A High Urinary Urobilinogen / Serum Total Bilirubin Ratio Reported in Abdominal Pain Patients Can Indicate Acute Hepatic Porphyria

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Research Article

Keywords: acute hepatic porphyria, urinary urobilinogen, serum total bilirubin, urinary porphobilinogen

Posted Date: November 16th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-587707/v2

Abstract

Background: Due to its variable symptoms and nonspecific laboratory test results during routine examinations, acute hepatic porphyria (AHP) has always been a diagnostic dilemma for physicians. Correct diagnosis mainly depends on the elevated urinary porphobilinogen (PBG) level, which is not a routine test performed in the emergency department. Therefore, identifying a more convenient indicator during routine examinations is important to improve the diagnosis of AHP.

Methods: In this retrospective study, we enrolled 12 AHP patients and 100 patients with abdominal pain of other causes as the control groups in Qilu hospital of Shandong University between 2015 and 2021. The clinical manifestations and laboratory result data including urinary urobilinogen/serum total bilirubin ratio were compared between these two groups. The diagnostic performance of urinary urobilinogen/serum total bilirubin ratio was measured as sensitivity, specificity, and accuracy. The cut-off for optimal clinical performance was determined by the receiver operator characteristic (ROC) curve. Results were considered significant at a $P < 0.05$.

Results: Compared with the control groups, AHP patients showed a significantly higher urinary urobilinogen level ($P < 0.05$). However, we showed that the higher urobilinogen level was caused by a false-positive result due to elevated urine PBG. Hence, we used serum total bilirubin, an upstream substance of urinary urobilinogen synthesis, for calibration. A remarkable increase in the urinary urobilinogen/serum total bilirubin ratio was observed in AHP patients. The area under the ROC curve of this ratio for AHP was 1.000 (95% confidence interval, 1.000–1.000, $P < 0.01$). A cutoff value of 3.22 for the urinary urobilinogen/serum total bilirubin ratio yielded a sensitivity of 100% and a specificity of 100% to distinguish AHP patients from the controls.

Conclusion: A reported high urinary urobilinogen level that was adjusted by the serum total bilirubin level (urinary urobilinogen/serum total bilirubin ratio) could be used as a sensitive and specific indicator for AHP in patients with abdominal pain.

Background

Acute hepatic porphyria (AHP) is a rare but life-threatening disease. There are four classes of AHP: acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP), and aminolevulinic acid dehydratase deficient porphyria (ADP)[1]. Since ADP is extremely rare and presenting a different laboratory results[2], we mainly focused on AIP, HCP and VP, which are more common classes with elevated urinary porphobilinogen (PBG) during attack, in the present study.

During the acute attack of AHP, abdominal pain is the most common presentation and the leading reason for the emergency visit[3]. However, due to the various symptoms and nonspecific routine laboratory test results, the diagnosis of AHP has always been a significant challenge for physicians, and a delay in diagnosis or even misdiagnosis is very common [3–5]. At present, the examination of urinary PBG is the key test in patients with suspected AIP, HCP and VP[6]. As an unconventional examination, the urine PBG is detected only when the physicians realize the possibility of AHP. Moreover, only several hospitals in mainland of China carried out the detection of urinary PBG which also greatly compromises the diagnosis of this rare disease[7].

The Watson-Schwartz test has been a widely used method for urinary PBG detection for more than 80 years. Ehrlich's reagent in the first step reacts with PBG and forms a red condensation product. However, PBG is not the only substance that can react with Ehrlich's reagent. Ehrlich's reagent also reacts with urobilinogen[8]. As such, in the second step of Watson-Schwartz test, chloroform is added to the solution to distinguish the PBG-Ehrlich compound from the urobilinogen-Ehrlich complex.

At present, the dipstick method is the most widely used method of urinalysis. The Ehrlich's reagent pad on the dipsticks is used for detection of urinary urobilinogen. The higher concentration of urinary urobilinogen, the darker color on the pad. Following the manufacturer’s instructions or using an automated dipstick reader, we can obtain semiquantitative results of urobilinogen. Since it could react with both PBG and urobilinogen, we suspected that the elevated urinary PBG during the attack of AIP, HCP and VP would cause a false-positive result of urinary urobilinogen. However, the upstream substance like serum bilirubin will not increased. In this retrospective study, we analyzed the urinary urobilinogen and serum bilirubin in the AHP patients and discovered for the first time that both the urinary urobilinogen and the urinary urobilinogen/serum total bilirubin ratio were greatly increased in the AHP group compared with the control groups, indicating its potential clinical value in diagnosing AIP, HCP and VP. Furthermore, the sensitivity and specificity of this ratio were assessed to evaluate the performance of the urinary urobilinogen/serum total bilirubin ratio as a convenient indicator during the acute attack of AIP, HCP and VP.

