Assessment of Hepatic Iron Overload in Paediatric Beta Thalassemia Major Patients using T2* Weighted Gradient Echo Magnetic Resonance Imaging

Likhitha N[1], Ravi N[2]

ABSTRACT

Introduction: Thalassemia is an inherited hematologic disorder which requires regular blood transfusion, this in turn will lead to tissue iron overload. Human body lacks a mechanism to excrete excess iron. Serum ferritin has been used as a marker for iron load in body but it is an acute phase reactant and not specific. Liver biopsy is the most reliable method for estimating organ iron overload; however, it is invasive and its accuracy is greatly affected by hepatic inflammation, fibrosis and uneven iron distribution. Main aim of this study was to study is to evaluate hepatic iron overload using T2* weighted Gradient Echo Magnetic Resonance Imaging and to study imaging pattern in such patients.

Material and methods: Magnetic resonance imaging (MRI) T2* weighted gradient echo was performed in patients with Beta thalassemia major patients due to its invasiveness and considered as more reliable method to detect iron overload in thalassemic patients.

Results: The association between haemoglobin and T2 levels for severe band was determined by Pearson's correlation, with the r value of 1.00, showing positive correlation, however the value was statistically significant, p=0.00.The association between ferritin and T2 for moderate band was determined by Pearson's correlation, with the r value of 0.99, showing positive correlation, however the value was statistically significant, p=0.009.

Conclusion: MRI T2* Gradient Echo, a sensitive and non – invasive method can accurately detect iron overload and help in chelation therapy in thalassemia patients and thereby reducing the mortality rate.

Keywords: Hepatic Iron, Paediatric, Beta Thalassemia, T2* Weighted Gradient Echo, Magnetic Resonance Imaging

INTRODUCTION

Thalassemia is an inherited hematologic disorder caused by decrease or absence of globin production.[1] These patients require frequent blood transfusion and has found to develop severe cardiopulmonary, liver, endocrine and other major organ dysfunctions. The overall prevalence of Beta thalassemia in India is 3-4% with an estimate around 10,000-12,000 children are born every year with Beta – thalassemia. Serum ferritin has been used as a marker for iron load in body, it is an acute phase reactant and it is not specific because its level can be raised in inflammation, infection and in liver damage.[2] Liver biopsy is the most reliable method for estimating organ iron overload; however, it is invasive and its accuracy is greatly affected by hepatic inflammation, fibrosis and uneven iron distribution.[2]

Serum markers of iron overload, serum ferritin and transferrin saturation, are inexpensive techniques to predict iron concentration in the body. These markers have been widely used to determine the need for starting iron chelation therapy and monitoring therapeutic response. However, these techniques cannot be used solely to monitor iron levels in the body.

In non-transfusion dependent thalassemia, gastro-intestinal absorption of iron increases several folds to accommodate the decrease of haemoglobin levels. Iron overload is toxic to cells, causing severe and irreversible organ damage.

Most patients with thalassemia would die by the age of 10 without red blood cell transfusions. However, once the capacity of transferrin to take up excess iron is surpassed, free iron appears and enter other organs through alternate pathways. Initially, the cells may utilize the excess iron by using it for the enzymes and mitochondria, as well as to store ferritin, but if it continues, ultimately the cellular capacity to utilize the free iron will be overwhelmed. As intracellular free iron is toxic, tissue damage may also occur, leading to morbidity and mortality. Humans have no active mechanism to excrete the excess iron from the body. Iron chelation therapy is therefore essential for the excretion of excess tissue iron and reducing morbidity and mortality.

Regular blood transfusion in thalassemia major patients causes iron overload because human body lacks a mechanism to excrete excess iron. Monitoring of iron overload is essential in establishing effective iron chelation regimen.[3] Chelation therapy, used for iron elimination, requires effective monitoring of the burden of iron, for which serum ferritin levels and liver iron content measured in liver biopsies are used as markers, but not reliable. MRI T2* weighted gradient echo is used for evaluation of tissue siderosis.

