

**RELATIONSHIP BETWEEN MAGNETIC RESONANCE
IMAGING LIVER T2* VALUE WITH ALANINE AMINO
TRANSFERASE (ALT) AND ALBUMIN IN TRANSFUSION
DEPENDENT THALASSEMIA PATIENTS DURING
CHELATION THERAPY**

DR. SHARMILA A/P ARRUMUGAM

**DISSERTATION SUBMITTED IN PARTIAL FULFILMENT
OF THE REQUIREMENT FOR MASTER OF MEDICINE
(RADIOLOGY)**



UNIVERSITI SAINS MALAYSIA

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

ALT	Alanine aminotransferase
DNA	Deoxyribonucleic acid
F	Female
FPN	Ferroportin
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HEP	Hepcidin
LPI	Labile plasma iron
M	Male
MTR	Malaysian Thalassemia Registry
MRI	Magnetic Resonance Imaging
NTBI	Non-transferrin bound iron
ROS	Reactive oxygen species
TDT	Transfusion Dependent Thalassemia
Tf	transferrin

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ABSTRAK

Latar belakang: Penyakit Talasemia adalah sejenis penyakit genetik di mana ianya berkaitan dengan kecacatan pada pembentukan sel darah merah. Penghasilan sel darah merah yang berkualiti dalam kuantiti yang mencukupi terjejas kerana mutasi pada gen yang mengawal penghasilan hemoglobin. Salah satu protein dalam sel darah merah ialah hemoglobin. Tugas penting hemoglobin adalah membawa bekalan oksigen ke tisu-tisu di dalam badan manusia. Talasemia dibahagikan kepada dua kumpulan utama berpandukan rantaian molekul hemoglobin yang terlibat, seperti dalam talasemia α , pengeluaran rantai α -globin terjejas, manakala dalam talasemia β , pengeluaran rantai β -globin terjejas. Untuk mereka yang menghadapi penyakit keturunan ini, kebanyakan mereka memerlukan transfusi darah secara berkala sepanjang hidup mereka. Tranfusi darah yang kerap ini menjadi penyebab kepada pengumpulan zat besi secara berlebihan di dalam organ -organ dalam badan, antaranya ialah hati. Zat besi ini yang terkumpul secara berlebihan ini menjadi punca kepada kerosakan hati. Namun, untuk mengurangkan pengumpulan zat besi ini, terdapat rawatan yang daapt membantu menyingkirkan zat besi yang berlebihan ini. Untuk membantu pemantauan tahap zat besi di dalam hati, pengimejan **MRI** T2* hati diaplikasikan. Ini bukan sahaja membimbing langkah rawatan, malah dapat memantau keberkesanan rawatan yang diberi. Namun kerana masih kekurangan teknologi MRI dan kosnya yang tinggi, ini sedikit sebanyak membantutkan rawatan dan susulan pesakit talasemia. Oleh yang demikian, kajian in dijalankan untuk melihat hubungan antara bacaan T2* MRI hati dengan ujian darah hati. Ini sedikit sebanyak dapat membantu untuk meyenaraikan pesakit yang memerlukan pengimejan ini dalam kadar secepat mungkin.

Metodologi: Kajian ini melibatkan pesakit talasemia yang mendapat transfusi darah secara berkala dan datang untuk pengimejan MRI hati di Jabatan Pengimejan Diagnostik, Hospital Seberang Jaya (HSJ), Pulau Pinang. Kadar zat besi yang berlebihan di dalam hati dinilai melalui bacaan yang diperoleh daripada T2* dan seterusnya diklasifikasikan sebagai tiada zat besi, ringan, sederhana, teruk dan sangat teruk. Fungsi hati dinilai melalui ujian darah yang diambil dalam masa enam bulan sebelum pengimejan, yang mana terdekat dengan tarikh pengimejan. Hubungan antara nilai T2* hati dengan ujian darah ALT dan albumin diuji berdasarkan ‘Pearson correlation’.

