Discussion

Only 6 of 46 beta thalassemia carriers (13%) were found to have coexisting iron deficiency as well. This frequency of coexistence was lower than that was noted by Hinchcliffe and Lilleyman (1995) who found coexisting iron deficiency in 26 out of 77(34%) BTT children in a large multicentric study among British Asian Children. N.Madan, Sikka, Sharma, Rusia in a Delhi based study noticed coincidental iron deficiency in 33 (37%) out of 88 BTT children. In a study based on Lohana children in Bombay (1989) Mehta found 20.8% suffering from coincidental iron deficiency. Other studies have shown the prevalence to vary between 17 and 47%. The lower frequency of iron deficiency in this study could have been due to the fact that this institute generally caters to the upper and lower middle class of the society, where deprivation of iron rich food may not be too prevalent. Although the present study amply proves that iron deficiency is present in a sizable fraction of the study group, a larger sample pooled across several centres catering to different strata of the society would have been more representative of frequency of coexistence of iron deficiency anaemia with beta thalassemia minor.

In this study, Iron deficiency was found to be present in 13% (6 out of 46) of BTT subjects as compared to 20% (5 out of 25) of the healthy non anemic controls. This result was not found to be statistically significant (p=0.66). When iron deficiency was compared between BTT children and healthy controls in 1-5 yr, 5-9 yr, 9-12 yr age groups, FISHER’S EXACT TEST returned statistically insignificant p values of 0.39, 0.74, and 0.60 respectively. 3 out of the 15 BTT children under 5 years of age were found to be iron deficient, maximum among the different age groups.

Similarly when ferritin levels of BTT children (28.6±16.1μg/dl) and healthy controls (22.3±8.4μg/dl) were compared with help of T TEST, insignificant p value of 0.07 was obtained, thereby indicating that any advantage in iron supply conferred by BTT does not protect against iron deficiency, at least in children.

Madan, Sikka, Sharma, Rusia (1998) in their Delhi based study, funded by ICMR, estimated iron status of 463 BTT subjects, and found out 27.2% prevalence of IDA among subjects with BTT, with prevalence being even higher among children(37%). Madan et al in their study also showed that iron deficiency occurs with high frequency in children suffering from BTT, especially in children below 5 years of age. They concluded that BTT confers no extra advantage in maintenance of positive iron balance. Hinchcliffe et al also opined, based on their study on british asian children, that it should not be presumed that trait protects iron status or that the two are mutually exclusive, at least in the early years. They found 26 (34%) out of the 77 children with BTT having coincidental IDA, with frequency of coincidence being highest in children under 5 years (48.5%). Galanello and Turco et al, (1990) indicated that children heterozygous for beta thalassemia have normal iron stores but are relatively protected against development of iron deficiency, but when IDA happened in children with BTT, its clinical expression was usually more severe than in those without BTT.

Recently Bruno Nobili in a Naples, Italy (2001) based study also found no difference in incidence of IDA in children with BTT compared to normal children. Economidou et al, in 1980 observed similarly that iron deficiency was a common finding among female carriers of reproductive age who were not receiving regular iron suplementations.

Chatterjea (1964), Hussien et al (1976), Hegde et al (1975) concluded that BTT imparts no additional protection against development of IDA. On the other hand, Mehta B.C. and his co workers, Flaming et al (1969), White et al (1986) also obtained results that highlighted that thalassemia trait actually protected an individual from developing IDA. In a series of studies based on interaction between BTT and IDA, Mehta et al showed that there was significant difference between incidence of Iron deficiency in BTT and that in normal population. They showed that this advantage that individual with BTT had over normal population regarding
Frequency of Coincident Iron Deficiency among Children with β Thalassemia Trait

maintenance of positive iron balance, existed among pregnant women (1988), those with only mild anaemia (1983), and in family members of transfusion dependent beta thalassemia major patients. However a survey in Bombay by Mehta, Gandhi and Kamath (1983) among Lohana children (6-15 years) failed to show significant difference in frequency of iron deficiency in BTT and non-BTT children. Lohanas are a community in western India with high prevalence rate of BTT (7%-17%).

Mehta and his co workers hypothesised that advantage conferred by BTT vis-a-vis iron status, namely increased iron absorption, has to be operative for a longer period to be of significance. Mehta concluded that the study group (6-15 yr) had not received enough time to improve their iron status by virtue of increased iron absorption from gut, in contrast to adult individuals with BTT living in iron deficient environment. This result was similar to the result obtained by the present study and explains why coincidental iron deficiency is highest among those BTT who are in 1-5 yr age group.

