Int J Clin Exp Med 2010;3(2):122-128
www.ijcem.com /IJCEM1001006

Original Article

Deferiprone challenge test for monitoring iron overload in hepatitis positive thalassemic major patients

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Received January 20, 2010, accepted April 15, 2010, available online April 30, 2010

Abstract: Viral hepatitis is common among β-thalassemia major (BTM) children in Pakistan. Transfusional iron overload in BTM is usually monitored by serum ferritin. But its levels are falsely raised in viral hepatitis and do not reflect the true iron body burden in thalassemic patients. The objective of the study was to develop a test for monitoring iron overload in 'Hepatitis B&C' positive BTM patients by urinary iron excretion (UIE) after oral deferiprone chelation as compared to serum ferritin. We recruited 130 BTM patients from the registry of Thalassaemia centre at Rawalpindi, Pakistan. The patients were grouped into Hepatitis positive and Hepatitis negative based on ELISA test. Serum ferritin levels were analyzed by kit on Access II. Each patient was given 75mg/kg of deferiprone at morning. Baseline UIE before deferiprone, and 4, 8 12 hours (hrs) UIE after deferiprone were analyzed on Selectra E. One hundred and thirty BTM patients aged 3 to 23 years comprising of Hepatitis positive (n=69) and Hepatitis negative (n=61) participated in the study. Hepatitis positive thalassemic patients had significantly high serum ferritin median (IQ) 4349 (2782-5927) μg/L than 3338 (2189-5506) μg/L in the Hepatitis negative (p=0.001). We did not find any significant change in UIE at 4, 8, and 12 hours between two groups after Deferiprone intake (p=NS). We observed significant positive correlation between serum ferritin and 4 hours UIE in Hepatitis negative patients (r=0.57; p=0.01) but not in the Hepatitis positive patients (r=0.16; p=NS). Deferiprone challenge with measurement of 4 hours UIE is cost effective and non-invasive test rather than serum ferritin for monitoring iron overload in Hepatitis' positive BTM patients.

Keywords: Thalassemia major, iron overload, hepatitis, serum ferritin, deferiprone challenge test, monitoring of iron overload, deferiprone challenge test

Introduction

The inherited disorder β-thalassemia major (BTM) is one of the common single gene defects in South Asia, Far East and West Africa [1]. Conventional treatment of ß-thalassemia is regular blood transfusions to keep the hemoglobin levels close to normal. Besides providing longevity to thalassemic patients, multiple transfusions without proper screening may introduce the Hepatitis B Virus and Hepatitis C Virus into the body of recipient [2]. Even by adopting proper screening procedures of blood transfusion, the incidence of HCV infection is still reported to be high (35%) in Pakistani BTM patients [3]. Additionally, multiple blood transfusions produce progressive tissue iron loading and toxicity [4].

Measurement of liver iron concentrations (LIC) in biopsy specimen is the gold standard for assessment of the total iron burden in thalassemic patients but its invasiveness has restricted its acceptability to patients [5]. According to the latest International practice guidelines, measurement of serum ferritin is still the first analyte of choice for monitoring iron overload in thalassemic patients in the absence of confounding factors [6-7]. Ferritin is an acute phase reactant protein and its levels are falsely raised in viral hepatitis [8]. Hence, not reflecting the true picture of iron overload in Hepatitis positive thalassemic patients. Although, Super conducting Quantum Interference Device (SQUID) and Magnetic Resonance Imaging (MRI) are new non-invasive methods of measuring

iron storage in the body, but the cost, and the complexity of these instruments have limited their use in the developing countries such as Pakistan [9-10].

Thus, it is critical to develop a cost effective and a non-invasive method for monitoring iron overload in Hepatitis positive BTM patients. Deferiprone (3-hydroxy-1,2-dimethylpyridin-4(1*H*)-one) is an orally active iron chelator, remained in clinical use for the last fifteen years [11]. The researchers assessed the effectiveness of Deferiprone by giving dosages of 50-100 mg/kg and observed urinary iron excretion (UIE) at a rate of 21-42 mg/kg/day in BTM patients [12]. However, UIE after deferiprone dose has not been used for monitoring iron overload in thalassemic patients.

In the scenario of the high prevalence of post-transfusional hepatitis and reduced availability of latest equipments for monitoring iron overload in Hepatitis positive patients, we proposed a novel deferiprone challenge test for assessment of total iron burden in these patients. The objective of our study was to establish a biochemical test for monitoring iron overload in 'Hepatitis positive' BTM patients by measuring UIE after oral deferiprone chelation in comparison with serum ferritin.

