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Cardiorenal Syndrome in Thalassemia Patients

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Abstract

Background Cardiorenal syndrome (CRS) is a serious condition with high morbidity and mortality. Secondary CRS (type 5 CRS) is characterized by the coexistence of cardiac abnormality and renal dysfunction occurring secondarily to a systemic condition. There is limited information about CRS in thalassemia. This study aimed to investigate the prevalence of secondary CRS in thalassemia patients and also associated risk factors.

Methods In this cross-sectional cohort study, thalassemia patients who attended the out-patient clinic of University hospital from October 2016 to September 2017 were enrolled onto the study. A criterion for diagnosis of secondary CRS is based on a system proposed by Ronco and McCullough. Cardiac abnormalities are assessed by clinical presentation, establishment of acute or chronic heart failure using definitions from 2016 ESC guidelines or from structural abnormalities shown in an echocardiogram. Renal dysfunction is defined by acute kidney injury or chronic kidney disease according to 2012 KDIGO guidelines. Clinical and laboratory findings from 2 consecutive visits, three months apart, were assessed.

Results Out of 90 thalassemia patients, 25 (27.8%) had secondary CRS. The multivariable analysis showed a significant correlation between CRS and extramedullary hematopoiesis (EMH) (odds ratio (OR) 20.55, p=0.016); thalassemia type [β 0 /β E vs β 0 /β 0 thalassemia (OR = 0.005, p=0.002)]; pulmonary hypertension (OR 178.1, p=0.001); elevated serum NT-proBNP (OR 1.028, p=0.022), and elevated 24-hour urine magnesium (OR 1.913, p=0.016). There was no correlation between CRS and frequency of blood transfusion, serum ferritin, liver iron concentration, cardiac T2*, type of iron chelating agents, and urine neutrophil gelatinase-associated lipocalin level.

Conclusions Secondary CRS is not rare in thalassemia patients. We also identified useful condition and markers for the detection of CRS in thalassemia cases.

Background

Thalassemia, characterized by mutation of a globin gene, is a common cause of congenital chronic hemolytic anemia in Southeast Asia including Thailand [1]. Severity of anemia varies by mutation type and the need of regular transfusion. The latter categorizes patients into transfusion dependent thalassemia (TDT) and non-transfusion dependent thalassemia (NTDT) [2–4]. The major cause of death in thalassemia is heart failure secondary to chronic iron overload, a condition known as iron overload cardiomyopathy. In the past, thalassemia patients with iron overload cardiomyopathy usually died within the 1st or 2nd decade of life [5–8]. However, an improvement of medical care and iron chelation therapy has led to a decrease in cardiac death and improved life expectancy in these patients [7–9]. Nevertheless, such patients are still at high risk of complications associated with chronic hemolytic anemia, especially heart and renal abnormalities.

Heart abnormalities are still mainly caused by chronic iron deposition in myocytes which results in an increase in oxidative stress, inducing myocyte injury, increased myocardial fibrosis and decreased cardiac

contraction [10]. Renal abnormalities in thalassemia manifest themselves through tubular dysfunction, glomerular dysfunction, hyperfiltration and renal stones caused by chronic hemolytic anemia and chelation therapy [11–16]. A coexistence of cardiac and renal abnormalities is known as 'Cardiorenal syndrome (CRS)'. CRS has been defined by Ronco et al.[17] as abnormalities of heart and kidney which can be categorized into five types. Types 1 to 4 CRS, known as primary CRS, are caused by either diseased a heart or kidney. Whilst type 5 CRS or secondary CRS is defined as systemic conditions leading to simultaneous injury and/or dysfunction of both heart and kidney. Thalassemia is classified as a systemic disease so the occurrence of cardiac and renal dysfunction is categorized as secondary, type 5 CRS. There is currently little information concerning secondary CRS in thalassemia. Therefore, this study aimed to determine the prevalence of secondary CRS in thalassemia patients and its associated risk factors.

Methods

Patient population

This was a cross-sectional cohort study. All thalassemia patients, including a TDT and NTDT population, from the hematology out-patient clinic at Chiang Mai University Hospital were enrolled from October 2016 to September 2017. Inclusion criteria were to have the underlying condition of thalassemia and be aged > 18 years. Exclusion criteria were congenital renal or heart disease and patients who had missing or inadequate 24-hour urine samples. Baseline characteristic data were collected. These included age, sex, thalassemia type, transfusion status, current medication and chelation therapy, iron status, previous cardiac T2* magnetic resonance imaging (MRI) and MRI for liver iron concentration (LIC), clinical history of heart failure, echocardiogram within 2 years, and any other complications. Clinical and laboratory assessments were performed on 2 consecutive visits which were at least three months apart. Laboratory tests included serum NT-proBNP, spot urine protein, spot urine albumin, spot urine electrolytes including calcium, 24-hour urine protein, and 24-hour urine electrolytes. This study was approved by the ethical review board of the Faculty of Medicine, Chiang Mai University (STUDY CODE: MED-2559-043461 Research ID: 4346, Approval number 046/2017). All the patients provided written informed consent.

