ORIGINAL ARTICLE

COMPARISON OF DIFFERENT TREATMENT MODALITIES OF CHELATION THERAPY IN BETA-THALASSEMIA MAJOR PATIENTS

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Background: Thalassemia has a high prevalence and carrier rate of 8–10% in Pakistan, repeated blood transfusions lead to iron deposition in organs. In this Prospective study, we have compared the efficacy of three chelation regimens being used in our country. It has been conducted at PBTS, Fatmid Foundation and Children Hospital Lahore. **Methods:** 60 thalassemia major patients, were divided into 3 groups according to their mode of chelation. Patients in group I were on oral iron chelator deferiprone, 7 days per week. Thalassemics in group II were on parenteral iron chelator deferoxamine given subcutaneously for 4 days a week, and group III patients were on combination therapy, deferiprone for 5 days & deferoxamine given twice weekly. The assessment of chelation was done by measurement of serum ferritin and 24-hour urinary iron excretion at the start of the study and then after six months of follow-up. To assess the hepatic iron, hepatic MRIs were also performed. **Results:** Ferritin levels were maximally decreased in group II, followed by group III, with no significant reduction in group I. However, a statistically significant difference in mean urinary iron excretion (increased) was seen in group III. The hepatic iron was very high in all three groups as shown by the hepatic MRI. **Conclusion:** Combination chelation therapy is the most effective chelation therapy in iron-overloaded patients. It helps to improve compliance and increases urinary iron excretion. Patients on DFX had the lowest degree of hepatic siderosis even though it was considerably higher than the normal population.

Keywords: Thalassemia; Iron chelating; Agents; Desferrioxamine; Drug therapy; Combination

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INTRODUCTION

Thalassemia is one of the most common high-frequency single gene disorders in the world.[1] It is particularly prevalent in the Mediterranean region. In Pakistan, the carrier rate is about 8–10%.[2] Beta thalassemia major presents within the first year of life. Regular and repeated blood transfusions in this condition result in excessive iron deposition in organs and tissues.[3] Without adequate chelation, there is a gradual accumulation of iron and the effects of tissue siderosis start to appear by the end of the first decade.[4]

Iron overload in the body is responsible for cardiac, liver and endocrine diseases. Conservative management of thalassemia is usually done by a moderate transfusion regimen, i.e., pretransfusion haemoglobin of the patient is kept at 9±1 mg/dl.[5] Management of iron overload in these patients requires regular administration of iron chelators and evaluation of serum ferritin levels at regular intervals.[6] Regular iron chelation should begin in these patients after transfusion of about 20 units of blood or when serum ferritin exceeds 1500 ng/ml.[3]

Hepatic iron concentration can be determined quite accurately by Hepatic MRI.[7] Number of investigators have shown an increase in hepatic signal intensity in response to chelation therapy.[8]

Evaluation of serum ferritin levels and 24-hour urinary iron excretion was done for all the patients with each chelation regimen. Hepatic MRIs for assessment of liver iron were also performed in all the patients included in this study. Based on the results of the above parameters comparison has been made between the three iron chelation regimens.

MATERIAL AND METHODS

The present study has been carried out to determine the efficacy of three iron chelation therapies oral, parenteral and combination therapies. In this study the serum ferritin levels and 24 hr urinary iron excretion levels of 60 thalassemia major patients were determined at the start of study and then after the duration of six months of follow up. Hepatic MRIs of these 60 patients were also performed to assess their hepatic iron status, without any invasive procedure.

It was a prospective, comparative study conducted at The Pathology Laboratory of Postgraduate Medical Institute Lahore, Institute of Blood Transfusion Services Punjab, Fatimid Foundation Lahore and the Radiology Department of Children Hospital Lahore.

Before the study all the patients were explained about the study and their consent was obtained.

This study was conducted between March 2012 to April 2013. A total of 60 transfusion-dependent thalassemia major patients were included in the study. Patients were selected from IBTS and Fatimid Foundation Lahore.