Keputusan: Terdapat korelasi yang signifikan di antara MRI T2* hati dengan ujian darah ALT, iaitu semakin tinggi bacaan ALT, semakin teruk kandungan zat besi di dalam hati, T2(r=-0.54). Namun, ujian darah tidak menunjukkan korelasi signifikan dengan MRI T2* hati, T2 (r=0.11).

Kesimpulan: Secara keseluruhan, kajian ini menunjukkan korelasi yang signifikan antara MRI T2* hati dengan ujian darah ALT, iaitu semakin tinggi bacaan ALT, semakin teruk kandungan zat besi di dalam hati. Walaubagaimanapun, disebabkan oleh sorotan literatur yang terhad, kajian yang lebih lanjut berkemungkinan akan menunjukkan hubungan korelasi antara hubungan MRI T2* hati dengan ujian darah albumin

Kata kunci: Talasemia Major; MRI T2 hati, kelebihan zat besi di hati, ujian darah ALT, ujian darah albumin*

ABSTRACT

Background: One of the commonest inherited disorders that affects the synthesis of haemoglobin is known as Thalassemia. It is classified depending on the abnormality that affects the synthesis of alpha and beta chains. Thalassemia patients are classified into TDT and non TDT. TDT patients who undergo monthly blood transfusion are predisposed to many bloods transfusion related complications such as iron overload mainly in organs such as cardiac, liver and pancreas. Many eventually succumb to their illness due to complications secondary to cardiac siderosis. The invention of iron chelation therapy does not only improve patients' quality of life but also prolonged their life. Nowadays, due an increase in survival rate, liver related complication secondary to liver siderosis is increasingly witnessed amongst TDT patients. The discovery of MRI T2* liver as method of measuring liver iron concentration has played a vital role in the management of these patients, mainly in optimising chelation therapy. The lack of MRI facility in Malaysia, especially in rural areas have hampered management of TDT patients suffering from iron siderosis. Prompt, efficient, and apt chelation therapy promises better quality of life, and this is only achieved with proper analysis of the severity of the liver siderosis. Serum liver function test amongst TDT patients is done regularly and this investigation is widely available and gives quick results. In the hope to hasten MRI T2* liver study among these patients, we hope to look for a relationship between serum ALT and albumin level with MRI T2* liver value. In doing so, we can facilitate the clinicians to use these blood results as a guide to screen potential patients with a possibility of severe liver siderosis and send them for MRI T2* liver at an earlier time. This in return will

help clinicians to expedite the chelation therapy and eventually help to reduce the risk of liver iron siderosis that leads to liver diseases.

Methods: A retrospective study was conducted in Hospital Seberang Jaya, Pulau Pinang, Malaysia with 358 patients who had been diagnosed as transfusion dependent thalassaemic patients and had undergone MRI T2* liver. MRI T2* liver was classified as MRI T2* liver values were taken with numerical values and categorised as, none (> 24), mild ($7.2 - 24$), moderate ($3.3 - 7.2$), severe ($2.2 - 3.3$) and very severe (< 2.2). ALT and albumin levels were taken as numerical numbers. The descriptive statistics for discrete variables (age, gender, race, status of splenectomy, the type of thalassemia) were presented as n=frequency (%). Pearson chi square test was used to examine the correlation of T2* MRI liver with ALT and albumin values, respectively in TDT patients who underwent chelation therapy.

Results: There were significant correlation between all related variables ($p < 0.050$). ALT was negatively moderate correlated to T2 ($r = -0.54$). Albumin was poorly positive correlated with T2 ($r = 0.11$). Further studies with a larger number of subjects would be beneficial in the future.

Conclusion: There were relationship between T2* MRI liver imaging findings with serum ALT and serum albumin amongst TDT patients, ALT was negatively moderate correlated to T2 ($r = -0.54$) whereas albumin was poorly positive correlated with T2 ($r = 0.11$). Further studies with a larger number of subjects would be beneficial in the future.

