

# HBsAg-positive patient with Dubin-Johnson Syndrome: a case report

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## SUMMARY

Dubin-Johnson Syndrome (DJS) is a rare autosomal recessive liver disorder characterized by chronic or intermittent conjugated, mild hyperbilirubinemia, and is caused by deficiency of multidrug resistance protein 2. DJS, is often an occult entity which until various factors accentuate hyperbilirubinemia, resulting in clinically apparent disease. In this study, we present a HBsAg-positive patient with DJS, who has been diagnosed based on elevated urinary coproporphyrin I isomer. The case was a 21-year-old male who complained about dizziness. His laboratory data revealed a chronic HBsAg sero-positivity and a mild conjugated/unconjugated hyperbilirubinemia; remaining analytical results were normal. Any pathological changes of liver and biliary tree were not observed on ultrasonography. Due to dark-brownish pigmentation demonstrated on liver biopsy reminded the DJS. Total porphyrin concentration in urine sample was within the reference range, diagnosis of DJS was confirmed by increased urine coproporphyrin I/total ratio (97%). Currently, DJS is diagnosed by demonstrating characteristic signs of the DJS on multiple diagnostic tests (e.g. histochemical examination of liver biopsy material and/or cholescintigraphic imaging). Although definitive diagnosis of this disorder mostly relies on ABCC2 gene mutation analysis, determination of urinary isomers of coproporphyrin may relatively be an easily accessible and strong biochemical indicator for diagnosis of DJS.

**Key words:** *Dubin-Johnson Syndrome, Hyperbilirubinemia, Coproporphyrin, Porphyrin*

## ÖZET

### HBsAg-pozitif olan Dubin-Johnson Sendrom'lu hasta : olgu sunumu

Dubin-Johnson sendromu (DJS) kronik veya aralıklı hafif konjuge hiperbilirubinemi ile karakterize ve "multidrug resistance protein 2" eksikliğinin neden olduğu, nadir görülen otozomal resesif geçişli bir karaciğer bozukluğudur. Çeşitli faktörlerin hiperbilirubinemiye tetikleyerek klinik olarak belirgin hastalığın ortaya çıkmasına yol açmalarına dek DJS genellikle gizli bir tablodur. Bu çalışmada biz idrar koproporfirin I izomer artışı gösterilerek tanı konulan bir HBsAg-pozitif DJS hastası sunmaktayız. Vaka baş dönmesinden yakından 21 yaşında erkek hasta idi. Hastanın laboratuvar sonuçları, kronik HBsAg sero-pozitifliği ve hafif direkt/indirekt hiperbilirubinemiye gösterdi, diğer analitik sonuçlar normaldi. Ultrasonografide karaciğer ve safra yollarında herhangi bir patolojik değişiklik gözlenmedi. Karaciğer biyopsisinde görülen koyu kahverengi pigmentasyon nedeniyle DJS düşünüldü. İdrar örneğindeki total porfirin konsantrasyonu referans aralığı içinde idi. DJS tanısı artmış idrar koproporfirin I/total oranı (% 97) tarafından doğrulandı. Günümüzde DJS tanısı, birden fazla tanı testi (örn. karaciğer biyopsi örneğinin histokimyasal incelemesi ve/veya kolesintigrafik görüntüleme) ile DJS'nun karakteristik belirtileri gösterilerek konulmaktadır. Bu hastalığın kesin tanısı çoğunlukla ABCC2 gen mutasyonu analizine dayanıyor olsa da, idrar koproporfirin izomerlerinin tayini DJS tanısı için nispeten kolay erişilebilir ve güçlü bir biyokimyasal gösterge olabilir.

**Anahtar kelimeler:** *Dubin-Johnson Sendrom, Hiperbilirubinemi, Koproporfirin, Porfirin*

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## Introduction

Dubin-Johnson Syndrome (DJS; OMIM No.237500), which was described in 1954, is a rare, benign, inherited metabolic disorder characterized by chronic conjugated hyperbilirubinemia, accumulation of a dark pigment in hepatocytes (1,2) and is caused by a deficiency of canalicular multispecific anion transporter/multidrug resistance protein 2 (cMOAT, MRP2) located in the canalicular membrane of hepatocytes (3). This protein mediates ATP-dependent transport of certain conjugated organic anions across the canalicular membrane of the hepatocyte and exports them from hepatocyte into bile.

