Case Report

Utility of Plasma Fluorometric Emission Scanning for Diagnosis of the First 2 Cases Reports of Variegate Porphyria: A Very Rare Type of Porphyrias in Thai

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Two Thai women who are siblings presented with a history of recurrent pruritic vesicles on dorsum of both hands and extensor surface of forearms where the sun-exposed areas are. The excoriated vesicles were healed with depressed scars. They had no previous history of intense abdominal pain, seizure, or psychiatric disorder. Urinary porphyrins were analyzed by High Performance Liquid Chromatography (HPLC). The level of coproporphyrin III was detected to be higher than the uroporphyrin level. Fluorescence emission scanning of both patients' plasma was performed and demonstrated typical emission peak at 626 nm, that confirmed the diagnosis of variegate porphyria.

Keywords: *Variegate porphyria, Plasma Fluorescence emission scanning, Thai*

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Heme biosynthesis is very important in nearly all living cells since it is essential for multiple electrons transport chain for energy recovery. Heme biosynthesis pathway requires porphyrin precursors and eight enzymes for heme formation[1]. Genetically determined partial deficiency of the second to the eighth enzymes along the pathway cause a group of diseases called "Porphyrias" (Fig. 1)[1,2]. This group of diseases results from an accumulation of porphyrin precursors and metabolic intermediates in the skin and other organs. Porphyrin molecules absorb visible light then generate free radicals with subsequent lipid peroxidation leading to cell damage. It causes a variety of characteristic clinical features[1,3].

Most forms of porphyrias are inherited as Mendelian autosomal dominant but some forms are recessive and can be acquired through exposure to porphyrinogenic drugs and chemicals. Based on the presence or absence of acute neurovisceral attacks, porphyrias can be classified as acute and non-acute porphyrias[1,2]. Acute porphyrias present with a

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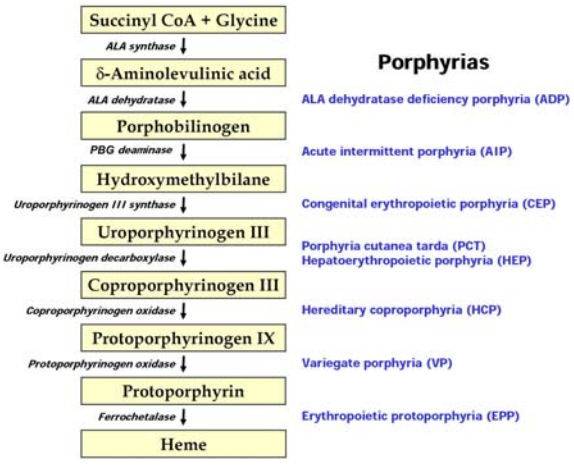

Fig. 1 The porphyrin-heme biosynthesis pathway and porphyrias[1,2]

variety of neurovisceral and psychiatric symptoms whereas nonacute porphyrias present with a variety of cutaneous findings including photosensitivity, increase skin fragility, vesicles, and bullae. Acute porphyrias include Acute Intermittent Porphyria (AIP), Variegate Porphyria (VP), Hereditary Coproporphyria (HCP), and ALA Dehydratase Deficiency Porphyria. Nonacute porphyrias consist of Porphyria Cutanea Tarda (PCT), the most common type of all porphyrias, Erythropoietic Protoporphyria (EPP), Congenital Erythropoietic Porphyria (CEP), and Hepatoerythropoietic Porphyria (HEP)[1].

Variegate porphyria is one of the acute hepatic porphyrias which is caused by a partial reduction of protoporphyrinogen oxidase, the seventh enzyme of the heme biosynthetic pathway[1]. Clinically, the patients present with acute neurovisceral symptoms including hypertension, severe abdominal pain, vomiting, constipation or bladder dysfunction. In addition to neurovisceral symptoms presentation as in acute intermittent porphyria, VP can manifest with photosensitive cutaneous symptoms simulating porphyria cutanea tarda[4]. In Thailand, abnormal porphyrin metabolism is very rare. As far as the authors' review, the first cases of AIP, EPP, Acute Hepatic Porphyria, HCP and CEP in Thailand were reported in 1973[5], 1985[6], 1989[7], 1993[8], and 1998[9] respectively. Although Acute Hepatic Porphyria, which was suspected to be VP or HCP, has been reported in 1989 but the definite diagnosis could not be made. Here the authors report two Thai siblings with classic cutaneous findings without systemic symptoms, which were finally confirmed to be variegate porphyria.