Definition and measurements

At the first visit, urine samples were collected from each patient for spot urine protein, albumin, creatinine, electrolytes and urinalysis. Blood samples were collected to test complete blood count (CBC), blood urea nitrogen (BUN), serum creatinine (SCr), electrolytes, ferritin, liver function test, serum NT-proBNP, lactate dehydrogenase (LDH), thyroid function test (TFT) and serum morning cortisol. All patients had a chest X-ray and an electrocardiogram at the first visit. At the next visit, at least three months later, in addition to all the same lab tests, 24-hour urine samples were collected to measure 24-hour urine protein, creatinine, and electrolytes. We chose at least three month follow-up to allow a certain definition and diagnostic criteria of chronic kidney disease. Estimated glomerular filtration rate (eGFR) was calculated by CKD-EPI formula.

Spot urine samples (3 ml) from both visits of every patient were also stored at -80^{o}C for urine neutrophil gelatinase-associated lipocalin (NGAL) assessment, a protein found in neutrophil granule that is mostly reabsorbed via proximal renal tubular cells. Elevation of NGAL reflected the degree of renal tubular cell damage. When all patients were included in this study, a measurement of urine NGAL levels was performed using a chemiluminescent microparticle immunoassay (ARCHITECT Urine NGAL assay, Longford, Ireland) [18].

Chronic excretion of urine NGAL > 5 ng/ml was defined as urine NGAL levels at the first visit and the three month visit were both higher than 5 ng/ml.

Definition of NTDT is defined by thalassemia disease that does not require regular transfusion for survival. However, the definition of transfusion varies among studies. We used the criteria of less than an average of three transfusions per year for the purpose of the study and less than 7 units of red cell in a year. Definition of TDT is defined by thalassemia disease that require regular transfusion every 4-8 weeks and transfusion free period not more than 8 weeks. Definition of TDT is the number of transfusions in past year greater than 3 units. If the number of transfusions in the past year was from 0-3 transfusion, the patient was classified as NTDT [19].

The diagnostic criteria used to assess heart failure in this study were based on the 2016 European Society of Cardiology (ESC) Guidelines for the diagnosis and treatment of acute and chronic heart failure [19]. These criteria classify patients into heart failure with a preserved ejection fraction (HFpEF), mid-range ejection fraction (HFmrEF) and reduced ejection fraction (HFrEF) as shown in **table 1**. Typical symptoms of heart failure include breathlessness, orthopnea, paroxysmal nocturnal dyspnea, reduced exercise tolerance, fatigue, tiredness, increased time to recover after exercise, and ankle swelling. Typical signs of heart failure include elevated jugular venous pressure, hepatojugular reflux, third heart sound (gallop rhythm), and laterally displaced apical impulse.

Diagnostic criteria for left ventricular hypertrophy was based on QRS voltage criteria i.e. R wave in V5/V6 plus S wave in V1/V2 exceeds 35 mm in height ($SV_{1-2} + RV_{5-6} > 35$ mm). Diagnostic criteria for right ventricular hypertrophy is right axis deviation with tall R-waves in RV leads; deep S-waves in LV leads. Left atrial abnormality is defined as P-wave > 120 msec. and wide notched P-wave > 40 msec. Right atrial abnormality is defined as upright P-wave in lead II > 2.5 mm [20].

Criteria for diagnosis of cardiomegaly from a standard chest X-ray is a cardiothoracic ratio > 0.5 [21].

Diagnostic criteria for acute kidney injury was based on the Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Acute Kidney Injury (AKI) 2012 [22], defined as increase in serum creatinine ≥ 0.3 mg/dl (≥26.5 μmol/l) within 48 hours or an increase serum creatinine ≥ 1.5 times from baseline within 7 days or urine output < 0.5 mg/kg/h for 6 hours.

Diagnostic criteria for chronic kidney disease according to the KDIGO 2012 Clinical Practice Guidelines for the Evaluation and Management of Chronic Kidney Disease [23] are abnormalities of kidney structure

or function, present for >3 months, including

- GFR <60 ml/min/1.73 m^2 or

- Presence of 1 or more markers of kidney damage: albuminuria (Albumin excretion rate (AER) ≥30 mg/24 hours; Albumin to creatinine ratio (ACR) ≥30 mg/g [≥3 mg/mmol]); urine sediment abnormalities; electrolyte and other abnormalities due to tubular disorders; abnormalities detected by histology; structural abnormalities, kidney stones detected by imaging; history of kidney transplantation.

According to Thalassemia International Federation guideline, we defined hemochromatosis by using

cardiac T2* technique and magnetic resonance imaging of liver to determine LIC. Hemochromatosis was diagnose when cardiac T2* less than 20 millisecond or LIC more than 7 mg.Fe/g dry weight. [3, 4]

In this study, a diagnosis of secondary cardiorenal syndrome (type 5 cardiorenal syndrome) [17] was established with the presence of both following criteria:

1. Heart failure and/or cardiac abnormalities which can be acute or chronic; heart failure diagnosed in line with the criteria listed above; cardiac abnormalities including LV remodeling and dysfunction, diastolic dysfunction; chronic abnormalities in cardiac function, and cardiomyopathy.
2. Kidney abnormalities including acute kidney injury and chronic kidney disease using criteria described above.