Materials and methods

We performed a quasi –experimental study on BTM patients at Army Medical College in collaboration with Thalassemia Center, Rawalpindi from January 2008 to June 2009. The study protocol was approved by institutional review and ethical committees of Army Medical College, Rawalpindi, National University of Sciences & Technology Pakistan.

A total of 154 BTM patients were randomly selected from the registry of Thalassemia Center, Rawalpindi, Pakistan. Eighteen patients showed unwillingness to participate and three were excluded due to chronic illness. Thus, 130 subjects with mean age 10 (ranged 3 – 23) years of either sex were enrolled after informed consent. Medical history, age of diagnosis, initiation and duration of blood transfusion, as well as chelation therapy were recorded. Diagnosis of BTM was re-confirmed with complete blood count and electrophoresis. Patients suffering from acute or chronic inflammatory conditions and thalassemia intermedia were excluded. Anthropometric data were collected.

Methodology

Five ml pre-transfused fasting blood samples were collected into plane tubes between 7:00-9:00 AM after an overnight fast. The serum was separated by centrifuging at 2000 g and stored at -80 ^{0}C until assayed. Baseline urinary sample was collected one hour after drawing blood samples on the same day. Patients were weighed. Deferiprone at a dose of 75mg/kg was administered orally. Then 4, 8 and 12 hours post dose urinary samples were collected in plain containers. Urine samples were measured for volume and stored at -20 ^{0}C till the time of analysis.

Biochemical analysis

Biochemical analysis was carried out in the Pathology Laboratotries of Army Medical College, Rawalpindi, Pakistan. HBsAg and AntiHCV serology was done by using Linear kit (Chemical,Barcelona, Spain) on ELISA Microplate Reader (Dia 710, UK). Serum ferritin was measured by Chemiluminescence based immunoassay Reagent Pack on ACCESS II (Beckman Coulter, USA). Urinary iron was estimated by Ferrozine colorimetric method described by Artiss et al [13] on a Selectra E(Vita Lab Netherland). Serum creatinine was analyzed by Jaffe's reaction [14] on a Selectra E. Coefficient of variation of assays was less than 5%.

Statistical analysis

The data were analyzed by using SPSS software version-17 (SPSS Inc, Chicago). Descriptive statistics including median, inter-quartile range (IQ), frequency and percentages were calculated. Mann Whitney-U test was applied for comparison of different analysis between two groups of the BTM patients. The cut off value of 4hr urinary iron excretion, sensitivity and specificity were estimated by Receiver operating characteristic curve (ROC). The spearman's correlation between serum ferritin level and urinary iron was determined. A p-value <0.05 was considered significant.

Results

Out of 154 BTM patients, 130 participated in the study. Baseline characteristics of the pa-

Table 1. Baseline characteristics and comparison of urinary iron excretion between hepatitis positive and hepatitis negative BTM patients

Parameters	Hepatitis Positive Median (IQ) n=61	Hepatitis Negative Median (IQ) n=69	p-value
Age(years)	10 (6-14)	9 (6-12)	0.39
Height(cm)	127 (109-137)	125 (118-134)	0.61
Weight(kg)	23 (20-30)	22 (20-28)	0.89
Hemoglobin(µg/dL)	8.4(6.4-9.6)	8.4(6.8-10)	0.58
Ferritin(µg/L)	4349 (2782-5927)	3338 (2189-5506)	0.01
Baseline UIE (µmol/L)	4.6 (2.85-6.6)	5.8 (3.2-8)	0.08
UIE of 1- 4hrs (µmol/L)	342 (231-508)	413 (280-602)	0.06
UIE of 4-8hrs((µmol/L)	169(113-297)	204(95-367)	0.27
UIE of 8-12hrs((µmol/L)	82(50-214)	77(52-120)	0.43
U ICR at 4hr (umol/mmol)	78 (49-139)	86 (49-160)	0.67
UICR at 8hr (umol/mmol)	42(18-98)	38(27-73)	0.74
UICR at 12hr (umol/mmol)	27(11-38)	22(12-34)	0.17