Cases Report

A 43 year-old Thai woman presented with a 10-year history of recurrent pruritic vesicles and bullae on dorsum of both hands and extensor surface of forearms.

The excoriated vesicles and bullae were healed with depressed scars. She had no previous history of intense abdominal pain, seizure, or psychiatric disorder. She had neither underlying disease nor taking any other medications, including oral contraceptive pills, tetracycline and furosemide. Physical examination revealed a solitary large bulla on the dorsal surface of her left hand (Fig. 2). Multiple discrete atrophic scars and milia were observed on the sun-exposed area of upper extremities. Hypertrichosis was presented on the forehead.

Her sister, a 38-year-old, also had a history of recurrent vesicles and bullae on sun-exposed area. Physical examination demonstrated only atrophic scars on sun-exposed area of the upper extremities with no sclerodemoid skin change or hypertrichosis (Fig. 3). The pedigree is demonstrated in Fig. 4.

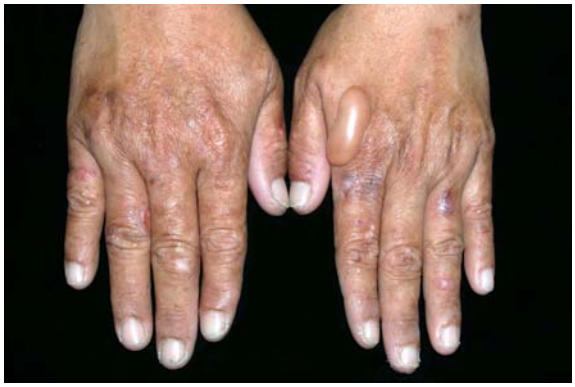

Fig. 2 A large bulla on dorsal surface of the left hand with scar formation (elder sister)

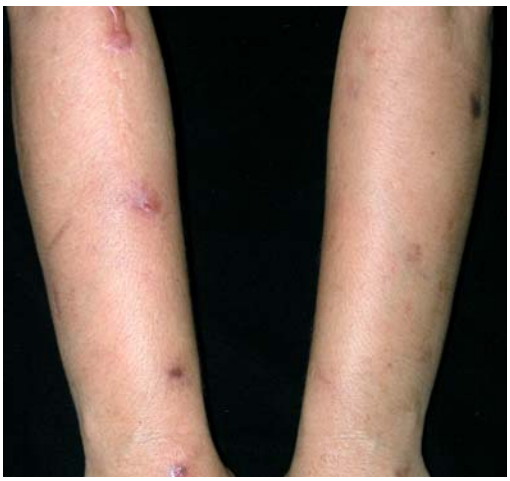

Fig. 3 Atrophic scars on sun-exposed area of upper extremities (younger sister)

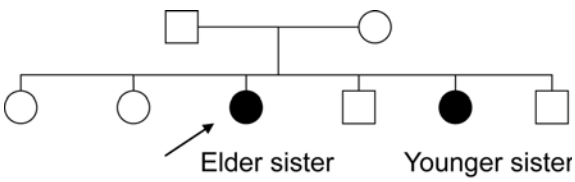

Fig. 4 Pedigree of the patients' family

Due to the history and clinical report of cutaneous photosensitivity without other systemic symptoms, the authors' first impression for both patients was porphyria cutanea tarda. Investigations for the elder sister revealed Hb 10.2g/dl, Hct 31.1%, WBC 5,200/mm^3 (N 52%, L34.5%, M9.4%, E3.8%), Plt308,000/uL. Blood chemistry for liver function test was within normal limit. Anti-HIV was negative, Hepatitis B antigen was negative, Anti-HCV was negative. The skin biopsy obtained from the blister on the dorsal aspect of the left hand revealed subepidermal bulla with festooning of dermal papilla. Periodic acid-Schiff (PAS) stain showed eosinophilic material around the blood vessel walls. Direct immunofluorescent study revealed granular pattern of immunoglobulin G along the dermal-epidermal junction and around superficial and deep blood vessel walls. The clinical appearance, histopathology, and direct immunofluorescence results favored diagnosis of porphyrias.

