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Urinary iron excretion for evaluating iron chelation efficacy in children with thalassemia major

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ABSTRACT

Background: In patients with thalassemia major, examination routinely used for the evaluation of iron load in Indonesia is serum ferritin, but it is strongly influenced by other factors such as infections, inflammation and vitamin C levels. Evaluation of urinary iron excretion is an important and easy method to indicate iron chelation efficacy.

Objective: To determine the efficacy of iron chelation therapy by urinary iron examination and to evaluate its correlation with the time of transfusion, serum ferritin level, transferrin saturation and T2* MRI.

Methods: Prospective cohort study was conducted in children with thalassemia major aged 7– < 18 years old who received DFP therapy. Twenty-four-hour urine collections were examined through inductively coupled plasma – mass spectrometry (ICP-MS). Patient's serum ferritin, transferrin saturation, peripheral blood, differential count and T2* MRI was documented during the study. Data analysis is based on urine iron level, body iron balance and the correlation between urine iron level, serum ferritin, transferrin saturation and T2* MRI and dosage of DFP.

Results: Thirty (55%) subjects showed a higher urine iron level on the day prior to transfusion (mean: 12,828 SD ± 12,801 μg/24 h) in comparison to post transfusion (mean: 10,985 SD ± 10,023 μg/24 h). All subjects had positive iron balance (mean 524 SD ± 230 mg). There were positive correlation between urine iron level and transferrin saturation ($r = 0.559$, $p = 0.01$) and serum ferritin ($r = 0.291$, $p = 0.03$), no correlation found with T2* MRI results.

Conclusions: There is a relationship to urinary iron excretion in response to chelation therapy and the degree of iron load.

1. Introduction

Correct and timely assessment of iron chelation efficacy is paramount for the adequate therapy of patients with chronic transfusion-dependent iron overload. MRI measurements of liver and cardiac iron concentration are the gold standards for assessment of tissue iron concentration but they are expensive and of limited availability [1–3]. Assessment of serum ferritin values is easily available but has limitations in providing information on the liver or cardiac iron load in individual cases due to factors such as inflammation, liver disorders, hemolysis, ineffective erythropoiesis and vitamin C level. In addition, changes in its values in transfusion-dependent patients are commonly slow and patients are frequently discouraged with their iron chelation therapy by the lack of significant reductions in values [4–6]. Urinary iron excretion is easily performed and provides useful indication as to whether therapy with chelators that promote significant iron excretion through the kidneys is efficacious in promoting negative iron balance. Most of the studies on urinary iron excretion have been conducted with deferoxamine and limited data are available on the factors that affect iron excretion during deferiprone therapy [7–10]. The current study was conducted to determine the extent of urinary iron excretion during deferiprone therapy and factors that may impact their values in patients with thalassemia major (TM).

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Table 1
Characteristic of subjects.

Characteristic	n = 55
Gender (boys/girls)	29/25
Age, mean (SD, range) years	12.38 (2.84, 7–17)
Transfusion duration, mean (SD, range) years	9.39 (3.5, 2–16)
Duration of DFP administration, mean (SD, range) years	6.69 (3.35, 1–12)
Nutritional status (n)	
Good nutrition	28
Under nutrition	22
Malnutrition	5
Transfusion 6 months prior to study, mean (SD, range) mL/kg	119.8 (25.3, 49.19–209.56)
Serum ferritin, mean (SD, range) ng/mL	3637.15 (1983.67, 2700–9667)
Transferrin saturation, mean (SD, range) %	82.69 (14.27, 38–100)
Hemoglobin pre-transfusion, mean (SD, range) g/dL	8.3 (0.7, 7.2–10.1)
Hemoglobin post-transfusion, mean (SD, range) g/dL	11.4 (0.8, 9.4–13.8)

Table 2
Results of cardiac, liver and pancreas T2* MRI.

