Assessment of Organ Specific Iron Overload in Transfusion-dependent Thalassemia by Magnetic Resonance Imaging Techniques

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ABSTRACT

The consequence of repeated blood transfusions in thalassemia is iron overload in different organs. Magnetic resonance imaging (MRI) is a reliable, non-invasive and accurate method for iron detection in various tissues, hence the introduction of MRI has revolutionized the management of these patients and improved the life expectancy of them. Cardiac MRI T2* has a profound effect not only on estimation of severity of cardiac siderosis, but on intensification of chelation regimens and survival of patients. Liver hemosiderosis is also a common morbidity among thalassemia patients, since the liver represents the dominant iron storage organ in the body; however, the relationship between total body iron and liver iron concentration (LIC) is challenging. Pancreatic iron overload occurs in 75-100% of patients with thalassemia major, but the association between pancreatic R2* and development of diabetes mellitus has not been established in the studies. On the other hand, there is a strong correlation between pancreatic R2* and cardiac R2*, so pancreatic R2* could predict left ventricular function. The most prevalent endocrinopathy in patients with thalassemia is hypogonadotropic hypogonadism which has been correlated with pituitary iron overload. Published data about kidney and adrenal MRI is limited, and further studies are needed to determine their clinical significance.

Introduction

Thalassemia syndromes are the most prevalent quantitative hemoglobinopathies in the world. The hallmark of β-thalassemia is reduced or absence of β chain synthesis resulting in excess of alpha chains and premature apoptosis of erythroid precursors in bone marrow. The severity of symptoms in β-thalassemia is related to the degree of imbalance between the β and non-β globin chains. In β-thalassemia major (β-TM), due to the complete absence of β-chain synthesis, patients present with severe anemia in early infancy. In patients with β-thalassemia intermedia (β-TI), the clinical severity of thalassemia varies among the mild symptoms of β-thalassemia minor and severe symptoms of β-TM. Repeated and regular long-life transfusions are the main part of the treatment in patients with transfusion dependent thalassemia (TDT) and the consequence of these transfusions is iron deposition in different organs. Therefore all of these patients need iron chelators to prevent organ hemosiderosis. With advances in the management of TDT, life expectancy of these patients has increased.

In recent years, quantitative measurement of iron by means of magnetic resonance imaging (MRI) has become an effective and reliable tool in the management of thalassemic patients. MRI is a non-invasive and accurate method for estimation of iron deposition in liver, heart and endocrine system. Iron deposition in vital organs begins from early childhood and increases over time. As a result, close monitoring of specific morbidities in TDT needs a sensitive estimation of iron since first decade of life.
In MRI images, different iron loaded tissues darken vastly and the half life of this darkening, could be used for assessment of the iron content. The results are expressed in milliseconds (ms) as T2* relaxation time. Organs with higher iron overload have shorter T2* relaxation times. R2* can be calculated from values of T2* as follows: R2 (Hz)=1000/T2 (ms).3 Therefore; R2 and R2* are directly related to iron content of tissues, while T2 and T2* are inversely proportional.4 Distribution of excess iron storage in different organs such as liver, heart and pancreas is completely heterogeneous, so liver iron cannot predict myocardial iron overload. In other words, liver iron is not a reliable and sensitive marker for identification of cardiac iron deposits. Moreover, iron chelation protocol could influence the distribution of iron in different organs in β-TM. Deferiprone (L1) specifically in combination with intravenous deferoxamine has more efficacy in iron removal of heart in comparison with deferasirox. Also deferasirox is more effective in iron removal of liver. Spleen is a storage site for excess iron, so splenectomy could accentuate iron deposition in vital organs.7

Markers of Iron Overload

Serum Ferritin

Serum ferritin is the most available biomarker for assessment of iron status. This intracellular protein releases from liver, so any liver disease such as hepatitis may lead to elevation of serum ferritin. Other conditions that could influence the level of serum ferritin include: infection, systemic inflammation, transfusion rate, ascorbate deficiency and rapid cell turnover. Ascorbate deficiency can lower serum ferritin and transfusion saturation.8 This test is inexpensive, but a rough estimation for prediction of liver iron burden. Meanwhile, serum ferritin level could not predict most morbidities in subjects with thalassemia intermedia. Moreover in TI patients, relying only on serum ferritin levels for initiation of iron chelation therapy may lead to delay in starting iron chelation.4 Serum ferritin level cannot be used as a precise and reliable marker for predicting cardiac iron content. In numerous articles, it has been demonstrated that there is a weak correlation between serum ferritin and cardiac MRI T2*.9,10 In this line, serum ferritin >2500 ng/ml has been associated with increased risk of liver cirrhosis, impaired growth, delayed puberty and cardiac disease.11