UIE =Urinary iron excretion, UICR = Iron creatinine ratio,

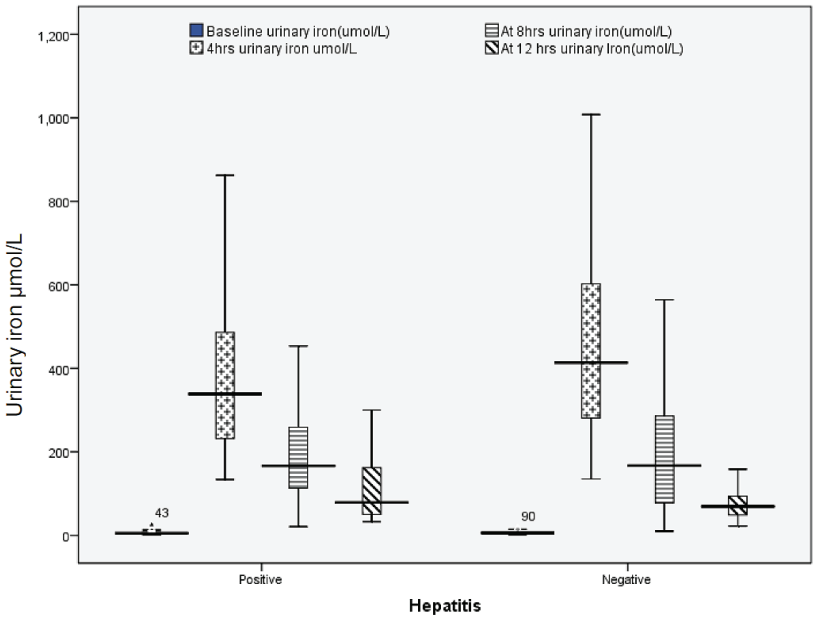

Figure 1. Comparison of baseline, 4, 8 and 12 hours urinary iron excretion between hepatitis positive and hepatitis negative BTM children (p=NS).

tients are shown in **Table 1**. They had age range 3 to 23 years and comprised of 73 (56%) males and 57 (44%) females. Sixty-nine BTM patients had negative serology and 61 (47%) patients were positive for Hepatitis C or B. Hepatitis positive patients had significantly high serum ferritin levels median (IQ) 4349 (2782-5927) µg/L as compared to the hepatitis negative patients