Variable	N
Cardiac MRI T2*	n = 55
> 20 ms, n (%)	52 (94.5%)
> 10–20 ms, n (%)	3 (5.5%)
8–10 ms, n (%)	0 (0%)
< 8 ms, n (%)	0 (0%)
Mean (SD, range) ms	32.9 (6.77, 15.78–50.91)
Liver MRI T2*	n = 55
> 6.3 ms, n (%)	4 (7.3%)
> 2.4–6.3 ms, n (%)	23 (41.8%)
1.4–2.4 ms, n (%)	17 (30.9%)
< 1.4 ms, n (%)	11 (20%)
Mean (SD, range) ms	3.19 (3.66, 0.83–24.98)
Pancreas MRI R2*	n = 55
< 30 ms, n (%)	11 (20%)
30–100 ms, n (%)	31 (56.4%)
> 100–400 ms, n (%)	13 (23.6%)
> 400 ms, n (%)	0 (0%)
Mean (SD, range) ms	69.55 (55.63, 16.3–212.3)

2. Methods

Fifty-five TM children at the Thalassemia Center at Dr. Cipto Mangukusumo Hospital, Jakarta, Indonesia were included in the study. All subjects were on a regular blood transfusion regimen, on iron chelation therapy (ICT) with deferiprone (DFP) at ≥75 mg/kg/day for at least 1 year and had serum ferritin levels > 2500 ng/mL on at least two consecutive assessments or transferrin saturation above 60%. Patients were excluded from the study if they have impaired kidney function (as evidenced by glomerular filtration rate < 90 mL/min/1.73 m^2), hepatic impairment (active hepatitis confirmed by serological tests, jaundice, ascites, or increase in AST or ALT > 4 times the normal upper limit).

Urinary iron examination was evaluated using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) with 24-hour urine sample. Serial urinary iron examinations were done on the day prior to transfusion, the transfusion day, the day after transfusion, the seventh day after transfusion and before the next transfusion. Hemoglobin level was examined before and after transfusion. Serum ferritin and transferrin saturation were evaluated using colorimetric enzyme-linked immunoassay. The cardiac, liver and pancreas iron load was determined

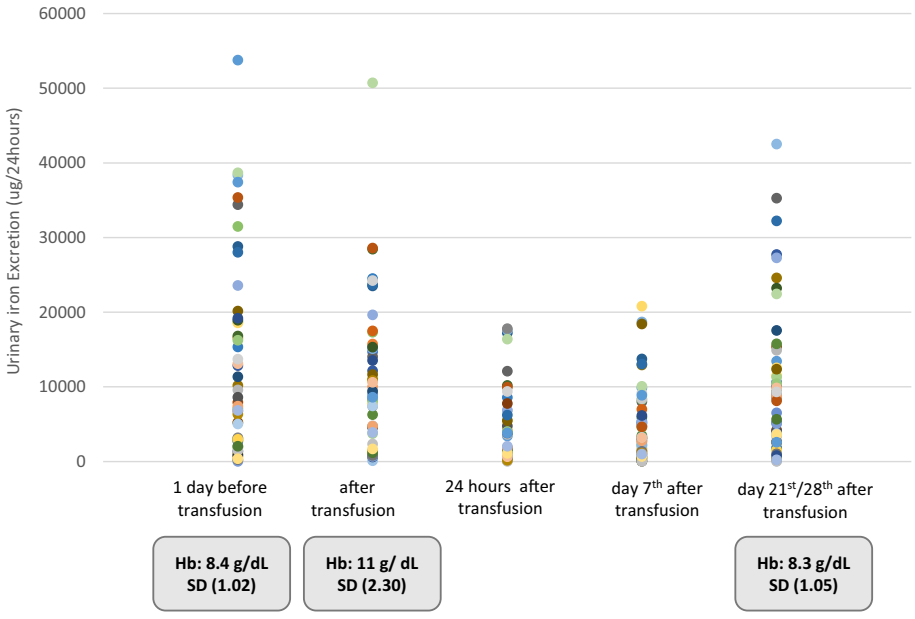

Fig. 1. Urinary iron excretion by the time of transfusion (level of hemoglobin).

