

Research Article

Correlation of Serum Ferritin with Hepatic Iron Overload among Nontransfusion Dependent Beta Thalassaemia with Haemoglobin E Disease Patients from Eastern India

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Abstract

Introduction: Iron over load estimation was attempted by MRI R2* and serum ferritin, in non -transfusion dependent HbE Beta Thalassaemia patients.

Methods: Seventy three patients selected on definite criteria were scanned and tested. Average age of the patients was 20.8 ± 11.14 years, 33 females and 40 male. Average hepatic iron concentration was determined to be 11.09 ± 11.74 mg/g of dry liver tissue and average serum ferritin was 972.44 ± 1121.51 ng/ml.

Results: 81% of the patients had hepatic iron concentration more than 2 mg/g of dry liver tissue, whereas only 41% had serum ferritin level >500 ng/ml, irrespective of age. The hepatic iron overload pattern was heterogenous, where age and sex were not well determined variable predictors of such iron overload. Though, higher hepatic iron load was seen more predictably in patients above the age of 10 years and LIC of 5 mg/g of dry tissue correlated with serum ferritin level of approximately 400 ng/ml.

Conclusion: It can be concluded that non transfusion dependence does not ensure non iron overload status and such patients should be scanned by using MRI R2* sequence to determine their hepatic iron status and start on excess iron chelation therapy if felt required.

Keywords: Thalassaemia; Haemoglobin; Anaemia

Introduction

Beta Thalassaemia with haemoglobin E disease is the commonest double heterozygous coexistence of quantitative and qualitative globin chain defect, causing haemolytic anaemia in the population of southeast Asia [1]. Over 700 structural haemoglobin variants have been identified, but only three, sickle haemoglobin, HbC and HbE, occur in high frequency in different population. This hereditary form of haemolytic anaemia seems to have differing presentation in most of the countries where it exists. It may be so mild, as never to present to the health care set up due to lack of physical problems, or present around 6 months of age with anaemia severe enough to require regular transfusion of blood for survival. This extremely heterogenous presentation exerts immense stress on the successful management, paving way to the various complications, adding to the morbidity and mortality of these patients. From the Thalassaemia model it is clear that the hallmark of phenotype is due to globin chain dysbalance arising of excess alfa chains and/or easy precipitability of the nonnormal, qualitatively defective beta globin chain - leading to intravascular haemolysis or ineffective erythropoiesis. It is very clear that the most important complications, like osteopathy, endocrinopathy, cardiomyopathy, neuropathy and splenomegaly, can all be related not only to the degree of anaemia but also the magnitude

of iron loading of the tissues. This iron over load related morbidity is due not primarily to blood transfusion, because most patients of this group are non-transfusion dependent, but increased absorption of iron from the gut.

Most, if not all patients, who suffer from even the mildest degree of anaemia, release supra physiological quota of erythropoietin due to relative tissue hypoxia, however clinically silent might the disease expression be. This leads to iron craving of the tissue hypoxic body, and excess absorption of the element from the gut. The ineffective erythropoiesis leads to inappropriately low hepcidin levels leading to up-regulation of ferroportin, hence augmenting intestinal iron absorption [2-4].

Non-transfusion dependent thalassaemic subjects have predominant ineffective erythropoiesis, this results in increased levels of growth differentiation factor 15, which also alters the expression of hypoxia inducible transcription factors. So a combination of Hepcidin suppression, increased iron absorption from the gut and increased release of recycled iron from the reticulo-endothelial system, the macrophage iron gets depleted, so the serum ferritin levels are relatively low and there is preferential portal and hepatocyte iron loading, which leads to an increase in liver iron concentration, which leads to discordance of iron overload as measured by serum ferritin and hepatic iron estimation. Citation: Chowdhury P, Saha M, Chowdhury D (2015) Correlation of Serum Ferritin with Hepatic Iron Overload among Non-transfusion Dependent Beta Thalassaemia with Haemoglobin E Disease Patients from Eastern India. J Mol Biomark Diagn 6: 231. doi: 10.4172/2155-9929.1000231

The clinical course of beta thalassaemia with haemoglobin E disease is punctuated by serious morbidity and mortality. Studies indicated that hypercoagulation to pulmonary hypertension, non-healing ulcers to paravertebral haematopoietic islands are complications, very commonly detected in non-transfusion dependent haemoglobinopathy patients, but until recently the iron overload part was overlooked.