A defect in the MRP2 protein is characterized by chronic conjugated hyperbilirubinemia and impaired hepatobiliary transport of non-bile-salt organic anions (3). The difficulty of transporting anionic conjugates from the hepatocyte in DJS patients is manifested clinically as jaundice; histologically as deposition of brown pigment granules in hepatocytes; radiographically as abolished or diminished excretion of administered anionic contrast dyes; on metabolic ground as retention of infused substances requiring conjugation prior to their efficient biliary excretion; and from the physiologic point of view as inversion of coproporphyrin isomers III and I ratio preferentially eliminated in urine (2).

The human ABCC2 gene is localized to chromosome 10q24, spans about 45 kb, and contains 32 exons (4). The first mutation of ABCC2 gene responsible for DJS was detected in 1997 (3). Subsequently, at least 15 different mutations in the ABCC2 gene have been reported in DJS patients (5,6). It has been described in all nationalities, ethnic backgrounds, and races. Although it's most common among the Iranian Jews, with a prevalence of 1:1300 (2) some cases have been reported in our country (7,8).

The DJS, inherited in an autosomal recessive manner with reduced penetrance, is often an occult entity until various factors and situations (eg, stress, pregnancy, excessive alcohol consumption, drug intake, or infections) accentuate hyperbilirubinemia, resulting in clinically apparent disease. Most patients are asymptomatic and have a normal life span. Occasionally patients complain of weakness and vague abdominal pain, and hepatosplenomegaly is rarely observed (1,2).

DJS is scarcely diagnosed in the neonatal period (9), usually manifests during puberty or in adults as mild conjugated hyperbilirubinemia, or intercurrent bouts of jaundice. Jaundice is evident by the time of puberty in about half, and by the age of 20 in about two-thirds, of affected persons (1,2). The results of liver function tests including enzymes are usually within normal limits although the bilirubin levels usually fluctuate. Bile acid excretion and serum bile acid levels are normal (1,2). The disease is completely benign and requires no treatment however, its correct diagnosis is required for prevention of unnecessary investigations.

The diagnosis of DJS is based on the presence of intracellular pigment granules in the liver (1,2), a delayed rise in the sulfobromophthalein (BSP) sodium test (10), highly disturbed excretion on  $^{99m}\text{Tc}$ -HIDA (hepatobiliary iminodiacetic acid) cholescintigraphy (11) and increased levels of coproporphyrin I isomer in the urine while total urine porphyrin output is normal (12), as well as the presence disease-associated mutation of the ABCC2 gene (3).

Herein we present a HBsAg-positive patient with DJS, who has been diagnosed based on elevated urinary coproporphyrin I isomer as well as the presence of pigmented granules within the cytoplasm of the hepatocytes.

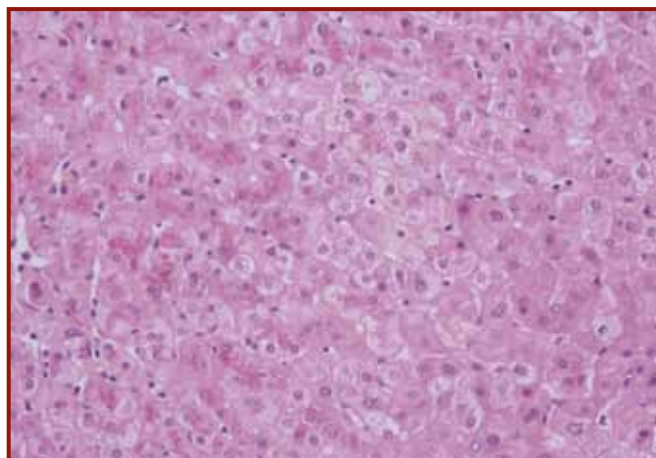
### Case Report

The case was a 21-year-old male who complained about dizziness at military conscription examinations. In physical examination, skin color, sclerae and vital signs were all normal. HBsAg positivity was detected 10 months ago, in his history. Needle biopsy was applied to the patient 2 times. The result of the first one was not assessed because of the insufficient material. At the second one hepatitis B carrierity and accumulation of brown pigment were detected and proposed for the evaluation of the probability of DJS patients.