It had been postulated that in a population where iron nutrition is good, with adequate iron intake and good bioavailability of iron, increased iron absorption (Bannerman and Callander (1964)), Crosby and Conrad (1964) may not offer significant advantage to beta thalassemia carriers. However, in a population with widespread iron malnutrition due to both poor dietary intake as well as poor bioavailability of iron, as in India, any factor that tends to increase iron absorption may be able contribute significantly to the improved iron nutrition. This hypothesis was put forward by Mehta et al to explain the disparity of results among studies conducted in India and abroad.

In the present study, MCV of β Thalassemia Trait (BTT) (63.56±3.34 fl, n=46) was found to be statistically highly and significantly different (t statistic =19.016)(p value=0.0000) when compared with MCV of healthy carriers (80.4±3.98, n=25), data being analysed by Student’s T Test, Table III

Microcytosis [MCV <75 fl] was found to be present in 100% of children with BTT as compared with 8% (2 out of 25) of non anemic healthy controls. This difference was found to be statistically highly significant. (p=1.33x10^-16, FISHER’S EXACT TEST). When BTT (n=40, trait without iron deficiency) and BTT+I.D. (n=6, β Thalassemia Trait with iron deficiency) were compared, significant differences were obtained regarding MCV levels. Comparing BTT (trait without iron deficiency) with healthy non anemic controls (n=25), values (mean ± s.d.) of MCV of both groups were found to be statistically significantly different from each other (p<0.0001).

Similar results were obtained by Madan et al in their study, thereby indicating the role of MCV as a primary screening tool for BTT. It also indicates that coexistence of iron deficiency makes microcytic cells of BTT even more smaller.

Hemoglobin concentration in the B Thalassemia Trait subjects with iron deficiency was not found to be significantly different from those carriers without iron deficiency [Table II]. This is in contrast to the result obtained by Madan et al who found significantly low haemoglobin concentration in iron deficient BTT subjects. This could be partly be due to lower number of iron deficient BTT children in the present study (n=6), as compared to 33 in the study by Madan et al.

Level of HbA2 In β Thalassemia Trait with Iron Deficiency A2: HbA2 >3.5% by HPLC/hemoglobin electrophoresis is thought to be diagnostic of beta thalassemia minor. In the present study, HbA2% of the iron deficient BTT (BTT+I.D.------5.1±0.49) and iron replete BTT (BTT---- 4.9±0.22) children were compared. The difference of HA2% (mean±SD) between the two groups was not statistically significant (p=0.43). The difference between the HbA2% of BTT+I.D. group and the healthy control group was found to be highly significant (p=0.0000). The above dataset indicates that in the present study presumed decrement of HbA2% in heterozygous beta thalassemics in face of iron deficiency did not preclude the diagnosis of carrier state.

Controversies are galore in medical literature about diagnosing BTT in a community with high incidence of IDA, owing to presumed dip in level of HbA2 that could of preclude the diagnosis of BTT. Wasi et al (1968), Kattamis(1972), reported that severe iron deficiency leads to a drop in HbA2 to normal level and is restored to its elevated level only after adequate iron supplementation.

Weatherall D J (2001), Steinberg (1993), found this fluctuation unusual and concluded that although the level of HbA2 level may fall, it still remains elevated in the range found in BTT. Madan et al (1998), Hinchcliffe, Lilleyman (1995), Mehta,Pandya (1987), and Saraya et al (1984) have all concluded that elevation of HbA2% was striking even in presence of iron deficiency in heterozygous BTT subjects.

Alperin et al (1977) found out that reduction in HbA2% corresponded with severity of anaemia . Mehta and Pandya in 1985 while studying the iron status of beta thalassemia carriers found out that HbA2 level of non-BTT subjects with iron deficiency could not be raised to 3.5% even after 12 weeks of iron therapy. They also observed that HbA2 level of none of BTT carriers was diminished below 3.5% even in face of severe iron deficiency. Madan et al had opined that coexistence of the both will not preclude the detection of BTT in majority of carriers, this may not be universal. They postulated that the DNA mutations responsible for sufficiently raised HbA2% even in presence of limiting factors like iron deficiency, may not be operational in other population groups. They have further suggested that utility of HbA2 as a confirmatory test of BTT in presence of iron deficiency needs to be reaffirmed in each population group.