Keywords: Transfusion Dependent Thalassemia, MRI T2 liver, liver siderosis, serum ALT, serum albumin.*

CHAPTER 1: BACKGROUND

1.1 Introduction

In Malaysia, prevalence of thalassemia is common. It is a common autosomal recessive disorder. In this disease, there is a reduction in oxygen carrying haemoglobin because there is abnormality in the production the subunits of haemoglobin. Depending on the affected chain, thalassaemia is referred as α -, β -, γ -, δ -, $\delta\beta$ -, or $\epsilon\gamma\delta\beta$ -thalassaemias. (Cappellini et al., 2014). The Malaysian Thalassaemia Registry (MTR) stated that as of 28 November 2018 there are 7984 alive patients with thalassaemia and 56.73% of them require regular blood transfusion. (Mohd Ibrahim et al., 2020). In view of the progressive advancement in the medical field, life expectancy of these thalassemia patients has tremendously improved as there is proper blood transfusion to combat anaemia as well as iron chelation therapy to deal with problems associated with blood transfusions related complications. Iron overload secondary to prolong blood transfusion causes significant morbidity and mortality amongst transfusion dependent thalassemia patients. Despite its benefits, blood transfusion causes hemochromatosis, predominantly in the heart and the liver. (Farhangi et al., 2017). Cardiac and liver siderosis are well-known risks for mortality in thalassemia patients who are on regular blood transfusion. However, proper iron chelation therapy has significantly contributed to a decrease in mortality secondary to organ siderosis amongst these patients. To achieve optimal efficacy of iron chelation, monitoring the iron level is essential. In recent years, advancement of medical imaging such as Magnetic Resonance Imaging (MRI) T2* has played a paramount role in monitoring iron siderosis in these two organs. By performing MRI T2* on the liver and the heart,

iron siderosis in these organs can be assessed. We can achieve optimal chelation for iron overload by subjecting these patients for MRI T2* of liver and heart. Regular MRI T2* surveillance will be ideal for the thalassemia patients who are on regular blood transfusion. Chelation therapy has successfully helped to lengthen the survival years of TDT patients and this contributes to an increase in morbidity and mortality related to liver siderosis and its complications. MRI T2* liver is less frequent mainly due to lack of facility. TDT patients are frequently tested for serum liver function tests, at least twice a year. On the contrary, the MRI T2* assessment, is done at least once a year due limited resources in Malaysia. We are conducting this study to observe the relationship between liver function blood serum parameters with severity of liver siderosis quantified by MRI T2*.

Iron storage in the body when has increased and exceeds the normal limit inflicts hepatocyte damage. This then causes cellular necrosis and subsequently leads to progressive fibrosis and ultimately cirrhosis. Progression of the disease is reflected by a deranged increase in serum liver function tests especially albumin and alanine aminotransferase. Liver synthetic activity can be reflected as changes in albumin level. On the other hand, alanine aminotransferase level can give an idea about hepatocellular damage. Evaluation of serum liver enzymes can evaluate the progression of liver siderosis causing hepatocellular damage. The severity of liver siderosis in TDT patients is usually evaluated using the gold standard method of MRI T2* liver assessment. Hence, we are hoping to correlate MRI T2* liver levels for liver siderosis with the serum albumin as well as serum alanine aminotransferase. Since MRI imaging modality is not easily available, especially in rural areas, it slows down the screening of MRI T2* liver for liver siderosis. From this study, using results of serum albumin and alanine aminotransferase that are frequently done

during their follow-up sessions, we aim to facilitate the screening of TDT patients earlier especially in whom we anticipate possibilities of severe liver siderosis. In doing so, we hope to identify TDT patients who need interval imaging during chelation therapy and guide physicians to optimize treatment. This in the hope that liver diseases secondary to liver siderosis can be significantly reduced.