Patient selected according to the inclusion & exclusion criteria of the study were divided into three groups depending on their mode of chelation therapy.

Inclusion criteria:

- Diagnosed Beta thalassemia major patients who were on regular blood transfusions.
- Age of the patients $\geq$ 10 years, (had received at least 20 units of blood)
- Patients who had serum ferritin above 1000ng/ml

Exclusion criteria:

- Thalassemics who had been diagnosed with renal dysfunction.

Group I included 20 transfusion-dependent thalassemia major patients who were receiving oral iron chelator Deferiprone at the dose of 50–75 mg/kg given in three divided doses per day 7 days a week.[1,18,19]

Group II included 20 thalassemia major patients who were on parenteral iron chelator Desferrioxamine given intravenously or subcutaneously at the dose of 30 – 40mg/kg for 4 days per week.[1,18,19]

Group III had patients who were on combination chelation therapy. They were being given deferiprone for 5 days per week and desferrioxamine twice weekly, at above-mentioned doses.[18,19]

Study parameters:

1. Serum Ferritin
2. 24 hr urinary iron excretion
3. Assessment of hepatic iron by MRI

Serum ferritin of the patients was determined by using Microparticle Enzyme Immunoassay (MEIA) for quantitative determination of ferritin in human serum. The kit used was AxSYM ferritin manufactured by Abbott Laboratories Longford, Ireland. In this method, ferritin present in the sample, enzyme-labelled antibody and microparticles all bind together to form an antigen-antibody complex. The substrate 4 methylumbelliferyl phosphate when added to this complex forms a fluorescent product which is measured by MEIA optical assembly.

Urinary iron was determined calorimetrically using Human Iron Kit, in this method urinary iron is measured by a monoreagent with LCF (lipid clearing factor). Chromazural B method (Callan and Cook 1982). Iron reacts with chromazurol B (CAB) and cetyltrimethylammonium bromide (CTMA) to form a coloured tertiary complex with an absorbance maximum of 623 nm. The intensity of colour produced is directly proportional to the concentration of iron in the sample.

Hepatic MRIs of the patients were performed by 1.5 Gyroscan NT - Philips MR system. MRI is a non-invasive imaging technique based on the principle of nuclear magnetic resonance. The changes in the MRI signal intensity appear as hypointense areas in liver, due to paramagnetic effect of iron. Based on this appearance of liver grouping of mild, moderate and severe hepatic siderosis was done by radiologists.[9]

RESULTS

Mean±standard deviation values of serum ferritin in group I, II and III at the start of the study were 4651.27 ng/ml±1600.44, 4712.60 ng/ml±1239.54 and 4029.23 ng/ml±1766.46 respectively.

Mean±standard deviation values of serum ferritin after six months of therapy were found to be 4800.58 ng/ml±1558.56, 3818.12 ng/ml±1421.64 and 3383.42 ng/ml±1904.77 for groups I, II and III, respectively.

On calculation the difference between the initial and final serum ferritin levels in these groups was found to be - 149.31 ng/ml for group I, 894.03 g/ml for group II and 675.81 ng/ml for group III as given in table 1 and figure 1.

By applying paired t-test the statistical significance of the difference between the initial and final serum ferritin values in each group was determined. For group I value of the paired t-test was found to be 1.74, but its *p*-value was >0.05 and not statistically significant. For group II value of the paired t test was 4.56 and the *p*-value was <0.001, thus statistically highly significant. For group III the test was 4.67, which coincided with *p*-value of <0.001.

The results of the paired t-test showed that the initial and final serum ferritin levels were significantly different in groups II and III.

One-way ANOVA applied on serum ferritin values obtained, gave the value of F calculated 3.76, the value of F critical was 3.15 Thus F calculated = 3.76 > F critical (2,57) = 3.15 so results are significant at the 5% of significance as shown in table 2.

