

Drug Metabolizing Enzyme CYP1A2 Status in Pediatric Patients with Hemoglobin E- β Thalassemia

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Objective: To evaluate the drug metabolizing enzyme CYP1A2 activity in pediatric hemoglobin E-beta-thalassemia patients, since CYP1A2 is responsible for the metabolism of a number of drugs. Alteration of its activity may have clinical consequences.

Material and Method: Twenty-three hemoglobin E-beta thalassemia pediatric patients and 24 age-matched controls were recruited in the present study. Caffeine in the form of a soft drink was orally administered as a test probe for CYP1A2 activity. Plasma collected at pre-dose and 6 hr after intake was analyzed for the levels of caffeine and paraxanthine, its major metabolite to represent the activity of CYP1A2.

Results: Biochemical markers, including blood glutathione and urinary lipid hydroperoxides indicated that patients were in an oxidant stress state. The plasma ratio of paraxanthine to caffeine was unchanged between patients and controls. Multiple regression analysis revealed that gender and the liver enzyme were the significant determinants of CYP1A2 activity (adjusted $r^2 = 0.48$, $p < 0.001$). Male gender was associated with higher activity of CYP1A2 activity.

Conclusion: The CYP1A2 activity is not apparently changed in thalassemia patients despite the presence of an oxidative stress state.

Keywords: Thalassemia, Oxidative stress, CYP1A2, Cytochrome p450

J Med Assoc Thai 2009; 92 (12): 1675-80

Full text. e-Journal: <http://www.mat.or.th/journal>

The thalassemias are a class of genetic disease of abnormal globin chain synthesis and remain a significant health problem in several regions of the world. Clinical manifestations are resulted from the decreased or absent production of normal globin chains of hemoglobin⁽¹⁾. Main subtypes are alpha and beta thalassemias. When beta thalassemia is inherited together with hemoglobin E (HbE), the globally very common hemoglobin variant, results in compound heterozygous condition; HbE-beta thalassemia (HbE- β -thal). HbE- β -thal is widespread throughout South Asia and South-east Asia⁽²⁾. Clinical symptoms may vary from a mild form to severe transfusion-dependent anemia but its natural history remains poorly defined⁽²⁾.

Oxidative stress is a condition that over production of oxidant species or reduction of endogenous antioxidant defense leads to potential damage⁽³⁾. Iron overload and increased oxidative status are important characteristics in thalassemia, which lead to oxidative tissue damage and organ dysfunction^(3,4). Hypogonadism, diabetes, cardiomyopathy, and liver dysfunction are among some of the oxidative stress implicated^(5,6). Cardiovascular changes including endothelial dysfunction, arterial stiffness, and hyperdynamic are very common even in uncomplicated patients⁽⁷⁻⁹⁾. Activity of CYP1A2, CYP2E2, and CYP3A4 are depressed in some liver diseases such as cirrhosis⁽¹⁰⁾. Inflammation, infections, and increased oxidant species have been shown to depress the activity of CYP1A enzymes⁽¹¹⁾. Therefore, it is interesting to evaluate whether thalassemia patients who are loaded with oxidative stress have depressed CYP1A activity, because CYP1A2 is responsible for

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the metabolism of a number of drugs including theophylline, caffeine, clozapine, imipramine, fluvoxamine, acetaminophen, and various aromatic amines in food and environmental contamination. Changes of its activity may have clinical implications. The present study was carried out in children, since they may not be confounded by overt liver pathology such as liver fibrosis, a complication usually found in adulthood⁽⁶⁾. For assessment of activity of CYP enzymes, caffeine was used as *in vivo* probes for assessing the activities of CYP1A2, because of its wide acceptance for safety and specificity^(12,13).

