

Estimation of Iron Overload-Implications of its Non-linear Correlation

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Abstract

Thalassaemias and haemoglobinopathies contribute the highest number of cases, as far as single gene disorders are concerned, where blood transfusion and iron chelation remain the mainstays of therapy. Depending upon the requirement of transfusion, thalassaemias have been classified into two categories: 1) Transfusion dependent, 2) Non-transfusion dependent.

Non-transfusion dependent thalassaemia patients, contrary to previous belief, suffer from iron overload. If this iron is estimated by the level of ferritin in serum, it does not linearly correlate to hepatic and/or cardiac iron. It was noted during our study that serum ferritin levels up to 300 ng/ml correlated with hepatic iron load of up to 3 mg/g of DLT almost linearly, within +0.5 SD of mean.

There were a few patients in whom the serum ferritin level compared to the corresponding hepatic iron content (as measured by MRI) was more than +1.0 SD, and in some patients it was even ≥ 2.5 SD. In addition to serum ferritin, which is also a marker of acute inflammation, CRP was also estimated. It was seen that in these patients, CRP was also high. They were investigated for Hepatitis B, C and work ups were done for Tuberculosis. Out of the 350 such patients who were examined, 18 patients (5.14%) had their level of serum ferritin ≥ 1.0 SD, 08 tested positive for Tuberculosis, and 05 tested Positive for Hepatitis C and 01 for Hepatitis B. 14 patients out of the 18 (77.77%), who were screened to be out of the limits of SD had some infectious pathology in addition to their primary disorder, which was detected due to this observed discrepancy. They are undergoing treatment as appropriate for the diagnosis.

In conclusion, in the range where the level of serum ferritin is linearly correlated to the corresponding hepatic iron, the value was >1.0 SD of mean, clinical investigations should be done to exclude possibilities of infections like Hepatitis and Tuberculosis.

Keywords: Thalassaemias; Iron overload; Infections; MRI; Serum ferritin

Introduction

Thalassaemias and haemoglobinopathies are the commonest single gene disorders reported worldwide. Their spectrum of phenotypic expression is extremely variable, ranging from individuals presenting as early as three months of age and requiring regular blood transfusions almost immediately, to completely non-transfusion dependent individuals where the patient (?) is so asymptomatic that he/she does not require to present themselves at the healthcare facilities at all, thus staying out of the patient census [1]. For the severe thalassaemics who require regular blood transfusions, iron chelation therapy is also required to get rid of the excess iron and intermediate free radicals from vulnerable organs.

This chelation therapy was guided by levels of serum ferritin as an easily accessible, available and economical marker of iron overload. Though it was long known to be a marker of acute phase reaction and iron deficiency, in the absence of more specific markers and despite

known drawbacks, its level and trend were accepted as evidence of iron overload [2].

The problem with maintenance arose when the non-transfusion dependent subjects presented with diabetes, heart failure and growth retardation despite maintaining near normal haemoglobin (thus excluding anaemic hypoxia as one of the causes). The iron overload parameter, serum ferritin, was also within normal limits, i.e., below or equal to 500 ng/ml.

This evoked suspicion as to the limitations of serum ferritin level as a parameter indicative of iron overload. This suspicion was confirmed when hepatic iron overload estimation was done by MRI. There was little correlation between iron overload estimation done by levels of serum ferritin and that of hepatic iron overload estimation done by MRI in iron overloaded subjects. To our surprise it was observed that serum ferritin values ≤ 300 ng/ml and Liver Iron Concentration (LIC) values, as estimated by MRI, ≤ 3 mg/g of DLT had linear correlation within ± 0.5 SD, in a population of 350 non-transfusion dependent thalassaemics.

Despite this near linear correlation, there were 18 individuals whose serum ferritin values exceeded the hepatic iron concentration correlate

and exceeded +1.0 SD to +2.5 SD. They were considered as outliers and investigation was conducted to find out the cause. As serum ferritin also contributes as a marker of Acute Phase Reactions, we desired to include infection, either acute or chronic, in explaining the situation and justifying the results. It was our intention to find out the cause of the outliers, the mitigation of such factor or factors, and its implication on the overall health of non-transfusion dependent thalassaemic individuals.

Objective of the Study

The objective of this study is to obtain positive correlation between Serum Ferritin levels vs. Liver Iron Concentration, and comparatively study the outliers and their infectious disease profile.