Magnetic resonance imaging (MRI) T2* weighted gradient echo used for detecting iron levels is non-invasive and

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reliable method for detecting iron overload in thalassemic patients and is helpful in early diagnosis to guide for iron chelation regimen. It is more sensitive and specific than liver biopsy.[4] Main aim of this study was to study is to evaluate hepatic iron overload using T2* weighted Gradient Echo Magnetic Resonance Imaging and to study imaging pattern in such patients.

MATERIAL AND METHODS

A Descriptive study was conducted on children 6-18 years who were diagnosed with Beta-thalassemia major presenting to Department of Radio Diagnosis, referred by the various attached hospitals of Bangalore medical college and research institute were included in the study. The study period was eighteen months from November 2017 to May 2019.

Sample size

Based on previous study by Eghbali.A[5], mean of hepatic T2* was 5.3+/-4.29 and

expected precision will be 1.2. Sample size calculation is n= $(Z_{\alpha}^2\ \sigma^2)/d^2$

where n = sample size, $Z_{\alpha=}$Standard value (1.96), σ = standard deviation (4.29),

d = Allowable error (1.2)

Substituting the same values in the equation, we get:

$$n\ (\text{Sample size}) = \frac{(1.96)^2 \times (4.29)^2}{(1.2)^2}$$

$$= \frac{(3.841) \times (18.404)}{1.44}$$

$$n = 49.09 \sim 5$$

Method of collection of data

After obtaining clearance and approval from the instituitional ethical committee, out of 50 sample size calculated, children who are fulfilling the inclusion and exclusion criteria and who gave informed consent were included in the study.

All the patients were subjected to T2* Weighted Gradient Echo MRI evaluation of hepatic iron overload and also blood sample was collected for measuring serum ferritin along with routine investigations.

MRI was performed by Seimens magnetom avento,1.5 tesla. Single breath T2*gradient echo sequence was taken.

1.5-T Siemens Avanto Magnetom MR system of Department of Radiodiagosis, Bangalore Medical College and Research Institute.

After plotting the values on excel sheet, T2*value is obtained and by using color coding we grade the patient

Normal (green) =>6.3ms.

Mild (yellow) = 6.3-3.8ms.

Moderate (orange) = 3.8-1.8ms.

Severe (red) = <1.8ms.

Serum ferritin level was measured by ELISA

The findings of MRI and serum ferritin was assessed simultaneously.

RESULTS

Study group consisted of 50 children aged between 6 years to 18 years and diagnosed with β Thalassemia Major. In the study group haemoglobin level was from 6.0 g% to 8.0 g% which was low with the recommended haemoglobin. Age group of 11 to 15 years (46%) constituted the major age group. In the total sample of 50, 22 were female (44%) and 28 were male (56%) children, who were considered for the study.

Thirty two children had normal T2* MRI (> 6.3ms), Eleven children had light(6.3 – 3.8ms), Four children had moderate (3.8 – 1.8ms), three children had severe

(<1.8ms), according to T2 relaxation grading. Study population was classified into four categories based on MRI T2* grading, majority of children 32(64%) had normal grading, 11(22%) children had light, 4(8%) children had moderate, 3(6%) had severe

The mean hemoglobin value of people in normal band was 7.16 with a standard deviation of 0.87, in light band was 7.87 with a standard deviation of 1.009, in moderate band was 8.25 with a standard deviation of 0.50, in severe band was 7.06 with a standard deviation of 0.11 (figure 1). The mean serum ferritin value of people in light band was 2577.5 with a standard deviation of 1750.09, in moderate band was 2985.5 with a standard deviation of 1516.66, in normal band was 2419.6 with a standard deviation of 1364.16, in severe band was 4878.0 with a standard deviation of 1217.63 (figure 2). The mean T2* grading of children in light band is 5.41 with a standard deviation of 0.66, in moderate band is 2.50 with a standard deviation of 0.57, in normal band is 10.96 with a standard deviation of 1.8, in severe band is 1.06 with a standard deviation of 0.11 (figure 3).