The sufficiency of 24-hour urine collection was checked using urinary creatinine (Cr), sex and body weight and the following equations:

Men = (urinary creatinine, mg/d) / (24 x body weight)

Women = (urinary creatinine, mg/d) / (21 x body weight)

If a ratio of Cr to body weight (BW) of <10.8 or >25.2 and a total urine volume <1000 mL/d with a urinary creatinine level <5 mmol/d, the urine collection was deemed incomplete.

Statistical analysis

Statistical analysis was performed using SPSS version 23.0. Calculation of sample size was performed by estimating a single proportion model. Previously published data on prevalence of renal and cardiac abnormalities was used to calculate sample size [24-26]. Estimated sample size from this was 90. Pearson Chi-square or Fisher's exact test was performed to calculate the correlation of CRS with other sets of categorized data. Normality was checked using Kolmogorov–Smirnov and Shapiro-Wilk methods. The statistical significance of differences for continuous data sets was calculated using the Student's t-test. A Mann–Whitney U-test was applied when appropriate. Multivariable analysis was performed by binary logistic-regression model. A p value of < 0.05 was considered significant.

Results

Ninety thalassemia patients were enrolled onto this study, 75 (83.3%) classed as transfusion dependent thalassemia and 15 (16.7%) as non-transfusion dependent thalassemia. Twenty-five patients (27.8%) had coexisting cardiac and renal abnormalities or secondary CRS. Cardiac abnormalities were detected in 35 patients (38.9%), all had structural cardiac abnormalities and 8 had clinical heart failure. Renal abnormalities were detected in 52 patients (57.8%), 51 of these had only chronic proteinuria and 1 had chronic proteinuria with eGFR below 60 mL/min. **(Table 2)** Eighty-eight patients (98.7%) had completeness of 24-hour urine collection.

The univariate analysis indicated that CRS was common in β^0/β^0 thalassemia when compared with other types of thalassemia (p=0.04) **(Table 3)**. The detail of thalassemia type was provided in **Table 4**. Thalassemia with secondary CRS also showed a significant correlation with pulmonary hypertension (PHT) (p<0.001), hemochromatosis (p=0.047) and presence of extramedullary hematopoiesis (EMH) (p=0.02). In addition, patients with CRS had a significantly higher level of serum NT-proBNP, 24-hour urine protein and 24-hour urine magnesium than those without CRS.

Patients with chronic excretion of urine NGAL at a level higher than 5 ng/ml showed a significant correlation with the occurrence of CRS in univariate analysis (OR 2.82, p=0.038) **(Table 5**), but not with multivariable analysis **(Table 6)**. Chronic excretion of urine NGAL > 5 ng/ml also showed a significant correlation with combined deferoxamine and deferiprone treatment (OR 3.69, p=0.011), female gender (OR 5.15, p<0.001), hemochromatosis (OR 3.01, p =0.012), elevated serum LDH (OR 2.81, p=0.018) and chronic proteinuria (OR 2.63, p=0.025). The 24-hour urine protein level was significantly higher in patients with chronic excretion of urine NGAL > 5ng/ml than those without (p=0.035).

A binary logistic-regression model was performed for the multivariable analysis and confirmed an independent correlation of CRS with the type of thalassemia [β^0/β^E VS. β^0/β^0 thalassemia (OR = 0.005, p=0.002)], presence of EMH (OR 20.549, p=0.016), presence of PHT (OR 25.455, p=0.016), elevated serum NT-proBNP (OR 1.028, p=0.022) and elevated 24-hour urine magnesium (OR 1.913, p=0.016) as shown in **Table 7**. There was no correlation of CRS with sex, age, splenectomy, transfusion status, mean hemoglobin level, mean serum ferritin, liver iron concentration, serum LDH, thyroid function, serum cortisol, hypogonadism, all echocardiogram parameters, cardiac T2* and type of iron chelation.

Discussion

Cardiac complications are common in thalassemia. The two most common manifestations are biventricular dilated cardiomyopathy and arrhythmia [7, 24, 25, 27]. In TDT patients, the prevalence of cardiac abnormalities has been reported as follows: iron overload 44%; LV dysfunction 8–19%; increased cardiac output/index 60%; abnormal ECG 46% (T wave abnormalities 34% and right bundle-branch block 12%); history of acute myocarditis 4.5% and heart failure 2.5-4% [3, 24, 28]. Elevated serum levels of NT-proBNP (> 125 ng/mL), compared with the normal population, have also been demonstrated in TDT

patients, a condition which is known to show a correlation with diastolic dysfunction [29–32]. In NTDT, Aessopos et al.[25] reported cardiac involvement including: congestive heart failure 5.4%; history of acute pericarditis 8.1%; pericardial thickening 34.5%; leaflet thickening 48%; endocardial calcification 20.9%; left-sided valve regurgitation (aortic 15.4%, mitral 47.2%), and pulmonary hypertension (peak systolic tricuspid gradient > 30 mmHg) 59.1%. In addition all patients had a high cardiac output and normal LV contractility.