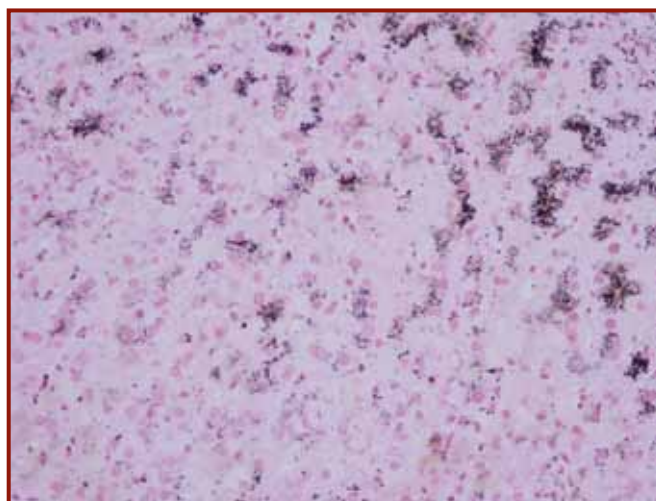
His laboratory data revealed HBsAg sero-positivity (HBsAg (+), HBeAg (-), HBV DNA (+,  $2.1 \times 10^5$ ), anti-HCV (-) and a mild conjugated/ unconjugated hyperbilirubinemia (0.75mg/dL and 1.2 mg/dL; respectively); remaining analytical results were otherwise normal. BSP test was not carried out. Any pathological changes of liver and biliary tract were not observed by ultrasonographic imaging. Cholescintigraphy with  $^{99m}\text{Tc}$ -HIDA was not carried out.

Granular, dark-brownish pigmentation demonstrated with routine haematoxylin-eosin (H-E) staining on liver biopsy reminded the DJS (Figure 1). The granules were positive with Masson-Fontana melanin staining (Figure 2) but negative with lipofuscin, PAS, iron, bile stains (data not shown).

Although total porphyrin concentration (19 nmol/ mmol creatinine) in spot urine sample was within the reference range (<35 nmol/ mmol creatinine); diagnosis of DJS was confirmed by increased urine



**Figure 1.** H-E staining of the liver tissue specimen shows the accumulation of brown granular pigment within the cytoplasm of hepatocytes.



**Figure 2.** Pigment showed positivity with Masson-Fontana stain (original magnification, 400x)

coproporphyrin I/total ratio (97%) (normal 25±6%) on porphyrin fractionation by HPLC (13) (Figure 3). Other members of his family were not accessed for screening.

### Discussion

In this case, diagnosis of the DJS was based on the increased levels of urinary coproporphyrin I/total ratio (97%) as well as the presence of pigmented granules within the cytoplasm of the hepatocytes.

Diagnosis of DJS should be made by demonstration of conjugated hyperbilirubinemia, BSP test, cholecintigraphy, histochemical examination of liver biopsy sample, urinary coproporphyrin isomer analysis and ABCC2 gene mutation analysis in addition to a detailed anamnesis.

Although serum total bilirubin level is usually around 1.5-6 mg/dl and more than half of it consist of conjugated bilirubin in patients with DJS, in our patient there was only a slight elevation in both conjugated and unkonjuged bilirubin levels.

Previously, delayed increase in the BSP test and evidence for the presence of intracellular brown granular pigments in the liver were used in diagnosis of DJS but, currently, BSP is no more available and is ignored because of possible side effects (14). Recently, oral or

intravenous cholecystographic, or cholescintigraphic imaging are being used as an alternative, less damaging and more effective diagnostic methods. Delayed liver visualisation and delayed filling of the gallbladder is typical for DJS at 99m-HIDA cholescintigraphy (11). However, these findings can be easily confused with signs of gallbladder disease if the patient presents with abdominal pain and may result in an unnecessary cholecystectomy; moreover, this technique is quite difficult to be done in childhood.