Materials and Methods

Type of study

This was a cross-sectional study undertaken in a period from March 2014 to February 2016.

1. Total number of patients: 350.
2. Disease Profile: Suffering from Beta Thalassemia with HbE Disease.
3. Male: 207, Female: 143.
4. Splenectomized: 269.
5. Age: 7–65 years.

Inclusion criteria

1. Suffering from Beta Thalassemia with HbE Disease.
2. Age between 7-65 years.

Exclusion criteria

1. Blood transfusion has been done within 6 months, and/or;
2. >20 units of blood have been transfused till date.

Authorizations for the study

Permissions were taken from the necessary authorities, the patients and the hospital ethics committee for this non-interventional study.

Tests Performed (Parameters tested/Data studied):

Haematological

Complete Blood count, Nucleated Red Cell count, Reticulocyte count

Bio-chemical

Liver Function Tests, Serum Creatinine, Serum Ferritin, C-Reactive Protein, Lactate Dehydrogenase, Fasting Blood Sugar

Serological

Tests for Hepatitis B, C, HIV

Radiological

Chest X-Ray, Ultrasonography of whole abdomen, Hepatic and Cardiac MRI for iron overload

Microbiological

Sputum for Acid Fast Bacilli, Culture of Bone marrow and body fluids by BD-Bactec System

Immunological

Mantoux Test

Notes

The above mentioned tests were performed only once for the purpose of the study; however, they may have been done again on a patient-to-patient basis for follow up.

Statistical Analysis

Standard Anova and Microsoft Excel 2013 software were used for the statistical analysis of the data, and correlations therein.

Study Proper

350 non-transfusion dependent thalassaemics were selected for this study. All the patients were suffering from Beta thalassaemia with HbE disease. There were 207 males and 143 females, with age ranging from 7 to 65 years. They had received no blood transfusion for at least 6 months preceding this study. None of them had received more than 20 units of blood as transfusion till date, and no one was on any type of iron chelation therapy. 269 patients were splenectomized, with prior vaccination against *Meningococcus sp.*, *Haemophilus influenzae* and *Pneumococcus sp.*, and were on prophylaxis with penicillin.

All the patients underwent the following blood-related laboratory investigations:

1. Complete Haemogram,
2. Nucleated Red Cells (NRBC),
3. Reticulocyte Count,
4. Liver Function Tests (LFT),
5. Lactate Dehydrogenase (LDH),
6. C-Reactive Protein (CRP),
7. Serum Ferritin, and
8. Serum Creatinine.

These were done using standard testing methods at the Department of Laboratory Medicine, Peerless Hospital.

Estimation of Hepatic Iron Overload by Magnetic Resonance Imaging (MRI) was done at the Department of Radiology, Peerless Hospital. The MRI was done on a Siemens 1.5T instrument using the Phantom protocol from Resonance Healthcare. The DICOM Images were sent to Resonance Healthcare for interpretation in a blind manner, and the results were expressed as mg/g of Dry Liver Tissue (mg/g DLT).

Serum Ferritin was measured by ChemiLuminisence Microparticle ImmunoAssay (CLMIA), on an Abbott Architect 1000i, with Abbott's serum ferritin estimation kit, from freshly collected and separated serum samples. Sample dilution was done as per approved protocol, and the results were expressed as nanogram/millilitre (ng/ml) [3,4].

These tests were done only once at the initiation of the study for entire group of Non-transfusion dependent HbE-Beta Thalasassaemia patients.

The results of Serum Ferritin and Hepatic Iron Overload as estimated by MRI were plotted against each other.

The outliers (i.e., the data points with >1.0 SD) were identified and their CRP levels were correlated and serological tests for Hepatitis B (HBsAg and Anti HBcAb), Hepatitis C (Anti HCV Ab) and HIV (p24 antigen capture assay and Anti-HIV 1 and 2 Antibody) were done.

Work ups were also done for Tuberculosis by Chest X-Ray, Mantoux Test, and Sputum for AFB. These were cultured on the BD- Bactec platform, from available tissue fluid and aspirated bone marrow.

The results were correlated: Serum Ferritin (SFr) vs. Hepatic Iron (LIC), and outliers were correlated with the help of the CRP level and Infectious Disease parameters.

Results

The mean serum ferritin was 648.71 ± 872.43 ng/ml, 479.36 ± 637.61 ng/ml in males and 789.83 ± 220.8 ng/ml in females.