Transferrin Saturation

Transferrin saturation is another marker for estimation of iron status. This marker is not practically useful in thalassemia patients due to repeated transfusions. These patients often have fully saturated transferrin and therefore non-transferrin bound iron (NTBI) significantly rises in them. In the majority of TM patients who receive regular transfusions, transferrin saturation exceeds 100%; hence heart and endocrine glands such as pancreas exclusively uptake NTBI, leading to iron accumulation in these organs. In addition, measurement of transferrin saturation accurately could not be relied when patients are receiving iron chelation and thalassemia patients must hold their iron chelator 24 hour prior to blood sampling for this test.12

Liver Iron Concentration (LIC)

LIC is the gold standard for the evaluation of total body iron. Approximately 70% of total body iron stores in the liver in thalassemic patients. LIC should be at least 3 mg Fe/g dry weight of liver. Liver iron content can be calculated by needle biopsy or MRI.15 Wood's formula measures iron content as: Fe mg/g=RIA+0.0254×0.202. Four classes of LIC have been described in thalassemic patients. Class I or normal: LIC <3 mg Fe/g dry weight of liver, class II or mild iron overload: 3-7 mg Fe/g of liver, class III or moderate overload: 7-15 mg and class IV or severe overload ≥15 mg Fe/g dry weight of liver.16 Needle biopsy is an invasive procedure and is not feasible to do it repeatedly. The accuracy of this technique is affected by hepatic inflammation or fibrosis and even unequal iron distribution throughout the liver.17 When significant fibrosis is present, liver biopsy could not help physician for assessment of response to the therapy.18

Magnetic Resonance Imaging

Detection of iron deposits in different organs has revolutionized the management of thalassemic patients. Early diagnosis of iron overload and also tailored chelation therapy are the most important benefits of MRI in clinical practice leading to increased survival of thalassemia patients.1 Several published data have demonstrated that various tissues in body have different iron kinetics, so liver MRI could not predict myocardial siderosis.19 To estimate iron content in each tissue accurately, organ specific MRI is necessary. Despite multiple data about liver and cardiac T2* or R2*, Studies about other organs such as pancreas, kidney, adrenal and pituitary gland are limited.

Liver MRI

Liver is the first organ for iron storage in thalassemia. When transferrin is fully saturated, the free iron as non-transferrin bound iron or NTBI appears in the circulation and then enters the tissues. Different cells in the body utilize the excess iron or store it as ferritin. Intracellular free iron damages tissues resulting in morbidity and mortality. Liver fibrosis occurs in approximately 30% of thalassemic patients due to iron overload.11 MRI T2* of liver is a valid, sensitive and accurate method for detection of iron deposition in the liver. In liver MRI, iron load is classified as normal (T2*>6.3 ms), mild (T2*: 2.7-6.3 ms), moderate (T2*: 1.4-2.7 ms) and severe (T2*<1.4 ms).20 The Thalassemia Clinical Research Network recommends performing annual T2* MRI of heart and liver after age of 10 years old and in patients with cardiac T2*<10 ms, every 6 months.13, 14 As mentioned before, due to poor correlation between liver and cardiac T2, evaluation of liver iron alone is not adequate for monitoring of chelation efficacy.21

Cardiac MRI

The mechanism of iron storage in the liver and heart is
completely different. Also, the degree of iron overload in the heart and liver is not identical.\textsuperscript{22} Iron stores in the cardiomyocytes in three forms: ferritin, hemosiderin and labile cellular iron (LCI) which is free iron. Accumulated iron damages cardiac tissue and LCI participates in the formation of free radicals. Free radicals could be neutralized by antioxidants, but their excess results in cardiac dysfunction. Indeed all of the thalassemic patients with left ventricle dysfunction have cardiac iron overload.\textsuperscript{23} In the cardiac MRI, iron overload was divided as: normal $>$20 ms, mild 14-20 ms, moderate 10-14 ms and severe $<$10 ms.\textsuperscript{24} Ferritin could not predict the cardiac iron content. Additionally, LIC does not have any significant correlation with cardiac T2*. The rate of iron accumulation in the liver and heart and also the rate of iron clearance between these two organs is completely different. In patients who are on intensive chelation, iron is removed faster from the liver.\textsuperscript{23}