It was discovered and concluded, that though non-transfusion dependent beta thalassaemia with haemoglobin E disease do not get regularly transfused for survival, they still develop significant iron overload. Spurred by various factors to which the end result suppression of hepcidin levels, increased absorption of iron from the intestine and increased release of recycled iron from the reticuloendothelial system [3]. This in turn leads to depletion of macrophage iron and relatively lower levels of serum ferritin, than what would be expected in transfusion dependent and non-transfusion dependent beta thalassaemia intermedia patients [5], which leads to preferential portal and hepatocyte iron loading with subsequent release into the circulation of free iron species that are capable of causing target organ damage and dyafunction. Other than this, such patients will eventually accumulate a minor fraction of this iron overload from occasional or infrequent transfusions [6]. Iron overload in such patients is a cumulative process and there is ample evidence of correlation between iron over load indices and advancing age. A considerable proportion of such patients would accumulate enough iron to precipitate organ dysfunction, which is more evidenced and followed in non-transfusion dependent thalassaenia patients by estimation of hepatic iron load, rather than the already underestimating serum ferritin levels [7]. These observations have clearly demonstrated that iron overload in non-transfusion dependent subjects, which should be promptly diagnosed and managed to prevent the occurrence of serious clinical complications [8].

Aims and Objectives

- To estimate the iron overload in non-transfusion dependent patients suffering from beta thalassaemia with haemoglobin E disease by serum ferritin.
- To estimate the iron overload in non-transfusion dependent patients suffering from beta thalassaemia with haemoglobin E disease by estimation of hepatic iron by MRI.
- To correlate increase in iron overload (both parameters) with increasing age.
- To correlate iron overload estimation by serum ferritin and LIC estimated by MRI.

Materials and Methodology

Non-transfusion dependent Beta Thalassaemia with Haemoglobin E disease patients, above the age of 3 years were selected.

They were included in the study if they were being followed up for at least five years irrespective of disease or co-morbidity status, which might or might not be related to the primary disease.

MRI scanning by R2* sequence was standardized on Siemens 1.5 T instrument by using the Phantom protocol from Resonance Healthcare – "Ferriscan", Australia, which is an FDA approved method for hepatic iron estimation.

MRI scanning by R2* sequence was done on all the patients and the DIACOM Images were sent to Resonance Healthcare for interpretation in a blind manner.

Serum Ferritin was measured from freshly collected and separated serum samples and tested by Automated Chemi-luminisence immuneassay (CLIA), Abbott Architect 1000i with Ferritin estimation Kit manufactured by Lilac Healthcare India Ltd. The samples were diluted 40 times before the assay procedure by kit manufacturer provided sample diluents and expressed as nanogram/mililitre.

Before entering this study all the non-transfusion dependent Beta Thalassaemia with Haemoglobin E disease patients were tested for Hepatitis B surface antigen and core antibody, anti-Hepatitis C antibody and HIV I and II antigen and antibody by commercially available CLIA Test kits marketed by Abbott and was tested on Abbott Architect 1000i platform. All patients who were found to be nonreactive to the above mentioned screening tests were included into the study, Patients who had history of claustrophobia and metallic implants were also excluded.

Total of 73 non-transfusion dependent Beta Thalassaemia with Haemoglobin E disease patients were included in the study. Their blood samples were collected one day in advance for serological testing and only those testing non-reactive were asked to come the next day. Blood sample was collected on the next day in empty stomach after 12 hours fasting for estimation of serum ferritin and was then asked to undergo hepatic MRI. This cross-sectional data that was obtained was used for further analysis and seeking of statistical correlation.

