Serum Ferritin in Thalassemia Intermedia

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Received: 4 February 2013 / Accepted: 27 April 2013 / Published online: 6 June 2013
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Abstract Serum ferritin is a useful monitoring tool for iron overload in thalassemia major. In resource poor settings access to modalities for assessment of iron overload are limited. This study was undertaken to assess the efficiency and usefulness of serum ferritin level in thalassemia intermedia (TI) patients. This was a cross sectional observational study. Seventy seven TI patients attending the pediatric hematology clinic were included. Fasting blood sample was taken from each patient in iron free vials for iron studies. Serum ferritin was estimated by immunometric enzyme immunoassay using Orgentec GmbH kits. Mean age of patients evaluated was 10.9 ± 5.03 (3–26) years. The mean age at diagnosis was 4.21 ± 2.3 (0.8–11) years. Mean serum ferritin was 486.54 ± 640.0 ng/ml (15–4,554). Thirty two (41.5 %) patients had a ferritin value of ≥ 500 ng/ml. Nine patients had a serum ferritin of $\geq 1,000$ ng/ml. Three of the subjects with a ferritin $>1,000$ ng/ml had never received a blood transfusion (BT) and in the other six, the number of BTs ranged from 1 to 8. Serum ferritin did not correlate with age, total number of BTs splenectomy status or BT in last one year ($p > 0.05$). In 41.5 % of TI patients, serum ferritin was ≥ 500 ng/ml. Age, BT and splenectomized status did not

affect ferritin level. We postulate interplay of other biological factors like HFE gene mutation, ferroportin, etc. to contribute to ferritin level and hence iron load in TI patients. Ferritin can possibly be used as screening and monitoring tool for iron load in TI patients when other modalities to assess iron overload are not easily available.

Keywords Thalassemia intermedia · Ferritin · Iron overload · Blood transfusion

Introduction

Thalassemia intermedia (TI) describes patients in whom the clinical severity of the illness encompasses a large grey zone between transfusion dependent β thalassemia major (TM) and β thalassemia minor. TI patients usually do not require regular blood transfusions (BTs). However, iron overload is a recognized feature of TI. The pathophysiology is attributed mainly to increased iron absorption from the gastrointestinal (GI) tract and the occasional transfusions. In TI, iron absorption is 3–10 times normal, which is 17–89 % compared to 14.8 ± 9.5 % in normal subjects, and increases after splenectomy [1, 2]. Studies have shown iron loading in non-transfused TI patients on standard mixed diet at 0.1 mg/kg/day amounting to iron load of up to 1–3.5 g/year and this results into rising ferritin with increasing age [2, 3]. Iron overload in TI patients is often detected late. Liver iron concentration and of late, CMR derived T2* are considered the best methods for estimation of iron overload. In low income countries these modalities are not easy to come by and serum ferritin is often the mainstay to measure iron overload. This study was undertaken to assess serum ferritin as a marker of iron load in TI patients.

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Patients and Methods

Patients

This study was carried out in the Pediatric Hematology Oncology unit of the Advanced Pediatric Centre at the Postgraduate Institute of Medical Education and Research, Chandigarh, which is a referral center in North India. Seventy seven patients with TI attending the pediatric hematology clinic were enrolled over a 15 month period (June 2008–August 2009).

TI was diagnosed when a patient fulfilled at least 2 of the following criteria. (i) Age at initial presentation ≥ 3 years. (ii) Administration of first red cell transfusion after the age of 3 years, before/after the diagnosis of thalassemia was established. (iii) Pretransfusion hemoglobin (Hb) level of ≥ 7.0 g/dl at the time of first transfusion and (iv) need for red cell transfusion < 5 times per year.

Inclusion Criteria

Patients diagnosed as TI, attending the outpatient clinic of pediatric hematology clinic.

Exclusion Criteria

Patients on iron chelation and those who had received BT within one month prior to performing the iron studies.

Methodology

This was a single time cross sectional observational study. Demographic data of 77 TI patients were recorded. This included:

1. Number of BTs received in the preceding year and mean Hb in the last one year.
2. Age, symptoms and Hb at presentation.
3. Height, Weight and examination findings.
4. Hb electrophoresis/HPLC findings of patient and parents.
5. Fasting blood sample was taken from each patient in iron free vials for iron studies. Iron studies included [i] serum iron [ii] ferritin [iii] transferrin saturation and the [iv] total iron binding capacity (TIBC).