1.2 Objectives

1.2.1 General Objective

To study the relationship between MRI T2* liver values and serum liver function tests in TDT patients who underwent chelation therapy.

1.2.2 Specific Objectives

1. To determine correlation of MRI T2* liver with ALT values in TDT patients who underwent chelation therapy.
2. To determine correlation of MRI T2* liver with albumin values in TDT patients who underwent chelation therapy.

1.3 Hypothesis

1. With increasing severity of liver iron overload in MRI T2*, the ALT will be increased
2. With increasing severity of liver iron overload in MRI T2*, albumin will be reduced.

1.4 Research Question

1. What is the relationship between MRI T2* liver imaging findings with serum ALT TDT patients.
2. What is the relationship between MRI T2* liver imaging findings with albumin in transfusion dependent thalassemia patients.

CHAPTER 2: LITERATURE REVIEW

2.1 Thalassemia and TDT

The term “thalassemia” is attributed to a genetic disorder whereby there is mutation of in the β -globin or α -globin gene, and are then named β thalassemia and α -thalassemia, respectively. (Cappellini et al.,2014.). In every erythrocyte, there is an oxygen-binding protein called haemoglobin. This haemoglobin plays a role in transportation of oxygen from the lungs to the tissues. The haemoglobin molecule is made of four polypeptide globin chains, beta, alpha, gamma and delta. In Thalassemia, mutations will cause reduction or absence of normal globin chain production. When there is a defect in the synthesis of normal globin, the ability of red blood cells to transport oxygen is reduced. Depending upon the degree of severity, thalassemia may be major, minor, or intermedia. The most common types in Malaysia are α and β thalassaemia. Most patients suffer from anaemia and regular blood transfusions are initiated if patients with thalassemia major are unable to sustain haemoglobin level of more than 7g/dl. According to the MTR, patient on regular blood transfusions are those patient who will need red blood cell (RBC) transfusions at a recurring interval from biweekly or once every 12 weeks. (Mohd Ibrahim et al., 2020). Blood transfusion is the mainstay in this disorder to lengthen their survival. Unfortunately, frequent blood transfusions lead to complications, particularly iron overload. This iron gets accumulated in heart, which can lead to cardiac dysfunction. This complication usually manifests as cardiomyopathy or heart failure. Amongst TDT patient, this is the commonest cause of mortality. (Farhangi et al., 2017). It is found that the liver is one of the many organs that are vulnerable to

early iron deposition. When iron is overloaded in the liver, this can lead to development of cirrhosis. This iron can be excreted via proper chelation, and hence introduction of chelating drug in many countries to tackle issues pertaining to iron overload in various organs. According to Shafie et al., they revealed that there are about 55 million thalassaemic carriers in Southeast Asia.

2.2 Iron overload in TDT patient

Iron is a crucial micronutrient in the body. It is mainly needed for cells producing cytochromes, haemoglobin, and myoglobin. However, when in excess it causes various problems in the body. In thalassemia patients who are undergoing regular blood transfusions, they usually face complications of iron overload in the body as human body cannot remove this essential nutrient. Over a period, there is an increase in the iron intake due to RBC transfusion in TDT patients. On the other hand, non TDT patient can have iron overload mainly due to an increment in the absorption of iron through the gastrointestinal tract. According to Remacha et al., 2013 approximately 200-250 mg of iron is in one pack of RBC.(Remacha et al., 2013). When iron is excessive, it mainly has a profound influence towards the spleen. The spleen is primarily affected as the RBC are broken down within macrophages of spleen. The other reticuloendothelial system that are involved are the hepatic macrophages. (Cappellini et al., 2014). The release of these intra-macrophagic iron will be progressive into the blood stream. It then reaches to the bone marrow to produce new RBC. (Cappellini et al., 2014). These irons will bind to transferrin, a transport protein of the iron.(Taher & Saliba, 2017.). In a normal situation, the usual iron saturation of plasma transferrin is less than 45%. However, as these are released into the blood stream there is a great rate of increment in iron saturation of plasma transferrin, which may reach to 100%. Now, there will be

