SERIAL SERUM FERRITIN LEVELS FOR MONITORING RESPONSE TO IRON CHELATION THERAPY IN PATIENTS OF BETA THALASSAEMIA MAJOR "PROSPECTIVE COHORT STUDY"

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ABSTRACT

Background: In Beta Thalassaemia Major repeated blood transfusion, ineffective erythropoiesis and increased gastrointestinal iron absorption lead to iron overload in the body. The management of the iron overload in these patients requires the administration of iron chelators continuously and evaluation of serum ferritin levels at regular intervals.

Aims & Objective: The aim of this study is to analyze serum ferritin level among Beta Thalassaemia Major **patients and** to see the effect of iron chelation therapy in transfusion dependent thalassemic patients.

Study Design: Prospective cohort study.

Study Setting & Duration: Seven (7) months from January to July 2014 at Hematology Day Care Center HMC Peshawar Pakistan.

Subjects & Methods: A total of 50 thalassemia major patients were selected and their blood samples were evaluated for serum ferritin assays by electrochemiluminescenc immunoassay technology (ECLIA) on cobas e 411 Roche special immunoassay analyzer. 3ml of venous blood was collected in a BD disposable syringe which was then transferred to serum vacutainer for estimation of serum ferritin levels. Serum ferritin assays were done on Day 0, then after 3 months & then after another 3 months of iron chelation therapy.

Results: The mean pre-Iron chelation therapy with desferoxamine (DFO) & Deferasirox (Asunra®) of serum ferritin was 3465.97 ng/ml ± std 2120.097 ng/ml and mean serum ferritin levels after 3 months & 6 months iron chelation was 2984.96 ng/ml ± std 1979.837 ng/ml and 2455.44 ng/ml ± std 1816.722 ng/ml respectively.

A highly significant difference ($P < 0.01$) was observed between the two i.e. 0 day & 3 months serum ferritin levels. Similarly highly significant difference ($P < 0.01$) was also seen at 3 months & 6 months intervals.

Conclusion: Serum ferritin is used for efficacy monitoring of iron chelation therapy and is a suitable method for ascertaining the iron metabolism situation. Determination of ferritin at the beginning of therapy provides a representative measure of the body iron reserves. Single or sporadic measurement of serum ferritin alone is a poor indicator of iron burden in the transfusion dependent patients like beta thalassemia major. Nevertheless serial studies in individual patients usually give an indication of whether the iron burden in that patient is static, increasing or decreasing . In addition the maintenance of serum ferritin level below 2500 μg/L is associated with improved survival free of cardiac disease in patients with thalassemia.

Key Words: Beta Thalassaemia Major, Ferritin, Iron Chelators, Iron overload.

INTRODUCTION

Beta thalassemia is a fairly common blood disorder worldwide, that reduces the production of hemoglobin. Beta Thalassemia occurs most frequently in people from mediterranean countries, North Africa, the Middle East, India, Central Asia and Southeast Asia.

Beta Thelassemia Major was first described in 1925 by Thomas Cooley and Lee.[1] In those days, thalassemia major patients rarely used to survive the first decade of life. Following the introduction of regular transfusion regimens in the 1960's, initially by orsini and later by wolman and piomelli, thalassemics survived into 2nd and 3rd decades[2,3,4,5,]. As a result of this improved survival due to transfusion therapy the problems of transfusional haemosiderosis became conspicuous.

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Transfusional haemosiderosis is the major cause of late morbidity & mortality in patients with thalassemia major.[6] Thus iron chelation therapy has a very important role in the management of a thalassemia major child. Since the 1960's desferoxamine (DFO) mesylate has been the " gold standard" iron chelator, improving the quality of life and prolonging the life of transfusion dependent thalassemics[7,8]. But the need for daily parentral infusions is an obvious disadvantage, decreasing compliance to therapy. Also for patients in the developing countries, regular chelation with DFO is extremely expensive. An orally effective and cheap drug with good safety profile will be the ideal iron chelator. Deferasirox though expensive at present is an orally effective chelator with reasonably good safety profile, was approved by the FDA for transfusional haemosidrosis in children above 2 years of age.[9] In clinical practice, chelation starts with desferrioxamine (desferol®) which is prescribed at 20 mg/kg/d by slow subcutaneous infusion over 8-12 hours after patients have received 12-15 transfusions and/or when the level of ferritin around 1000 ng/ml. Deferasirox (Asunra®) is a once daily, oral, iron chelator approved for the treatment of chronic iron overload resulting from blood transfusions. The effectiveness of deferasirox in reducing or maintaining body iron has been demonstrated in studies involving large numbers of patients with variety of transfusion dependent anemias.[10, 11]