Material and Method

Subjects

Twenty-four healthy control subjects (10 males and 14 females), with an average age of 11.0 ± 1.3 (mean \pm standard deviation, SD) years were enrolled in the present study. They were considered healthy upon physical examination by blood chemistry for the liver, kidney function tests, hematological laboratory tests, and hemoglobin typing. Twenty-three transfusion-dependent HbE- β thal pediatric patients (14 males and 9 females), with an average age of 12.2 ± 2.7 years were recruited from the Department of Pediatrics, Srinagarind Hospital, Faculty of Medicine, Khon Kaen University. All patients had been previously characterized for beta-globin gene mutations. The patients received leukocyte-poor packed red cells approximately once a month if the pre-transfusion hemoglobin levels were less than 9 g/dl and all received deferoxamine as iron chelation therapy. Current medications of thalassemia subjects were reviewed and none of them was known to affect the CYP1A2. Of the twenty-three patients, eight were splenectomized and 15 were non-splenectomized (intact spleen). None of the controls and patients had a history of sensitivity to caffeine. The present study protocol was approved by the Ethics Review Committee of Khon Kaen University. Informed consent was obtained from the parents of all participants.

Study protocol

The subjects were asked to abstain from food or drinks containing caffeine for at least 3 days before the day of the present study. All subjects fasted overnight and the present study was conducted the next morning. Blood samples (5 ml) and urine samples were collected from each subject before they were given a soft drink (Coca-Cola®) containing caffeine at the dose approximately 2 mg/kg body weight, and other blood samples were obtained 6 hours later. The blood

samples were kept on ice and immediately centrifuged to obtain plasma. All samples were kept frozen at -20°C until assays.

Assays of oxidative stress and antioxidants

Assay of glutathione (GSH) was performed essentially according to a previously described method⁽¹⁴⁾. Urinary hydroperoxides were estimated by ferrous ion oxidation xylene orange (FOX)⁽¹⁵⁾ using H_2O_2 as standard. The lipid hydroperoxides were estimated from incubating samples with catalase before adding FOX reagent. Plasma malondialdehyde (MDA) was assessed as thiobarbituric acid reactive products.

Assay of caffeine and metabolites

Caffeine and its major metabolite, paraxanthine, in plasma were determined by high performance liquid chromatography (HPLC) according to the authors' previously described method⁽¹²⁾. Briefly, 300 μl of plasma samples were precipitated with 400 mg of ammonium sulfate, then the mixture was extracted with dichloromethane: isopropanol (85:15). The organic phase was dried and reconstituted with 300 μl of a mobile phase consisting of 10 mM ammonium acetate buffer, methanol and tetrahydrofuran (90:8.5:1.5) and injected into HPLC column (Luna 5 μm C_{18} column, 150 x 4.60 mm; Phenomenex, CA, USA). Column eluent was monitored by a UV spectrophotometer at 276 nm.

Statistical analysis

All results were expressed as mean \pm standard deviation (SD). Plasma ratio of paraxanthine to caffeine or caffeine metabolic ratio (CMR) was examined for normal distribution by the Shapiro-Wilk test and described as mean and 95% confidence interval (95% CI). Comparisons between the two groups were performed with Student's t-test or rank-sum test as appropriate. The relationship between metabolic ratios and other parameters were analyzed by Pearson's correlation. Stepwise regression was used to identify significant determinants for predicting CMR. Comparisons among subgroups in controls and thalassemia subjects were performed by analysis of variance with Student-Newman-Keuls method. P-value of less than 0.05 was considered statistically significant.

Results

Hemoglobin was decreased but serum ferritin and transaminase enzymes were increased in

Table 1. Characteristics data of control and hemoglobin E β -thalassemia subjects. Blood samples from the patients were obtained at the pre-transfusion period

	Controls (n = 24)	Thalassemia subjects (n = 23)	p-value
Body mass index (kg/h ²)	23.7 \pm 4.8	22.0 \pm 4.8	NS
Hemoglobin (g/dl)	12.7 \pm 0.7	8.0 \pm 1.0	<0.001
Serum ferritin (ng/ml)	56.0 \pm 24.0	2,230.0 \pm 1,496.0	<0.001
Total bilirubin (mg/dl)	0.4 \pm 0.1	2.6 \pm 0.8	<0.001
Uric acid (mg/dl)	6.3 \pm 1.1	5.4 \pm 1.3	NS
Creatinine (mg/dl)	0.63 \pm 0.1	0.51 \pm 0.10	NS
AST (U/l)	36.7 \pm 6.6	57.0 \pm 31.6	0.01
ALT (U/l)	16.7 \pm 4.3	35.4 \pm 20.8	0.01
Blood GSH (μ M)	575.0 \pm 21.0	448.0 \pm 20.4	<0.001
Plasma GSH (μ M)	1.70 \pm 0.67	1.86 \pm 0.39	NS
Urinary lipid hydroperoxide (μ M)	2.1 \pm 1.4	3.9 \pm 1.4	0.01
Plasma malondialdehyde (μ M)	3.3 \pm 0.5	3.8 \pm 1.1	0.04