Results

Out of a total of 73 non-transfusions dependent Beta Thalassaemia with Haemoglobin E disease patients were included in the study, there were 41 male and 32 female subjects. The average age was 20.8, ranging from 3 to 54 years. Average age of males was 20.62 with a range of 3 to 46 years and that for females was 19.78, ranging from 5 to 54 years. Average iron overload as denoted by level of serum ferritin was 972.45 \pm 1121.5 ng/ml and the average hepatic iron overload as measured by Hepatic MRI-R2* sequence was 11.27 ± 11.70 mg/g of dry liver tissue. The data was analyzed after segregating the whole population into 6 groups depending upon age, Group I – up to 10 years, Group II – more than 10 to 15 years, Group III – more than 15 to 20 years, more than 20 to 25 years, Group IV – more than 25 to 30 years, Group V – more than 30 to 35 years and Group VI – more than 35 years.

In Group I, there were 11 subjects, 5 males and 6 females, average age being 6.9 years with a range of 3 to 9 years, average age among males was 6.2 years with a range of 3 to 9 years and in females, the average age was 7.5 years with a range of 5 to 9 years.

In Group II, there were 10 subjects, 8 males and 2 females, average age being 13.2 years with a range of 11 to 15 years, average age among males was 13.25 years with a range of 12 to 15 years and in females, the average age was 13 years with a range of 11 to 15 years.

In Group III, there were 14 subjects, 7 males and 7 females, average age being 17.4 years with a range of 16 to 20 years, average age among males was 17.9 years with a range of 17 to 19 years and in females, the average age was 17 years with a range of 16 to 20 years.

In Group IV, there were 18 subjects, 9 males and 9 females, average age being 22.9 years with a range of 22 to 25 years, average age among

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males and females was the same - 22.9 years with a range of 26 to 28 years, average age among males was 6.2 years with a range of 3 to 9 years and in females, the average age was 7.5 years with a range of 5 to 9 years.

In Group V, there were 10 subjects, 7 males and 3 females, average age being 27.3 years with a range of 26 to 30 years, average age among males was 26.7 years with a range of 26 to 28 years and in females, the average age was 28.67 years with a range of 27 to 30 years.

In Group VI, there were 10 subjects, 5 males and 5 females, average age being 38.6 years with a range of 32 to 54 years, average age among males was 39.8 years with a range of 36 to 46 years and in females, the average age was 37.5 years with a range of 32 to 54 years.

In Group I, the mean serum ferritin being 648.71 \pm 872.43 ng/ml, mean serum ferritin level among males was 479.36 \pm 637.61 ng/ml and in females – 789.83 \pm 220.8 ng/ml.

In Group II, the mean serum ferritin being 1434.51 ± 2109.1 ng/ml, mean serum ferritin level among males was 1501.9 ± 2326.76 ng/ml and in females – 1165 ± 1398.66 ng/ml.

In Group III, the mean serum ferritin being 509.7 \pm 346.2 ng/ml, mean serum ferritin level among males was 388.78 \pm 420.77 ng/ml and in females – 630.61 \pm 220.18 ng/ml.

In Group IV, the mean serum ferritin being 1085.22 \pm 782.08 ng/ml, mean serum ferritin level among males was 949.56 \pm 776.76 ng/ml and in females – 1220.89 \pm 809.31 ng/ml.

In Group V, the mean serum ferritin being 1525.98 \pm 1207.88 ng/ml, mean serum ferritin level among males was 1681.69 \pm 1271.39 ng/ml and in females – 1162.67 \pm 1197.16 ng/ml.

In Group VI, the mean serum ferritin being 1065.5 \pm 1146.48 ng/ml, mean serum ferritin level among males was 1634.4 \pm 1392.84 ng/ml and in females – 496.6 \pm 456.44 ng/ml.