formation of plasma non-transferrin bound iron. This plasma non transferrin bound iron has strong affinity towards parenchymal cells of the liver, heart, and pancreas and will be then stored at the cells. (Cappellini et al., 2014). When iron is collected in all these cells, they then potentiate different clinical complications of iron overload. (Taher & Saliba, 2017). The metabolism of these non-transferin bound iron will produce a result called reactive oxygen species, ROS. These ROS has tremendous effect on the cellular level changes and death of target tissues. In the usual manner, transferrin binds 2 molecules of Fe^{3+} and subsequently binds to two transferrin receptors, namely transferrin receptor 1(TfR1), commonly found in erythroid precursors, the liver, and the myocardium and transferrin receptor 2 (TfR2), found in the liver and intestine. After undergoing endocytosis, the transferrin sets Fe^{3+} free and later it is reduced to Fe^{2+} . The iron is more attracted to TfR1 by at least 25 times more as compared to TfR2.(Taher & Saliba, 2017). According to (Taher & Saliba, 2017.), the building up of iron loading continues in the liver as the TfR2 are deficient of an iron responsive element. On the other hand, the TfR1 is suppressed with increased transferrin saturation. These changes are depicted in Figure 1.

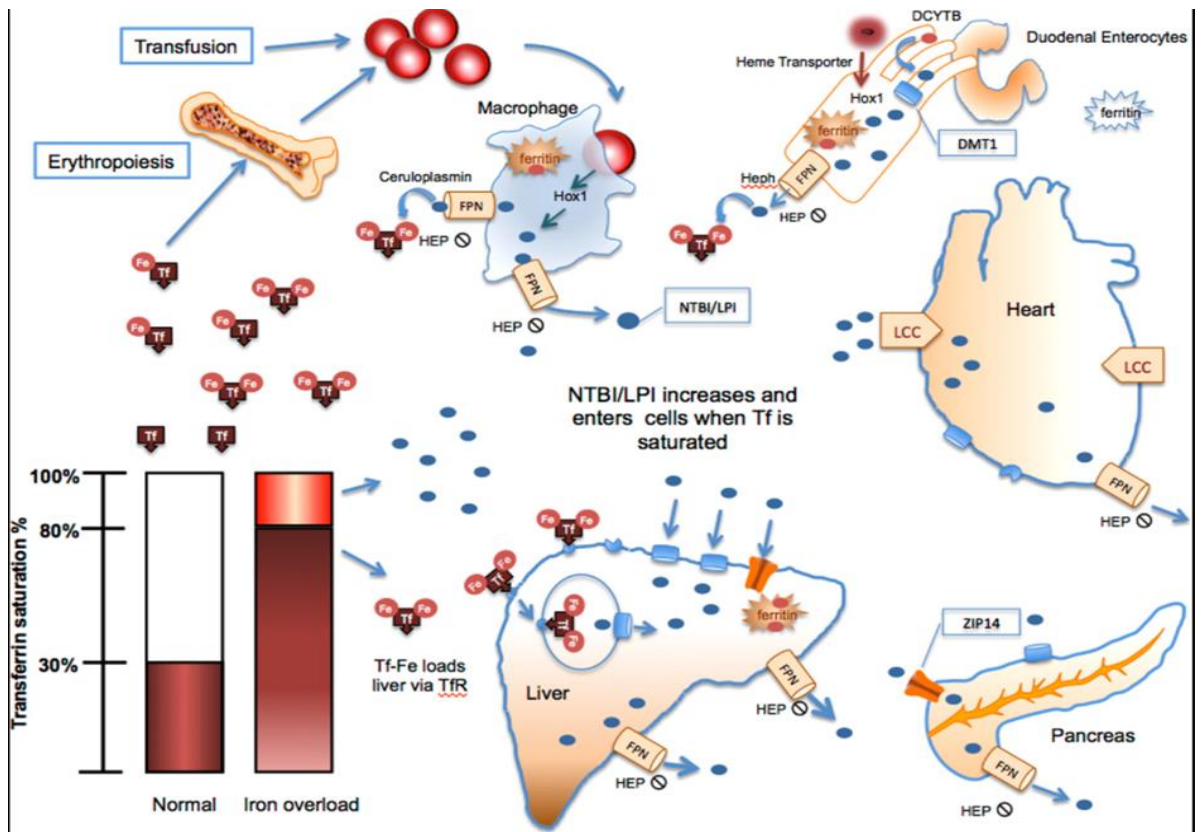


Figure 1 : Iron homeostasis in transfusional iron overload. Red cells phagocytosis by reticuloendothelial macrophages, where the hemoglobin is degraded by heme oxygenase (Hox1) and Fe is exported via ferroportin (FPN) and binds to transferrin (Tf). When Tf becomes saturated, non-transferrin-bound iron (NTBI) and labile plasma iron (LPI) can enter organs through the divalent metal transporter (DMT1), ZIP14, and L-type calcium channels (LCC). LPI and labile cellular iron are highly reactive species of NTBI that can cause direct oxidant damage. Diferric transferrin enters the marrow and liver through the transferrin receptors 1 and 2. Heme and ferric iron enter the gut and are exported by FPN. Hepcidin (HEP) blocks export of Fe through FPN.

