

**LABILE PLASMA IRON ASSAY IN THALASSEMIA - A REVIEW OF LITERATURE**

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Article Received on 10/05/2020

Article Revised on 01/06/2020

Article Accepted on 22/06/2020

**ABSTRACT**

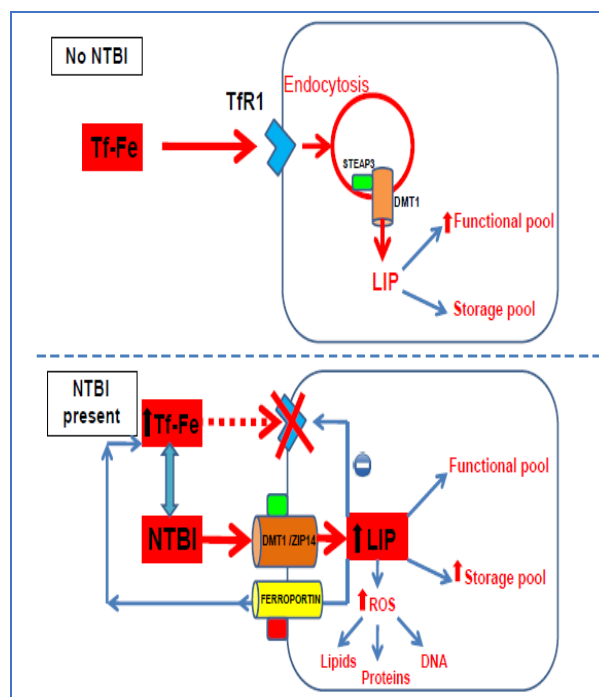
Blood transfusion and iron overload are unavoidable issues in thalassemia. There is always a quest for a better and sensitive test for iron overload which can detect tissue iron overload efficiently and earlier than available markers. Labile plasma iron assay (LPI) which measures the non transferrin bound iron (NTBI) component of plasma is a potentially intuitive marker as it directly reflects tissue iron overload which is the cause of mortality in such cases. Present in research setting for almost a decade, the test is yet to be widely adopted by clinicians in routine patients setting. The limited availability, standardization and inherent limitations could be the possible causes. However, the assay appears promising and in future it may serve to monitor the trend of tissue iron overload during long-term follow up where it may be better than ferritin levels.

**INTRODUCTION**

Labile plasma iron or LPI levels are newly found surrogate markers of iron overload which have gained attention recently in thalassemia major patients. It represents that component of non-transferrin bound iron which is capable of freely permeating into organs and causing tissue iron overload.<sup>[1]</sup> Sustained higher levels of LPI over time compromise functions of vital organs (eg heart and liver) and affect patient survival.<sup>[1]</sup> This has led to increasing new interest recently to measure this component in iron overloaded patients in order to initiate early treatment.

**Labile Plasma Iron In Health and Disease**

In physiological condition, free iron which appears in plasma is completely scavenged by transferrin however in iron overload situation, greater influx of iron and altered binding kinetics leads to appearance of some portion of non transferrin bound iron even when transferrin is not completely saturated.<sup>[2]</sup> Labile plasma iron (LPI) represents that component of non transferrin bound iron which is capable of freely permeating into organs and inducing tissue iron overload (as demonstrated in Figure1).<sup>[3]</sup>



**Figure 1: Diagram showing entry of LPI into cells and production of reactive oxygen species.**

Various pathological conditions like hemochromatosis, transfusion related iron overload in conditions like thalassemia, myelodysplastic syndromes, diabetes mellitus aplastic anemia, alcoholic liver disease etc have shown to have high labile plasma iron/NTBI.<sup>[4,5,6,7]</sup> Sustained levels of LPI over time can compromise

functions of vital organs (eg heart and liver) and affect patient survival.<sup>[6]</sup> This has led to increasing interest in past few years to measure this component by various assays in different conditions. Till now LPI has mostly been studied in Thalassemia patients on chelation. Table 1 shows the levels of NTBI/LPI in various conditions as reported by Carmine TC et al.<sup>[6]</sup>