Ferritin was estimated by immunometric enzyme immunoassay (ELISA kit manufactured by ORGENTEC, KUPFER BERG TERRASSE 17–19, D-55116, Mainz, Germany). Estimation of serum iron, TIBC is a modification of the recommendation of the International Committee for Standardization in Haematology (ICSH) [4]. Percentage transferrin saturation was calculated by the formula; serum iron ($\mu\text{g/dl}$)/TIBC ($\mu\text{g/dl}$).

Institutional ethics committee approval was taken for this study. An informed consent was taken from parents for children under 18 and from patients who were older than 18 years.

Statistical Analysis

Data were analyzed in SPSS-15 software. For analysis, patients were divided into three age groups: group A: < 8 years, group B: 8–12 years and group C: ≥ 13 years. Descriptive statistics were calculated for all measurable data. Comparison between 3 groups was done for various parameters using ANOVA followed by post hoc test (SNK procedure). We applied non parametric test (Mann–Whitney test) for comparison of two groups with ferritin < 500 or ≥ 500 ng/dl for various parameters. Bivariate correlations (Spearson's correlation) were worked out. A ' p ' value of < 0.05 was taken as significant.

Results

Forty nine boys and 28 girls constituted the 77 patients studied. The mean age was 10.9 ± 5.03 (3–26) years. The number of subjects in each group was: group A (< 8 years): 24; group B (8–12 years): 29 and group C (≥ 13 years): 22. The sex distribution was equal among the groups.

Table 1 Demographic profile of patients

Mean age	10.9 ± 5.03 years (3–26)
Mean age at diagnosis	4.21 ± 2.3 years (0.8–11)
Mean Hb at diagnosis	6.09 ± 1.61 g/dl (2–9.4)
Primary presenting symptom	
Pallor	32 (41.5 %)
Fever	16 (20.7 %)
Jaundice	10 (12.9 %)
Abdominal distension	5 (0.06 %)
Hemolytic facies at diagnosis (clinician documentation)	39 (50.6 %)
Mean age of first BT	5.2 ± 1.78 years (2–11)
Mean no. of BTs	1.64 ± 4.5 (0–30)
Splenectomy	19 (24.6 %)
Mean age of splenectomy	8.0 ± 2.4 years (5–15)
Patients with serum iron > 70 $\mu\text{g/dl}$	19 (24.6 %)
Patients with serum ferritin > 250 ng/ml (normal range)	43/77 (55.8 %)
Patients with serum ferritin > 500 ng/ml	32/77 (41.5 %)

The mean age at diagnosis was 4.21 ± 2.3 (0.8–11). Most patients (51.2 %) were diagnosed between 3 and 8 years of age. The common presenting symptoms included pallor, fever and jaundice (Table 1). Two patients were diagnosed as a result of family screening. The mean hemoglobin (Hb) at diagnosis was 6.09 ± 1.61 g/dl. Forty four patients (57.1 %) presented with a Hb between 5 and 7 g/dl at diagnosis. Fourteen (18 %) patients presented with severe anemia (Hb <5 g/dl) while 3 (0.04 %) had a Hb >9 g/dl at diagnosis. Forty four (57.1 %) received BTs at some point of time. The age at first BT was 5.2 ± 1.78 (2–11) years. The number of BTs ranged from 1 to 30, the mean being 1.64 ± 4.5 (the extreme number removed to avoid skew). Eighty percent patients received <5 BTs. Five patients received 5–10 BTs and only 2 patients received >10 BTs (22 and 30). Nineteen patients (24.6 %) had a splenectomy. Splenectomy was done at a mean age of 8.0 ± 2.4 (5–15) years of age.

Values of serum iron, TIBC, transferrin saturation and ferritin are given in Table 2. Mean serum ferritin was 486.54 ± 640.0 ng/ml, the range being 15–4,554 ng/ml. Mean serum ferritin level in different age groups was (i) group A: 279.88 ± 291.3 ng/ml (19–1,291) (ii) group B: 585.17 ± 830.4 ng/ml (14–4,554) and (iii) group C: 573.54 ± 591.0 ng/ml (15–2,000).