Majority of the children in all the age group were graded

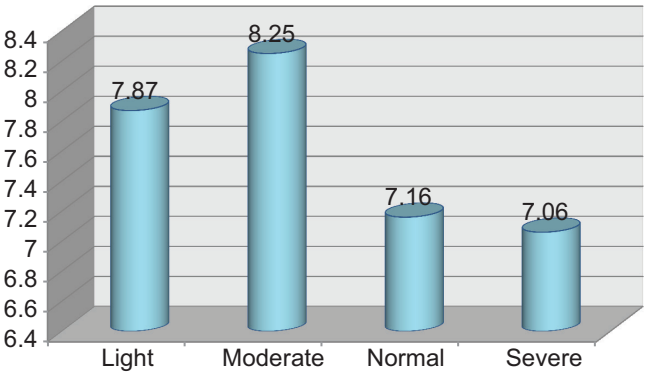


Figure-1: Mean value of hemoglobin based on MRI T2 grading

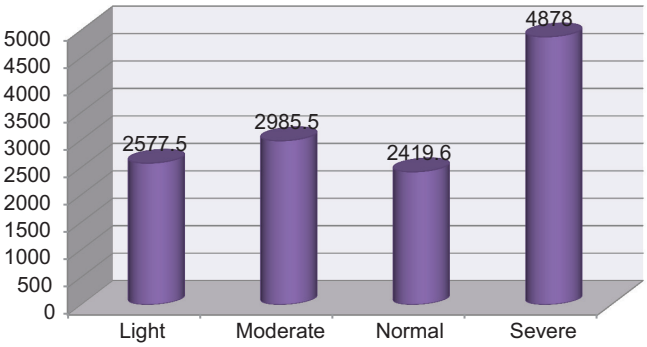


Figure-2: Mean value of serum ferritin based on MRI T2 grading

normal by MRI T2 grading. However on application of Fischer exact test, the value was found to be just significant (figure 4). Majority of the males and females were in the normal grading by MRI T2 followed by lighter band in both females and males. However this was statistically not significant by chi-square with a P value of 0.30. (figure 5).

STATISTICAL ANALYSIS

SPSS (Statistical Package for Social Sciences) version 20. [IBM SPASS statistics (IBM corp. Armonk, NY, USA released 2011)] was used to perform the statistical analysis Data was entered in the excel spread sheet. Descriptive statistics of the explanatory and outcome variables were calculated by mean, standard deviation for quantitative variables, frequency and proportions for qualitative variables. Inferential statistics like fischer exact test was used for categorical variables and pearson's correlation was used to correlate haemoglobin, Serum Ferritin and T2 scores. The level of significance was set at 5%