It is estimated that 1.5% million people of the world population i.e. 200 million people are carriers of the Beta thalassemia gene. Iron overload is the life limiting complication commonly found in thalassaemics.[12] The progressive iron overload in Beta Thalassemia major patients is the consequence of ineffective erythropoiesis, increased gastro-intestinalabsorption of iron, lack of physiologic mechanism for excreting excess iron and above all multiple blood transfusions. A unit of red blood cells transfused contains approximately 200-250 mg of iron while the body cannot excrete more than 1 mg of iron per day. The iron which exceeds the iron binding capacity of transferin appears in the plasma as non-transferin bound iron (NTBI) which is highly toxic to tissues.[13]

This "Free" iron can catalyze the formation of very injurious compounds such as the hydroxyl radical (OH) from compounds such as hydrogen peroxide, which are normal metabolic byproducts (Fenton reaction).[14] The hydroxyl radical is highly reactive and attacks, lipid, proteins & DNA.[15] The main source of iron in thalassaemia is transfused blood so that the iron intake from this source must be taken into account before chelation therapy starts in this respect it is important to consider the indication for regular blood transfusions these include the inability of the patient to maintain a hemoglobin (HB) level above 7 g/dL, impairment of growth, bone deformities and progressively enlarged spleen. Regular transfusions should be maintained in order to keep pre-transfusion Hb concentration between 9-9.5g/dL.

The estimation of serum ferritin levels is the most commonly employed test to evaluate iron overload in Beta thalassemia major. The association between serum ferritin and levels of body iron are well established and the test is easy to perform compared with other tests for iron overload.[16]

Ferritin is a macromolecule with a molecular weight of at least 440 KD (depending on the iron content) and consists of a protein shell (apoferritin) of 24 subunits & an iron core containing an average of approximately 2500 Fe +++ ions (in liver & spleen ferritin).[17]

At least 20 isoferritins can be distinguished with the aid of isoelectric focusing.[18]

This microheterogeneaty is due to differences in the contents of the acidic H and weekly basic L subunits. The basic isoferritins are responsible for the long term iron storage function and are found mainly in the liver spleen & bone marrow.[19] Acidic isoferritins are found mainly in the myocardium, placenta & tumor tissue. They have a lower iron content & presumably function as intermediaries for the transfer of iron in various syntheses.[20, 21, 22]

The determination of ferritin is a suitable method for ascertaining the iron metabolism situation. Determination of ferritin at the beginning of therapy provides a representative measure of the body's iron reserves.[23] The present study was thus designed with an objective to see the effect of iron chelation therapy in patients of beta thalassemia major.

SUBJECTS & METHODS

In this prospective cohort study a total of 50 thalassemia major patients admitted in Haematology Day Care Center of HMC Peshawar were selected in seven (7) months time from January to July 2014. Serum ferritin assays were analyzed by ECLIA technology on state of art equipment cobas e411 Roche immunoassay analyzer at main lab Institute of Kidney Disease, on day 0, three months & another three months intervals after starting the iron chelation therapy. About 3 ml of patient's blood sample was collected by a clean venepuncture. The blood was allowed to clot. To obtain serum ferritin estimation, blood was centrifuged at 5000 revolution per minute (rpm) for 5 minutes in an electric centrifuge to obtain serum. Ferritin levels were performed by ECLIA technology alongwith normal & abnormal controls (precicontrol varia PCV_1 and PCV_2 elecys immonoassay on cobas e 411 analyzer.

Patient's Selection: A total of 50 cases of Beta thalassaemia major were included in this prospective cohort study. This study was conducted at Haematology Day Care Center HMC Peshawar, from January to July 2014. The patients registered for transfusion were randomly selected.

Inclusion Criteria

All thalassemia major patients who were on chelation therapy.

Known cases of beta thalassaemia major that had been transfused at least 10-15 units of blood, irrespective of their age and sex was included in this study.

Exclusion Criteria

1. Patients who had been transfused less than 10 units of blood as part of their management were excluded.
2. Non-transfusion dependent patients.
3. All other hematological disorders who do not need lifelong transfusion therapy.

