Long-Term Follow-up of Erythrocyte Porphobilinogen Deaminase Activity in a Patient With Acute Intermittent Porphyria: The Relationship between the Enzyme Activity and Abdominal Pain Attacks

Hiroaki TANAKA, Kan USUDA, Eri TANIDA, Masafumi IMANISHI and Koichi KONO

Division of Preventive and Social Medicine, Department of Hygiene and Public Health Osaka Medical College, Takatsuki-city, Osaka 569-8686, Japan

Key Words: Acute intermittent porphyria (AIP), porphyria attack, abdominal pain, erythrocyte porphobilinogen deaminase activity (PBG-D), urinary heme precursors.

ABSTRACT

The relationship between the onset of abdominal pain attack and the urinary levels of δ-aminolevulinic acid, porphobilinogen, uroporphyrin, and the activity of erythrocyte porphobilinogen deaminase was studied on a monthly basis over a four-year period in a 29-year-old male patient with acute intermittent porphyria.

A close relationship is seen between the onset of pain episodes and sharp decreases of porphobilinogen deaminase activity. The activity normalizes as the patient improves, suggesting that this enzyme is a more sensitive monitor for acute intermittent porphyria attacks than the urinary parameters currently used for its diagnosis.

Our results suggest that month-by-month testing of porphobilinogen deaminase activity in acute intermittent porphyria patients is a good practice to predict episodes of acute abdominal pain before its onset, also allowing estimation of possible promoting factors and in establishing optimal specific therapies.

INTRODUCTION

Acute intermittent porphyria (AIP) is an autosomal dominant hereditary inborn error of the heme biosynthetic pathway (Chromosome 11q24.1-q24.2; Dominant). The condition can be exacerbated through a multitude of environmental factors. The disease is clinically manifested with severe abdominal pain, confusion, and seizures which may be life threatening [1]. Abdominal pain occurs in 90-95% of the attacks [2].

Clinical signs of AIP include excess of urinary heme precursors such as δ-aminolevulinic acid (ALA), porphobilinogen (PBG), uroporphyrin (UP), coproporphyrin (CP) and a decrease in erythrocyte porphobilinogen deaminase activity (PBG-D) [3].

The key point of AIP management is to detect a crisis as early as possible in order to appropriately treat it and to avoid inappropriate environmental factors that may exacerbate it [4]. Maintaining good nutrition, particularly with respect to carbohydrates and avoiding non-genetic factors such as infections...
or exposure to certain prescription drugs such as sulfa drugs, is important to prevent AIP attacks. Heme therapy is recognized as the most specific and effective AIP therapy [5]. Although there are established diagnostic criteria and clinical practice guidelines for the management of AIP, a reliable method to predict AIP attacks has not been reported. Furthermore, it is known that AIP patients show an abnormal urinary profile of ALA, PBG, UP and PBG-D and that this profile shows wide variations from status to status within the same subject [6].

The present study was designed to follow the relationship between onset episodes of abdominal pain and the urinary levels of ALA, PBG, UP and the activity of PBG-D over a long-term period of observation of a single male AIP patient.

**MATERIALS and METHODS**

**Subject and AIP attack history**

The study was carried out on male AIP patient, individual III-10 shown in Figure 1. The subject was born in 1973 in a rural Japanese town “M”. The patient was recruited for the study in October 2002 and remained under close observation until June 2006.

The first abdominal pain attack occurred in December 2002 as a result of using acetaminophen and chlorpheniramine maleate. Urine samples taken at the time showed a large excess of heme precursors. During hospitalization, the patient was kept under the infusion of 10% glucose (1500-2000-ml/day) until his complete recovery. Between 2003 and 2005 the patient experienced five crises. During this 3-year period his job required heavy physical work and a large burden of mental stress. After the unfavorable working conditions were settled in autumn 2005 the patient’s abdominal pain attacks subsided.

**Urine sample preparation**

Urine samples were collected from patient III-10 once per month during the four-year study period. The samples were maintained in dark test bottles and stored in a deep freezer until analysis. At that time, the samples were thawed by incubating at 37°C and dissolved by shaking (Termo Mini TM-100). To 700 µl of urine, an equal volume of 0.08% iodoacetic acid (Wako Pure Pharmacy) was added and the mixture was then centrifuged at 11808 G (10,000 rpm) for 10 minutes. The supernatant was used for UP analysis by HPLC and for ALA and PBG analysis by means of the Urata-Granik method [7].