Renal abnormalities in thalassemia manifest as tubular dysfunction and glomerular hyperfiltration. One mechanism associated with tubular dysfunction in thalassemia is due mainly to renal tubular cell hypoxia caused by chronic anemia that contributes to tubular cell death and fibrosis. Another contributory factor is the occurrence of iron deposition in glomeruli, proximal tubules and interstitium which results in glomerulosclerosis, tubular atrophy, and interstitial fibrosis [33]. Evidence of tubular dysfunction includes hypercalciuria (12.9–22%), proteinuria (8.6–89%), phosphaturia (9.2%), magnesiumuria (8.6%), hyperuricosuria (38-82.4%) and microalbuminuria (29%) [26, 34, 35].

Secondary CRS has been mainly studied in the setting of sepsis, a unique condition of secondary CRS. Other important systemic conditions associated with secondary CRS are systemic amyloidosis and systemic lupus erythematosus (SLE). Although many mediators and signaling pathways have been found in association with secondary CRS, the exact mechanisms involved in secondary CRS are still unclear and vary with specific systemic conditions. In cases of sepsis, CRS is associated with decreased cardiac contractility resulting from cardiac ischemia and injury, and AKI caused by renal ischemia, inflammation and endothelial injury. In systemic amyloidosis, cardiac involvement is characterized by amyloid fibril deposition that can lead to congestive heart failure and cardiac conduction abnormalities. Renal amyloid deposition frequently occurs with heavy proteinuria. SLE is a systemic condition in which immune complex and complement deposition play a major role in pathogenesis of tissue injury, commonly involving the kidney. If this process involves both the kidney and heart, patients finally develop secondary CRS [36].

In thalassemia, a highly prevalent condition in countries of Southeast Asia including Thailand, there is restricted data regarding coexisting renal and cardiac abnormalities or CRS. This study has demonstrated that the prevalence of secondary CRS in Thalassemia was 27.8%. To the best of our knowledge, our study was the first study that has demonstrated the prevalence of secondary CRS in thalassemia. The occurrence of CRS showed a correlation with the type of thalassemia (β^0/β^0 thalassemia), EMH, PHT, increased 24-hours urine magnesium and elevated serum NT-proBNP. However, the cut points for BNP and NT-proBNP in our study were below standards for those with CKD and heart failure in previous studies [37, 38]. A correlation between secondary CRS and EMH/ PHT may be explained by the presence of chronic severe anemia with inadequate transfusion that resulted in cardiac and renal hypoxia and dysfunction manifested by elevated serum NT-proBNP levels and magnesiumuria. Chronic anemia-associated systemic hypoxia generally enhances cardiac compensation by initially increasing cardiac output which ultimately leads to pathologic cardiac remodeling such as chamber enlargement if the anemia not corrected. Chronic activation of the renin-angiotensin-aldosterone system (RAAS) may be a

major key to treating secondary CRS in thalassemia patient since cardiac and renal hypoxia generally stimulates RAAS. It is noteworthy that persistent RAAS activation can induce myocardial fibrosis, renal tubular damage, efferent arteriole constriction causing glomerular hypertension, proteinuria and renal fibrosis, thereby leading to cardiorenal syndrome [39].

The degree of tissue hypoxia may explain why CRS showed a correlation with β^0/β^0 thalassemia. β^0/β^0 thalassemia is caused by total deletion of the beta globin gene resulting in a severe misbalance of the alpha and beta globin chains and severe anemia. β^0/β^E thalassemia sufferers however, have less severe anemia than β^0/β^0 thalassemia because some beta globin chains, despite them being a defective form, are still produced. Although hemoglobin E is an abnormal form of hemoglobin and made of abnormal globin chains, it can carry and deliver oxygen to tissues.

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDA, a low molecular weight protein, majorly found in neutrophil granules and renal tubular cells. NGAL is involved in iron transportation, iron binding and renal cell repair. It is freely filtrated through the glomeruli then mostly reabsorbed in the proximal renal tubules. NGAL can be elevated in inflammatory conditions and renal diseases such as: autosomal dominant polycystic kidney disease; immunoglobulin A nephropathy; HIV nephropathy; contrast-induced nephropathy; urinary tract infections and renal tubular injury. Elevation of urine NGAL is associated with decreased estimated GFR, urinary albumin excretion, increased serum NT-proBNP levels and may be potentially used for early detection of AKI [40, 41]. However, several studies have shown inconclusive results as regards urine NGAL as a predictor of CKD progression [42–47].

Clinical studies, in cardiac surgery settings, after contrast infusion, critical illness, and traumatic patients, have demonstrated an association between NGAL and early detection of AKI. Both urine and serum NGAL were elevated preceding elevation of serum creatinine in AKI patients. Elevation of urine or serum NGAL has also been observed in CRS especially in cases of type 1 CRS and may have been beneficial in the diagnosis of type 5 CRS [17, 48–52].

