Study of Serum ferritin levels in β-Thalassemia major children

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Abstract

Background: Thalassemias one of the most common inherited single-gene disorder in the world. Every year approximately 100,000 thalassemia major children are born all over the world, and there are about 65,000-67,000 β thalassemia major patients in India, with around 9,000-10,000 cases being added every year. Measurement of serum ferritin level can give idea regarding starting of Iron chelation therapy, which will reduce the concentration of serum ferritin and effective in preventing iron induced tissue injury and prolonging life expectancy.

Method: This study was conducted on 60 children between 3 to 17 years, being regularly transfused at department of Pediatrics, Kamala Raja Hospital, Gajra Raja Medical College, Gwalior, for period of 1 year from 2011 to 2012, after taking the informed consent from the parents and explaining them the purpose of study. Detailed history weretaken and serum ferritin level weremeasured by ELISA based serum ferritin assay kit.

Results: Serum ferritin level was found to be elevated in all the patients of beta thalassemia major with range from 1050 to 5029 µg/l and with a mean value of 3879µg/l. Out of 60 patients, 30 (50%) patients had serum ferritin level below 2000μg/l, 20 (33.3%) patients had serum ferritin value between 2001 to 4000 µg/l and rest 10 (17.7%) patients had values above 4000 µg/l. Mean value of serum ferritin was found to be higher in patients who received frequent blood transfusion.

Conclusion: Majority of the patients had very high ferritin levels, with a mean value of 3879 µg/l. 50% patients had serum ferritin levels more than 2000μg/l. This cut off value reflect either inadequate chelation therapy or non-affordability of parents to purchase oral chelation therapy.

Key words: β-Thalassemia major, Serum ferritin, Blood transfusion

Introduction

Thalassemia is one of the most common inherited single-gene disorder in the world. Reportedly there are about 240 million carriers of beta thalassemia worldwide and in India alone, the number is approximately 30 million with a mean prevalence of 3.3%. Every year approximately 100,000 thalassemia major children are born all over the world, and there are about 65,000-67,000 β thalassemia major patients in India, with around 9,000-10,000 cases being added every year[1,2]

The name Thalassemi is derived from a combination of two Greek words: Thalassa meaning the sea, i.e. the Mediterranean, and anemia (“weak blood”)[3]. It is characterized by deficient or absent synthesis of normal globin chains owing to inherited mutations of β- globin genes, where more than 200 mutations, mostly point mutations are seen in β thalassemia. There is an excess of alpha globin chains relative to beta and gamma globin chains and alpha globin tetramer formed that cause damage to both developing RBC and mature RBC. The gamma and delta globin are produced in increased amount leading to an elevated HbF and HbA2. Since β chains are not present in fetal hemoglobin, beta thalassemia major does not manifest itself in newborns. Beta thalassemia presents at 6 months of age when adult hemoglobin has replaced fetal hemoglobin.

The clinical course is characterized by severe anemia, failure to thrive, typical facies (also called chipmunk facies/thalassemic facies), marked hepatosplenomegaly,
cachexia and pathological bone fractures [4]. The most effective approach to reduce the burden on the society and reduce the disease incidence is through implementation of a carrier-screening programs offering genetic counseling, prenatal diagnosis and selective termination of affected fetus [5].

The liver is the major site of iron overload, containing 70% or more of body iron content. Liver iron correlates closely with total body iron in Transfusional iron overload.

Estimation of direct liver iron concentration is the most accurate method of estimation of iron overload. But in our set up this method was not available. Indirect method with serum ferritin level measurement is reliable, easy to perform, low cost, and had no side effects [6].

Cure of beta thalassemia major is available with bone marrow transplantation but because of lack of facility, affordability and awareness, cure of these children is very difficult; so now a day's regular blood transfusion and continuous iron-chelation therapy is the mainstay of treatment available for these children.

In India, most thalassemic children are under transfused and do not get appropriate iron-chelation therapy because the cost of treating a thalassemia child is approximately one lakh per year.

The aim of the present study is to assess the serum ferritin levels in multi-transfused β Thalassaemia major patients, so that timely corrective measures can be adopted.

Material & Methods

Study design- Prospective and hospital-based study.

Study center- Department of Pediatrics, Kamala Raja Hospital, Gajra Raja Medical College, Gwalior, Madhya Pradesh, India.

Period of study- June 2011 to June 2012

Study population- This study was conducted on 60 confirmed β thalassemia major children between 3 to 17 years, registered in Gwalior thalassemia society, being regularly transfused at department of Pediatrics, Kamala Raja Hospital, G R Medical College, Gwalior.

Ethical clearance was obtained from the institutional ethical committee.

Sample collection- The known cases of β thalassemia major children between 3-17 years, that had been transfused in Kamala Raja Hospital were selected by purposive sampling method. Subjects were classified into three age groups: 3-5 years, in between 6-10 years and 11-17 years.

Inclusion criteria
• Child suffering from β-thalassemia major only as confirmed by Hbelectrophoresis.
• Age of thalassemic child should be between 3-17 years.
• Attending department of pediatrics, Kamala Raja Hospital, Gajra Raja Medical College, Gwalior.

