p.Gly693Arg Homozygote Mutation in Dubin-Johnson Syndrome with Atypical Liver Biopsy due to Reactivation of Hepatitis B Concomitant with Persistent Loss of Kidney Function

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ABSTRACT

Dubin-Johnson syndrome is a rare genetic disease that causes impaired transport of bilirubin. In most cases, there will be no symptoms. However, some people might develop jaundice due to certain conditions. In this case, we would like to present a 54-year-old male patient with Dubin-Johnson syndrome confirmed through genetic analysis showing homozygote mutation of p.Gly693Arg, with no apparent bile deposition in liver biopsy and reactivation of hepatitis B. The Patient had no symptoms since birth and was recently found to have an increased level of direct bilirubin. Further inspection showed a familial pattern of the disease. This is a unique case of homozygote mutation with p.Gly693Arg with atypical presentation of liver biopsy and reactivation of hepatitis B with no clinical manifestation.

Keywords**:** *Dubin-Johnson syndrome, homozygote mutation, p.Gly693Arg mutation, Hepatitis B reactivation.*

INTRODUCTION

Dubin-Johnson syndrome (DJS; OMIM 237500) was first reported by Dubin and Johnson in 1954 with 12 cases of persistent idiopathic jaundice and unidentified pigment in the hepatocyte.[1] It is a rare disease with an estimated prevalence of less than one case per 100,000 individuals globally.[2,3] Most individuals diagnosed with DJS are young adults with no symptoms. DJS is an autosomal recessive disorder causing defective production or loss of function of multidrug resistance-associated protein (MRP2) due to a variety of mutations in the ATP-binding cassette subfamily C member 2 (ABCC2) gene.[4]

DJS is commonly present with no symptoms, with the slightest jaundice being its main symptom. Other symptoms, such as weakness and stomach pain, could also be present. Jaundice might be intermittent due to concurrent disease, pregnancy, or use of oral contraceptives.[5] Laboratory examination would find low-grade elevation of conjugated bilirubin in the blood and changes in the metabolism of coproporphyrins, thus increasing urinary excretion of coproporphyrin I relative to coproporphyrin III. Liver biopsy might show accumulation of dark, coarse, granular, melanin-like pigment in the centrilobular hepatocyte with no hepatic injury.[2,3] DJS are usually misdiagnosed, especially in individuals with persistent mild elevation of conjugated bilirubin. Therefore, it is important to rule out other hepatobiliary disorders that might cause liver injury. We aimed to describe a case

of complex hereditary hyperbilirubinemia with a misleading presentation, highlighting the need for in-depth genetic testing.

CASE ILLUSTRATION

A 54-year-old male patient came for a routine medical examination. The patient has a history of jaundice since childhood with unclear etiology. He was diagnosed with hepatitis B after a routine medical check-up in 2005. In 2016, he collapsed and appeared to be jaundiced after a marathon run. Laboratory test in June 2022 **(Table 1)** showed a remarkable hemogram and liver function test. There was an increase in total bilirubin with an elevation in conjugated bilirubin. Kidney function test showed eGFR of 77 ml/min/1.73m^2. Hepatitis B panel showed reactive HbsAg, reactive anti-Hbe, and HBV DNA of 4.01 x 10^2 IU/mL. Transient elastography was performed and found moderate fibrosis (10 kPa). Tenofovir alafenamide and ursodeoxycholic acid treatments were initiated.

The patient is the last child of seven siblings. His first sibling was suspected to have Dubin-Johnson syndrome, but it was not confirmed by a genetic test. The second sibling was diagnosed with hepatitis of an unknown etiology, cirrhosis, hypertension, and kidney failure. The third sibling was diagnosed with hepatitis of an unknown etiology and kidney failure. The fifth and sixth siblings were diagnosed with kidney failure. The first, second, third, fifth, and sixth siblings passed away due to kidney failure.

In August 2022, another blood test was performed, and it showed remarkable results of renal function and liver function test, although total bilirubin remained elevated (**Table 2**).

After monthly monitoring, in September 2022, the total bilirubin level remained high (5.34 mg/dL). Further examination with magnetic resonance cholangiopancreatography (MRCP) and abdominal MRI with contrast was planned. MRCP revealed mild stricture in the right and left intrahepatic duct without dilation **(Figure 1).** Entecavir treatment was added due to the persistent level of bilirubin. In November 2022 (**Table 3**), another full laboratory test was performed. The result of complete blood counts,

Table 1. The result of the laboratory test performed in June 2022.

Complete Blood Count	**Results**	**References**
Hemoglobin	15.6 g/dL	13.1-17.3
Erythrocyte	5.23x10^6/mcL	4.20-5.60
Hematocrit	45.8%	39-50
MCV	87.6 fl	81-101
MCH	29.8 pg/cell	27-35
MCHC	34.1 g/dL	32-36
RDW	12.4%	11.5-14.5
Thrombocyte	237,000/mcL	150-450
Leucocyte	7,100/mcL	3600-10600
Erythrocyte Sedimentation Rate	17 mm/h	0-20
Blood Differential Count	Results	References
Eosinophil	2.1%	0-3
Basophil	0.3%	0-2
Segmented neutrophil	60.5%	50-70
Bilirubin	Results	References
Total	4.95 mg/dL	<1.3
Direct	3.86 mg/dL	
Indirect	1.09 mg/dL	
Liver Function Test (LFT)	Results	References
AST	17 U/L	0-37
ALT	18 U/L	0-50
GGT	19 U/L	<66
ALP	90 U/L	53-128
Hemostasis	Results	References
Prothrombin Time (PT)	14.5 s	12.7-15.9
International Normalized Ratio (INR)	1.00	

hemostasis parameters, and electrolytes remained remarkable. Kidney function test showed an increase in the level of cystatin-C. Urinalysis also found albuminuria of +1 (25mg/dL). Antinuclear antibody (ANA) test found a speckled pattern with 1:100 titers. Multiple bilateral kidney cysts (Bosniak I) were found on abdominal MRI with contrast. A biopsy of the liver was planned. The patient was also referred to a nephrologist, and a kidney biopsy was recommended.