**Table 1: Mean concentration of NTBI/LPI in various pathological conditions (Carmine et al).**

Clinical condition	NTBI / LPI	Conc (µmol/L)
Genetic hemochromatosis	NTBI	0.76 +/- 0.50
Hereditary hemochromatosis	LPI	4.0 +/- 16.3
Beta thalassemia major	LPI	1.7 +/- 8.6
Diabetes mellitus	NTBI	0.62 +/- 0.43
Patient undergoing chemotherapy	NTBI	10.6 +/- 6.6
End stage renal disease	NTBI	0.1 +/- 13.5

### Labile Plasma Iron In Thalassemia

Despite steady advances in iron chelation therapy, iron mediated cardiotoxicity remains a leading cause of mortality in these patients.<sup>[8]</sup> Almost all the therapeutic approaches in these cases are directed towards reducing the tissue iron burden to a minimum and the constant endeavour is to detect the storage iron burden at the earliest and by most effective means. The existing marker which is routinely used for the same is serum ferritin levels. However, ferritin measurements, being affected by many confounding factors, is a less sensitive and less desired test.<sup>[9]</sup> Recently, Labile plasma iron assays (LPI) have been found to correlate well with ferritin levels and have been also found to be useful in assessing the short term efficacy of chelation.<sup>[10,11,12]</sup> At present, the research studies of LPI in Thalassemia major have concentrated around the following 3 major aspects;

#### A. LPI as a measure of iron overload in Thalassemia major patients

Tests which are more commonly used to measure iron overload in patients of Thalassemia major include serum ferritin levels, transferrin saturation, Liver biopsy to measure LIC and organ specific MRI.<sup>[9]</sup> CT scan and SQUID have not gained popularity due to their low sensitivity, limited availability and high cost. Hitherto, the methods used to measure iron overload do not differentiate the nature and source of iron that circulates in the plasma. Labile plasma iron assay specifically measures this component of iron load which is responsible for parenchymal iron loading and hence making it an intuitively appealing biomarker This subfraction of NTBI assayed periodically can be used to monitor iron overload.<sup>[13]</sup> This is demonstrated by a decrease in LPI over time in thalassemia intermedia patients on chelation by Cabantchik et al.<sup>[13]</sup> The authors found that although the basal LPI levels decline relatively faster than other iron related parameters, it

correlated linearly with serum ferritin.<sup>[13]</sup> The data regarding the use of LPI to measure iron burden in chronically transfused patients is however limited and needs further studies.

#### B. LPI as a measure of efficacy of chelation therapy

Labile plasma iron due to its high redox activity is rapidly inhibited by exogenously added chelator and is probably the earliest measurable parameter affected by chelator ingress into body fluids; in contrast to generally used indices of body iron stores, such as serum ferritin or transferrin saturation which respond to chelator treatment over a period of weeks to months.<sup>[13,14,15,16]</sup> Thus monitoring daily LPI levels have been shown to offer following advantages in previous studies:

- Rapid assessment of chelation protocol to control this potentially toxic fraction of iron.
- Indexing the duration of chelator action via detection of LPI re-emergence in the course of treatment.
- Possibility of streamlining the customization of chelation dosages, frequency and protocol.

As the kinetics of LPI elimination coincides with the kinetics of chelator intake and clearance, LPI measurements have been found to be useful in determining chelation regimens which could maintain it below a basal level on a diurnal basis and thereby, prevent tissue iron overload.

#### C. LPI as a surrogate markers for early cardiac damage

Cardiac complications are still responsible for 54% of the deaths in patients with Thalassemia major. It is important to diagnose cardiac damage early in the course so that chelation therapy may be intensified in time and can prevent further damage. Cardiac 2 D echocardiography usually detects the abnormalities late when the patient is already symptomatic.<sup>[17]</sup> Serum ferritin is a poor marker for cardiac iron load and does not correlate well with tissue iron load. Since, extrahepatic iron loading, as in cardiac iron, occurs through unregulated transport of non-transferrin bound iron species, labile plasma iron measurements are potentially attractive metrics for predicting cardiac response. T2\* MRI have shown to have high predictive value to detect early iron deposits before functional impairment occurs and remains gold standard till now.<sup>[18]</sup> Piga et al (2009) explored the hypothesis that LPI levels may be associated with relevant clinical outcomes and found that patients with heart disease had significantly higher LPI levels.<sup>[17]</sup> Conversely, they found that none of the patient without high LPI or transferrin saturation below 70% had heart disease or symptom suggesting the possibility of LPI levels as early marker of cardiac damage.<sup>[17]</sup>