In literature review, the cutoff level point for the level of urine NGAL for AKI or CKD progression varies from 10 to 500 ng/ml depending on patient population and conditions but to date there is no generalized standard cutoff for urine NGAL [18, 40, 41, 48, 53]. In our study, the univariate analysis indicated that a chronic urine NGAL level higher than 5 ng/ml showed a correlation with female gender, combined deferoxamine (DFO) and deferiprone (DFP) treatment, hemochromatosis, elevated serum LDH, chronic proteinuria, and increased 24-hour urine protein. Proteinuria in thalassemia may be contributed by glomerular or tubular injury, but evidence from our study supports tubular injury as urine NGAL was a tubular damage marker and was not elevated in cases of glomerular injury [54, 55]. The association between hemochromatosis and urine NGAL excretion may be due to iron toxicity that produces free radicals to injure renal tubular cells. Combined use of iron chelators, DFO and DFP, results in a greater severity of iron overload and renal tubular injury. This may explain its correlation with chronic urine NGAL excretion. Moreover, both DFO and DFP may be directly toxic to renal tubular cells as they synergistically enhance iron excretion via the kidney. This therapy also results in increased iron accumulation in renal

tubular cells that causes tubular cell damage [13, 56]. In this study we found that threshold of > 5 ng/mL was associated with CRS. The threshold of urine NGAL in our study was quite low compared to other study [18, 57]. But as previously mentioned, the cut point of urine NGAL can be varies depending on patient population. But all studies were in agreement that NGAL could be a useful marker for predicting tubular damage and AKI [58].

A limitation of this study is that it is a cross-sectional study with only a 3 month follow-up. Prospective long term follow-up is more likely to detect more detailed dynamics of disease and other complications, in particular those related to renal abnormalities and changes in eGFR. Moreover, a prospective long term study may be useful to define the timing of cardiac and renal abnormalities and more specific types of CRS, especially CRS type 2 and 4. In this study, data collection of renal abnormalities was based on laboratory criteria while renal symptoms may have not developed yet.

Apart from conventional renal markers such as serum creatinine and eGFR, which are not sensitive enough for early detection of renal injury, only a single novel kidney injury biomarker, urine NGAL, was used in the present study. Using a panel of biomarkers may give a higher level of sensitivity for the detection of renal abnormalities. One interesting alternative marker to provide an early measure of renal damage is 'renal functional reserve' as some thalassemia patients tend to have glomerular hyperfiltration that may reflect a functional reserve response to stress. Chronic stress to the kidney could result in low renal functional reserve despite a normal eGFR or serum creatinine level [58]. However, the cut-off of urine NGAL in this study quite low compared to levels described in previous studies. These may have caused false positive readings of elevated urine NGAL. Renal ultrasound was not routinely done in all patients unless there is a clinical indication/suspicion, this may miss structural diseases of the kidneys and urinary tract as well as kidney stone. There were also two important confounding factors regarding study into CRS in thalassemic patients. First, iron chelation therapy especially deferasirox can cause renal failure and proteinuria. However, this factor may not effect to our study since we found only one patient who had an eGFR below 60 ml/min/1.73 m^2. Second, heart failure symptoms can mimic anemic symptoms, but to prevent this happening we used physical examination to give us a clear focus when looking for signs of heart failure help to us to diagnose and heart failure with accuracy. We also recorded symptoms after blood transfusion, which should be improved if they had anemic symptoms.

Conclusions

Secondary CRS is not rare in thalassemia patients, especially in cases of β^0/β^0 thalassemia. This condition shows a correlation with the presence of EMH and PHT. Elevated serum NT-proBNP and magnesiumuria may also be useful markers for the detection of CRS in thalassemia cases.

Abbreviations

CRS: cardiorenal syndrome; EMH: extramedullary hematopoiesis; OR: odds ratio; TDT: transfusion dependent thalassemia; NTDT: non-transfusion dependent thalassemia; MRI: magnetic resonance

imaging; LIC: liver iron concentration; CBC: complete blood count; BUN: blood urea nitrogen; SCr: serum creatinine; LDH: lactate dehydrogenase; TFT: thyroid function test; eGFR: estimated glomerular filtration rate; NGAL: neutrophil gelatinase-associated lipocalin; HFpEF: heart failure with a preserved ejection fraction; HFmrEF: heart failure with a mid-range ejection fraction; HFrEF: heart failure with a reduced ejection fraction; KDIGO: Kidney Disease: Improving Global Outcomes; AKI: Acute Kidney Injury; AER: albumin excretion rate; ACR: albumin to creatinine ratio; Cr: creatinine; BW: body weight; PHT: pulmonary hypertension; SLE: systemic lupus erythematosus; RAAS: renin-angiotensin-aldosterone system

Declarations

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Authors' Contributions

S.M. designed the research, collected, summarized, analyzed clinical data and wrote the paper; A.T. designed the research, obtained researched grant, collect data, analyzed data, wrote the paper, gave critical comment and corresponding author; S.L. designed the research, wrote the paper, approved the final version to be published and revised it critically for important intellectual content; A.P., K.F, .T.R., E.R., C.C., L.N., P.C. wrote, approved the final version to be published, gave critical comment. "All authors read and approved the final manuscript."