the thalassemia group ($p < 0.05$) when compared to controls (Table 1). However, these parameters including ferritin and liver function test were not significantly different between splenectomized and non-splenectomized subjects. It was noted that the liver enzymes, aspartate amino transferase (AST) in HbE- β -thal patients were varied with a range from 22-159U/l.

For oxidant parameters, there was a significant decrease in whole blood GSH in HbE- β -thal patients compared with controls, whereas there were a comparable levels of plasma GSH (Table 1). Levels of urinary lipid hydroperoxides and plasma MDA were significantly increased in thalassemia subjects.

The ratio of paraxanthine to caffeine was used as an index of CYP1A2 activity⁽¹³⁾. Caffeine ratios between control and thalassemia groups were not significantly different: (mean: 95% confidence limit) 0.76: 0.66-0.85 and 0.75: 0.63-0.87, respectively. Within the thalassemia group, CMR in splenectomized patients was also comparable to the spleen-intact subjects, (mean: 95% confidence interval 0.75: 0.59-0.91 and 0.75: 0.54-0.97), respectively. On the other hand, CMR in male patients were higher than female patients (mean: 95% confidence interval 0.86: 0.68-1.04) and 0.59: 0.53-0.65), respectively, $p < 0.05$ (Fig. 1). Similarly, CMR in healthy male subjects were higher than the female. To identify significant determinants for CYP1A2 activity, the relevant variables were included in an analysis: *i.e.* gender, body mass index, hemoglobin level, serum ferritin, plasma and blood glutathione, splenectomy status, urinary lipid hydroperoxides and plasma MDA. The only significant determinants were

gender ($\beta = -0.47$, $p < 0.01$) and AST ($\beta = -0.006$, $p < 0.01$) with adjusted $r^2 = 0.48$ ($p < 0.001$). The partial regression plots of caffeine metabolic ratio and gender or AST are shown in Fig. 2A and 2B. It is implied that male subjects are associated with a higher activity of CYP1A2 than female subjects, whereas, increased liver enzyme is associated with the depressed CYP1A2 activity.

Discussion

The antioxidant and oxidant status in HbE- β -thal patients in the present study is consistent with

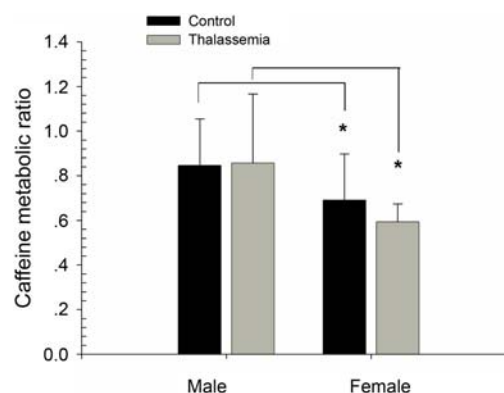


Fig. 1 Caffeine metabolic ratio of control and HbE- β thalassemia patients stratified by gender status. Bars represent mean \pm SD n, control: male and female subjects; 10 and 14, respectively and thalassemia subjects: 14 and 9, respectively