In Group I, the mean hepatic iron overload as measured by Hepatic MRI-R2* sequence was 7.05 ± 8.9 mg/g of dry liver tissue, level among males was 5.8 ± 8.76 mg/g of dry liver tissue and in females – 8.1 ± 2.81 mg/g of dry liver tissue.

In Group II, the mean hepatic iron overload as measured by Hepatic MRI-R2* sequence was $15.47 \pm 14.3 \text{ mg/g}$ of dry liver tissue, level among males was $15.43 \pm 15.4 \text{ mg/g}$ of dry liver tissue and in females – $15.6 \pm 13.43 \text{ mg/g}$ of dry liver tissue.

In Group III, the mean hepatic iron overload as measured by Hepatic MRI-R2* sequence was 5.02 ± 4.53 mg/g of dry liver tissue, level among males was 3.5 ± 3.37 mg/g of dry liver tissue and in females – 6.54 ± 5.27 mg/g of dry liver tissue.

In Group IV, the mean hepatic iron overload as measured by Hepatic MRI-R2* sequence was 12.37 ± 11.45 mg/g of dry liver tissue, level among males was 12.88 ± 12.64 mg/g of dry liver tissue and in females – 11.87 ± 10.86 mg/g of dry liver tissue.

In Group V, the mean hepatic iron overload as measured by Hepatic MRI-R2* sequence was 19 ± 16.28 mg/g of dry liver tissue, level among males was 21.34 ± 16.95 mg/g of dry liver tissue and in females – 13.53 ± 16.34 mg/g of dry liver tissue.

In Group VI, the mean hepatic iron overload as measured by Hepatic MRI-R2* sequence was 14.02 ± 13.02 mg/g of dry liver tissue, level among males was 19.62 ± 16.32 mg/g of dry liver tissue and in females – 8.42 ± 6.04 mg/g of dry liver tissue.

Discussion and Conclusion

One of the initial publications which recognized iron overload to be frequently present in thalassaemia intermedia patients was by Florelli et al in 1990, commenting 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, and deduced that they were spread over a wide range and did not correlate with other parameters, though hepatic iron over load as a parameter was not considered as it was unavailable12. 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 ones. In the current study, iron over load by MRI as evidenced by LIC of >2 mg /g dry weight was documented in 59 out of 73, i.e., 81% of subjects of age between 3 to 53 years.

In the article published by Taher et al in 2011, the mean iron overload in 168 beta thalassaemia intermedia patients, the mean age was 35.2 ± 12.6 years, of whom 42.6 were males, the average liver iron concentration was 8.4 ± 6.7 mg Fe/g dry weight [9]. Whereas, Mazza et in 1995, 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 also recorded linear correlation of serum ferritin with liver iron content [10]. In another comparative study by Papakonstantinou et al. in 1995, where 40 transfusion dependent thalassaemia patiens' iron over load status was estimated by LIC, serum ferritin and histologic grading of siderosis and was compared, LIC ranged from 2.32 to 18 mg/g dry weight and this value correlated well serum ferritin levels [11]. In the current study of 73 non transfusion dependent HbE/Beta thalassaemia patients, the mean age was 20.8 \pm 11.13 years, of whom 43.8% were females. The average LIC was 11.27 ± 11.7 mg/g dry weight and serum ferritin was 972.45 ± 1121.5 ng/ml (Figure 1).



Figure 1: Relationship between serum ferritin and age.

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 [12]. 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] (Figure 2).