Thirty two children had a ferritin value of more than 500 ng/ml. Intergroup comparison was done between patients with a ferritin of <500 ng/ml and with a ferritin of ≥ 500 ng/ml (Table 3). There was no difference in serum ferritin value with regards to age, BTs and Hb. Higher transferrin saturation was seen in those with a ferritin of ≥ 500 ng/ml.

Nine patients had a serum ferritin of $\geq 1,000$ ng/ml. The mean age of these patients was 13.2 (6–26) years. Three of these patients had never received a BT. In the other six patients, the number of BTs ranged from 1–8. Interestingly, 4 children were ≤ 10 years of age. One child had a ferritin of 4,554 at 10 years having received only eight BTs. Conversely, in the whole cohort one 12 year old child who had received 30 BTs had a serum ferritin value of 520 ng/ml.

Comparison of Factors Which can Affect Iron Overload Status

Age, number of BTs, splenectomy status, BT in last one year, height percentage (CDC standard) was compared

Table 2 Values of serum iron, TIBC, transferrin saturation and ferritin

Parameter	Iron (μ g/dl)	TIBC (μ g/dl)	Transferrin saturation (%)	Ferritin (ng/ml)
Normal range	50–70	200–450	20–40	15–250
Mean value	136.0 ± 62.1	340.14 ± 3.7	40.70 ± 17.8	486.54 ± 640.0
Range	35–392	183–626	11–77	15–4,554

along with serum ferritin level by applying non parametric correlation test. Serum ferritin value did not correlate with any of the above parameters. However a positive correlation with serum transferrin saturation was found.

Intergroup Comparison of Factors Affecting Iron Load Status

The number of patients who received BTs in each group was similar. The number of BTs per patient in each group was comparable ($p = 0.392$). Mean value of serum ferritin was higher in group B (585.2 ± 830.4) and C (573.5 ± 591.0) as compared to group A (279.9 ± 291.3) but the intergroup difference was not statistically significant ($p = 0.163$). There was no statistical difference in the serum ferritin values between the two gender groups. No difference in serum ferritin was observed between the splenectomized and non splenectomized patients ($p = 0.183$).

Discussion

The mean age of diagnosis of TI in our study was 4.21 ± 2.3 years. A multicenter study from US has reported the mean age of diagnosis in TI to be 3.5 years [5]. Only 41.5 % patients presented with pallor. The mean Hb level in our study population was 6.09 ± 1.61 g/dl. Tyagi et al. [6] reported a mean Hb at presentation of 7.4 g/dl. A large multicenter study from Italy reported a mean Hb

Table 3 Comparison between patients with serum ferritin <500, and those with ≥ 500 ng/ml (applied statistics—Mann–Whitney test)

Parameter	Ferritin <500 ng/ml ($n = 45$)	Ferritin ≥ 500 ng/ml ($n = 32$)	<i>P</i> value
Age (years)	10.0 ± 4.6	11.5 ± 5.5	0.264
Total number of BTs	0.7 ± 1.3	2.83 ± 6.6	0.272
Number of BTs in last one year	0.4 ± 0.6	0.57 ± 0.9	0.979
Mean Hb (over previous 1 year) (g/dl)	7.2 ± 1.4	7.0 ± 1.5	0.820
Iron (μ g/dl)	126.40 ± 66.2	157.7 ± 46.1	0.006
TIBC (μ g/dl)	356.06 ± 98.7	308.3 ± 73.7	0.089
Transferrin saturation (%)	35.5 ± 15.8	52.6 ± 16.8	0.000

of 8.9 ± 1.4 g/dl [7]. Our mean Hb was lower at presentation. This was attributed to folic acid deficiency as the level rose to >7.0 g/dl after supplementation. Eighteen percent had severe anemia at presentation and needed blood. However, subsequently regular transfusion was not required. We attribute this to a delayed presentation and folic acid deficiency. No correlation was observed between mean age at diagnosis and Hb level. Two third (67 %) of our patients had BTs during the initial 12 years of life. The moderate to severe phenotypes would be likely to present earlier to the pediatric clinic while the milder ones possibly present late to the adult clinic and need lesser transfusions.

Ferritin has been reported to increase with age in non-transfused thalassemia patients especially when the iron overload is excessive [8]. Gumruk et al. [9] in their study of 43 patients in the age group of 3–36 (mean 13.4 ± 7.5) did not demonstrate any correlation with age. Similarly, Pakbaz et al. [10] also did not find any correlation of serum ferritin with age. Our study did not show correlation of age and ferritin level, although a higher trend was seen with increasing age.