Exclusion criteria
• Abnormal liver functions.
• Presence of infections at the time of sample collection.
• Any haemoglobinopathy other than beta thalassemia major.

Methodology and Sampling methods: Detailed history (age, sex, history of blood transfusion, history of iron-chelation therapy) as per the proforma was taken. 3ml of blood was collected by vein puncture and allowed to clot. The separated serum was used for the estimation of ferritin levels using ELISA based serum ferritin assay kit (Acculite ferritin kit) by chemiluminescence immunoassay method.

Principle of serum ferritin estimation: The essential reagents required include high affinity and specific antibodies, with different and distinct epitope recognition, in excess and native antigen. In this procedure, the immobilization takes place during the assay at surface of an opaque chemiluminescent reaction cell through the interaction of streptavidin coated on the opaque reaction cell exogenously added biotinylated monoclonal antibody coupled to the analyte of interest. Upon mixing monoclonal biotinylated antibody, the enzyme labeled antibody and test serum containing the native antigen and the antibody, without competition, to form a soluble sandwich complex.

Statistical Analysis: All the statistical analysis were conducted by using Epi Info™ software version 3.5.3. Data were analyzed to determine association between variables. The association between age, sex, frequency of blood transfusion and serum ferritin level were established. Chi-square test was used to compares the groups. A two-tailed pvalue of <0.05 was considered to be statistically significant.
Results

This study included 60 patients suffering from beta thalassemia major, with 11 (18.3%) females and 49 (81.7%) males. The study subjects were classified into three age groups: 3-5 years, 6-10 years and 11-17 years (Table-1). The mean age was 9.45±4.9 years with a range of 3–17 years. The median age at the first transfusion was six months (range 4–16 months). The most frequently observed patients were Sindhi (86%), followed by the Muslim (10%) and Others (4%). Blood group analysis revealed Group O to be the most common amongst our sample (45%). A positive family history of thalassemia major was recorded in 67.5% of the patients, and 55.5% of them gave a history of consanguineous marriage of parents. History of splenectomy was found in 10(17.7%) children.

Table-1: Age and sex wise distribution of beta thalassemia major patients.

| Age group (in years) | Male | Female | Total |
|----------------------|------|--------|-------|
| 3-5                  | 25   | 4      | 29    |
| 6-10                 | 10   | 4      | 14    |
| 11-17                | 14   | 3      | 17    |

Out of total 60 children, most were in the age group of 3 to 5 years.

The mean serum ferritin concentration was 3879 µg/l (range 1050 –5029µg/l). Mean serum ferritin of children aged between 3-5 years was 1667 ± 297, while in children of more than 10 years mean serum ferritin was 3744 ± 715.

Table-2: Age wise distribution of mean serum ferritin level in beta thalassemia major patients.

| Age group (in years) | Serum ferritin (Mean±SD) | Total patients |
|----------------------|--------------------------|----------------|
| 3-5                  | 1667 ± 297               | 29             |
| 6-10                 | 2675 ±719                | 14             |
| 11-17                | 3744 ± 715               | 17             |

The serum ferritin concentration was less than 2000µg/l in 30 patients (50%), between 2001–4000 µg/ in 20 patients (33.3%) and rest 10 patients (17.7%) had serum ferritin levels >4000 µg/l. Majority of the patients with high serum ferritin concentration (>4000µg/l) were aged more than 10 years (Fig-1). Mean serum ferritin concentration of the females patients was 2986µg/l while in male patients it was 3245µg/l. There was no significant difference of mean serum ferritin concentration between two groups (p = 0.54).

![Figure-1: Range of serum ferritin level observed in beta thalassemia major patients](image_url)

Serum ferritin values were analysed based on average total number of transfusions. Patients with transfusion more than 150 times had ferritin level greater than the patients who had transfusions less than 150 times and this difference was statistically significant (p value <0.001)(Table-3).
Table-3: Correlation between mean serum ferritin level and number of blood transfusions

| Number of blood transfusions | Serum.ferritin (mean±sd) | p.value |
|-----------------------------|--------------------------|---------|
| <150                        | 2109 ± 734               | <0.001  |
| >150                        | 3956±925                 |         |

Patients with transfusion more than 150 times had ferritin level greater than the patients who had transfusions less than 150 times and this difference was statistically significant (p value <0.001)

Out of 60 children 42(70%) were taking some form of oral chelation therapy, rest 18(30%) children were not receiving chelation therapy. **Fig-2** showed a linear relationship between the age of thalassemia major patients and total number of blood transfusions received so far. With the increase in age, the cumulative number of blood transfusions received will increase, as is depicted in the graph.

**Fig-2: Age group wise average total no of blood transfusion in beta thalassemia major children**

Graph shows a linear relationship between the age of thalassemia major patients and average total number of blood transfusions.