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author, A.T., adisak.tan@cmu.ac.th upon reasonable request. The data are not publicly available due to privacy or ethical restrictions

Ethics approval and consent to participate

Subjects (or their parents or guardians) have given their written informed consent.

This study was approved by the ethical review board of the Faculty of Medicine, Chiang Mai University (STUDY CODE: MED-2559-043461 Research ID: 4346, Approval number 046/2017).

Consent for publication

Not applicable

Disclosure Statement

The authors have no conflicts of interest to declare.

Competing interests

The authors declare that they have no competing interests

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Appendix

Table legends

Table 1 Criteria for diagnosis of heart failure (required all 3 criteria)

Table 2 Cardiac and renal abnormalities

Table 3 Baseline characteristics

Table 4 Thalassemia type

Table 5 Chronic excretion of urinary NGAL > 5 ng/ml

Table 6 Multivariable analysis of chronic urine NGAL > 5ng/ml

Table 7 Multivariable analysis correlation with Cardiorenal syndrome

*No figure in this manuscript

Tables

Table 1 Criteria for diagnosis of heart failure (required all 3 criteria)

Criteria	HFrEF	HFmrEF	HFpEF
1	Symptoms +/- signs*	Symptoms +/- signs*	Symptoms +/- signs*
2	LVEF < 40%	LVEF 40-49%	LVEF ≥ 50%
3	-	1. Elevated natriuretic peptides** 2. One of the following 2.1 relevant structural heart disease (LVH and/or LAE) 2.2 diastolic dysfunction	

* Signs may be not found in early heart failure especially HFpEF, and in patients with previous diuretic use.

**Cutoff, BNP > 35 pg/ml, NT-proBNP > 125 pg/ml

Table 2 – Cardiac and renal abnormalities

	CRS (N=25)	No CRS (N=65)
Cardiac abnormalities	25	10
Structural cardiac abnormalities n(%)	25 (100)	10 (15.4)
Heart failure n(%)	8 (32.0)	0
Renal abnormalities	25	27
Chronic proteinuria n(%)	24 (96.0)	27 (41.5)
eGFR < 60 n(%)	1 (4.0)	1 (1.5)

Table 3 – Baseline characteristics

	Total (N=90)	CRS (N=25)	No CRS (N=65)	OR	*P*-value
Median age yrs (range)	29 (16-58)	30 (21-57)	28 (16-58)		0.256
Sex				1.51	0.748
Male	31 (34.4%)	4 (7.5%)	12 (22.6%)		
Female	59 (65.6%)	12 (22.6%)	25 (47.2%)		
Mean arterial pressure mmHg (±S.D.)	76.3 (±8.8)	74.2 (±6.6)	77.1 (±9.5)		0.262
Thalassemia type					
β^0/β^E thalassemia	57 (63.3%)	11 (12.2%)	46 (51.1%)	0.325	0.018
β^0/β^0 thalassemia	26 (28.9%)	12 (13.3%)	14 (15.6%)	3.36	0.013
Hemoglobin H disease	7 (7.8%)	2 (2.2%)	5 (5.6%)	1.043	0.961
Splenectomy				2.71	0.055
Yes	54 (60.0%)	19 (21.1%)	35 (38.9%)		
No	36 (40.0%)	6 (6.7%)	30 (33.3%)		
Type of iron chelation					
Deferoxamine (DFO)	41 (45.6%)	12/25 (48.0%)	29/65 (44.6%)	1.15	0.773
Deferiprone (DFP)	65 (72.2%)	18/25 (72.0%)	47/65 (72.3%)	0.99	0.977
Deferasirox (DFX)	13 (14.4%)	2/25 (8.0%)	11/65 (16.9%)	0.43	0.281
Combined DFO + DFP	25 (27.8%)	7/25 (28.0%)	18/65 (27.7%)	1.01	0.977
Combined DFP + DFX	4 (4.4%)	0 (0.0%)	4/65 (6.2%)	-	0.573
Pre-transfusion Hemoglobin (Mean(±S.D.), gm/dL)		7.18 (±1.14)	7.28 (±1.01)		0.70
Ferritin (Mean(±S.D.), ng/mL)		1577.6 (±226.6)	1877.0 (±172.8)		0.30
Transfusion dependent				2.88	0.219
Yes	75 (83.3%)	23 (25.6%)	52 (57.8%)		
No	15 (16.7%)	2 (2.2%)	13 (14.4%)		
Presence of EMH				4.59	0.02
Yes	31 (34.4%)	15 (16.7%)	16 (17.8%)		
No	59 (65.6%)	10 (11.1%)	49 (54.4%)		
Presence of pulmonary hypertension				25.50	<0.001
Yes	17 (19.3%)	14 (15.9%)	3 (3.4%)		
No	71 (80.7%)	11 (12.5%)	60 (68.2%)		
Hemochromatosis				2.60	0.047
Yes	42 (47.2%)	16 (18.0%)	26 (29.2%)		
No	47 (52.8%)	9 (10.1%)	38 (42.7%)		
Electrocardiogram					0.138
Normal sinus rhythm	62 (69.7%)	15 (16.9%)	47 (52.8%)		
Abnormal rhythm	7 (7.9%)	1 (1.1%)	6 (6.7%)		
ST segment change	20 (22.5%)	9 (10.1%)	11 (12.4%)		
Echocardiogram					
Left ventricular ejection fraction [LVEF, Mean(±S.D.), %]		64.8 (±8.17)	65.0 (±4.67)		0.949
Right ventricular systolic pressure [RVSP, Mean(±S.D)., mmHg]					
Mean pulmonary arterial pressure [mPAP, Mean(±S.D.), mmHg]		67.1 (±35.68)	49 (±2.82)		0.509
Tricuspid velocity [TV, Mean(±S.D.), m/s]					
E/A ratio of tricuspid valve [Mean(±S.D.)]					
E/E' ratio of tricuspid valve [Mean(±S.D.)]		50.1 (±42.2)	23.0 (±5.0)		0.324