Methods

Study design and participants

We performed a retrospective study and reviewed patients with AHP who were admitted to our hospital between 2015 and 2021. The study protocol was approved by the Medical Ethics Committee of Qilu Hospital, Shandong University, China (approval number:KL2021027). Informed consent was obtained from all the patient who participated in this study. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patients were diagnosed as AHP (including AIP, CHP and VP) if they met the following diagnostic criteria[7],(i) acute attack symptoms: severe abdominal pain in the absence of significant abdominal tenderness and neuropathic symptoms such as seizures; and (ii) a positive result for urinary

PBG (N=10) or a genotype with a mutation in the hydroxymethylbilane synthase, protoporphyrinogen oxidase and coproporphyrinogen oxidase genes (N=4).

According to studies of AHP in China, the common misdiagnosis of AHP includes intestinal obstruction, pancreatitis, appendicitis, cholecystitis, and gallstones[9]. We randomly enrolled 100 patients with intestinal obstruction, pancreatitis, appendicitis, and gallbladder diseases as the control group. Patients with appendicitis (N=25) and gallbladder diseases (N=25) were surgically treated. Their classic symptoms and postoperative pathology results confirmed the diagnosis. The diagnosis of pancreatitis (N=25) was established by the presence of more than two of the following criteria: (i) abdominal pain consistent with pancreatitis; (ii) serum amylase and/or lipase greater than three times the upper limit of normal; and/or (iii) characteristic findings from abdominal imaging (e.g., exudation around the pancreas) [10]. Intestinal obstruction (N=25) was confirmed by clinical signs and symptoms and a triad of multiple air-fluid levels, distention of small bowel loops, and the absence of gas in the colon during the abdominal imaging plain upright abdominal radiography or CT scan[11]. Patients with a history of tumor, hemolytic disease, liver cirrhosis, splenomegaly, and autoimmune hemolytic anemia were excluded.

Data collection

The medical history, physical examination, and blood and urinary biochemical results were collected from the Electronic Medical Records of the patients.

The blood biochemical tests included hemoglobin (range 130–175 g/L), alanine transaminase (range 21–72 U/L), aspartate transaminase (range 17–59 U/L), total bilirubin (range 3–22 μmol/l), conjugated bilirubin (range 0–5 μmol/l), unconjugated bilirubin (range 0–19 μmol/l), amylase (range 30–110 U/L), serum sodium (range 137–145 mmol/l). These tests were performed using standard hospital laboratory techniques. BC-5390CRP (Mindray Biomedical Electronics Co., Ltd, China) was used for the hemoglobin examinations. The remaining serum markers were measured using a Vitros 5600 (Ortho Clinical Diagnostics, USA). Urinalysis was carried out using urine test strips and a Urilyzer Auto (Analyticon Biotechnologies AG, Germany).

Statistical analyses

All the statistical analyses were performed in SPSS (version 24.0, SPSS, Chicago, IL, USA). Normally distributed continuous data (assessed by the Shapiro-Wilk test) are presented as mean ± SD and were analyzed by the independent sample *t*-test. Non-normally distributed data are presented as median (percentage) and were analyzed by the Mann-Whitney U test. The diagnostic performance was measured as sensitivity, specificity, and accuracy. The cut-off for optimal clinical performance was determined by the receiver operator characteristic (ROC) curve. Results were considered significant at a $P < 0.05$.