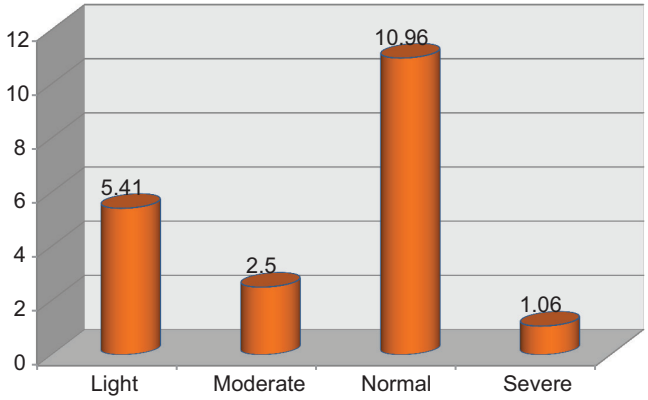


Figure-3: Mean value of T2 scores based on MRI T2 grading

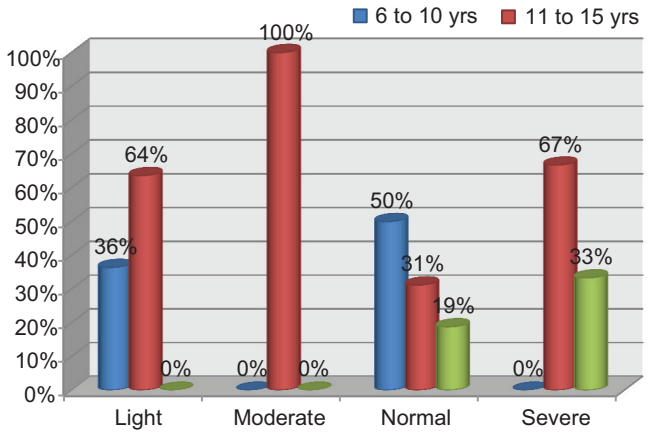


Figure-4: Cross-tabulation of age and MRI T2 grading

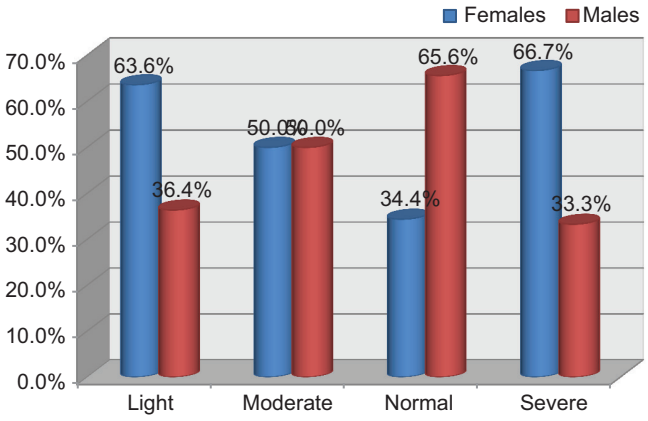
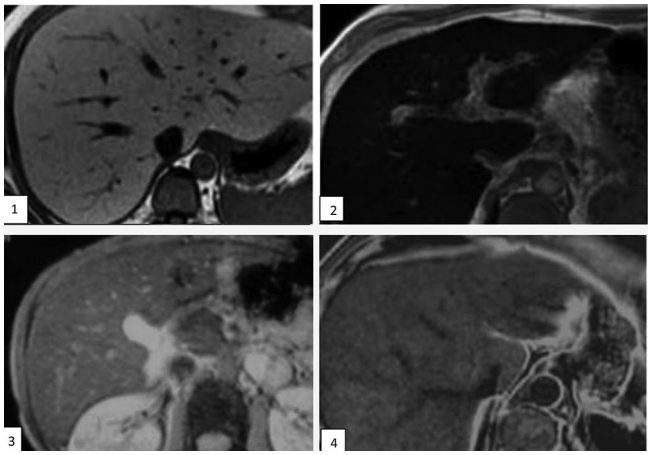


Figure-5: Cross-tabulation of gender and MRI T2 grading

DISCUSSION

Magnetic resonance (MR) imaging is the most sensitive and specific imaging modality in the diagnosis of parenchymal iron overload in thalassemia patients on regular blood transfusion. The susceptibility effect caused by the accumulation of iron leads to signal loss in the affected tissue, particularly with the T2* weighted sequences, which makes the diagnosis of iron overload possible in a non-invasive way, thereby avoiding repeated biopsies. The accumulation of iron ions in the tissues, because of the super paramagnetic properties of the iron, causes local distortion in the magnetic fields and relaxation of the spins which results in shortening of the longitudinal relaxation time(T1) and the transverse relaxation time(T2), and particularly the transverse relaxation time as affected by magnetic field inhomogeneity(T2*). This effect causes a loss of signal intensity in the affected organs that is proportional to their on deposition. Dual sequences MR imaging (gradient in and out phase) demonstrates decreased signal intensity in the affected tissues on the in-phase images compared with the out-phase images (images-1). This occurs because the echo time of the in-phase sequence is usually higher than that of the out –phase sequence; therefore, the in-phase pulse sequence is more sensitive to iron deposits because of the increasedT2* effect In the current study, the single breath T2* gradient echo sequence was preferred for its short scanning time making it more convenient in young age and the resulting signal intensity was compared to that on T2WI.