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In our study, correlation coefficient of 0.99 was obtained in the age group of 3 to 10 years of age among females 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 Fe/g of dry liver tissue, whereas among both sexes of this age group the correlation coefficient was 0.64, where the mean serum ferritin level was 648.7 ng/ml and the LIC was 7.05 mg Fe/g of dry liver tissue. Similar correlation was obtained in the age group 11 to 15 years in both sexes, which was 0.887, where serum ferritin level was 1434.5 ng/ml and

LIC of 15.47 mg Fe/g of dry liver tissue, but the data is not statistically valid because the SD is more than the mean (Figure 3). Statistically significant correlation was obtained in the age group 25 to 30 years in both sexes, which was 0.93, where serum ferritin level was 1525.98 \pm 1207.9 ng/ml and LIC of 19 \pm 16.27 mg Fe/g of dry liver tissue, among males of this age group was 0.899, where serum ferritin level was 1681.68 \pm 1271.3 ng/ml and LIC of 21.34 \pm 16.94 mg Fe/g of dry liver tissue, among females the correlation was 0.99, with the mean serum ferritin level being 1162.66 \pm 1197.15 ng/ml and LIC of 13.53 \pm 16.34 mg Fe/g of dry liver tissue, but this was not statistically significant because the SD was more than the mean.



It is evident from the results of the current study, that there is definite evidence of iron overload among non-transfusion dependent HbE/Beta thalassaemia patients. The conventional method of estimation of the trend of iron over load by serum ferritin level is it a static measurement or a trend is misleading, when compared to LIC by MRI. Serum ferritin level of 38 has been shown to have LIC of 16.4 or 256 and 21.4, also LIC of 1.9 recorded Serum ferritin level of 1590 ng/ml. Though 36% in age group below 10 years had LIC above 5 mg/g dry tissue, same patients had iron over load also evidenced by serum

ferritin level of >500 ng/ml. In the >11 to 15 year age group 80% had evidence of iron overload as evidenced by LIC>5 but 60% had serum ferritin >500 ng/ml. in the >16 to 20 years age group 37% had LIC of >5 but 47% had serum ferritin >500 ng/ml. in the age group >20 to 25 years, 84% had LIC >5 and 74% had serum ferritin >500 ng/ml, 36.8% had LIC >10 and serum ferritin >1500 ng/ml.



In the >25 year age group 65% had LIC>5 and 50% had LIC>10 and 65% had serum ferritin >500 but only 20% >1500 ng/ml. In the overall study, of the 16 out of 73 patients (21%), who had LIC <2 mg/g dry tissue the average serum ferritin was 275.85 ng/ml and 11 out of 73 (15%), who had LIC >2-3 mg/g dry tissue, the average serum ferritin was 236.8 ng/ml, who had LIC >3 - 4 mg/g dry tissue, the average serum ferritin was 555.8 ng/ml who had LIC >4 - 5 mg/g dry tissue, the average serum ferritin was 475.27 ng/ml and overall patients who had LIC <5 mg/g dry tissue, the average serum ferritin was 489.63 ng/ml. So it can be concluded that LIC of <5 correlates with serum ferritin level of approximately 500 ng/ml and serum ferritin of less than 300 ng/ml definitely correlates with LIC of <2 mg/g of dry tissue, but above that range no correlation between level of ferritin and LIC was obtained (Figure 4). There was also no correlation between age and iron over load as evidenced serum ferritin and/or corresponding increase in hepatic iron at lower ages and lower serum ferritin and LIC at higher ages.



Figure 5: Correlation of serum ferritin with LIC of <5 mg/g of dry tissue.

So it is recommended that serum ferritin can be regarded as a parameter of iron overload in otherwise uncomplicated patients as long as it is below 300 ng/ml, but is suspicion of iron over load is high, as may be thought of from presenting complicating symptoms, it should always be correlated with LIC estimation by MRI. Age should not be a criteria for estimation of iron overload in this group of patients because higher age do not correlate with higher iron overload neither did lower age correspond with less iron overloaded state. It can

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also be proposed that due to the erratic nature of the iron load as regards the liver, the lower age limit of MRI in this group of patients may be brought down to 3 years for effective screening and prevention of end organ damage (Figure 5).

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