Results

Demographic and clinical characteristics of the AHP patients

Between 2015 and 2021, 12 AHP patients were admitted into our hospital. The mean age at diagnosis was 27.7±6.8 years old, with a female/male ratio of 11:1. These patients had 4.8 ± 1.9 acute attacks with a maximum of eight attacks before the correct diagnosis. Abdominal pain (100%), pain in the extremities (50.0%), seizures (33.3%), and dark urine (16.7%) were common clinical presentations. During the physical examinations, tachycardia and hypertension were observed in 75.0% and 58.3% of the patients, respectively. None of the patients reported skin lesions. In seven of the patients, AHP was triggered by hormonal variations during pregnancy or the menstrual cycle. In one patient, AHP was triggered by alcohol intake. No clear causes were recorded for the remaining four patients (Table1).

Table 1
Clinical profile of the patients with acute hepatic porphyria

Case	Sex	Age	Precipitating factors	Abdominal pain	pain in the extremities	seizures	dark urine	skin lesions	Blood pressure (mmHg)	Pulse rate/min	Watson-Swartz test	Gene mutation
1	Female	20	Menstruation	Yes	No	No	No	No	149/87	112	Positive	N/A
2	Male	24	Alcohol	Yes	Yes	Yes	No	No	107/62	101	Positive	N/A
3	Female	37	Pregnancy	Yes	No	No	No	No	146/94	86	Positive	Yes (HMBS)
4	Female	28	Pregnancy	Yes	Yes	Yes	No	No	137/80	104	Positive	N/A
5	Female	32	Menstruation	Yes	No	Yes	No	No	111/87	116	Positive	N/A
6	Female	19	Unknown	Yes	Yes	No	Yes	No	144/113	123	N/A	Yes (HMBS)
7	Female	23	Menstruation	Yes	Yes	No	No	No	169/120	112	Positive	N/A
8	Female	35	Menstruation	Yes	No	No	Yes	No	161/89	86	Positive	N/A
9	Female	29	Unknown	Yes	No	No	No	No	97/68	122	Positive	N/A
10	Female	27	Unknown	Yes	No	No	No	No	150/78	78	Positive	N/A
11	Female	18	Unknown	Yes	Yes	No	No	No	129/72	123	N/A	Yes (HMBS)
12	Female	25	Menstruation	Yes	Yes	Yes	No	No	129/92	81	Positive	Yes (HMBS)

HMBS: Hydroxymethylbilane Synthase

Interestingly, half of the AHP patients were first diagnosed by endocrinologist during a consult for hyponatremia. For the other six cases, four were diagnosed by neurologists, and two were diagnosed by a gastroenterologist and surgeon. During their previous visits, these patients had been misdiagnosed as intestinal obstruction (83.3%), undifferentiated abdominal pain (75.0%), appendicitis (8.3%), and seizure (16.7%). Appendectomy was performed in one patient at the primary hospital and seizures happened after the surgery.

Urinary urobilinogen was elevated in AHP patients, which was a false-positive result caused by urinary PBG

One hundred patients with abdominal pain caused by other diseases were enrolled in the control groups, and their laboratory results were compared with those of the AHP patients. During the acute attack, the AHP patients showed significantly lower hemoglobin, serum sodium, and serum chlorine, and higher urinary urobilinogen levels compared with control groups (Table 2). Urobilinogen is a product of bilirubin metabolism by anaerobic bacteria in the intestine. However, we found most of the AHP patients showed normal serum bilirubin levels in the present study (Table 2), which made it difficult to explain the elevated urinary urobilinogen values. Thus, we suspected that the elevated urinary urobilinogen level in AHP patients could have been a false-positive result caused by other substance that can react with Ehrlich's reagent on the dipstick. According to previous reports, sulfonamides, p-aminosalicylic acid, and drugs containing Azo dyes (nitrofurantoin, riboflavin, methyldopa) can also react with Ehrlich's reagent, but none of the patients in the current study had a history of taking these drugs.

Table 2
Comparison of laboratory results between acute hepatic porphyria patients and patients with abdominal pain due to other causes.