**Fig-3 showed that frequency of blood transfusions received per month also goes up. Most of the patients aged 3-5 years receive blood transfusions only once a month.**

Most of the beta thalassemia major children age group 3-5 years, received blood transfusions only once a month, while children in 6-10 years and 11-17 years age group, received blood transfusion twice or thrice a month (**Fig-3**)

**Discussion**

Thalassemia is one of the commonest hemolytic anemia in India. Serious complications due to iron overload still occurs in Thalassemia. Transfusional hemosiderosis is the major cause of late morbidity and mortality inpatients with thalassemia major. It can be prevented by use of iron-chelation therapy which will reduce the concentration of serumferritin and is effective in preventing iron induced tissue injury and prolonging life expectancy.
Parameters used to monitor iron overload include serum ferritin, liver biopsy, SCQID, and T2 MRI assessment of liver and cardiac iron, in conjunction with functional testing such as echocardiography and measures of endocrine function. Dual energy CT for measurement of liver iron has also been performed in some centres [7]. Each of these parameters has a different role. While Serum ferritin is the most commonly used parameter for monitoring iron overload [8]. It correlates with cardiac impairment and survival [9] but can be elevated by many confounding factors, including acute phase reactions such as infections, inflammation or malignancy, or by hepatic damage. The study group comprised of 60 children of β thalassemia major being regularly transfused at department of Pediatrics, Kamla Raja Hospital, G.R Medical College, Gwalior. Out of the 60 β thalassemia major patients, 29 patients belong to age group 3-5 years, 14 patients belong to age group 6-10 years, and 17 patients belong to age group 11-17 yrs. Males 49 (81%) were more than females 11(19%), similar to the result obtained by Torres et al[10]Najafipour et al[11] and Nadeem Ikran et al[12] in which the males were 60%, 64% and 64% and females were 40%, 36% and 36% respectively.

Serum ferritin level was found to be elevated in all the patients of beta-thalassemia major with range from 1050 to 5029 µg/l with a mean value of 3879µg/l. The age of patients at the time of diagnosis ranged from 4 months to 16 months with a mean of 10 months. The interval between successive transfusions varied between one week to four weeks in different patients. Nadeem Ikran et al (2004)[12] conducted study to know the levels of serum ferritin in patients of beta thalassemia. In a total of 75 cases of thalassaemia major studied in this series, 48 were males and 27 females with a male to female ratio of 1.77: 1. The age of patients at the time of diagnosis ranged from 6 months to 2½ years with a mean of 1 year and 4 months. The age at the time of this study ranged between 2 years 5 months to 21 years. The interval between successive blood transfusions varied between 7 days to 5 weeks in different patients. The frequency of transfusion had gradually increased. The mean serum ferritin levels were 3390 +135.6 ng/ml. Only two patients (2.67%) had serum ferritin levels of less than 1000 ng/ml. Sixteen patients (21.34%) had serum ferritin levels between 1000 – 2500 ng/ml, while 57 patients (76%) had values more than 2500 ng/ml. Results are almost similar to our study.

Table-4: Summary of the studies showing mean serum ferritin levels in beta thalassemia major patients:-

| S No. | Author | Country       | Year | Mean serum ferritin level(µg/l) |
|-------|--------|---------------|------|---------------------------------|
| 1.    | Nadeem Ikran et al [12] | Pakistan     | 2004 | 3390 µg/l                      |
| 2.    | Lucas et al[13]        | Sri-lanka     | 2000 | 5743 µg/l                      |
| 3.    | Shah et al[14]         | Western India | 2010 | 3456 µg/l                      |
| 4.    | Al Jaouni et al[15]    | Saudi Arabia  | 2010 | 3272.5 µg/l                    |
| 5.    | Cunningham et al[16]   | North America | 2004 | 1696 µg/l                      |
| 6.    | Choudhry VP et al[17]  | India         | 2004 | 6723 µg/l                      |
| 7.    | Riaz et al[18]         | Pakistan      | 2011 | 4236 µg/l                      |
| 8.    | Rehman M et al[19]     | Pakistan      | 2004 | 2861 µg/l                      |
| 9.    | Present study          | Gwalior, India| 2012 | 3879 µg/l                      |

Conclusion

Majority of the patients had very high ferritin levels, with a mean value of 3879 µg/l. 50% patients had serum ferritin levels more than 2000µg/l. Thus it can be concluded from the present study that serum ferritin concentration which is considered to be a marker for liver iron concentration was found to be increased in all the beta thalassemia major patients. serum ferritin is a easy, cost effective and best indirect test for determination of iron overload.

What this study adds to existing knowledge?

Iron chelation therapy should be start when serum ferritin level exceeds 1000µg/L, with the aim of keeping serum ferritin levels below 1000µg/Lto avoid the systemic effects of iron overload.

Contributions

- Dr Sunita Koreti and Dr Bablu Kumar Gaur wrote first draft of the manuscript.
- Dr G Das and Dr Ajay Gaur helped in supervised data collection and analysis.
- Dr Bablu Kumar Gaur made final correction of the manuscript before submission.
- All authors approved submission of the manuscript and own responsibility of the manuscript.
- None of the authors have any conflict of interest.

Ethical approval-obtained

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