Cardiac T2* is a valid and useful method either in early diagnosis of iron overload (even in asymptomatic patients) or in monitoring of chelation therapy. Moreover, cardiac MRI can assess both atrial and ventricular function.\textsuperscript{25} Modell and colleagues found a significant increase in the survival rate of patients with thalassemia major as a result of decrease in cardiac iron overload. They suggested that the introduction of T2* MRI in order to detect the severity of myocardial iron was the main reason of improved survival of thalassemia patients.\textsuperscript{26} One of the limitations of cardiac magnetic resonance (CMR) imaging is the patients who have pacemakers, defibrillators or implantable pump in the chest. Patients with port-a-cath (titanium port) or stents have no contraindication for doing MRI. Cardiac T2* MRI can assess the iron as deposited in the form of ferritin and hemosiderin, but not the labile cellular iron which is toxic and dangerous.\textsuperscript{21} In advanced stages of myocardial hemosiderosis, progressive LV dysfunction and reduction of LV ejection fraction occurs and finally symptomatic heart failure develops.\textsuperscript{27} Both systolic and diastolic function have significant associations with cardiac T2* figures.\textsuperscript{28}

Kirk et al. have found a significant correlation between myocardial iron and risk of heart failure and arrhythmia in patients with thalassemia major.\textsuperscript{10} Once heart failure occurs, despite aggressive chelation, the outcome of thalassemia patients would be poor.\textsuperscript{29}

**Pancreatic MRI**

Pancreatic siderosis has been reported in 75-100\% of thalassemia patients.\textsuperscript{29,30} In Pancreatic R2, iron overload is classified into 4 groups; normal pancreas: R2<30 Hz, mild iron overload: R2: 30-100 Hz, moderate iron overload: R2: 100-400 Hz and severe iron overload: R2>400 Hz.\textsuperscript{32} In T2* method, the minimum threshold of normal value was considered 26 ms.\textsuperscript{33} Iron deposition in the pancreas often is demonstrated as hypointensity in MRI T2*.\textsuperscript{34}

Evaluation of pancreas R2 is challenging in older thalassemic subjects due to fatty infiltration of pancreas.\textsuperscript{6} Spleenectomies result in difficulties in measurement of pancreatic R2* since the splenic artery is a useful landmark.\textsuperscript{32} Papakonstantiou and co-workers found that liver iron concentration has a weak correlation with pancreatic or cardiac siderosis.\textsuperscript{31} Noetzli and colleagues have demonstrated similar results.\textsuperscript{35} In a study from Iran on 164 patients with thalassemia a moderate correlation between pancreas R2* and LIC have been shown.\textsuperscript{29} Assis and colleagues found a weak correlation between cardiac and pancreatic R2* with LIC.\textsuperscript{3} On the other hand, Meloni and colleagues found a positive correlation between cardiac iron overload and cardiac hemosiderosis. These authors have demonstrated that pancreatic R2* is a strong predictor for left ventricular function.\textsuperscript{39} Noetzli and co-workers showed a strong correlation between cardiac and pancreatic R2*. A pancreas R2*>100 Hz is reported as a strong predictor for cardiac siderosis.\textsuperscript{35} Wood et al. showed that iron accumulation in the pancreas occurs much earlier than heart.\textsuperscript{3} Another important feature of pancreatic MRI is the association between pancreatic iron overload and risk of developing diabetes mellitus. The incidence of impaired glucose tolerance test and diabetes mellitus varies from 9 to 24\% among thalassemic patients. One of the most probable etiologies of diabetes mellitus in thalassemia is direct toxic effects of iron on beta and acinar cells. However, many authors have suggested that increased peripheral resistance to insulin is responsible for impaired glucose tolerance in these patients.\textsuperscript{1} In a study on 130 patients with transfusion dependent thalassemia no correlation was found between diabetes incidence and pancreatic T2.\textsuperscript{37} Li et al. declared pancreatic volume as a better predictor for impaired glucose metabolism in comparison with pancreatic iron overload.\textsuperscript{38} In contrast; in other studies, Matter and De Sanctis established a correlation between diabetes mellitus and pancreatic iron measured by MRI.\textsuperscript{39,40} Noetzli et al. showed that pancreas R2* is the most useful predictor of glucose dysregulation, but cardiac R2* is more specific for impaired glucose metabolism. However cardiac R2* is insensitive for mild impaired glucose regulation. Also despite impaired cardiac function, beta cell dysfunction is not completely reversible and hence, predisposes patients to overt diabetes as they age. Moreover, pancreatic R2* could predict cardiac risk much better than hepatic iron assessment and also identifies the risk of dysfunction of other endocrine glands. Isolated pancreas R2*>100 Hz is highly specific for glucose dysregulation and therefore intensification of chelation therapy as soon as possible in order to reduce damage to the beta cells. Measurement of pancreas R2* can be performed simultaneously with cardiac and liver study without no significant increase in the cost and time of imaging.\textsuperscript{41}