Statistical Analysis

Data Analysis was carried out using SPSS version 16. Descriptive statistic was given in the form of frequency distribution and percentages. For quantitative data, mean and SD etc were calculated. To see the significance between the results before and after treatment with iron chelation therapy student t test was applied. Level of significance was chosen as 0.05 and P-value was calculated, and the results were tabulated in the form of tables.

OPERATIONAL DEFINITIONS

Iron overload.

As iron accumulates the serum ferritin concentration rises and value of > 200μg/L (women) and 300μg/L (men) suggest iron overload. 400 μg/L (ng/mL) ferritin is used as the threshold value.

Removal of iron is essential in patients with transfusion dependent anemias such as thalassemia major to prevent death from iron overload, usually due to cardiac failure or arrhythmia.

Serum ferritin

Serum ferritin is useful in monitoring changes in body iron. Storage iron occurs in two forms ferritin & hemosiderin. Ferritin is colorless and is finely dispersed in tissues. It is composed of a spherical outer shell of an iron free protein, apoferritin and an inner core of trivalent iron. Single or sporadic measurement of Serum ferritin alone is a poor indicator of iron burden in the transfusion dependent patients. Nevertheless serial studies in individual patients usually give an indication of whether the iron burden in that patient is static, increasing or decreasing. In addition the maintenance of serum ferritin level below 2500 μg/L is associated with improved survival free of cardiac disease in patients with thalassemia.

IRON CHELATION THERAPY

Iron chelation therapy is inevitable to prevent the consequences of transfusion hemosidrosis in Thalassemia major patients. Desferioxamine (DFO) is the main agent licensed in all countries in clinical use at present. It is not absorbed orally and after parentral injection by continuous I.V or slow subcutaneous infusions which allow more prolonged exposure of the drug to the chelatable iron. DFO is a trihydroxamine acid (Hexadendate), one molecule binding covalently to all six oxygen sites on one ferric iron to form the red chelate, ferrioxamine. This is excreted in urine & bile. A regime of 40mg /kg is given as 8—10 hours subcutaneous infusion on a minimum of 5 days a week. Deferasirox tablet has to be taken dispersed in water or orange juice at least 30 minutes before food. It is given for two days a week once daily.

RESULTS

The results of the study are tabulated in tables 1-4. Data were analyzed statistically by applying student t-test. The difference between mean of serum ferritin levels that occurred in thalassemia major patients before and after the iron chelation therapy with desferoxamine (DFO) & Deferasirox (Asunra®) are shown in table 1.

In table 2 the serum ferritin levels at day 0 before starting Iron chelation therapy and after 10-15 post transfusions there is none of patients who show serum ferritin level of < 1000 ng/ml. 17 (34%) patients have serum ferritin level between 1000-2500 ng/ml while 33(66%) of patients have serum ferritin level > 2500 ng/ml.

Table.1

S.No	Parameter	Pre Iron Chelation therapy serum ferritin level		Post Iron chelation therapy serum ferritin level	
		Mean ± sd	(P Value)	Mean ± sd	(P Value)
1	Serum ferritin level Day-o	3465.97 ng/ml ± 2120.097 ng/ml	.000		
2	Serum ferritin level 3 months			2984.96 ng/ml ± 1979.837 ng/ml	.000
3	Serum ferritin level 6 months			2455.44 ng/ml ± 1816.722 ng/ml	.000

Table.2

Serum Ferritin in Patients with Beta Thalassaemia Major Day-0	
Levels	**Number of Patients**
< 1000 ng/ml	0
1000-2500 ng/ml	17 (34%)
>2500 ng/ml	33 (66%)

Table.3

Serum Ferritin in Patients with Beta Thalassaemia Major 3 Months	
Levels	**Number of Patients**
< 1000 ng/ml	0
1000-2500 ng/ml	29 (58%)
>2500 ng/ml	21 (42%)

Table.4

Serum Ferritin in Patients with Beta Thalassaemia Major 6 Months	
Levels	**Number of Patients**
< 1000 ng/ml	4 (8%)
1000-2500 ng/ml	29 (58%)
>2500 ng/ml	17 (34%)

In table 3 after 3 months of iron chelation therapy the serum ferritin levels show that none of the thalassemics patients have serum ferritin level < 1000 ng/ml while 29 (58%) patients have serum ferritin level between 1000-2500 ng/ml and 21 (42%) have serum ferritin level > 2500 ng/ml.