	Cholecystitis and gallstones (N=25)	Intestinal obstruction (N=25)	Pancreatitis (N=25)	Appendicitis (N=25)	Acute hepatic porphyria (N=12)
Male/female ratio	13:12	10:15	13:12	10:15	1:11
Age	67.00(60.50,69.00)**	52.96±16.04**	52.48±15.48**	42.80±17.97**	27.73±6.77
Hemoglobin	133.64±16.08*	133.16±18.25*	140.29±21.35**	137.04±20.37*	107.75.±13.79
ALT	37.00(20.50,137.00)	22.50(20.00,27.75)**	63.50(33.50,233.50)	25.00 (20.00, 42.00) **	41.50(32.50,83.00)
AST	28.00(23.50,150.00)	26.00(21.00,34.00)*	47.00(32.00,199.00)	23.00(19.50,33.00)*	43.00(29.50,106.60)
Serum TBIL	16.00(10.50,35.50)	14.00(11.00,20.50)	20.00(12.50,32.00)	16.00(10.00,22.00)	13.00(10.90,19.50)
Serum DBIL	0.00(0.00,0.00)	0.00	0(0.00,0.00)	0.00	0.00
Serum IBIL	9.00(7.00,18.00)	11.00(7.00,16.00)	13.00(8.50,23.50)	11.00(7.00,16.00)	11.38±7.34
Serum amylase	69.50(57.25,83.00)	72.00(59.00,72.00)	531.00(238.00,1021.50)**	75.50±34.00	65.25±27.04
Serum sodium	136.43±3.37**	135.46±3.71**	136.25±3.08**	137.42±4.34**	128.17±8.93
Urinary urobilinogen	0.00(0.00,0.00)**	0.00(0.00,0.00)**	0.00(0.00,0.00)**	0.00(0.00,0.00)**	109.55 ± 57.03

ALT: alanine transaminase; AST: aspartate transaminase; DBIL: direct bilirubin; IBIL: indirect bilirubin; TBIL: total bilirubin;*P < 0.05, ** P < 0.01.

PBG, which is greatly increased during an acute attack of AIP, VP and HCP, can also react with Ehrlich's reagent. So we suspected that the elevated urinary urobilinogen level in AHP group could have been a false-positive result caused by the urinary PBG and Watson-Schwartz test was used to confirm this speculation (Figure 1). After an equal volume of Ehrlich's reagent was added, the urine sample of AHP patient turned red and proved the existence of PBG-Ehrlich compound or urobilinogen-Ehrlich complex (Figure 1C). And then chloroform was added to the solution to distinguish these two substances. Red color in the aqueous phase of the test tube after adding chloroform illustrated the existence of the PBG-Ehrlich compound, whereas no color in the chloroform phase excluded the existence of the urobilinogen-Ehrlich complex (Figure 1D).

Performance of urinary urobilinogen/serum total bilirubin ratio as an indicator for AHP

Because urinary urobilinogen was a false-positive result caused by urinary PBG, we used serum total bilirubin for calibration. A remarkable increase in urinary urobilinogen/serum total bilirubin ratio in AHP patients (6.19 ± 1.88 vs. 0.00 ± 0.0, P < 0.01) was observed when compared with that in the control groups (Figure 2). The performance of urinary urobilinogen/serum total bilirubin ratio as an indicator for AIP, HCP and VP was assessed by generating a ROC curve (Figure 3). Since one patient did not undergo the urinary examination, only data from eleven AHP patients were used for the ROC analysis. With the 100 samples from abdominal pain patients of other causes as the controls, the area under the ROC curve of urobilinogen/serum total bilirubin ratio for AHP was 1.000 (95% CI, 1.000–1.000, P < 0.01). When using the maximum paired sensitivity and specificity values from the ROC curve, we determined a cutoff value of 3.22 for the urinary urobilinogen/serum total bilirubin ratio in indicating AIP, HCP and VP from abdominal pain patients. The sensitivity, specificity, PPV, and NPV of the urinary urobilinogen/serum total bilirubin ratio to identify patients with AHP were 100%, 100%, 100%, and 100%, respectively.

Discussion

AHP is an autosomal dominant genetic disorder caused by the defect of the enzymes during heme biosynthesis pathway[12]. Due to the partial deficiency of these enzymes, excess amounts of 5-aminolevulinic acid (ALA) and PBG accumulate and cause dysfunction of the autonomic system and neuropathy[13]. Common presentations of AHP include abdominal pain (85–95%), constipation (48–84%), extremity pain (50–52%), nausea and vomiting (43–88%), tachycardia (28–80%), and hypertension (36–54%)[3]. Bullous skin lesions may be present during an attack of VP or HCP[14]. In severe cases, seizures, hallucination and respiratory distress might also present and patients could require intensive care[15]. In our study, 100% of patients complained of abdominal pain, which was much higher than that reported in previous studies. This might be due to a potential missed diagnosis of AHP patients with other symptoms caused by the poor awareness of physicians in our hospital.