![Fig. 1 Genealogy of patient III-10. There are seven acute intermittent porphyria patients in this family.](image-url)
Analysis of urinary UP

An HPLC (Intelligent HPLC System LC-800 series, JASCO, Japan) was equipped with a system controller (model 801-SC); autosampler (855-AS), pump (880-PU), degasser (DG-3510), column ovens (860-CO), fluorescence detector (FP-210, excitation wavelength 404 nm, emission wavelength 620 nm), chromatography Data System (805-GI), column (Finepak SILC18-5, 4.6mm I.D. x 150 mm) and column filter (IRIKA, pore size 0.45µm, Mobile phase: A = 80% acetonitrile, 7% acetic acid and 50 nM ammonium acetate. B = 10% acetonitrile, 4% acetic acid and 50 nM ammonium acetate).

The temperature was set at 40°C. The HPLC injection was 20µl. A standard curve was obtained by using Porphyrin acids chromatographic marker kit and CP fluorescence standard (Porphyrin Products USA).

Analysis of urinary ALA and PBG

Amberlite®, CG-50 (GFS Chemicals, Inc.) and Dowex-1-X8 polystyrene ion-exchange resin were used for ALA and PBG analysis by Urata-Granik method.

Determination of erythrocyte PBG-D activity

Every month during the entire study period 2-ml venous blood samples were collected into sterile Venoject heparin vacutainers, diluted 1:35 with ice-cold distilled water and then left in a refrigerator for hemolysis.

After centrifugation at 11808 G for 10 minutes, 350µl of supernatant was taken for PBG-D activity analysis. The PBG-D activity was assayed by converting porphobilinogen into uroporphyrin as described by Meyer et al. [8].

RESULTS

Figure 2 shows the ALA trend during the 4-year observation period. During this period, the mean ALA value ± sd was 51 ± 15 mg/L, within the range 35-100 mg/L. Figure 3 shows the corresponding PBG trend. Its mean ± sd value was 85 ± 16 mg/L, range 60-130 mg/L. No synchronized-spikes of these two values were evoked by recurrent AIP attacks.

The UP trend is shown in Figure 4. The mean ± sd value was 1.0 ± 0.1 mg/L, range 0.8-1.2 mg/L. As shown in this figure, a sharp increase of 15-20% above the mean value coincided with each AIP attack.

Although there were ALA, PBG and UP positive and negative spikes over one standard deviation that
coincided with onsets of abdominal pain attacks, the relationship could not be clearly established as these spikes seemed to appear after a certain time lag.

The trend of PBG-D during the 4-year observation period is shown in Fig. 5. Within the range 14.5-22.5 nmol UP/ml RBC/hr, the mean ± sd value was 18.4 ± 2.3 nmol UP/ml RBC/hr. As shown in this figure, negative spikes of 10-20 % from the mean were observed concomitantly with each AIP attack. Major PBG-D negative spikes over one standard deviation occurred during the 2004 - 2005 period of recurrent abdominal pain attacks. A strong PBG-D upward shift >20 nmol UP/ml RBC/hr was observed after the fall in 2005 (Figure 5). This event also coincided with improvement of the patient’s working conditions.

**DISCUSSION**

**Clinical features of AIP**

Acute intermittent porphyria is an autosomal dominant disorder caused by insufficient activity of PBG-D, the third enzyme in the heme biosynthetic pathway, hydroxymethylbilane synthase, also known as uroporphyrinogen I-synthase.

The estimated prevalence of the disorder is 5 -10 per 100,000 inhabitants [1]. In Japan, since first reported by Sato and Takahashi in 1920 until December 2002, a total of 827 cases of porphyrias have been diagnosed on the basis of clinical and/or laboratory findings. Of these, 463 were males, 358 females, and 6 of unknown gender. Symptomatic porphyria is thought to be three times more frequent in females than in males [9].

The decrease of the PBG-D activity results in bone marrow and liver accumulation of the heme precursors ALA, PBG and UP. Eventually, they enter circulating blood and are excreted in the urine and feces. Although the effect of an abnormal porphyrin precursor profile is unknown, AIP is suspected to be the primary cause of several of the symptoms that characterize a crisis: abdominal pain, vomiting, confusion, hysteria, peripheral neuropathies and seizures. In general, deficiency of PBG-D by itself is not sufficient to produce a crisis. Other risk factors include some drugs and poor nutrition.