Wood et al (2005) studied similar aspects and concluded that though persistent elevations of LPI was found to have negative prognostic value as far as cardiac

outcomes were concerned, declining LPI levels did not guarantee a favourable cardiac outcome in the long run.<sup>[19]</sup> As per their recommendations, LPI measurements at best can be used as short term (few days) surrogates of chelation compliance and cardiac risk. The long term predictive value of cardiac damage by Labile plasma iron remains unproved as of now.

### Measurement of Labile Plasma Iron

Due to the ambiguous nature of NTBI/LPI, a universally accepted gold standard method is not available to measure the same or any subfraction of it. Various researchers have applied a range of analytical approaches to estimate overlapping subfractions of NTBI.<sup>[20]</sup> The various approach to measuring NTBI/LPI are as below;

1. Indirect estimation of NTBI with antitumor antibiotic bleomycin
2. Chelation of NTBI with chelator followed by its separation and estimation using various analytical techniques
3. Measurement of intracellular chelatable iron by fluorescence metalo sensor
4. Direct estimation of NTBI/LPI with iron sensitive fluorescent probe.

Last method is the most common method adopted for the measurement of labile plasma iron fraction which is a fluorescent based technique requiring fluorimeter as detection system.<sup>[10,20]</sup> The advantage of measuring LPI is that it is measured on native plasma/serum and no external iron mobilising agent or filtration step is required. This assay measures the iron specific redox activity in the serum using a reducing agent (ascorbate) and an oxidizing agent (atmospheric O<sub>2</sub>) to generate reactive oxygen species from endogenous oxidants and labile catalytic iron. Reactive oxygen species were detected by an oxidation-sensitive probe DHR (dihydrorhodamine).<sup>[20]</sup> Comparison of the generated fluorescence in the presence and absence of an iron chelator (deferiprone or deferoxamine) makes the assay specific for iron, which can then be measured from a standard curve generated from known iron concentrations.

### Limitations of Labile Plasma Iron Levels

The fluorescence based assay requires a specialised instrument (Fluorometer) for measurement of LPI levels. In the absence of any standard recommendations regarding the methodology to assess the Labile plasma iron and complete absence of quality checks, the current methods are far from perfection.<sup>[21]</sup> The methods described by Esposito et al required patients to withhold chelation on the morning of the assay which is not always easy to achieve in busy clinical practice.<sup>[10]</sup> Also, plasma must be quickly separated and sent to the laboratory at the earliest. Factors that can influence the LPI measurements include antioxidant and iron-binding activities of sera.<sup>[10,21]</sup> The total antioxidant activity of human plasma/serum has been estimated in the range of 1 mM<sup>5</sup> and can be influenced by a variety of factors

including diet and clinical conditions. Therefore, it is possible that sera containing similar concentrations of NTBI might have different levels of LPI, due to masking by antioxidants. Also, hemolysis, lipemia and icterus may affect the final reading by altering serum colour and turbidity.<sup>[20]</sup>

### Labile Plasma Iron Assays – Current Status & Future Possibilities

Blood transfusion is a necessary evil for thalassemia patients. One single test for measuring iron overload and predicting cardiac damage, which is cheap and have high sensitivity is yet to be discovered. Labile plasma iron assay appears potentially attractive candidate due to its ability to measure the potential hazardous fraction of iron directly responsible for tissue iron overload. It can serve as an effective alternative to serum ferritin in cases where ferritin appears to be fallacious (particularly with liver function derangement in such patients). LPI levels may serve to monitor the trend of tissue iron overload during long-term follow up where they may be better than ferritin levels. However, due to lack of standardized testing, recommendations and availability, it is currently limited to research field only. The extent to which labile plasma iron profiles can contribute towards improving the treatment of iron overload remains to be established by long term prospective studies.

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