		329.7 (±95.7)	256.7 (±48.6)		0.090
		1.21 (±0.31)	1.55 (±0.54)		0.139
		11.9 (±2.2)	11.7 (±3.0)		0.899
Hypothyroidism	26/90 (28.9%)	7/25 (28.0%)	19/65 (29.2%)	0.94	0.908
Diabetes mellitus	5 (5.6%)	2/23 (8.0%)	3/62 (4.6%)	1.80	0.615
Adrenal insufficiency	7 (7.8%)	4/21 (16.0%)	3/62 (4.6%)	3.94	0.09
Serum NT-proBNP [Median(IQR), ng/ml]	137.01 (78.3 - 195.8)	141.6 (65.3-217.9)	99.47 (37.5-161.4)		0.028
Mean serum Creatinine (±S.D., mg/dl)	0.57 (±0.17)	0.55 (±0.2)	0.58 (±0.15)		0.37
Different in serum creatinine at 0 and 3 months (Mean±S.D., mg/dl)	0.0689 (±0.055)	0.0688 (±0.057)	0.069 (±0.055)		1.00
24 hours urine [Median(IQR)]					
Protein (mg/24hr.)	193.0	273.0	175.0		<0.001
Potassium (mmol/24hr.)	(103.0-183.0)	(186.2-359.8)	(102.8-247.2)		0.214
Phosphorus (mg/24hr.)	26.9	30.3	25.6		0.931
Magnesium (mEq/24hr.)	(17.8-36.0)	(19.0-41.6)	(17.4-33.9)		0.008
Calcium (mg/24hr.)	471.7	449.9	486.4		0.247
	(309.4-634.1)	(213.3-686.5)	(332.5-640.5)		
	4.1	5.48	3.85		
	(2.8-5.4)	(3.9-7.0)	(2.7-5.0)		
	58.9	50.8	72.85		
	(13.1-104.7)	(1.8-99.8)	(26.6-119.1)		
Mean urinary NGAL [Median(IQR), ng/ml]	9.55	11.67	9.0		0.143
	(3.8-15.3)	(3.5-19.9)	(4.4-13.6)		
Chronic Urinary NGAL > 5ng/ml				2.82	0.038
Yes	49 (54.4%)	18 (20%)	31 (34.4%)		
No	41 (45.6%)	7 (7.8%)	34 (37.8%)		
Mean liver iron concentration (±S.D.,mg/g)	13.78 (±5.89)	13.26 (±6.24)	14.02 (±5.80)		0.70
Mean cardiac T2 star (±S.D, millisecond)	37.20 (±12.23)	36.76 (±16.37)	37.42 (±9.98)		0.96

Table 4 Thalassemia type

	TDT	NTDT	P value
Thalassemia type			
β^0/β^E thalassemia	48 (53.3%)	9 (10%)	
β^0/β^0 thalassemia	25 (27.8%)	1 (1.1%)	<0.001
Hemoglobin H disease	2 (2.2%)	5 (5.6%)	
Sex			
Male	26 (28.9%)	5 (5.6%)	0.921
Female	49 (54.4%)	10 (11.1%)	
Splenectomy			
Yes	50 (55.6%)	4 (4.4%)	0.004
No	25 (27.8%)	11 (12.2%)	
Hemochromatosis			
Yes	39 (43.8%)	3 (3.4%)	0.021
No	35 (39.3%)	12 (13.5%)	
Cardiorenal syndrome			0.171
Yes	23 (25.6%)	2 (2.2%)	
No	52 (57.8%)	13 (14.4%)	
Mean ferritin [Mean(±S.D.),ng/ml]	1,785 (±1,302)	1,771 (±1,417)	0.701
Pre-transfusion Hemoglobin (Mean(±S.D.), gm/dL)	7.15 (±0.96)	7.76 (±1.34)	0.039