* Significant difference between two indicated groups, $p < 0.05$

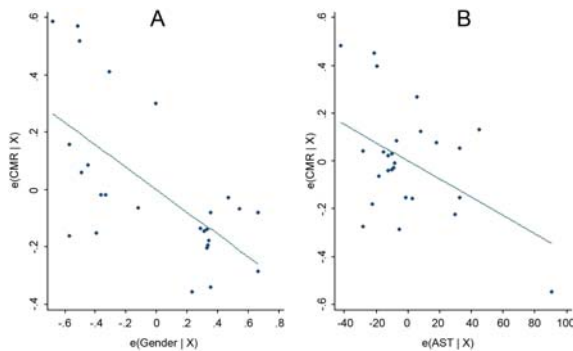


Fig. 2 Partial regression plots of caffeine metabolic ratio and gender or AST, adjusted by AST or, gender respectively, in HbE- β thalassemia patients. The plots are between the partial residual of CMR “e (CMR | x)” and partial residue of gender “e (Gender | x)” (A) and partial residual of CMR and partial residue of AST: “e (AST | x)” (B)

many previous studies^(4,9) *i.e.* patients were in the oxidative stress status as evidenced by the decrease in GSH and increases in levels of lipid hydroperoxides and MDA. Caffeine metabolic ratio, which has been shown to reliably represent the CYP1A2 activity *in vivo*⁽¹³⁾, was used to evaluate the presented patients. Although thalassemia major is afflicted with chronic oxidative stress and systemic inflammation⁽¹⁶⁾, CYP1A2 activity in these patients remained unchanged when compared with age-matched healthy subjects. There was no correlation between caffeine metabolic ratio and parameters of oxidants or hematological measurements.

The present study is in contrast with previous studies in that increased oxidative stress, inflammation, and reactive oxygen species were associated with the decreased activity and expression of CYP1A and CYP3A^(17,18). In particular, inflammatory cytokines suppressed the expression and activity of various CYP enzymes and are ultimately associated with oxidative stress^(19,20). Moreover, the authors' recent study reported that CYP2E1 and CYP3A4 activities by using chlorzoxazone and cortisol as metabolic probes, respectively were increased in pediatric thalassemia patients⁽⁴⁾. In addition, clearance and peak blood levels of paracetamol have been reportedly increased in adult thalassemia patients⁽²¹⁾, whereas paracetamol is primarily conjugated by hepatic UDP-glucuronosyl-transferases-1A1⁽²²⁾. The increased or unaltered CYP activities or clearances of the probe drugs which are metabolized by different

drug metabolizing enzymes suggest that thalassemia patients in the present study may be under good medical condition, and not imposed with systemic inflammation. Moreover, the increased hepatic clearance of the probe drugs may probably resulted from an increased hepatic blood flow primarily due to hyperdynamic circulation response to anemia⁽²³⁾. An unchanged caffeine metabolic ratio may be due to both chlorzoxazone and paracetamol being relatively high hepatic extraction drugs whereas caffeine is a well-known low hepatic extraction drug^(13,24,25). Therefore, change in hepatic blood flow has only a little effect on drugs with low hepatic extraction.

Regression analysis had revealed significant determinants of the CYP1A2 activity including gender and liver enzyme AST. This result is consistent with previous reports in that the CYP1A2 activity is higher in men than in women^(12,26). This implies that the young thalassemia patients retain the characteristic of gender associated CYP1A2 activity, despite many thalassemia major patients showing some degree of endocrinopathy^(5,6,9).

The current study was carried out in pediatric patients whereas liver function may be still well-preserved when compared to the adult thalassemia. It remains to elucidate the CYP enzyme activities in adult patients, where the degree of liver injury or iron overload may be greater than the young patients in the present study. It is concluded that based on caffeine test probe, CYP1A2 activity in HbE- β thalassemia major may be unchanged, however, pharmacokinetics of individual drugs with different characteristics of hepatic drug extraction are warranted to further investigation.

Acknowledgement

This work was supported by a grant-in-aid from the Faculty of Medicine, Khon Kaen University.