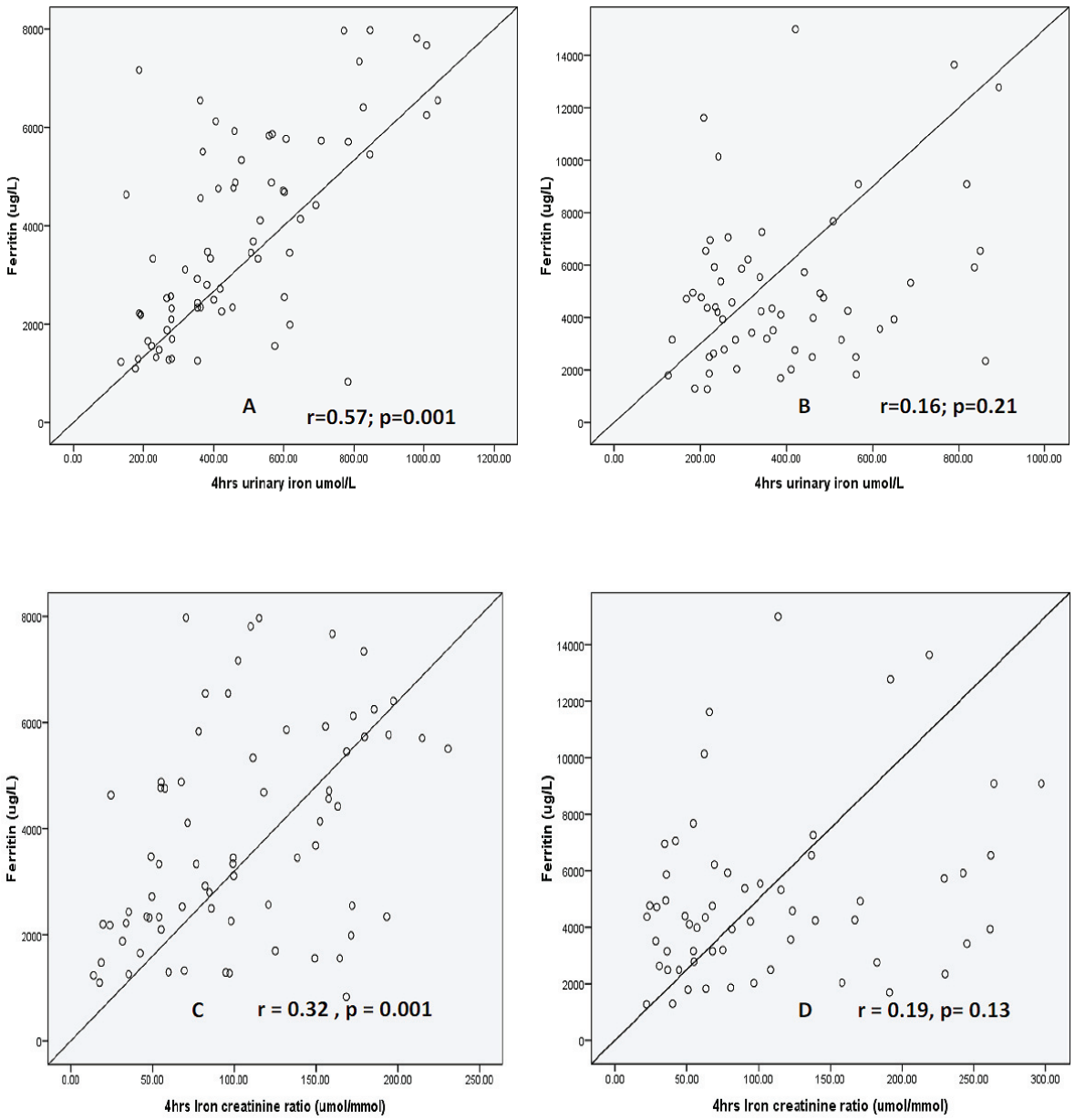

Figure 2. Spearman's correlation between serum ferritin and 4hours urinary iron excretion/4hours urinary iron creatinine ratio in hepatitis negative (A&C) and hepatitis positive (B&D) BTM patients.

3338 (2189-5506) µg/L. We did not find any significant change in baseline UIE before deferiprone intake as well as 4, 8 and 12 hours after deferiprone intake between two groups (**Figure 1**). Peak UIE was observed at 4 hours after deferiprone in Hepatitis positive group 342 (231-508) µmol/L and 413(280-602) µmol/L in Hepatitis negative group (p <0.06).

Hepatitis negative patients had shown a significant correlation between serum ferritin and UIE at 4, 8 and 12 hours after deferiprone challenge (p=< 0.01) but no correlation was observed in Hepatitis positive patients (**Table 2**). Moreover, the best correlation was observed between serum ferritin and urinary iron (r=0.57; p<0.001) at 4 hours (**Figure 2A**). Similarly a positive correlation between serum ferritin and urinary iron creatinine ratio (UICR) was observed in hepatitis negative group at 4 hours (**Figure**

Table 2. Spearman correlation between serum ferritin, urinary iron excretion and iron creatinine ratio in Hepatitis positive and Hepatitis negative groups of BTM patients

Parameters	Hepatitis Positive n= 61		Hepatitis Negative n=69	
	r- value	p- value	r- value	p- value
Baseline UIE (µmol/L)	0.19	0.14	0.14	0.23
UIE of 1- 4hrs (µmol/L)	0.16	0.21	0.57*	0.001
UIE of 4-8hrs (µmol/L)	0.16	0.20	0.43*	0.001
UIE of 8-12 hrs (µmol/L)	0.21	0.11	0.38*	0.001
4hr urinary ICR ratio (µmol/mmol)	0.19	0.13	0.32*	0.001
8hr urinary ICR µmol/mmol)	0.21	0.10	0.20	0.08
12hr urinary ICR(µmol/mmol)	0.05	0.68	0.14	0.24

UIE =Urinary iron excretion, UICR= Urinary Iron creatinine ratio, BTM = Beta thalassaemia major.

2C) but not in hepatitis positive group.

Receiver operating characteristics curve (ROC) was made at 4 hours UIE and recommended levels of serum ferritin < 2500µg/L for the BTM patients. The Area under curve (AUC) for 4 hours urinary iron is shown in **Figure 3**. The cutoff value of 4 hours urinary iron was found at 290µmol/L with 72% sensitivity and 60% specificity for hepatitis positive patients. The patients tolerated the deferiprone well and no side effects were observed except nausea and vomiting in two patients.