Ferritin level is expected to increase with rise in the number of BTs. In our study no difference was seen in the ferritin level between patients who received BTs vis a vis those who did not. This is in sharp contrast to the findings of Gumruk et al. [9] who showed significant correlation between the levels of serum ferritin in the group who received BTs versus the group which did not, the values being 399 ± 20 and 219 ± 18 ng/ml, respectively. In addition, their study also showed significant correlation between the number of BTs and serum ferritin level. However our study did not endorse these findings. It is interesting to note that in our study, a 10 year old patient had a ferritin level of 4,554 ng/ml after having received eight transfusions while another 12 year old child had a ferritin of 520 ng/ml after having received 30 BTs (both tested twice for serum ferritin level). Nine children had a ferritin of $>1,000$ ng/ml. We could not make a definite conclusion as to the cause of a higher ferritin in these nine children by studying the existing variables.

Splenectomy has been reported to influence ferritin level. A higher ferritin level in a splenectomized individual as compared to an individual with intact spleen has been described [11]. The mechanism for higher iron overload in splenectomized patients is postulated by the fact that the intact spleen may be a reservoir of excess iron and possibly has an excess scavenging effect on iron-free fractions, including non-transferrin-bound iron [12]. We did not find any difference in the ferritin level between splenectomized versus non splenectomized patients. This outcome is similar to findings by Gumruk et al. [9] where no difference was observed in serum ferritin levels between splenectomized ($n = 15$) and non splenectomized ($n = 28$) TI patients.

Factors which have been postulated to affect iron load include the HFE gene mutation (Cys282Tyr and His63Asp), ferroportin (Val162del mutation of the SLC11A3 gene), β_2 microglobulin, transferrin and Nramp (SLC11A1) [13]. Mutation of HFE gene is one of the noted risk factors for iron overload in TI patients. However, conflicting reports are described in literature [14–17].

We have used an indirect method (serum markers) to assess iron load status. Direct methods (liver biopsy, cardiac iron quantification, SQUID) are more reliable. Non-invasive imaging methods like MRI have shown promising results and periodic iron load assessment using CMR-derived T2* relaxation time is now being advised [18]. It is currently the mainstay for the quantitative assessment of cardiac iron deposition [19, 20]. Serum ferritin underestimates actual iron load in TI patients as compared to TM patients. This is due to the combination of ineffective erythropoiesis and chronic anemia/hypoxia which results in hepcidin suppression, leading to increased iron absorption and increased release of recycled iron from the RE system. This results in depletion of macrophage iron, preferential portal and hepatocyte iron loading and may explain the relatively low levels of serum ferritin encountered in TI [21]. By contrast, in transfused TM patients, iron is preferentially distributed to the RE system, stimulating ferritin synthesis and its release to the circulation, resulting in high serum ferritin levels [10].

Optimal cut-off level of ferritin or liver iron concentration (LIC) in TI patients to start iron chelation therapy is not well defined. Iron chelation is recommended to be started when LIC exceeds 7 mg/g dry weight of liver tissue [22]. This has been shown to correspond to serum ferritin of 400–500 ng/ml [23] and it is the recommended threshold of starting chelation in TI patients [21].

From this data we feel that one can recommend monitoring TI patients with serum ferritin in the absence of better modalities. This would help identify patients who are probably iron overloaded and need further management.

Conclusions

Significant proportion of TI patients (41.5 %) had serum ferritin value of >500 ng/ml irrespective of age and blood transfusion status. The number of patients with iron overload would possibly be higher as serum ferritin is considered to underestimate iron overload especially in TI patients. The number of BTs and splenectomized status do not appear to affect serum ferritin level in TI patients. We postulate interplay of other biological factors like HFE gene mutation, ferroportin, etc. to contribute to iron overload in TI patients. Serum ferritin may not be a very good indicator of iron overload status in TI; however it can be

used as a screening and monitoring tool for iron overload in TI. This simple screening test has helped us to identify patients with a ferritin of >500 ng/ml who would need close monitoring and probably chelation in the near future. Iron status should be monitored regularly in TI patients irrespective of age and number of BTs so that iron overload can be detected and treated early to avoid long term complications. Further studies are required to establish exact mechanisms of iron overload in TI patients.

Conflict of interest The authors state no conflict of interest in this manuscript.

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