References

1. Wasi P, Pootrakul P, Fucharoen S, Winichagoon P, Wilairat P, Promboon A. Thalassemia in southeast Asia: determination of different degrees of severity of anemia in thalassemia. *Ann N Y Acad Sci* 1985; 445: 119-26.
2. Fucharoen S, Ketvichit P, Pootrakul P, Siritanaratkul N, Piankijagum A, Wasi P. Clinical manifestation of beta-thalassemia/hemoglobin E disease. *J Pediatr Hematol Oncol* 2000; 22: 552-7.
3. Chakraborty D, Bhattacharyya M. Antioxidant defense status of red blood cells of patients with beta-thalassemia and Ebeta-thalassemia. *Clin Chim*

- Acta 2001; 305: 123-9.
4. Somparn N, Kukongviriyapan U, Tassaneeyakul W, Jetsrisuparb A, Kukongviriyapan V. Modification of CYP2E1 and CYP3A4 activities in haemoglobin E-beta thalassemia patients. *Eur J Clin Pharmacol* 2007; 63: 43-50.
 5. Gulati R, Bhatia V, Agarwal SS. Early onset of endocrine abnormalities in beta-thalassemia major in a developing country. *J Pediatr Endocrinol Metab* 2000; 13: 651-6.
 6. Cunningham MJ, Macklin EA, Neufeld EJ, Cohen AR. Complications of beta-thalassemia major in North America. *Blood* 2004; 104: 34-9.
 7. Aessopos A, Farmakis D, Tsironi M, Diamanti-Kandarakis E, Matzourani M, Fragodimiri C, et al. Endothelial function and arterial stiffness in sickle-thalassemia patients. *Atherosclerosis* 2007; 191: 427-32.
 8. Cheung YF, Chan GC, Ha SY. Arterial stiffness and endothelial function in patients with beta-thalassemia major. *Circulation* 2002; 106: 2561-6.
 9. Kukongviriyapan V, Somparn N, Senggunprai L, Prawan A, Kukongviriyapan U, Jetsrisuparb A. Endothelial dysfunction and oxidant status in pediatric patients with hemoglobin E-beta thalassemia. *Pediatr Cardiol* 2008; 29: 130-5.
 10. Tanaka E. Clinical importance of non-genetic and genetic cytochrome P450 function tests in liver disease. *J Clin Pharm Ther* 1998; 23: 161-70.
 11. Morgan ET. Regulation of cytochromes P450 during inflammation and infection. *Drug Metab Rev* 1997; 29: 1129-88.
 12. Kukongviriyapan V, Senggunprai L, Prawan A, Gaysornsiri D, Kukongviriyapan U, Aiensa-Ard J. Salivary caffeine metabolic ratio in alcohol-dependent subjects. *Eur J Clin Pharmacol* 2004; 60: 103-7.
 13. Fuhr U, Rost KL. Simple and reliable CYP1A2 phenotyping by the paraxanthine/caffeine ratio in plasma and in saliva. *Pharmacogenetics* 1994; 4: 109-16.
 14. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal Biochem* 1969; 27: 502-22.
 15. Gay CA, Gebicki JM. Perchloric acid enhances sensitivity and reproducibility of the ferric-xylenol orange peroxide assay. *Anal Biochem* 2002; 304: 42-6.
 16. Aggeli C, Antoniadis C, Cosma C, Chrysohoou C, Tousoulis D, Ladis V, et al. Endothelial dysfunction and inflammatory process in transfusion-dependent patients with beta-thalassemia major. *Int J Cardiol* 2005; 105: 80-4.
 17. Barker CW, Fagan JB, Pasco DS. Down-regulation of P4501A1 and P4501A2 mRNA expression in isolated hepatocytes by oxidative stress. *J Biol Chem* 1994; 269: 3985-90.
 18. Kourylko O, Fradette C, Arcand M, du SP. Modulation of CYP1A2 and CYP3A6 catalytic activities by serum from rabbits with a turpentine-induced inflammatory reaction and interleukin 6. *Drug Metab Dispos* 2006; 34: 27-35.
 19. Morel Y, Barouki R. Repression of gene expression by oxidative stress. *Biochem J* 1999; 342 (Pt 3): 481-96.
 20. Morgan ET. Regulation of cytochrome p450 by inflammatory mediators: why and how? *Drug Metab Dispos* 2001; 29: 207-12.
 21. Tankanitlert J, Howard TA, Temsakulphong A, Sirankapracha P, Morales NP, Sanvarinda Y, et al. A pharmacokinetic study of paracetamol in Thai beta-thalassemia/HbE patients. *Eur J Clin Pharmacol* 2006; 62: 743-8.
 22. Forrest JA, Clements JA, Prescott LF. Clinical pharmacokinetics of paracetamol. *Clin Pharmacokinet* 1982; 7: 93-107.
 23. Aessopos A, Farmakis D, Tsironi M, Deftereos S, Tassiopoulos S, Konstantopoulos K, et al. Hemodynamic assessment of splenomegaly in beta-thalassemia patients undergoing splenectomy. *Ann Hematol* 2004; 83: 775-8.
 24. Prompila N, Wittayalertpanya S, Komolmit P. A study on the pharmacokinetics of chlorzoxazone in healthy Thai volunteers. *J Med Assoc Thai* 2007; 90: 160-6.
 25. Rawlins MD, Henderson DB, Hijab AR. Pharmacokinetics of paracetamol (acetaminophen) after intravenous and oral administration. *Eur J Clin Pharmacol* 1977; 11: 283-6.
 26. Ou-Yang DS, Huang SL, Wang W, Xie HG, Xu ZH, Shu Y, et al. Phenotypic polymorphism and gender-related differences of CYP1A2 activity in a Chinese population. *Br J Clin Pharmacol* 2000; 49: 145-51.