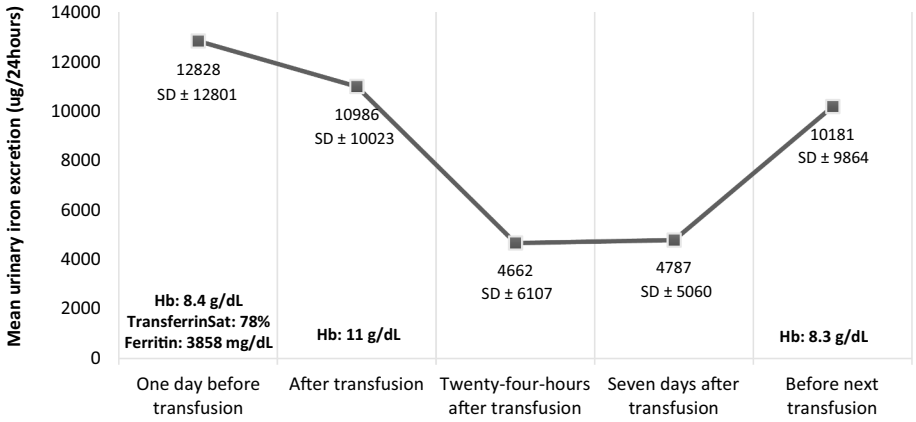

Fig. 2. Mean urinary iron excretion by the time of transfusion (level of hemoglobin).

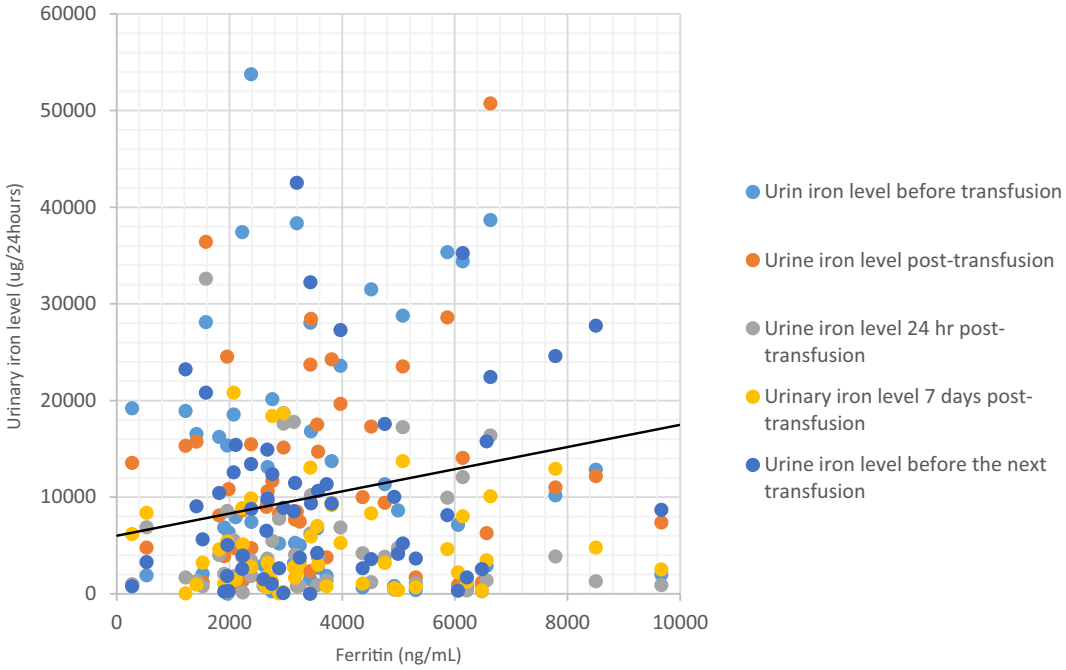

Fig. 3. Correlation between urinary iron excretion and serum ferritin ($r = 0.291$, $p = 0.03$).

by T2* MRI (Siemens® MAGNETOM Avanto 1.5 T, total imaging matrix (TIM) 76 × 18 and analysed using CMRtools™). Serum ferritin and MRI assessments were conducted within 5 ± 3.5 months prior to or after the urinary iron excretion assessments. Ethical approval for this study was granted by the Faculty of Medicine, University of Indonesia prior to the start of the study.