Adapted from Coates, 2014

2.3 Hepatic iron overload in TDT

Liver is the largest internal organ. It has multiple functions in the body. One of the most important functions is it has the greatest ability to keep excess iron. In usual scenario, the normal iron concentration is less than 20 $\mu\text{mol/g}$ of dry weight. (Kwo et al., 2017a). In the body, iron plays an important role as an essential micronutrient for the human body. It includes roles such as transporting oxygen, oxidative phosphorylation, and many other enzymatic activities. Hepatocytic iron overload is favourable for development of liver parenchymal disease and further complications like cirrhosis or hepatocellular carcinoma. The ROS formed due to excessive iron in the liver, when has reached certain level causes hepatocytes to go through cell death. Eventually fibrosis ensues. When oxidative process begins, these hepatocytes are not able to cope, these are then engulfed by Kupffer cells. This event then causes certain consequences such as synthesise of inflammatory cytokines as well as growth factors. Following these, hepatic stellate cells (HSC) get activated. A great contributor to liver fibrosis is HSC. (Xy et al., 2022). When the liver is exposed to repeated cycles of ROS generation, inflammation and hepatocyte cell death together with activation and increasing amount of HSC, these can lead collectively to liver cirrhosis (Imbert-Bismut et al., 1999). Once hepatocyte damage is established, cellular necrosis ensues and is reflected by increase in serum transaminase activities: alanine aminotransferase and aspartate aminotransferase. The development of liver fibrosis and liver cirrhosis then follows. (Cappellini et al., 2014). Jensen et al., 2003, conducted a study to look for relationship between hepatocellular injury and serum levels of aminotransferases. They concluded that 54% of their patients who had increasing hepatocellular cell damage secondary to transfused iron had raised serum ALT and