สถานภาพการทำงานของ CYP1A2 ในเด็กป่วยฮีโมโกลบินอี-เบตาธาลัสซีเมีย

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วัตถุประสงค์: ประเมินการทำงานของเอนไซม์เปลี่ยนแปลงยา CYP1A2 ในเด็กป่วยฮีโมโกลบินอี-เบตาธาลัสซีเมีย เนื่องจากเอนไซม์ CYP1A2 รับผิดชอบเปลี่ยนแปลงยาจำนวนมาก การเปลี่ยนแปลงการทำงานอาจ มีผลกระทบต่อทางคลินิก

สถานที่ทำการวิจัย: ภาควิชาเภสัชวิทยา และภาควิชากุมารเวชศาสตร์ โรงพยาบาลศรีนครินทร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

วัสดุและวิธีการ: เด็กป่วยฮีโมโกลบินอี-เบตาธาลัสซีเมียจำนวน 23 คน และเด็กสุขภาพปกติ 24 คน ที่มีคุณสมบัติคล้ายกับเด็กป่วย ให้ดื่มคาเฟอีนในรูปแบบเครื่องดื่มน้ำตาลมเพื่อเป็นสารทดสอบต่อการทำงานของ CYP1A2 ทำการเก็บปัสสาวะก่อนและหลังดื่ม 6 ชั่วโมง เพื่อวิเคราะห์ระดับคาเฟอีนและ พาราแซนทีนซึ่งเป็นเมแทบอไลต์ของคาเฟอีน

ผลการศึกษา: ตัวบ่งชี้ทางชีวเคมีได้แก่ระดับกลูตาโรอินในเลือดและลิปิดไฮโดรเปอร์ออกไซด์ในปัสสาวะในเด็กธาลัสซีเมียมีค่าสูงขึ้นซึ่งบ่งถึงภาวะเครียดออกซิเดชัน ไม่พบความเปลี่ยนแปลงในสัดส่วนของพาราแซนทีนต่อคาเฟอีน ในระหว่างเด็กป่วยและเด็กปกติ การวิเคราะห์ด้วยเส้นทศถอยพบว่าเพศและระดับเอนไซม์มีความสัมพันธ์อย่างมีนัยสำคัญกับการทำงาน CYP1A2 (adjusted $r^2 = 0.48$, $p < 0.001$) เพศชายมีการทำงานของเอนไซม์สูงกว่าผู้หญิง

สรุป: การทำงานของเอนไซม์ CYP1A2 ไม่มีการเปลี่ยนแปลงในเด็กธาลัสซีเมียแม้ว่าจะมีภาวะเครียดออกซิเดชันปรากฏก็ตาม