Acute intermittent porphyria has been fully characterized by excess of ALA, PBG, UP and decreases of PBG-D during the chronic stage [10]. The abdominal pain crisis, neuropathies, constipation and other various clinical symptoms characterize the intermittent acute stage [11]. Although these are important diagnostic criteria of AIP, the long-term trends of ALA, PBG, UP, PBG-D and their shifts during the transition from chronic to acute stage have not been reported in detail.

**UP, ALA, and PBG relationship to AIP attack**

By HPLC chromatography we have established that the mean value of UP in the patient under study was about 1000µg/ ��, which is at least one order of magnitude higher than the reference ranges of total porphyrins (20-120µg/ ��), UP (6-45µg/ ��), and CP (9-63µg/ ��) reported by de Rover et al. [12].

Sudden increases of 15-20 % above the mean values of the chosen markers were observed after an AIP attack albeit with a certain time lag. The mean values of ALA was ≈50 mg/ ��(range 35-100mg/ ��) and that of PBG ≈90 mg/ ��(range 60-130mg/ ��), also markedly higher than the 2.39 ± 0.87mg/ �� reference ranges of ALA reported by Tomokuni et al. [13] and of PBG (<2 mg/ ��) reported by Nuttall [14]. These increases, however, were not synchronous with AIP attacks.

In a 2-year study on AIP patients, Aarsand et al reported within-subject changes in relation with reported reference values of 50% for ALA and 70% for PBG [6]. Our results for ALA and PBG seem to be within a reasonable fluctuation range. Additionally, as these indicators are not related to an AIP attack onset, these results suggest that UP can be a good indicator to evaluate previous AIP attacks, and that ALA and PBG would be more suitable to routine evaluation throughout both chronic and acute stages of the disease.

**Relationship between PBG-D and AIP attack**

The mean value of PBG-D here reported of about 18.4 nmol UP/ml RBC/hr is lower than the low-end reference range of 20.9-63.2 reported by Santos et al [15]. Kostrzewska et al examined PBG-D during

---

**Fig. 5** Trend of erythrocyte porphobilinogen deaminase activity (PBG-D) during the four-year observation period. See legend to Fig. 2 for explanation of the superimposing.
attacks of AIP and concluded that PBG-D showed high levels of >24.3 nmol UP/ml RBC/hr during attacks and they decreased during convalescence and remission [16]. The Kostrewska report well supports our findings of PBG-D negative spikes associated to AIP attacks observed in this study.

A strong upward shift of PBG-D was observed in close correspondence to the patient’s working condition improvement after 2005 autumn, with the PBG-D value reaching the lower limit (22.0 nmol UP/ml RBC/hr) of the range reported as normal by Santos et al [15]. The ALA, PBG and UP remained unchanged after the patient’s improvement.

These results suggest that the activity of PBG-D is more sensitive to the conditions prevailing during a crisis than ALA, PBG and UP. Therefore, it can be concluded that PBG-D is a good indicator to predict or detect the AIP attack before its onset.

**PBG-D observation and other strategies for AIP attack prevention**

At present, the majority of AIP patients receive treatment with good prognosis. The incidence of serious complications and mortality of the disease is low, but the acute crisis potentially leads to life threatening [17]. Repetitive acute attacks [18,19] or poor management during this stage [20] could be fatal. Thus, by monitoring the PBG-D activity the acute phase of the disease can be prevented and even avoided by appropriate clinical care.

As the acute attack of AIP might be precipitated by various promoting factors during surgery and anesthesia, including fasting, starvation, dehydration, stress, infection, and medications [21], an important foundation for the prevention of AIP crises, porphyric patients should not be prescribed unsafe drugs, their stress level should be reduced and, in general, maintain them in the best possible living conditions.

As the heme is the final biosynthetic product, intravenous heme therapy, usually at 3mg/kg-day for 4 days is good clinical practice to regularize porphyrin biosynthesis [5, 22].

**Conclusion**

Presently PBG-D testing is performed for the diagnosis of chronic or acute AIP. We propose that monthly range PBG-D monitoring is crucial for the prediction of the acute phase in AIP patients by using the negative deviation of PBG-D from the mean value as a clinical benchmark to predict and prevent AIP attacks. Physicians treating porphyric patients should use monthly testing schedules to estimate the potential risk of an AIP attack and to establish opportune heme therapy.

**REFERENCES**


Received May 28, 2007
Accepted June 18, 2007