raised serum transaminases were noticed in hepatocyte iron concentration (HIC) more than 300 $\mu\text{mol/g}$. Another interesting finding in Jensen et al., 2003, was that rising serum ALT levels only appreciated in patients whose iron saturation level was above 75%. Once liver parenchymal disease sets in the scarring will slowly take place, which is the liver fibrosis, and this is seen in HIC value of more than 400 $\mu\text{mol/g}$ according to study conducted by Angelucci et al., 2002. Liver cirrhosis is then the precursor for deadly diseases like HCC. Another reason for iron toxicity is the presence of labile iron in the form of plasma non-transferrin bound iron (NTBI), which is known to largely produce ROS. (Cappellini et al., 2014). According to Jensen et al., 2003, they deduced that before chelation therapy, raised aminotransferase level indicates hepatocellular injury, and this patients the liver iron concentrations exceeded 300 to 400 $\mu\text{M/g}$. Various method for assessment of iron overload has been introduced in TDT patients. This includes serum blood investigations such as serum iron level, transferrin saturation, serum ferritin and serum liver function test. From the imaging aspect, Magnetic Resonance Imaging (MRI) T2* of the liver has been used throughout the world. The ultimate gold standard assessment is liver biopsy, however it is currently replaced by MRI T2* liver. The serum iron investigations that are commonly done, reveals high level and complete saturation of transferrin in early years of transfusion amongst TDT patient. It is found that there is weak relationship between ferritin and iron levels in the body; Moreover, ferritin is an acute-phase reactant and will give a false increase in certain patients with chronic infectious diseases. Ferritin levels are also raised in malignancies and inflammatory disorders making it a poor indicator for iron overload level. Conditions like deficiency in ascorbate can reduce the levels of ferritin which again will have an impact if we look at this parameter to decide iron overload level in TDT patients. (Gianesin et al., 2012). Biopsy of the liver

tissue has been well advocated as the gold standard for diagnosing iron overload. Unfortunately, this procedure has many disadvantages. It is an invasive procedure with complications like post biopsy bleeding that if unable to be controlled can be fatal. It also hampers proper diagnosis as iron distribution is heterogeneous in the liver, and this indirectly may lead to poor site selection for the correct reflection of excessive iron assessment in the liver. Through imaging study such as Magnetic Resonance Imaging T2* (MRI-T2*), iron overload can be estimated accurately. It is not only non-invasive but fairly reliable study. (Gianesin et al., 2012).

2.4 Assessment of iron concentration in liver using T2* Magnetic Resonance Imaging of liver (MRI-T2*)

The gold standard for measurement of liver iron content is via liver biopsy. As previously mentioned, this procedure is not only invasive but may not yield accurate results as hepatic siderosis usually has heterogeneous spread making it difficult to correctly pick an area for biopsy. In recent years, the adapted method for assessment of liver siderosis is by MRI T2* liver, (Brittenham, 2015) (Farhangi et al., 2017). Many studies have demonstrated a good correlation between liver iron measured using MRI T2* and that measured by liver biopsy (Farhangi et al., 2017),(Brittenham, 2015) (Gianesin et al., 2012). According to World journal of gastroenterology,2012 , Zamani et al study showed liver iron concentration from liver biopsy revealed significant correlation with hepatic T2* ($r > 0.9$).

MRI has ability to pick up iron signal in the body as iron is a material that influences T2*, T2 and T1. It shortens the relaxation time in T2*, T2 and T1. When iron is present, and is exposed to MRI study, the iron will cause shortening of proton relaxation times, particularly T2, an effect termed susceptibility-induced relaxation.

(Gillis et al., 1999). An axial slice of the liver using multi echo gradient-echo images is obtained (Hankins et al., 2009). On the axial slice, the individual echo time (TEs) as well as region of interest based on the signal intensity in the liver is identified. We then manually will draw in the region of interest measuring up to 0.5 to 3.0cm. Through an automated algorithm, at <http://www.isodense.com/ic>, as seen in Figure 2, we can arrive to a value. (Git et al., 2015). In certain cases where there are fewer than three sets of data, the operator can manually adjust the results if truncation failed. This automated algorithm can be assessed by using modern web browsers (<http://www.isodense.com/ic>) (Figure 2). During the earlier days, cardiac MR techniques adapted T2 measurement by the spin echo (SE) sequences to measure the liver iron deposits. Unfortunately, this showed undesirable reading of myocardial iron content as these readings were influenced by many other factors such as flow, noise and motion artifact as well as hardware constraint. (Mavrogeni et al., 1998).