The key issue in AHP management is to suspect the diagnosis[16], and the elevation of urinary PBG is the main diagnostic criteria for AHP including AIP, HCP and VP [14]. However, the urine PBG detection is only ordered and performed when AHP is suspected by physicians. In our study, we found that 50% of the patients were diagnosed by endocrinologist, which was rare in the published articles whose authors usually were from the neurology, general surgery or emergency departments. This finding highlights the importance of experienced physicians for AHP diagnosis. However, as a rare disease, awareness of AHP in China is limited[7]. According to a previous investigation carried out in a tertiary hospital in China, the misdiagnosis rate

of AHP was 70% in the hospital[9]. Further, case reports are barely reported from secondary or communal hospitals. Thus, a simple way for AHP screening is needed to help physicians notice the possibility of AHP.

Here, for the first time, we reported the important role of the urinary urobilinogen/serum total bilirubin ratio in the screening of AIP, HCP and VP. Compared to the abdominal pain patients of other causes, the AHP patients showed a significantly higher urinary urobilinogen level and urinary urobilinogen/serum total bilirubin ratio. Urobilinogen is a product of bilirubin metabolism by anaerobic bacteria in the intestine. Up to 20% of the urobilinogen produced daily are reabsorbed from the intestine and undergo enterohepatic recirculation. The majority of the reabsorbed urobilinogen is taken up by the liver and then re-excreted into bile, while a small amount is excreted in the urine and being detected as urinary urobilinogen[17]. In healthy people, the urinary urobilinogen in urinalysis is negative since the amount of urobilinogen is too low to be detected. However, in certain diseases, such as hemolytic anemia, hepatic jaundice, and biliary disease, the serum bilirubin level is greatly elevated and leads to the excessive production of urobilinogen. Thus, a positive result shows in the urinalysis[18, 19]. However, our study demonstrated that most of the AHP patients showed normal serum bilirubin levels, consistent with the literature[9, 15, 20–22]. Therefore, we believe that the elevated urinary urobilinogen level in AHP group was a false-positive result caused by the urinary PBG, which was proved by Watson-Schwartz test (figure 1). When chloroform is added, the red PBG-Ehrlich compound is located in the aqueous phase while the red urobilinogen-Ehrlich complex is located in the chloroform phase[16].

However, a false-positive result can occur due to urinary PBG since it is difficult to use chloroform to distinguish the red product on the dipstick. To avoid this, we used serum total bilirubin, which is a routine examinations and generally performed in patients with abdominal pain, for calibration. In the AHP group in this study, the urinary urobilinogen increased greatly (caused by PBG) while the serum total bilirubin was normal or slightly elevated. This led to a great increase in the urinary urobilinogen/serum total bilirubin ratio. Via ROC curve analysis, we found that the cutoff point for AHP diagnosis was 3.22. So in patients with typical clinical symptoms such as abdominal pain, the AHP diagnosis should be considered when the urinary urobilinogen/serum total bilirubin ratio was above 3.22 and further investigation of urinary PBG and ALA should be carried out to confirm the diagnosis.

The potential clinical use of the urinary urobilinogen/serum total bilirubin ratio suggests the importance of urinalysis in the diagnosis of AIP, HCP and VP. Dark urine is very common in AHP patients and sometimes becomes the first clue for AHP in the intensive care unit[5, 23]. However, in the emergency department, dark urine color is often overlooked both by patients and physicians. In our study, all the urine specimens showed amber color during urinalysis, but only two patients mentioned a change of urine color in their complaints. In addition, a menstrual period is a common predisposing factor of an acute attack, but urinalysis is often avoided both by the physicians and female patients during their periods. Therefore, we strongly suggest that all patients with abdominal pain undergo urinalysis, and the urinary urobilinogen/serum total bilirubin ratio should be calculated to indicate a diagnosis of potential AIP, HCP and VP.

Our study has several limitations. Since AHP is a rare disease, we only enrolled 12 patients from one single center in this study. Due to the limited sample size, there might be variations in the cut-off point. In this retrospective study, although we enrolled patients whose diagnosis of diseases other than AHP were confirmed by postoperative pathological results, typical imaging and laboratory findings, no urinary PBG detection was done to fully rule out the possibility of AHP in the control groups, even though most of their urinary urobilinogen result was negative. Future multi-center studies with larger sample sizes are needed.