In table 4 the serum ferritin levels after 6 months of iron chelation therapy 4 (8%) patients have serum ferritin levels < 1000 ng/ml while 29 (58%) patients have serum ferritin levels between 1000-2500 ng/ml and 17(34%) patients have serum ferritin levels > 2500 ng/ml. These results clearly indicate that there is significance decrease in the levels of serum ferritin after regular iron chelation therapy with desferoxamine (DFO) & Deferasirox (Asunra®).

In a total of 50 cases of thalassaemia major studied in this series, 33 (66%) were male and 17 (34%) were female.

DISCUSSION

The results of serum ferritin levels showed significant decrease in post iron chelation therapy at 3 months & 6 months intervals after starting iron chelation therapy with desferoxamine (DFO) & Deferasirox (Asunra®). The difference between the mean of serum ferritin levels that occurred in Thalassemia Major Patients before & after iron chelation therapy (ICT) were calculated. A highly significant difference ($P < 0.01$) was observed between the two i.e. day 0 & 3 months. Similarly a highly significant difference ($P < 0.01$) was also seen at 3 months and 6 months.

Serum ferritin measurement is a readily available test but it has to be emphasized that a single estimation of serum ferritin level correlates poorly with hepatic iron concentration. Also it is influence by vitamin C deficiency (lowers ferritin) and hepatitis (increases ferritin) both of which are seen in thalassemics. But serial ferritin measurement are predictive of complications like iron induced heart disease. We found in our study that the difference between mean of serum ferritin levels that occurred in Thalassemia Major patients before and after iron chelation therapy when we compared day 0 results with 3 months and 3 months with 6 months results shown in Table 1, in which the mean serum ferritin level showed significant decrease in post iron chelation therapy of thalassemia patients.

Removal of iron is essential in patient with transfusion dependent anaemias such as thalassemia major to prevent death from iron overload usually due to cardiac failure or arrhythmia.

DFO is a hexidentate iron chelator produced by streptomyces pilosus was first used for treatment of transfusion hemosidrosis in 1962 [24]. With four decades of clinical experience, it has been definitely proven beyond doubt that DFO when administered as prolonged parenteral infusions either intravenously or subcutaneously can achieve negative iron balance [24,25,26,27,28].

DFO is the current "gold Standard" chelator against which newer chelators are measured. Olivieri et al in 1994 demonstrated that in those with serum ferritin less than 2500 μg/L. cardiac disease free survival is 91 % after 15 years of chelation with DFO. But in those with ferritin above 2500 μg/L cardiac disease free survival is less then 20 % after 15 years. Hence chelation therapy should be initiated as soon as serum ferritin rises above 1000 μg/L.

Long term chelation can reverse the functional complication due to iron overload like liver fibrosis, arrhythmia, abnormalities detected by echocardiogram. But the complications due to extensive tissues damage like frank diabetes hypothyroidism and myocardial sclerosis cannot be reversed.

Deferasirox is a tridendate synthesis orally effective iron chelator developed by computer modeling[29]. Deferasirox is an extremely effective once a day chelator with no significant toxicities requiring reduction of dose or withdrawal of the drug.

Effective management of iron overload in thalassemia requires monitoring both for iron toxicity and the effects of excessive chelation. Careful monitoring together with adherence to established regimens using desferoxamine (DFO) results in 78% survival rate 40

years of age with steadily improving survival as progressive cohorts receive chelation earlier in life. The early uses of desferoxamine in an amount proportional to the transfusional iron load reduces the body iron burden and helps protect against diabetes mellitus, cardiac, disease and early death in patients with thalassemia major.[30]

A prospective study of 30 children on deferasirox from Iran demonstrated evidence of decreased glamarlar filtration rate and renal tubular dysfunction but the mean serum creatinine level stayed less than 1 mg/dl over a 6 months fallow up [31]. Monitoring of renal function is recommended[32].

CONCLUSION

The emergence of new orally effective iron chelator gives hope to both patients as well as physicians caring for thalassemia patients. Though the current (Gold standard)iron chelator DFO is effective in attaining negative iron balance majority of the thalassemia major patients on transfusion therapy suffer from consequences of iron overload. Though the long term efficacy of newer chelator remain to be proven the case of administration and reduced toxicity will surely improve the compliance. Also combining them with DFO will reduce and the number of days of DFO required and thus improve compliance to DFO. A Govt. Funded program for supplying chelating agents to these patients will help to improve the survival and quality of life of thalassemia major patients.

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