The mean hepatic iron overload was 7.05 ± 8.9 mg/g of DLT, 5.8 ± 8.761 mg/g of DLT in males and 8.1 ± 2.81 mg/g of DLT in females.

In our study, a correlation coefficient of 0.99 was obtained between serum ferritin and hepatic iron concentration, where the mean serum ferritin level was 220.8 ng/ml and the LIC was 2.8 mg/g DLT.

Results were divided into groups for convenience:

1. >1.0 SD for Serum Ferritin level where corresponding LIC level is <2 mg/g of DLT:

- a. 6 out of 268 patients (2.238%),
- b. Mean level of serum ferritin in this group was 275.85 ng/ml.

2. >2.5 SD of Mean for Serum Ferritin level where corresponding level of LIC is >2-3 mg/g of DLT:

- a. 12 out of 82 (14.63%),
- b. Mean level of serum ferritin was 236.8 ng/ml (Figures 1 and 2).

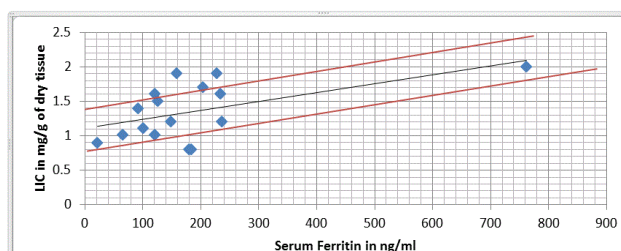


Figure 1: Correlation between Serum Ferritin and LIC SFr <2 ng/ml.

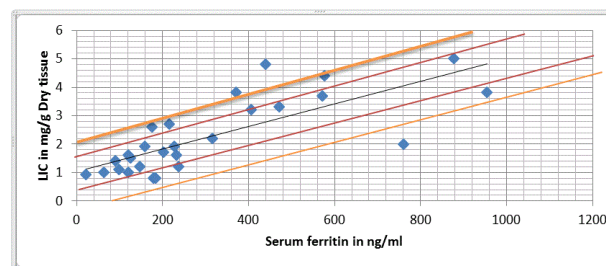


Figure 2: Correlation between Serum Ferritin and LIC, Sfr <3 ng/ml.

Out of the 6 patients who had serum ferritin more than +1.0 SD for LIC of <2 mg/g of DLT, all 6 had a CRP level of 11 ± 2.8 mg/L, and 17 patients who had serum ferritin more than +2.5 SD for LIC >2-3 mg/g of DLT, 12 had a CRP level of 27 ± 3.4 mg/L [5-7].

Out of the 6 patients whose serum ferritin levels were ≥ 1.0 SD for corresponding LIC, all patients had CRP level above 10 mg/L. Their Hepatitis B, C and HIV antibody yielded non-reactive results. Chest X-Rays (P-A view) were suspicious in 3 patients, 3 had palpable, non-tender and matted lymph nodes in the cervical region, and Mantoux test done with 5 TU (Tuberculin Test) tested Positive [7,8]. They were started on anti-tubercular therapy and are on the way to recovery (Figure 3).



Figure 3: Patients with serum ferritin levels of ≥ 1.0 SD for corresponding LIC.

Out of the 12 patients whose serum ferritin levels were >2.50 SD for corresponding LIC, all patients had CRP level above 20 mg/L. 2 had suspicious Chest X-Ray, with one having a right sided pleural effusion

with pleural reaction. When Mantoux test was done with 5 TU (Tuberculin Test), both tested Positive. 5 patients screened positive for Anti Hepatitis C antibody when tested by CLIA yielding reactive results, PCR by Roche Taqman System was also positive, and tests for Hepatitis B by HBsAg and Anti HBCAb by CLIA yielded reactive results, and PCR by the above mentioned method was also positive. None tested reactive for HIV by either of the methods [9-11].

They were started on corresponding anti-viral and anti-tubercular therapy (Figure 4).



Figure 4: Patient before and after completion of treatment for Tuberculosis.