Conclusions

In patients with abdominal pain, the reported high urinary urobilinogen level (from the Ehrlich's test) adjusted by the serum total bilirubin level (the urinary urobilinogen/serum total bilirubin ratio) can be used as an indicator for AIP, HCP and VP. With a cutoff point of 3.22, this ratio had a specificity and sensitivity of 100% and 100%, respectively. When the ratio is higher than 3.22, further investigation of urinary PBG, ALA or a genetic test is recommended. This finding may greatly improve the diagnosis of AIP, HCP and VP, especially in primary or secondary hospitals where physicians have limited experience of this rare disease.

Abbreviations

AHP: acute hepatic porphyria; AIP: acute intermittent porphyria; HCP: hereditary coproporphyria; VP: variegate porphyria; ADP: aminolevulinic acid dehydratase deficient porphyria; PBG: porphobilinogen; ALA: 5-aminolevulinic acid; HMBS: Hydroxymethylbilane Synthase; ALT: alanine transaminase; AST: aspartate transaminase; TBIL: total bilirubin; DBIL: direct bilirubin; IBIL: indirect bilirubin.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Medical Ethics Committee of Qilu Hospital, Shandong University, China (approval number:KL2021027). Informed consent was obtained from all the patient who participated in this study. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed in the present study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

None.

Authors' contributions

YL designed and conducted this study and was a major contributor in writing the manuscript. CS collected the data and drafted the manuscript. SS analyzed the data. All authors read and approved the final manuscript.

Acknowledgements

The authors wish to thank the physicians in the Department of Endocrinology, Department of Neurology, Department of Gastroenterology for diagnosing the AHP patients in this study.

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Figures

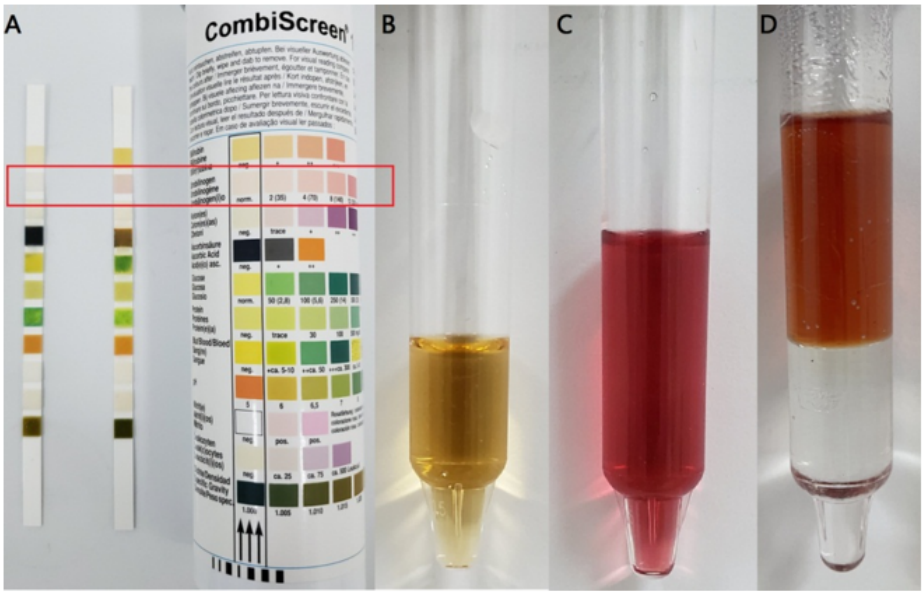

Figure 1

Elevated levels of urinary urobilinogen in acute hepatic porphyria patients were false-positive results caused by urinary PBG. (A) A positive urinary urobilinogen result reported by the dipstick (the right dipstick in the red box). (B) Fresh urine sample of acute hepatic porphyria patient. (C) Urine sample turned red after adding an equal volume of Ehrlich's reagent. (D) Red color in the aqueous phase of the test tube after adding chloroform illustrated the existence of the PBG-Ehrlich compound, whereas no color in the chloroform phase excluded the existence of the urobilinogen-Ehrlich complex.