**Kidney MRI**

Despite multiple data on MRI of liver and heart, there are only a few published articles regarding the use of this modality for assessment of kidney iron overload in thalassemia patients. In addition to repeated transfusions, one of the main sources of iron deposition in kidneys is intravascular hemolysis. Free hemoglobin is filtered by glomeruli and then taken up by megalin-cubilin receptors which are located in proximal and distal tubules. This phenomenon leads to characteristic pictures in MR
images of kidneys. Renal cortical area become dark, but the medulla does not change at all. Wood could not show any correlation between kidney R2* and LIC in hemolytic conditions which is ascribed to different mechanisms of uptake, transport and clearance of iron between various organs.14 Chronic anemia, iron overload and toxicity of iron chelators are the main possible mechanisms of kidney damage in thalassemia.12 Oxidative stress secondary to iron deposition in the kidneys results in proximal tubular dysfunction as well as some degrees of glomerular dysfunction in younger children.43 Early detection of high risk patients for kidney dysfunction among thalassemia has a key role in preventing kidney damage progression and reduction of the incidence of end stage renal disease.44 In recent years, kidney MRI has become a non-invasive, accurate ad specific method for detection of iron overload in transfusion dependent thalassemia. Both methods, MRI gradient echo (T2*) and spin echo (T2) have been used to measure iron deposits. Hemosiderin molecules cause local disturbances in the magnetic field leading to a rapid MRI signal decay rate. The amount of T2* signal decay rate depends to the tissue iron concentration. Therefore, the presence of iron in different tissues results in reduction of field homogeneity and low T2 signal.45 In a recent study on 120 transfusion dependent thalassemia patients, a moderate correlation is reported between kidney MRI T2* and serum ferritin, whereas the correlation between kidney and cardiac and liver MRI T2* was weak.46 Shein and colleagues found the same results in sickle cell patients. They proposed that kidney iron toxicity may occur at concentrations below MRI detection limits.47 The correlation between kidney hemosiderosis and early markers of kidney dysfunction among thalassemia patients is reported to be controversial.48 In a large retrospective study on 821 TM and TI patients, 19.6% of them had kidney hemosiderosis according to kidney MRI. There is a report of a weak negative correlation between kidney hemosiderosis and serum ferritin; however, the correlation between kidney T2* and liver and heart T2* was weakly positive.49 Meloni and colleagues, in a study on 119 TM patients, demonstrated that 33.6% of them had kidney hemosiderosis. They defined kidney iron overload as T2*<36 ms. They documented a correlation between kidney, heart and liver T2*.50 Regarding these conflict results, it seems that direct kidney imaging is a more accurate and sensitive technique for assessment of kidney iron overload. In other words, hepatic and cardiac T2* are not reliable predictors for evaluation of kidney iron overload. However the practical importance of kidney T2* and also its correlation with early markers of tubulopathy and glomerulopathy needs further longitudinal studies.