The initial quantitation of urine porphyrins from both patients' random urine specimen using fluorometric methods demonstrated higher levels of coproporphyrin than uroporphyrin. Therefore, the diagnosis of PCT was unlikely. The authors confirmed the screening urinary test for both patients by high performance liquid chromatography (HPLC), which is the most reliable technique for quantification of each porphyrin. The results still demonstrated an increase in urinary coproporphyrin III levels (Table 1). The authors remaining differential diagnosis was VP and HCP.

To differentiate between VP and HCP, plasma of both patients was scanned by fluorescence

Table 1. Quantitation of urinary porphyrins from random urine specimens of both patients using HPLC

Porphyrins	Reference values* (μmol/mol creatinine)	Elder sister	Younger sister
Uroporphyrin	0.2-2.7	2.4	1.4
Heptacarboxyl porphyrin	0.0-0.2	0.1	0.1
Hexacarboxyl porphyrin	0.0-0.2	0.2	0.1
Pentacarboxyl porphyrin	0.0-0.5	0.5	0.7
Coproporphyrin I	1.0-13.0	14.1	11.1
Coproporphyrin III	1.7-24.0	66.1	52.1

* The reference values of urinary porphyrins in normal Thai subjects are from the Department of Biochemistry, Faculty of Medicine Siriraj Hospital[14]

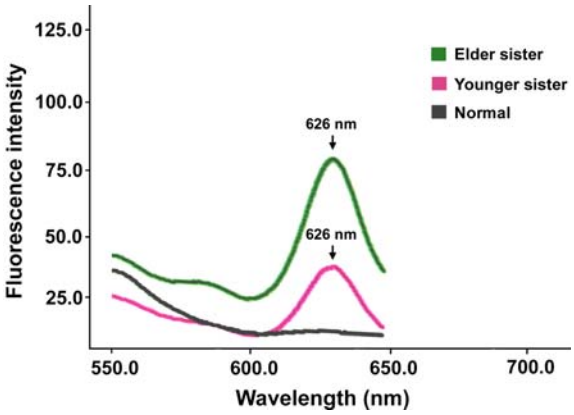

Fig. 5 Plasma fluorescence emission scanning of both patients, excitation wavelength at 405 nm, demonstrated typical emission peak at 626 nm

emission spectroscopy at the excitation wavelength of 405 nm. Their plasma demonstrated a typical emission maximum at 626 nm, characteristic of VP (Fig. 5). Based on their histories, clinical features, and laboratory investigations, the definite diagnosis of VP was made.

Discussion

Variegate porphyria (VP) is a rare disease. It is found worldwide but the most prevalent was reported in South Africa (3 per 1000)[4]. It transmits through autosomal dominant with incomplete penetrance[4]. VP results from accumulation of protoporphyrinogen due to a reduction of protoporphyrinogen oxidase enzyme activity to approximately 50%[10]. As the name implies, VP can present in different forms. While the majority of the patients (40-60%) present with cutaneous manifestation, only 10-20% present with merely acute attack and 10% present with both neurovisceral and cutaneous lesions. One-third of the patients remain asymptomatic[2,3]. Manifestations of acute attack include severe abdominal pain, vomiting, hypertension, and neuropathy.

Many factors can precipitate the acute attacks such as various drugs, alcohol, smoking, dieting, or fasting as well as variations in steroid hormone levels[1-3].

The exact pathogenesis of neurovisceral signs in acute attack is still unclear but porphyrin precursors ALA and PBG are believed to play a role on neurotoxic effects[1].

The presented patients had a recurrent history of vesicles and bullae on sun-exposed areas. The cutaneous lesions cannot be distinguished

between PCT, VP, and HCP. VP and HCP can also present only cutaneous lesion without acute attack[3]. It is important to make an accurate diagnosis since the treatment strategies of these diseases are different. Phlebotomy and antimalarials are used effectively in PCT but not in VP[1].