Macroscopically black liver and histopathologically brown pigmented granules with H-E staining were demonstrated at the liver biopsy of the patient. Although what we know about the nature of these brown granules in DJS is insufficient, evidence currently indicates the anionic metabolites of tyrosine, phenylalanine and tryptophan(15). These coarse, dark-brown pigmented granules share some physiochemical properties with lipofuscin and melanin, variably positive with PAS stain, and stains black with the Fontana stain (16).

Although a macroscopically black tissue sample as well as a histological liver sample with brown pigmented granules is not an infrequent sign in patients with DJS, in fact it is not unique to this disorder. Other conditions for black liver include DJS-like liver

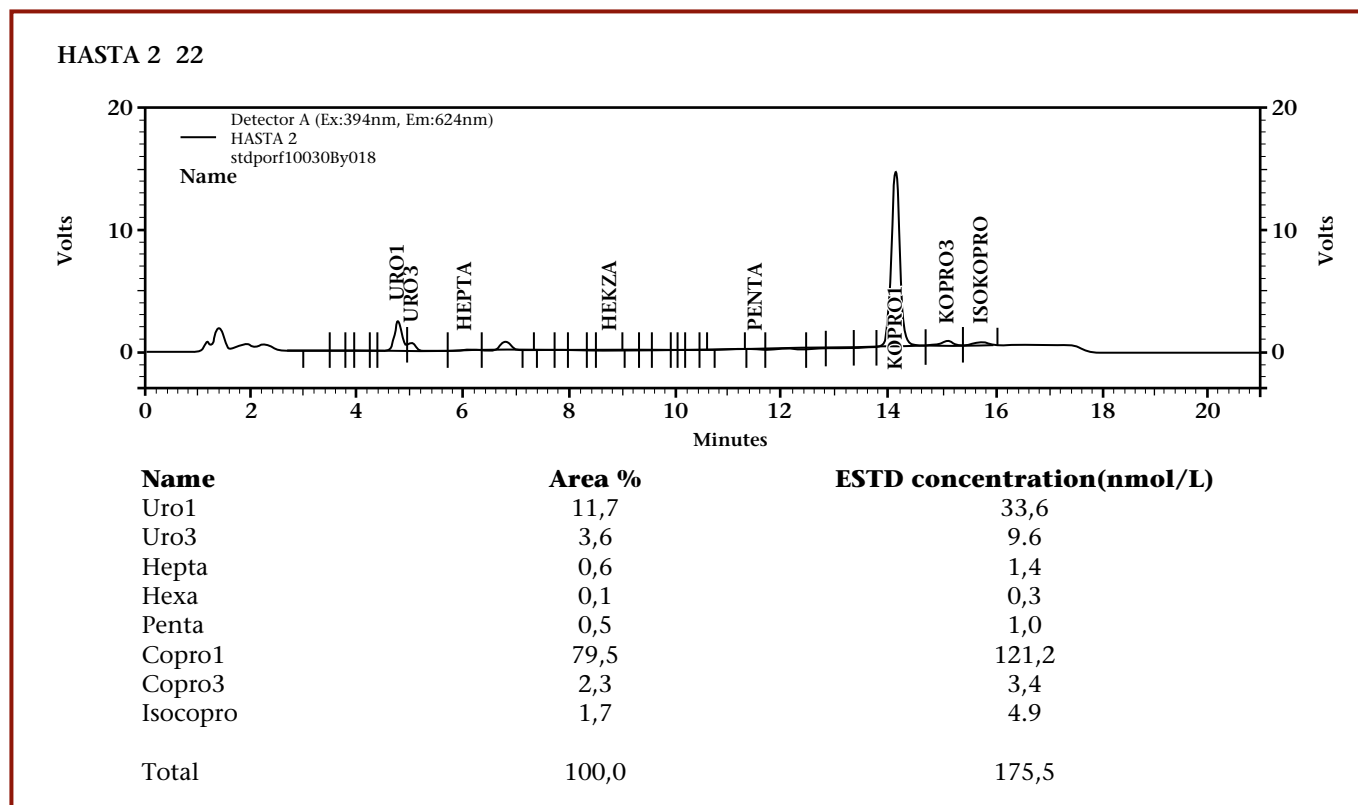


Figure 3. Urine porphyrin fractionation by HPLC

with normal level of serum bilirubin (17) and primary hyperoxaluria (18). Furthermore, intensity of brown granules is variable in liver samples of patients with DJS and does not correlate with serum bilirubin levels (2). Besides, these granules are generally deficient in early childhood period although their presence had been demonstrated in neonatal period of some cases with DJS (19).

Some variability in pigmentation may be due to occurrence of coincidental disease such as acute viral hepatitis. These pigments vanish during the course of acute viral hepatitis infections while reappears at coalescence period (20). In chronic hepatitis, pigment granules may be reduced or even disappear gradually and also may develop deep jaundice in patients with DJS (21,22). Brown pigments were observed at histopathologically examination of liver sample of the presented case with HBsAg seropositivity. Although we don't have the more detailed viral hepatitis panel of this patient, who was diagnosed with chronic hepatitis, the histopathologic finding contrasts with previous publications. A new article, as far as we detect, that show coexistence of HBsAg and DJS is published recently (23). In this study, unlike DJS patients uncomplicated by chronic hepatitis B, myelin-like bodies were found stored in transmission electron microscopy sections of DJS patients complicated by chronic hepatitis B. We were not able to make electron microscopy in this patient.