The desired area for liver iron estimation is taken as an area of the liver measuring approximately 0.5cm to 3.0cm (regions-of-interest, ROIs), and this area should be homogeneous and without vessels traversing. (Papakonstantinou et al., 2009) (Christoforidis et al., 2009) (Ooi et al., 1999]

2.5 Quantification of liver iron concentrations by MRI T2* liver using an online open-source tool

Further management of iron siderosis in various organs has shown tremendous progression since the emergent of MRI T2* liver that was able to quantify the liver iron concentrations. For this reason, quantification software are needed to obtain

accurate values to reflect iron overload in patients. Most of this software are expensive and not easily available. In Malaysia, the brilliant development of an online open-source software tool by Git, K.A. et al., which was validated against CVI 42 version 5.1 (Circle Cardiovascular Imaging, Calgary, AB) has impacted the progression of MRI T2* liver reading. (Git et al., 2015). This online software is compatible with most web browsers. This is currently used in most hospitals in Malaysia and runs in web browsers, <http://www.isodense.com/ic> (Figure 1). (Git et al., 2015)

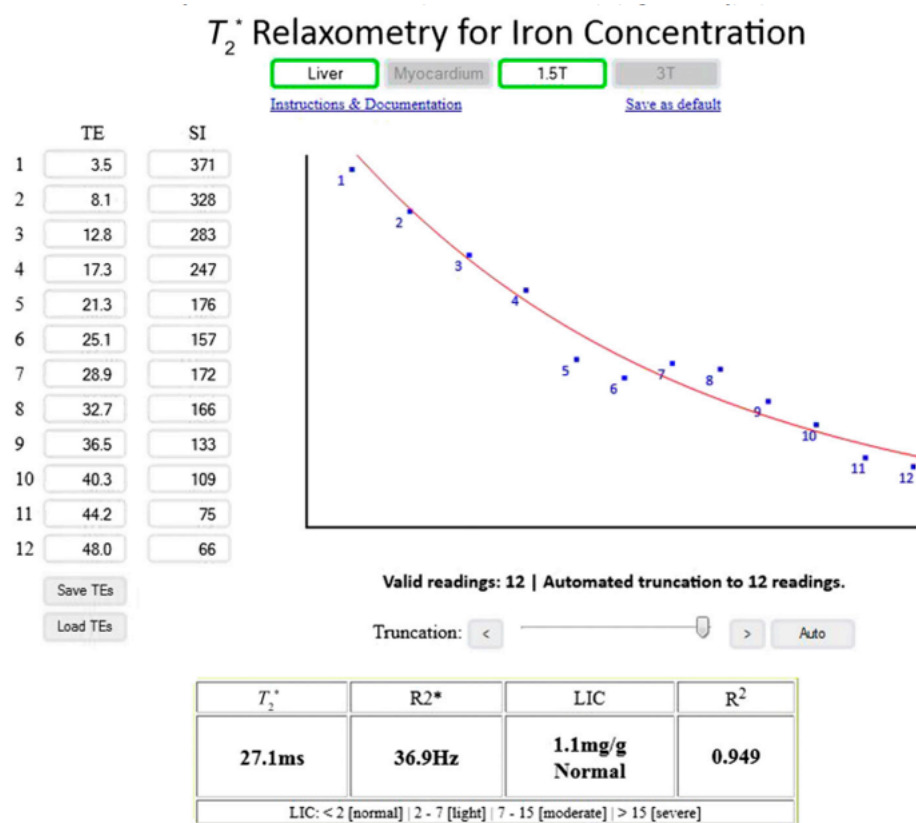


Figure 2 : A sample calculation performed with the online tool that allows for automated truncation and provides result in T2*, R2 and final liver iron concentration, LIC, SI Signal intensity, TE Echo time.