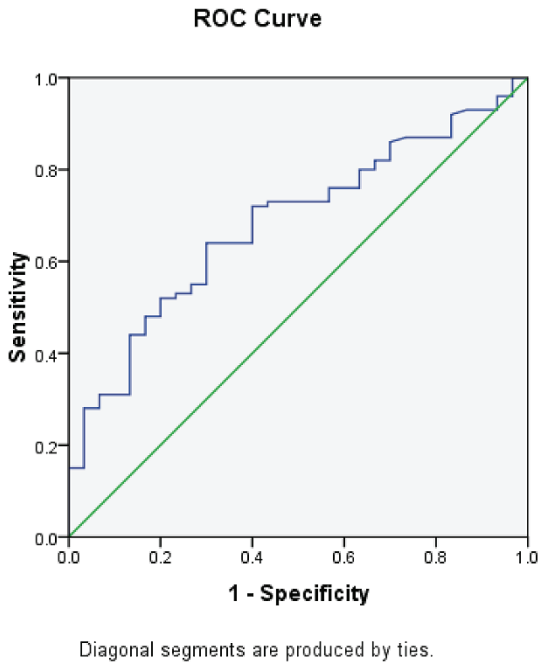

Figure 3. The ROC curve for 4 hours urinary iron excretion and recommended serum ferritin levels for assessment of iron overload in the hepatitis positive BTM patients.

Discussion

High prevalence of viral hepatitis (47%) among Pakistani thalassemic patients is attributable to the use of improperly screened blood supply. Among transfusion related infections, viral hepatitis is at the top [2]. Rehman & Lodhi also reported high prevalence of viral hepatitis (37%) in Pakistani thalassemic patients [15]. Ferritin is an iron storage protein and used for monitoring of iron overload in response to chelation therapy. We observed significantly high serum ferritin levels in Hepatitis positive thalassemic patients as compared to Hepatitis negative patients. This rise could be due to acute phase protein reaction in response to hepatic inflammation [16]. Our study has established that in contrast to serum ferritin, urinary iron excretion after deferiprone challenge test was not significantly different in either group (**Figure 1**). This finding signifies that urinary iron levels remain unaffected in viral hepatitis hence, favoring our hypothesis of using urinary iron as unbiased biochemical marker for monitoring iron overload in Hepatitis positive thalassemic patients. Deferiprone has been used as an iron chelator since 1992 [17]. Studies have shown that considerable amount of iron is excreted after com-

parable doses of deferiprone [18]. By using a dose of 75mg/kg we also observed maximum urinary iron excretion in our thalassemic patients. Our study results are in close concordance with results published by Jirasomprasert et al. [19] for exhibiting maximum UIE at 4 hours. They demonstrated that all of deferiprone together with iron was rapidly excreted in urine within 12 hours with a peak excretion between 4-8 hours. Unlike Jirasomprasert et al, we could not measure Deferiprone levels in our patients. Our study results have shown that the 4 hours urinary iron excretion levels provide the best correlation with serum ferritin levels in Hepatitis negative patients in comparison with 8 hours and 12 hours. These correlations with serum ferritin in Hepatitis negative patients indicate that we can use either serum ferritin or 4 hours urinary iron excretion or spot UICR in negative patients but alternatively, only 4 hours urinary iron or spot UICR after deferiprone can be used as a reliable biochemical marker for monitoring iron overload in Hepatitis positive patients. We also determined a cutoff value of 4hr urinary iron with reference to serum ferritin in Hepatitis positive patients. International recommendation for serum ferritin regarding prediction of complications in thalassemia major is > 2500µg/L [20]. The cutoff value of 4 hours urinary iron can be considered as a predictor of high risk Hepatitis positive thalassemic major patients. According to our study, the patients possessing 4 hours urinary iron levels less than 290µmol/L are comparatively at low risk of acquiring complications. This value possesses moderate sensitivity with respect to serum ferritin and may overcome the challenge of using serum ferritin in the presence of confounding factors like viral hepatitis. Although, different non-invasive methods (SQUID and MRI T2*) have been introduced in the developed countries for regular monitoring of iron overload but their cost and complexity have limited their use [7]. Monitoring of iron overload through urinary iron is an alternative of serum ferritin as a non-invasive and economical biomarker. Our study is giving comparative efficacy of urinary iron after deferiprone with serum ferritin in Hepatitis positive patients. But diagnostic sensitivity and specificity is still to establish by comparing it with gold standard (Live biopsy). We did not correlate urinary iron levels with established reference gold standard because of unwillingness of patients for liver biopsy. The study is applicable to all thalassemic patients irrespective of age and sex.

Conclusion

Deferiprone challenge with measurement of 4 hours UIE is a cost effective and non-invasive test rather than serum ferritin for monitoring iron overload in Hepatitis B&C' positive BTM patients in the developing countries where SQUID and MRI T2*are not accessible.

Acknowledgement

This study was a part of the Project No 20-837/ R&D/07/319 entitled, "Quantitative Evaluation of Iron Overload among Pakistan β-Thalassemic Major Children" supported by Higher Education Commission (HEC) Pakistan.

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