Post Hoc analysis of the group means was done with Tukey at 5% group III was found to be significantly different from Group I. The population mean of the third group differs from the population mean of other groups, however, the difference in the first and second groups was not statistically significant as shown by table 3.

The mean±standard deviation values of urinary iron excretion in groups I, II and III at the start of the study were 3.69±0.94, 5.43±1.38 and 5.49±1.28 mg/day respectively. After six months of therapy, the UIE levels were found to be 3.74±0.92, 5.81±1.72 and 6.36±1.02 mg/day for groups I, II and III respectively as shown in Table 4. The UIE was highest in group III as shown in figure 2.

After hepatic MRI of these patients, they were classified as having mild moderate and severe

siderosis. In group I, 18 patients had severe and 2 had moderate hepatic siderosis. In group II 15 patients had severe and 4 patients had moderate hepatic siderosis whereas only one patient was categorized as having mild hepatic iron overload. Moderate and one patient with mild hepatic siderosis as shown in figure 3.

Table-1: Initial and final serum ferritin values of groups I, II and III. And the difference between the two (i – f) in each group.

Groups	Serum ferritin (ng/ml) Intial (i) ±SD	Serum ferritin (ng/ml) Final (f) ±SD	Difference (i-f)
I	4651.27±1600.44	4800.58±1658.56	149.31
II	4712.60±1239.54	3818.12±1421.64	894.03
III	4029.23±1766.46	3383.42±1904.77	675.81

Group I *p*-value > 0.5 (NS). Group II *p*-value <0.001 (HS) Group III *p*-value <0.001 (HS).

Table-2: Result of one-way ANOVA F test for final (F) serum ferritin levels for groups I, II and III.

F calculated (F test)	F critical (2, 57) read from F table
3.76	3.15

F calculated >F critical, α=0.05, *p*<0.05 (S)

Table-3: Tukey's Post Hoc Analysis between groups I, II and III.

Comparative groups	Post Hoc Analysis	*p*-value
Group I vs Group II	< than the critical value	> 0.05 (NS)
Group II vs Goup III	< than the critical value	>0.05 (NS)
Group III vs Group I	> than the critical value	<0.05 (S)

NS=Non-significant, S=significant.

Table-4: Mean±SD of 24-hour UIE of patients in groups I, II, & III.

Groups	UIE mg/day Initial (i) at the start of the study	UIE mg/day Final (f) after six months.
I	3.69±0.87	4.28±0.89
II	5.43±1.38	5.81±1.72
III	5.49±1.28	6.36±1.02

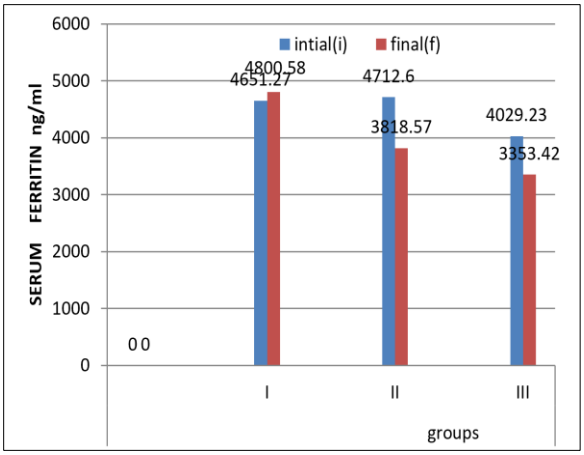

Figure-1: comparison of initial (i) and final (f) serum ferritin values in group I, II and III.