Adrenal MRI

One of the most important endocrinopathies in thalassemia is adrenal insufficiency. Its prevalence is reported to vary between 13-61 percent depending on diagnostic tests and cut-off values. Symptoms of adrenal insufficiency are non specific such as arthralgia, muscle pain, chronic fatigue and gastrointestinal complaints. Many of these symptoms could be related to thalassemia itself, therefore diagnosis of adrenal insufficiency only by clinical symptoms is not possible. In stress situations such as surgery or sepsis, adrenal crisis may occur, hence diagnosis of adrenal insufficiency is critical.51 Iron deposition in adrenal glands is defined as hypointensity on the T2* sequences. Focal or diffuse hypointensity in the adrenal glands may be due to calcification, hemorrhage or granulomatous disease. Drakunaki and co-workers performed a study on 35 TM cases and found hypointense adrenal glands on T2* GRE sequence in 68.6% of cases which is due to the paramagnetic properties of iron. They demonstrated a positive correlation between degree of liver and adrenal hemosiderosis. Moreover, there was no significant correlation between adrenal signal intensity and patient age or serum ferritin level.52 A study on 21 patients with thalassemia major and 11 subjects as healthy control group showed that there was no significant correlation between serum ferritin and adrenal T2. They demonstrated that there was no significant correlation between adrenal gland and liver T2* in β-TM patients, but moderate between adrenal and cardiac T2*.53 The difference between Drakunaki and Guzelbey study could be explained with the difference in method of detection of iron deposition. In the Drakunaki study, the method of iron assessment was semi quantitative, but in the Guzelbey study, the technique was quantitative.54 Due to the paucity of studies regarding the detection of iron deposition in adrenal glands in β-thalassemia, further studies are necessary in order to evaluate the precise correlation between adrenal MR imaging features and biochemical tests of adrenal insufficiency.

Pituitary Gland MRI

The prevalence of hypogonadotropic hypogonadism among patients with thalassemia major is reported to be about 50%. Hypogonadism due to iron overload is the most prevalent endocrine disorder in thalassemic patients.14 Early detection of pituitary hemosiderosis is critical, since hypogonadism even after intensive chelation is not completely reversible.53 Pituitary iron overload is defined as T2*<5.9 ms.54 Ferritin shortens both T1 and T2 relaxation times, but hemosiderin only shortens T2. T1 is not appropriate for detection of iron overload in different tissues of the body. Gradient-echo (GE) sequences have shown more sensitivity in comparison to Spin-echo (SE) images. In addition, the duration of GE sequencing is shorter, hence the probability of motion artifacts reduces.55 In majority of the cases, hypogonadism is actually diagnosed after puberty. Significant iron deposition in the pituitary gland occurs in the first and second decade of life and results in an irreversible damage in early adulthood. Growth failure and delayed puberty are the most common symptoms. Pituitary iron overload and loss of pituitary volume were correlated directly with development of hypogonadism. Reduction of pituitary volume has specificity but not sensitivity for the following hypogonadism. Noetzi et al. demonstrated a linear relationship between pituitary R2 and LIC or serum
Also, there was a correlation between pituitary iron with pancreatic and cardiac iron. It has proposed that the kinetic of pituitary iron loading is between the liver and pancreas. Cardiac iron is suggested as a predictor for pituitary iron overload, but is most strongly associated with pancreas R2+.

In a study on 84 thalassemia patients, pituitary T2* values were correlated with hepatic and cardiac T2* values, serum ferritin level and age of the patients. It has shown that splenectomy can accentuate pituitary and cardiac iron accumulation. Christoforidis et al. in a study on 30 TM patients couldn’t reach a correlation between pituitary iron and age of the patients despite finding a moderate correlation between pituitary iron and serum ferritin. Pituitary MRI has a key role in early detection of iron deposition in pituitary gland and intensification of chelation therapy in order to omit the development of hypogonadism.

Conclusion
The advent of MRI for detection of iron accumulation in different organs had a significant effect on survival of thalassemia patients. Due to differences in uptake, transport, storage and kinetic of iron in various organs, the organ specific iron detection in different organs has a key importance in these patients. Despite the existence of multiple published data on cardiac and hepatic MRI, there are limited data about pancreas, pituitary, adrenal and kidney MRI in the literature.

Cardiac and hepatic hemosiderosis are common complications in TDT with profound impact on survival of the patients. Pancreatic hemosiderosis may be found in majority of thalassemia cases and pituitary iron overload occurs in approximately half of the patients. There are limited data about kidney and adrenal MRI with uncertain clinical significance. Larger clinical experience with MRI of these two organs is advised in order to identify their role in routine management of thalassemia patients.

Conflict of Interest: None declared.

References