Present study is done on 50 children diagnosed β Thalassemia major. Age of the children ranged from 6 years to 18 years, mean age being 11.64 years**.** Study done by Trehan A et al[6] showed mean age at presentation in the whole cohort 17.2 ± 19.9 months, with 50% presenting in the first year of life. Children presenting directly to their centre had a mean age of



Images-1: T2* GRE MRI image in mild iron overload. 2.T2* GRE MRI image in severe iron overload. 3.T2* GRE MRI image in normal patient. 4.T2* GRE MRI image in moderate iron overload.

13.2 ± 9.7 months with 64% being diagnosed within the first year of life. Age groups were divided into three categories, 6 to 10 years age group had 20 children that constitute 40%,11 to 15years age group had 23 children constituted 46% and above 15years had 7 children constituted 14% of the total study population**.** Study done by Al-Kherbash HA et al[7] showed 33% of the patients in the age group 7–10 years, followed by the age group 3–6 years. The mean age of the cases was 7.68±5.8 years. Of the study population 22 are female (44%) and 28 are male(56%) children are considered for the study**.** Study done by Trehan A et al showed a male to female ratio of 2.5:1. Indian studies by Bandyopaadhyay B et al.[8] reported a male preponderance of 68% and study by Chhotray GP showed a male preponderance of 69.5%. The studies from the middle east, west and mediterranean showed equal sex distribution. The male preponderance in India is attributed to gender bias. Studies across India have shown that boys are more likely to be taken to a healthcare facility than girls when they fall sick

Mean distribution of hemoglobin is 7.40 with standard deviation 0.92, Mean distribution of serum ferritin is 2647.18 with standard deviation 1533.84, mean distribution of T2* is 8.47 with standard deviation 3.82

Study population was classified into four categories based on MRI T2 grading, majority of children 32(64%) had normal grading, 11(22%) children had light, 4(8%) children had moderate, 3(6%) had severe. The mean hemoglobin value of people in normal band was 7.16 with a standard deviation of 0.87, in light band was 7.87 with a standard deviation of 1.009, in moderate band was 8.25 with a standard deviation of 0.50, in severe band was 7.06 with a standard deviation of 0.11 (figure 1). The mean serum ferritin value of people in light band was 2577.5 with a standard deviation of 1750.09, in moderate band was 2985.5 with a standard deviation of 1516.66, in normal band was 2419.6 with a standard deviation of 1364.16, in severe band was 4878.0 with a standard deviation of 1217.63 (figure 2). The mean T2* grading of children in light band is 5.41 with a standard deviation of 0.66, in moderate band is 2.50 with a standard deviation of 0.57, in normal band is 10.96 with a standard deviation of 1.8, in severe band is 1.06 with a standard deviation of 0.11 (figure 3). Majority of the children in all the age group were graded normal by MRI T2 grading. However on application of Fischer exact test, the value was found to be just significant (figure 4). Majority of the males and females were in the normal grading by MRI T2 followed by lighter band in both females and males. However this was statistically not significant by chi-square with a P value of 0.30. (figure 5).The results of study done by Chaosuwannakit N et al[18] is compared with the results of the present study in the table below

The study done by Chaosuwannakit N et al, Serum ferritin levels showed a significant negative correlation with the liver T2* values ($p = 0.01$, $r = 0.318$). No significant correlation was observed between serum ferritin level and cardiac T2* MRI ($p = 0.68$, $r = 0.06$). Nevertheless, a significant correlation was observed between serum ferritin and liver iron concentration evaluated by MRI ($p = 0.04$, $r = 0.68$).