HPLC is the technique for quantification of each porphyrin subtype. It is the most reliable method to differentiate VP from PCT. In VP, the level of urinary coproporphyrin is equal or higher than uroporphyrin opposite to PCT, which the level of urinary uroporphyrin is higher than coproporphyrin. The presented patients had an elevated ratio of urine coproprophyrin to uroporphyrin, so PCT can be excluded.

A simple and quick method to differentiate VP from HCP is using a fluorometric emission scanning of the plasma. After metal-free porphyrins absorb the Soret band, they emit characteristic fluorescence spectra between 550 and 680 nm. This principle can be used to classify each type of porphyria (Table 2)[11]. Under excitation wavelength of 405 nm, VP demonstrates a sharply defined fluorescence emission maximum at 626 $\pm$ 1 nm while HCP shows emission spectrum at 618-622 nm[11-13]. The authors did fluorometric emission scanning of the presented patients' plasma. Their plasma showed a fluorescence emission maximum at 626 nm, confirmed the diagnosis of VP. Management of VP includes educating patients to avoid precipitating agents for the acute attack. The porphyrinogenic drugs known to precipitate the acute attack include barbiturates, corticosteroids, ethanol, griseofulvin, and oral contraceptives. There is no specific treatment for the cutaneous symptoms, however photoprotection by seeking shade when going out, wearing photoprotective clothing, a wide-brimmed hat and applying opaque inorganic (physical) sunscreen, are recommended. Both of the presented patients were recommended to avoid precipitating drugs and to do adequate photoprotection. At the time of this writing, their cutaneous lesions were well-controlled and they still had no symptoms of acute attacks.

Table 2. Characteristics of fluorescence emission spectra of Porphyrin-Containing plasma[11]

Excitation (nm)	Emission (nm)	TYPE
405	626-628	Variegate porphyria
405	636	Erythropoietic protoporphyria
405	618-622	Normal subjects
		Non-porphyria patients
		Acute intermittent porphyria
		Hereditary Coproporphyria
		Congenital erythropoietic porphyria
		Porphyria cutanea tarda

In conclusion, the authors reported two cases of variegate porphyria, which is the very rare type of porphyrias in Thais. Only cutaneous manifestation cannot be rule out for VP. Detailed history and laboratory testing are essential in arriving at the diagnosis. Simple screening test by plasma fluorometric emission scanning is introduced for diagnosis and typing of porphyrias.

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ประโยชน์ของการตรวจพลาสมาโดยใช้ fluorometric emission scanning เพื่อวินิจฉัยผู้ป่วย วาริเกทพอร์ฟัยเรีย 2 รายแรกในประเทศไทย

ลีนา จุฬาโรจน์มนตรี, ชนิษฎา ตู้จินดา, ชัชวาลย์ ศรีสวัสดิ์, นิโลบล เนื่องตัน, ศราวุธ จั่นหนู, สุรินทร์ ขันหยก

ผู้ป่วยหญิงไทย 2 รายซึ่งเป็นพี่น้องกัน มาตรวจด้วยอาการเป็นตุ่มน้ำพองใสที่หลังมือและแขนด้านนอก ซึ่งเป็นบริเวณที่ถูกแดด เป็น ๆ หาย ๆ มาหลาย ปี โดยตุ่มน้ำเมื่อหายแล้วจะทิ้งรอยแผลเป็น ผู้ป่วยทั้ง 2 ราย ไม่เคยมีประวัติปวดท้องอย่างรุนแรง, อาการชัก หรือ อาการทางจิตเวช ผลการตรวจสารพอร์ฟัยริน ในปัสสาวะด้วยวิธี high performance liquid chromatography พบว่ามี คอโปรพอร์ฟัยริน สูงกว่า ยูโรพอร์ฟัยริน และผลการตรวจพลาสมาโดยวิธี fluorescence emission scanning ให้ emission spectrumสูงสุด ที่ 626 นาโนเมตร ผู้ป่วยทั้ง 2 ราย จึงให้การวินิจฉัย ที่แน่นอนได้ว่าเป็น วาริเกทพอร์ฟัยเรีย