2.1. Statistical analyses

The data analysis was conducted using SPSS version 21.0 for Windows. Normality test were conducted on existing data using Kolmogorov-Smirnov test. Relations of variables were evaluated by unpaired *t*-test, Pearson correlation test and Kruskal-Wallis test. A *p*-value of < 0.05 was considered significant.

3. Results

Demographics of the patients at time of their urinary iron excretion assessments are presented in Table 1. In summary, the patients were young (mean 12 years old), have been receiving blood transfusions for approximately 10 years and ICT with DFP for approximately 7 years. In general, the patients had mild to severe iron load as measured by their serum ferritin, liver and pancreatic T2* MRI values as presented in Table 2. Only 3 patients (5.5%) had mild excessive iron in their heart. Conversion of liver T2* MRI value to LIC was done with formula presented by Wood et al. [30] Statistical test shows a strong and significant correlation between the liver T2* MRI and the converted LIC value ($r = 0.737$, $p < 0.001$). Median for LIC value in all of the samples were 10.79 (3.81–30.8) with no significant correlation between LIC and urinary iron ($r = 0.012$, $p = 0.791$).

There was a significant variation in urinary iron excretion based on the time of its assessment in relation to the time of the blood transfusions; urinary iron excretion was the highest immediately prior to the transfusion and the lowest between 2 and 7 days after transfusions (Figs. 1 and 2).

As presented in Fig. 3, there was a significant but low correlation between urinary iron excretion and serum ferritin ($r = 0.291$, $p = 0.03$) and no significant correlation with T2* MRI results of the liver, heart or pancreas (Fig. 5). The greatest correlation was with transferrin saturation ($r = 0.552$, $p = 0.001$) (Fig. 4).

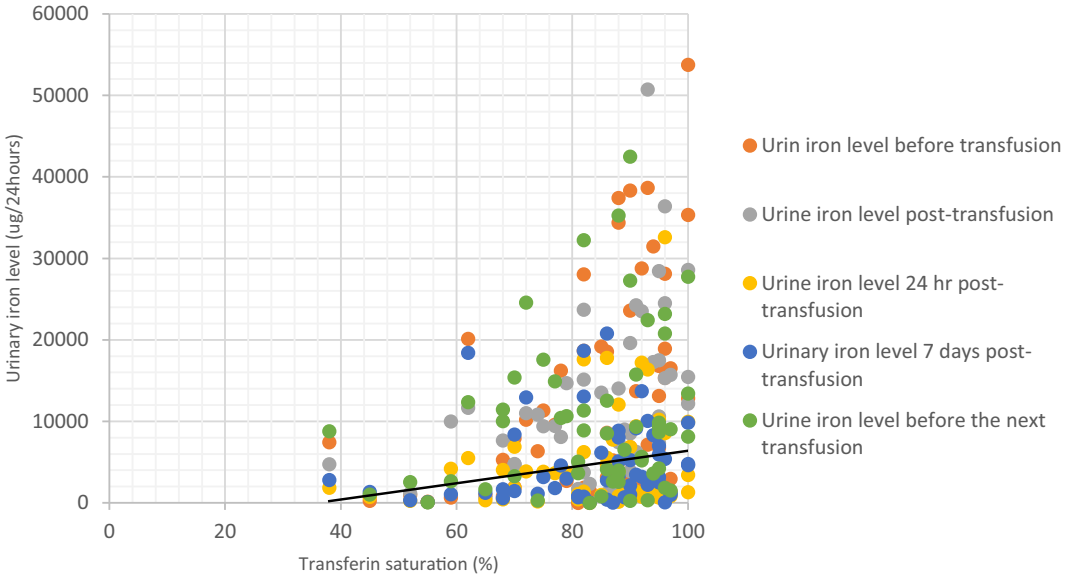

Fig. 4. Correlations between urinary iron excretion and transferrin saturation ($r = 0.552$, $p = 0.001$).