TDT -transfusion dependent thalassemia, NTDT – non-transfusion dependent thalassemia

Table 5– Chronic excretion of urinary NGAL > 5 ng/ml

	Chronic excretion of urinary NGAL > 5 ng/ml			
	Yes (N = 49)	No (N =41)	OR	*P*- value
Sex			5.15	<0.001
Male	9 (18.4%)	22 (53.7%)		
Female	40 (81.6%)	19 (46.3%)		
Thalassemia type				0.207
β^0/β^E thalassemia	30 (33.3%)	27 (30.0%)		
β^0/β^0 thalassemia	15 (16.7%)	11 (12.2%)		
Hemoglobin H disease	4 (4.4%)	3 (7.3%)		
Splenectomy	29 (59.2%)	25 (61.0%)	0.92	0.055
Type of iron chelation				
Deferoxamine (DFO)	25 (51.0%)	16 (39.0%)	1.62	0.255
Deferiprone (DFP)	39 (79.6%)	26 (63.4%)	2.25	0.088
Deferasirox (DFX)	6 (12.2%)	7 (17.1%)	0.67	0.516
Combined DFO + DFP	19 (38.8%)	6 (14.6%)	3.69	0.011
Combined DFP + DFX	2 (4.1%)	2 (4.9%)	0.83	0.855
Presence of EMH	21 (42.9%)	10 (24.4%)	2.32	0.066
Presence of pulmonary hypertension	12 (24.5%)	5 (12.8%)	2.20	0.168
Presence of LVH	11 (22.4%)	10 (25.0%)	0.86	0.778
Presence of heart failure	7 (14.3%)	1 (2.4%)	6.67	0.067
Hemochromatosis	29 (59.2%)	13 (32.5%)	3.01	0.012
Elevated serum LDH	33 (68.8%)	18 (43.9%)	2.81	0.018
Elevated serum NT-proBNP	21 (42.9%)	18 (43.9%)	0.96	0.921
24 hours urine [Median(IQR)]				
Protein (mg/24hr.)	221.0 (143.4-298.6)	157.0 (80.3-233.8)		0.035
Sodium (mmol/24hr.)	126.0 (76.6-175.4)	127.0 (80.4-173.6)		0.471
Magnesium (mEq/24hr.)	4.9 (3.1-6.7)	3.8 (2.5-5.0)		0.094
Chronic Proteinuria	33 (67.3%)	18 (43.9%)	2.63	0.025
Cardiorenal syndrome	18 (36.7%)	7 (17.1%)	2.82	0.038
Mean liver iron concentration (±S.D.,mg/g)	13.98 (±5.65)	13.4 (±6.34)		0.793
Mean cardiac T2 star (±S.D, millisecond)	38.0 (±14.1)	36.0 (±9.19)		0.591
Echocardiogram				
Left ventricular ejection fraction (LVEF, Mean±S.D., %)	66.2 (±6.4)	62.1 (±8.4)		0.198
Right ventricular systolic pressure (RVSP, Mean±S.D., mmHg)				
Mean pulmonary arterial pressure (mPAP, Mean±S.D., mmHg)				
Tricuspid velocity (TV, Mean±S.D., m/s)	56.0 (±17.7)	99.0 (±72.2)		0.093
E/A ratio of tricuspid valve (Mean±S.D.)				
E/E’ ratio of tricuspid valve (Mean±S.D.)				
	53.0 (±39.6)	18.2 (±0.4)		0.192
	308.9 (± 64.5)	316.5 (±135.5)		0.853
	1.21 (±0.34)	1.45 (±0.44)		0.240
	12.7 (±2.38)	10.05 (±0.92)		0.087

Table 6 - Multivariable analysis of chronic urine NGAL > 5ng/ml

Correlation with chronic urine NGAL > 5ng/ml	Odds Ratio (OR)	*P*-value
Female	8.05	0.03
Splenectomy	0.55	0.35
EMH	3.64	0.07
Combined DFO+DFP	2.96	0.11
Hemochromatosis	2.01	0.22
24 hr. urine protein	1.00	0.18
24 hr. urine magnesium	1.38	0.06
Cardiorenal syndrome	2.57	0.34
Presence of heart failure	0.42	0.34
Elevated serum LDH	1.80	0.32

Table 7 - Multivariable analysis correlation with Cardiorenal syndrome

Correlation with Cardiorenal syndrome	Odds Ratio (OR)	*P*-value
Thalassemia type		
β^0/β^E VS. β^0/β^0 thalassemia	0.005	0.002
β^0/β^E thalassemia VS. hemoglobin H disease	0.003	0.714
Splenectomy	8.287	0.099
Extramedullary hematopoiesis	20.549	0.016
Hemochromatosis	3.078	0.252
Pulmonary hypertension	178.1	0.001
Increase serum NT-proBNP	1.028	0.022
24 hour urine protein	0.995	0.142
24 hour urine magnesium	1.913	0.016