Analysis of Literature

Iron overload being frequently present in thalassaemia intermedia patients was documented by Florelli et al. [12], who commented that it becomes evident mainly after the second and third decades of life, which was heterogeneous in nature. 38 patients were evaluated as regards levels of transferrin saturation, serum ferritin and desferrioxamine induced urinary iron excretion. Hepatic iron estimation by non-invasive means was not considered as it was unavailable [12]. Iron overload in un-transfused beta thalassaemia intermedia patients had been estimated to be 1-3.5 g/year compared with 2 to 12 g/year in regularly transfused patients.

In the article published by Taher et al. [13], the mean iron overload in 168 beta thalassaemia intermedia patients, where the mean age was 35.2 ± 12.6 years and of whom 42.6% were males, the average liver iron concentration was 8.4 ± 6.7 mg Fe/g of DLT [9].

Mazza et al. reported iron overload in 33 thalassaemia patients by serum ferritin, which ranged between 276 and 8013 ng/ml and liver iron content was measured by MRI which ranged between 1.6 to 31.0 mg/g dry weight. They recorded linear correlation of serum ferritin with liver iron content [10].

In another comparative study by Papakonstantinou et al. [11], where 40 transfusion dependent thalassaemia patients' iron overload status was estimated by LIC, serum ferritin and histologic grading of siderosis, LIC ranged from 2.32 to 18 mg/g dry weight and this value correlated well with serum ferritin levels [11].

Taher et al. had reported in a cross-sectional study of 168 patients, that positive correlation existed between LIC of >6 mg/g dry weight with endocrine and bone disease, in Beta thalassaemia intermedia patients [13]. They have also concluded that a serum ferritin level of <300 ng/ml was highly predictive of Liver Iron Concentration of <3 mg Fe/g of dry weight. Liver Iron Concentration of 1-2 mg Fe/g of dry liver tissue was considered normal [13].

It was published by Taher et al. [13] that level of serum ferritin did not correspond to actual iron overload and was underestimated in non-transfusion dependent thalassaemia patients. This was also the inference by our group in another publication [14]. It was also pointed out by them that serum ferritin level and LIC did possess a linear relationship in these subjects within the range of 300 ng/ml of serum ferritin and 3 mg/g of DLT of LIC. This was also proved by our group in another publication [15]. There was no explanation as to why there were some outliers among the same group where there was variable degree of discrepancy between the serum ferritin and LIC, from 0.05-1.0 SD to >2.5 SD.

We thought that since serum ferritin was an acute phase reactant in addition to being a surrogate marker of iron overload status, it should be helpful if we could also study CRP, which, being another acute phase reactant, might be able to detect if this elevation was due to infection or not.

All patients whose serum ferritin level was above the mean for the LIC for that corresponding value also had CRP values higher than normal. When they were tested for common infections in this part of the world like tuberculosis and transfusion-transmitted diseases like Hepatitis B, C and HIV, of the 18 patients who had outlying values for serum ferritin for the corresponding LIC and higher than normal CRP, 8 were detected positive for tuberculosis, 5 for Hepatitis C and 1 for Hepatitis B. The ones who had tested positive for tuberculosis had serum ferritin in the range of 1.0-2.5 SD for that corresponding LIC, but the ones who tested positive for Hepatitis B and C infections had serum ferritin >2.5 SD for that corresponding LIC. All the patients who had tested positive for tuberculosis had been splenectomized and were on prophylaxis with penicillin.

Conclusion

To conclude, it should be emphasized that, generally, as a rule of thumb, the LIC when multiplied by 100 should give the corresponding serum ferritin in non-transfusion dependent subjects, if the LIC is ≤ 3 mg/g DLT.

Primary result

$$\text{SFr} = 100 \times \text{LIC (when LIC} \leq 3 \text{ mg/g DLT)}$$

Clinical notes

If the SFr is more than expected by 150, start investigating for tuberculosis in developing countries, and more so if the person is splenectomized.

If the SFr is more than expected by 200, start investigating for transfusion transmissible diseases, moreover if Alanine Aminotransferase and Aspartate Aminotransferase is high or has suddenly become higher by >3 times of the last reading, the same sudden increase is applicable for SFr.

Strengths and Weaknesses of the Current Study

Strengths

It gives an interpretation of iron overload from serum ferritin and its correlation to liver iron concentration and goes beyond mere iron overload estimation, with enunciating a defined relationship of serum ferritin to liver iron concentration, and, primarily, defining

relationships between discrepancies of serum ferritin to liver iron concentration correlation and infectious diseases which the patient may be suffering from.

Weaknesses

The primary weakness of this study is its small sample size.

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