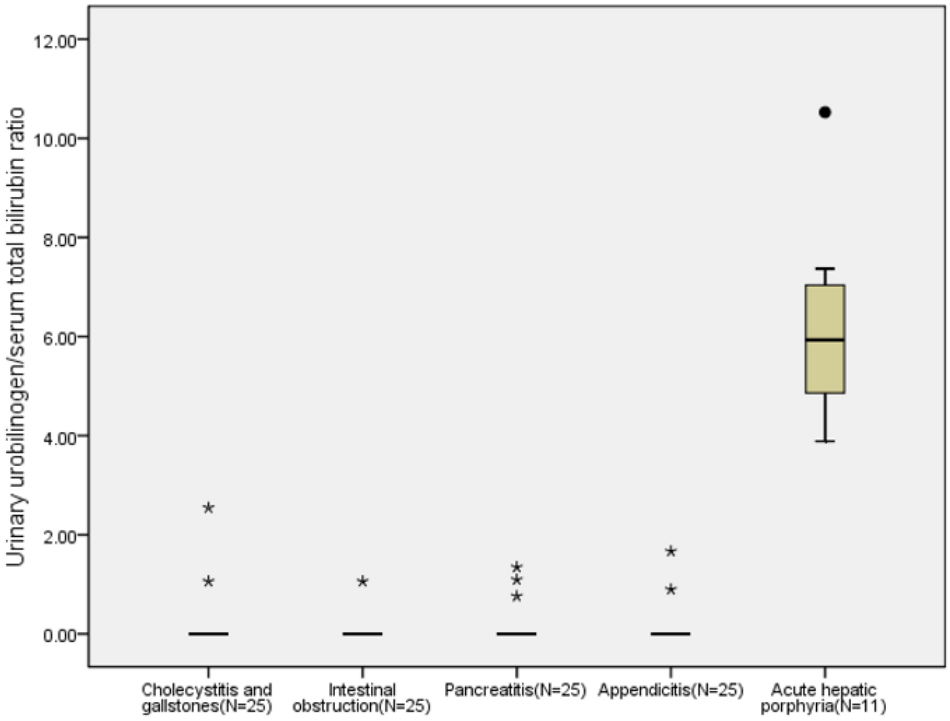

Figure 2

Comparison of urinary urobilinogen/serum total bilirubin ratio between acute hepatic porphyria patients and abdominal pain patients from other causes. A remarkable increase in urinary urobilinogen/serum total bilirubin ratio was observed in acute hepatic porphyria patients when compared with that in the control groups (6.19 ± 1.88 vs. 0.00 ± 0.0, P < 0.01). Data are presented as mean ± SD median (percentage). An independent sample t-test was used for statistical comparisons.

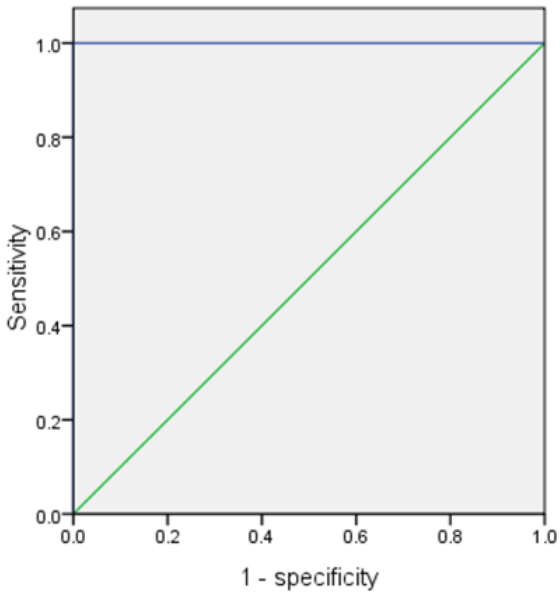

Figure 3

Diagnostic performance of urinary urobilinogen/serum total bilirubin ratio for acute hepatic porphyria. ROC curves were plotted using data from the acute hepatic porphyria patients and abdominal pain patients of other causes to assess the performance characteristics of urinary urobilinogen/serum total bilirubin ratio for acute hepatic porphyria. AUC was 1.000 with a 95% CI of 1.00–1.00. Cutoff point = 3.22