Poor negative correlation between serum ferritin level and T2* MRI liver was observed by Eghbali et al. and Fahmy et al.[19] also. On the other hand, Azarkeivan et al.[20], Kolnagou et al.[21], and Zamani et al.[22] showed moderate correlation, and in contrast to our study, Assis et al.[23] demonstrated excellent correlation between serum ferritin and T2* MRI liver. The association between haemoglobin and serum ferritin levels determined by pearsons correlation[24], with a correlation coefficient (r) value of 0.07, however this was not stastistically significant, p = 0.62.The association between haemoglobin and T2 determined by pearsons correlation, with a correlation coefficient (r) value of -0.26, however this was not stastistically significant, p = 0.06. The association between serum ferritin and T2 determined by pearsons correlation, with a correlation coefficient (r) value of -0.22, however this was not stastistically significant, p = 0.11. The association between haemoglobin and ferritin levels for lighter band was determined by Pearson's correlation, with the r value of -0.10, showing negative correlation, however the value was not statistically significant, p=0.75. The association between Haemoglobin and T2 for lighter band was determined by Pearson's correlation, with the r value of -0.30,showing negative correlation, however the value was not statistically significant,p=0.42. The association between Serum ferritin and T2 for lighter band was determined by Pearson's correlation, with the r value of 0.99, showing positive correlation, however the value was not statistically significant,p=0.70. The association between haemoglobin and ferritin levels for moderate band was determined by Pearson's correlation, with the r value of 0.51, showing positive correlation, however the value was not statistically significant, p=0.48. The association between haemoglobin and T2 for moderate band was determined by Pearson's correlation, with the r value of 0.57, showing positive correlation, however the value was not statistically significant, p=0.42. The association between ferritin and T2 for moderate band was determined by Pearson's correlation, with the r value of 0.99, showing positive correlation, however the value was statistically significant, p=0.009**.**

The association between haemoglobin and ferritin levels for normal band was determined by Pearson's correlation, with the r value of 0.14, showing positive correlation, however the value was not statistically significant, p=0.43. The association between haemoglobin and T2 levels for normal band was determined by Pearson's correlation, with the r value of 0.06, showing positive correlation, however the value was not statistically significant, p=0.71. The association between ferritin and T2 levels for normal band was determined by Pearson's correlation, with the r value of 0.06 showing positive correlation, however the value was not statistically significant, p=0.72**.** The association between haemoglobin and ferritin levels for severe band was determined by Pearson's correlation, with the r value of 0.49, showing positive correlation, however the value was not statistically significant, p=0.66. The association between haemoglobin and T2 levels for severe band was determined by Pearson's

correlation, with the r value of 1.00, showing positive correlation, however the value was statistically significant, p=0.00. The association between ferritin and T2 levels for severe band was determined by Pearson's correlation, with the r value of 0.49, showing positive correlation, however the value was not statistically significant, p=0.66.

CONCLUSION

In spite of chelation therapy and proper monitoring of thalassemia patients, liver siderosis is a common and persistent problem. Though liver biopsy can accurately detect iron overload, it is invasive, painful and associated with a significant risk of morbidity and mortality. Serum Ferritin though widely available and cost effective, does not accurately detect hepatic iron overload. Magnetic Resonance Imaging (MRI) measurements have become increasingly accurate for determining hepatic iron deposition and have generally supplanted direct tissue biopsy for assessing the presence of iron overload and quantifying its severity. In our study haemoglobin and Serum ferritin levels had a poor correlation with T2 scores on all MRI T2 grades and was statistically not significant, except for positive correlation (r = 0.99) of serum ferritin with T2 scores on Moderate MRI T2 grading which was statistically significant (p = 0.009). Therefore MRI T2* Gradient Echo, a sensitive and non – invasive method which can accurately detect iron overload and help in chelation therapy in thalassemia patients and thereby reducing the mortality rate. Hence MR imaging has to be conducted at least once to monitor the deposition of iron in the liver.

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