4. Discussion

This study uses urinary iron excretion to determine the efficacy of DFP in removing iron has shown that there is more iron excretion just before transfusion and that if the DFP was only excreted in the urine, all patients would be in positive iron balance. There was no significant correlation with the degree of iron load as determined by other iron overload measurements.

Most of subjects (50.9%) were well nourished, 40% subjects were under-nourished and 9.1% subjects were malnourished even though no subject shows any clinical signs of malnutrition. There was no significant relation between nutritional status and transferrin saturation ($p = 0.053$) but post-hoc test showed significant relation of transferrin saturation between subjects with good nutrition and subjects with malnutrition ($p = 0.034$). This differences could be caused by lower protein levels in patients with malnutrition [11,12].

Mean serum ferritin in this study was 3858 (270–9667) ng/mL. Since most of the patients had a high serum ferritin level, it indicated that poor acceptance of ICT and lack of availability of iron chelators because of cost restraints remain the greatest obstacle to iron control. Serum ferritin levels > 2500 ng/mL has been suggested as a risk factor associated with an increased risk of heart disease, liver cirrhosis, impaired growth and delayed puberty [13,14]. In this study, 30 (55%) subjects were found to experience a reduction in urinary iron excretion immediately after transfusion which then increased before the next transfusion (Figs. 1 and 2). This fits in with the concept that in transfused patients with reduced erythropoiesis and iron load, the daily breakdown of red cells results in 20 mg of iron that cannot be reused. With the case of urinary iron and pancreatic iron overload – higher urinary irons mean there are more iron being excreted thus leaving none deposited on the organs (represented by the facts that there are no heavy iron loads in pancreatic T2* MRI).

As a patient becomes more anemic, the erythroferrone is increased and this suppresses hepcidin production that allows iron release from macrophages and possibly more gastrointestinal iron absorption. This results in more free iron in the circulation, explaining the high iron excretion before transfusion [15,16]. This, in conjunction with the mechanism of ICT, especially DFP which binds free iron and removes it mostly through the urine [17–19] results in higher pretransfusion excretion [27]. This result is similar to the study by Pippard which showed that urinary iron excretion reaches its peak at the lowest hemoglobin levels [7].

There is a correlation between the urinary iron excretion to the serum ferritin ($p = 0.031$, $r = 0.291$) and transferrin saturation ($r = 0.552$, $p = 0.001$) in this study. This condition mirrored other studies and it might be related to the fact that urinary iron excretion can increase when the amount of labile iron in the body increases, represented by an increase in serum ferritin or transferrin saturation [20,21,28]. Our patients generally preferred DFP compared to DFO due to its oral administration. Both its acceptability and better efficacy in removing cardiac iron can explain the reason for the low incidence of excess cardiac iron among our patients [22–24]. Other explanation regarding the lack of cardiac iron in this cohort can also be explained with the low ratio of transfusional iron to bone marrow activity. Patients in this study have low pre-transfusion hemoglobin levels which means that there is significant erythropoiesis and only modest iron burdens from transfusion. Patients in this study are also quite young and the amount of transfusion they received throughout the years may not be sufficient for significant cardiac iron loading [29].