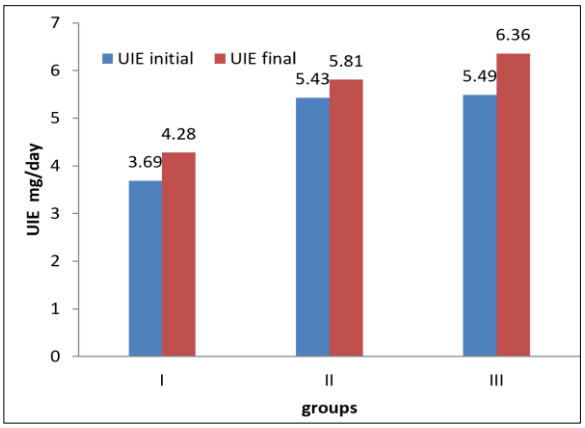

Figure-2: comparison of means of 24 hr UIE in thalassemia major patients in groups I, II, and III.

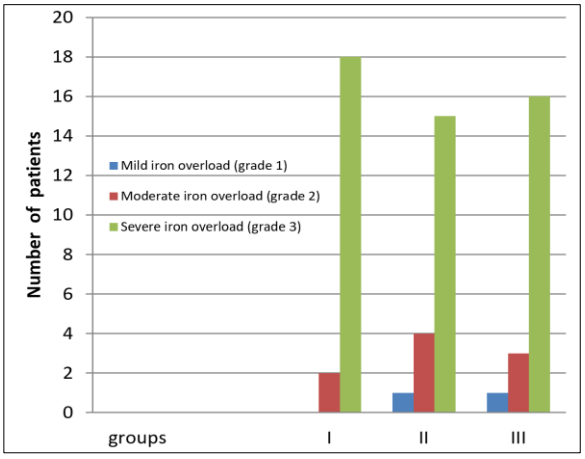

Figure-3: Mild, moderate and severe iron overload in the three chelation groups, as assessed by hepatic MRI.

DISCUSSION

Iron overload is a complication occurring in thalassemia major patients as a result of repeated and regular blood transfusions. This excessive iron is responsible for cardiac, liver and endocrine diseases.[10] Effective chelation therapy can prevent or reverse iron toxicity related to iron overload.[11] In the present study the efficacy of various chelating regimes was determined and compared with each other. Therapy with desferrioxamine is a well-established effective therapy for iron chelation in thalassemia major patients.[12] However, its mode of administration is a strong impediment to following the therapy regularly, especially in developing countries like Pakistan. In our country in addition to the mode of administration, the high cost of the chelator is also a major limiting factor in its use.[13]

In this study rise in serum ferritin values after 6 months of chelation therapy with oral iron chelator Deferiprone L1 was recorded, which conforms with the results of a few earlier workers.[10,14] L1 was found to be

effective only in balancing the iron input due to blood transfusion by increasing the excretion of urinary iron. In our local setup, the major limiting factor is the cost-effectiveness of the drug. Patients are unable to keep up with the requirements of rigorous chelation therapy due to poverty and lower education levels.

Few earlier reported studies demonstrated a fall in serum ferritin values only after at least 18 months of therapy.[15] The rise in serum ferritin concentration has been attributed to the rapid glucoronization of the drug in the liver making it ineffective to chelate the stored iron in the body.[12] The results obtained from different centres which quantitatively determined the body's iron burden in patients receiving long-term deferiprone therapy, do raise concerns that deferiprone alone may not provide adequate sustained control of the body's iron in a substantial proportion of patients with thalassemia major.[5,10]

Recently there have been some interesting studies demonstrating the increased efficacy of L1 in reducing myocardial iron as compared to DFO.[16] With the availability of MRI T_2^* technique it has been possible to determine the myocardial iron concentration. The above-mentioned studies have shown that oral DFP is more effective in the removal of cardiac iron than DFO.

The results of combined therapy have been found quite encouraging as also noticed by a few other workers.[17] The benefits of combination iron chelation therapy completely outweigh the monotherapy. Combination chelation therapy improves compliance, improves organ-specific iron removal, minimizes/reduces toxicity and enhances iron removal in an additive effect. The synergistic and additive mechanism in which the oral chelator mobilizes tissue iron and then exchanges it with the parental agent has been proposed.[18]

Recently a prospective controlled trial which enrolled asymptomatic patients with myocardial siderosis, previously treated with DFX showed a significant reduction of cardiac iron deposits and improvement in contractility, in those patients to whom deferiprone was also added as an iron chelator along with DFO.[18] Cardiac benefits were also observed in beta-thalassemia major patients with mildly reduced ventricular function, who had significant improvement in contractility after combining deferiprone with desferrioxamine.[19] Myocardial hemosiderosis is the leading cause of mortality in multi-transfused patients. The improvement of cardiac function with combination therapy is of tremendous benefit as established in above mentioned studies.