DOI: 10.9790/0853-14412026 www.iosrjournals.org 23 | Page
Garewal and Marwarha\textsuperscript{22} (1994) had highlighted that coexistence of alpha thalassemia trait may decrease HbA2 levels in BTT. While the present study has brought forward certain salient points regarding the interaction between thalassemia minor and iron deficiency anaemia, much scope remains for improvement. A larger sample size collected across several centers would have been more representative. In the hindsight it also seems that inclusion of other parameters like RBC count, MCH, and HbA2/cell would have made the study more comprehensive, but at the same time would have made the study more complicated and would in no way help in identifying the frequency of coincidence of iron deficiency anaemia with beta thalassemia minor. Financial constraints, have in a way, precluded the use of HPLC for all the subjects.
V. Conclusion

While the present study has brought forward certain salient points, a multicentric study with larger sample size would make the results more representative of the entire population. However it can be safely opined that, Iron deficiency is no less common in children with β Thalassemia Trait than in those without. Any advantage conferred by BTT in prevention of Iron deficiency is probably trivial, and so a combined state of BTT and iron deficiency should be searched for in presence of microcytic anaemia . Iron supplements should be given in case of coincident iron deficiency in children with BTT as timely intervention can preclude the psychomotor adverse effects of iron deficient. Microcytosis(MCV<75 fl) can be regarded as a sensitive screening tool for β Thalassemia Trait. The percent elevation of HbA2 in BTT was striking even in presence of iron deficiency and concern about the reliability of HbA2 quantitation in presence of iron deficiency does not appear to be relevant and the test can be used confidently as a discriminant.

Reference


Table I: Comparison among incidence of Iron deficiency in Beta Thalassemia Carriers and Healthy Controls

<table>
<thead>
<tr>
<th>AGE</th>
<th>SEX</th>
<th>BETTA THAL MINOR</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO IRON</td>
<td>WITH IRON</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DEFICIENCY</td>
<td>DEFICIENCY</td>
<td></td>
</tr>
<tr>
<td>1-5 YRS</td>
<td>MALE</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>FEMALE</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>5-9 YRS</td>
<td>MALE</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>FEMALE</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>9-12 YRS</td>
<td>MALE</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FEMALE</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>40</td>
<td>6</td>
<td>20</td>
</tr>
</tbody>
</table>

Table II: Hematological profile of 3 different groups of patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Significance (p&lt;0.0001)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BT (n=26)</td>
<td>BT + I.D. (n=26)</td>
<td>CONTROL (n=25)</td>
<td>1 vs 2</td>
</tr>
<tr>
<td>Hb (gm/d)</td>
<td>10.12±0.49</td>
<td>9.5±0.72</td>
<td>12.67±0.76</td>
<td>NS</td>
</tr>
<tr>
<td>MCV (pg)</td>
<td>64.15±3.11</td>
<td>59.66±2.03</td>
<td>80.48±3.98</td>
<td>S</td>
</tr>
<tr>
<td>HbA2%</td>
<td>5.10±0.49</td>
<td>4.9±0.22</td>
<td>2.46±0.39</td>
<td>NS</td>
</tr>
<tr>
<td>Ferritin (mcg/dl)</td>
<td>31.31±15.5</td>
<td>10.61±0.49</td>
<td>22.30±8.42</td>
<td>S</td>
</tr>
</tbody>
</table>
BTT=Β Thalassemia Trait, I.D = Iron Deficiency, N.S = Not Significant, S = Significant, Student’s T Test was used for statistical analysis

**Table III: Comparison of different parameters in Beta Thalassemia Carrier and Healthy Controls**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO OF OBSERVATIONS</th>
<th>MEAN</th>
<th>S.D.</th>
<th>STATICAL TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age structure among Beta Thalassemia Carrier and Healthy Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTT</td>
<td>46</td>
<td>6.94</td>
<td>2.98</td>
<td>( t = 0.2013, \ p = 0.8410 ) (non significant)</td>
</tr>
<tr>
<td>CONTROL</td>
<td>25</td>
<td>5.89</td>
<td>3.11</td>
<td></td>
</tr>
<tr>
<td>HbA2 % among Beta Thalassemia Carrier and Healthy Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTT</td>
<td>25</td>
<td>2.46</td>
<td>0.94</td>
<td>( t = 24.08, \ p = 0.0000 ) (highly significant)</td>
</tr>
<tr>
<td>CONTROL</td>
<td>46</td>
<td>5.11</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>MCV among Beta Thalassemia Carrier and Healthy Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTT</td>
<td>25</td>
<td>80.4</td>
<td>3.98</td>
<td>( t = 19.016, \ p = 0.0000 ) (highly significant)</td>
</tr>
<tr>
<td>CONTROL</td>
<td>46</td>
<td>63.56</td>
<td>3.34</td>
<td></td>
</tr>
<tr>
<td>Ferritin level (μg/dl) among Beta Thalassemia Carrier and Healthy Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTT</td>
<td>25</td>
<td>22.3</td>
<td>8.4</td>
<td>( t = 1.8223, \ p = 0.0727 ) (non significant)</td>
</tr>
<tr>
<td>CONTROL</td>
<td>46</td>
<td>28.6</td>
<td>16.1</td>
<td></td>
</tr>
</tbody>
</table>