An important aspect that can be considered from these results is the good compliance with the ICT received by the subjects. Where necessary, modifications of either the dose, or the combination they were receiving were made in accordance to that which would best suit the subjects. There was no significant correlation between urinary iron excretion and T2* MRI results of heart, liver or pancreas [25,26]. The limitations of this study are the fact that we did not account for the amount of iron excretion of DFP from feces. This varies between patients and within the same patient. To know really how much is excreted requires full balance studies. These are not feasible because it requires careful diet and collection of urine and feces. The process of 24 hour urine and feces collection also pose numerous challenges due to the countless factors that will affect the amount of iron being excreted. Though numerous studies had also mentioned how unreliable iron excretion measurement from urine and feces is, this method might still show a little glimpse on how iron is being excreted from the body.

5. Conclusions

Urinary iron excretion, on subjects using DFP, had a correlation with time of transfusion (level of hemoglobin), serum ferritin and transferrin saturation. All subjects had positive iron balance, if urinary excretion alone were considered.

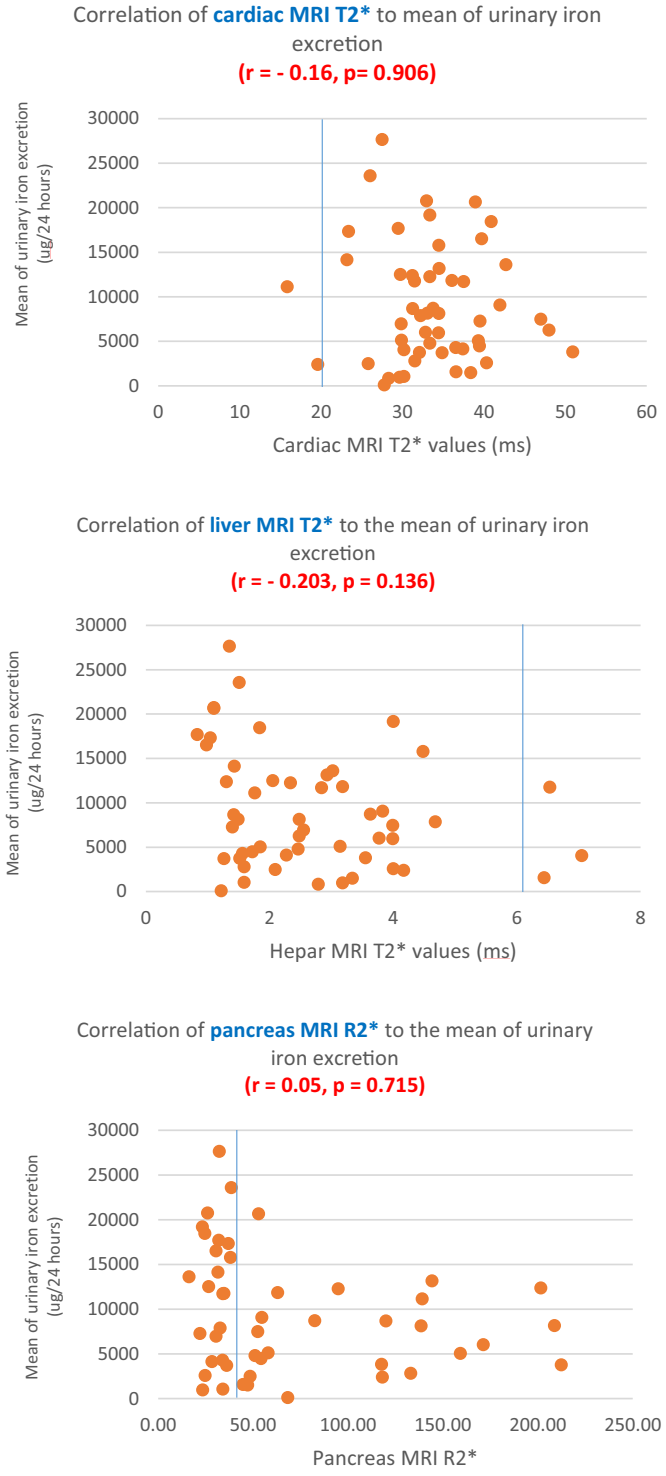

Fig. 5. Correlation between T2* MRI results and urinary iron excretion.

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