The mean serum ferritin values in the present study were 4800.58 ng/ml for group I, and 3818.57 ng/ml for group II and group III. The mean serum ferritin value for all the patients included in the study was 3390 ng/ml. Whereas Cunningham *et al* in 2004 reported mean serum ferritin levels in beta-thalassemia patients of North America to be 1696 ng/ml. the difference is largely attributed to the difference in health care between the two regions.

UIE is an important test to monitor iron overload during treatment. Its measurement helps in assessing the presence or absence of a negative iron balance. To ascertain the efficacy of chelating regimens in the groups which were studied it was observed that UIE was highest in the group that was receiving a combination of DFO and DFP. Wonke *et al* also noticed the additive effect of combination therapy on urinary iron excretion.[18] Thus demonstrating additive and synergistic mechanisms of action of L_1 and DFO in removing excessive body iron.

The present study demonstrates a linear correlation of UIE with serum ferritin. These results are confirmatory with the observations of other workers.[20]

The change in the serum ferritin level during chelation therapy generally parallels the changes in the liver's iron concentration.[14] In the present study, hepatic MRIs of all the patients were performed to assess their hepatic iron overload. Hepatic MRI has been a very effective and non-invasive technique for determining the liver's iron burden as compared to previous invasive techniques. The effectiveness of hepatic MRI for assessing hepatic iron in multi-transfused thalassemia major patients has been demonstrated by other workers.[21]

With recent advances in radiology, measurement of hepatic iron using special MRI software T_2^* has been performed with great accuracy. In a large number of studies conducted worldwide, it has been shown that the grading of liver hemochromatosis by MRI is significantly correlated with the grading of hemochromatosis by liver biopsy.[18,21] In this study grading of patients' hepatic iron, overload was done in terms of mild, moderate and severe iron overload as previously done by various workers.[9] About 85% of patients included in the study showed severe iron overload on hepatic MRI, 13% of patients had moderate and less than 2% had mild iron overload. In the present study, it has been observed that MRI provides a practical grading system for hepatic iron overload based on the severity of the picture, albeit arbitrarily assigned.

CONCLUSION

1. Combination chelation therapy is the most effective chelation therapy in iron overloaded multi-transfused patients.
2. Combination chelation therapy improves compliance, increases hepatic iron removal and enhances iron removal as an additive effect.
3. Desferrioxamine therapy is most effective in reducing hepatic siderosis even though it is still considerably higher than that of the normal population.

4. Hepatic MRI is a very effective non-invasive technique for determining the body's iron burden as compared to previous invasive procedures and thus can help in guiding chelation therapy.

Further suggestions:

1. Few earlier reported studies demonstrated a fall in serum ferritin level only after at least 18 months of therapy.[15] Thus long-term studies for determining this effect should be conducted.

2. A study conducted by Galalanello *et al* in 2010 showed improvement in cardiac function and better survival in patients who were being treated with combination chelation therapy as compared to oral or parenteral therapy alone. Any study regarding the effects of combination chelation therapy on cardiac siderosis would be worthwhile indeed.

3. The unavailability of T_2^* MRI software in Pakistan is a major limitation in determining myocardial iron overload. Hopefully, the availability of T_2^* MRI software in future will open new venues for research in this field.

AUTHORS' CONTRIBUTION

GU: Literature search, study design, data collection, write-up. MUR: Supervision, proofreading. SK: Data collection, write-up. MSH: Data analysis and interpretation.

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