Determination of urinary coproporphyrin isomers is another approach for the diagnosis of DJS. When coproporphyrin III > coproporphyrin I is seen in normal human urine samples, the ratio of coproporphyrin I to total coproporphyrin is seen as around 50% and >80% in heterozygous and homozygous subject with DJS, respectively (12). In our patient, coproporphyrin I to the total coproporphyrin ratio was 97%,

and this indicates that the patient carry a homozygous mutation in the ABCC2 gene.

In a neonate with cholestasis, significant liver attenuation on abdominal computed tomography along with urinary coproporphyrin I isomer to total coproporphyrin ratio greater than 80% strongly indicates neonatal DJS and in this case an essential invasive liver biopsy can easily be eliminated (24).

Recent studies have shown that, urine coproporphyrin I to total coproporphyrin ratio is affected by ABCC2 polymorphism and reflects in vivo activity of MRP2 (25, 26).

Porphyria metabolism disorder may also be seen in Rotor's Syndrome (RS, OMIM No.237450) during which hereditary jaundice with a predominating conjugated hyperbilirubinemia is seen (2). For discrimination of these two hereditary syndromes characterized with conjugated hyperbilirubinemia, histopathological examination of liver biopsy material, cholescintigraphic imaging and DNA analyses along with urinary coproporphyrin isomer analyses are considered as of great benefit (Table I) (2, 27).

Currently, DJS is diagnosed by demonstrating characteristic signs of the DJS on multiple traditional diagnostic tests (e.g. histochemical examination of liver biopsy material and/or cholescintigraphic imaging).

Although definitive diagnosis of this disorder mostly relies on ABCC2 gene mutation analysis, determination of urinary isomers of coproporphyrin may relatively be an easily accessible and strong biochemical indicator for diagnosis of DJS.

### Kaynaklar

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**Tablo I. Principal differential characteristics of DJS and RS (2, 27).**

	<i>Dubin- Johnson syndrome</i>	<i>Rotor syndrome</i>
Appearance of liver	Black pigmentation	Normal
Histology of liver	Dark pigment, predominantly in centrilobular area; otherwise normal	Normal, no increase in pigmentation
Gallbladder visualization (cholescintigraphy)	Delayed visualized liver, Delayed or no visualization of gallbladder	Neither the liver nor biliary tract is visualized
Urinary total coproporphyrin Urinary Coproporphyrin-I isomer/total coproporphyrin ratio (normal: 25± 6%)	Normal >80%	Elevated < 80%
Mutated gene	ABCC2	?

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