Table 2. The result of the laboratory test performed in August 2022.

Renal Function	**Results**	**References**
Blood Urea Nitrogen (BUN)	33 mg/dL	12.8-42.8
Creatinine	0.92 mg/dL	0.67 – 1.17
eGFR	94 ml/min/1.73 m^2	
Bilirubin	Results	References
Total	5.39 mg/dL	<1.3
Direct	4.51 mg/dL	
Indirect	0.88 mg/dL	
Bilirubin	Results	References
Total	4.95 mg/dL	<1.3
Direct	3.86 mg/dL	
Indirect	1.09 mg/dL	
Liver Function Test (LFT)	Results	References
AST	15 U/L	0-37
ALT	15 U/L	0-50
GGT	19 U/L	<66
ALP	90 U/L	53-128

Table 3. The result of the laboratory test performed in November 2022.

Complete Blood Count	**Results**	**References**
Hemoglobin	16.2 g/dL	13.1-17.2
Erythrocyte	5.43x10^6/mcL	4.20-5.60
Hematocrit	47.9%	39-50
MCV	88.2 fl	81-101
MCH	29.8 pg/cell	27-35
MCHC	33.8 g/dL	32-36
RDW	12.4%	11.5-14.5
Thrombocyte	285,000/mcL	150-450
Leukocyte	7.120/mcL	3600-10600
Hemostasis	Results	References
Prothrombin time (PT)	14s	12.7-15.9
International Normalized Ratio (INR)	0.96	0-2
Activated partial thromboplastin time (aPTT)	40s	26.4-42.6
Renal Functions	Results	References
BUN	27 mg/dL	12.8-42.8
Creatinine	0.97 mg/dL	0.67 – 1.17
eGFR	68 ml/min/1.73 m^2	
Cystatin-C	1.13 mg/dL	0.56-0.98
Hepatitis Panel	Results	References
Anti-HAV	Nonreactive	Nonreactive
Electrolytes	Results	References
Uric acid	4.9 mg/dL	<7
Sodium	135 mmol/L	136-145
Potassium	4.3 mmol/L	3.5-5.1
Chloride	101 mmol/L	98-107
Calcium	9.3 mg/dL	8.4-10.2
Phosphate	2.8 mg/dL	2.7-4.5
IgG	2,090 mg/dL	650-1600
Autoimmune Panel	Results	References
ANA Profile	Speckled, 1:100 titer	< 1:100

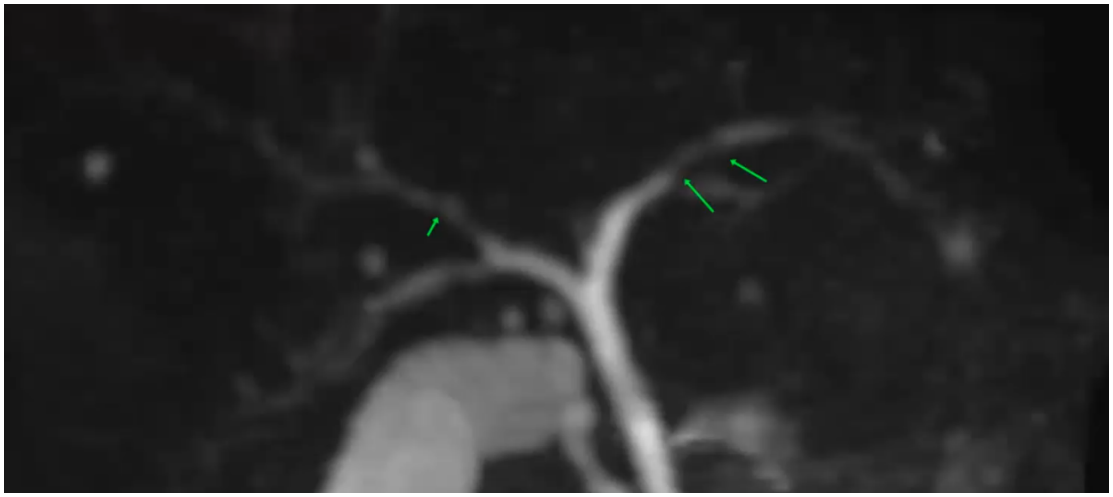

Figure 1. MRCP of the biliary tree showing mild stricture in the branches of the left and right hepatic duct (green arrow).

In December 2022, a liver biopsy was performed and showed moderate steatosis with mild focal lobular inflammation and deposition of coarse brown granular pigment in hepatocytes predominantly in the centrilobular area. No significant inflammatory cells were found in the portal tract, but there was mild focal fibrosis. Fontana-Masson and Prussian blue stain were negative **(Figure 2)**. Kidney biopsy showed glomerulonephritis with focal mesangial hypercellularity and global sclerosis in 2/13 of the glomeruli **(Figure 3).** Immunofluorescence of the kidney showed unclear deposition of IgG, IgA, IgM, C3, and C1q. Silybin-phospholipids 120 mg and vitamin E 400 IU were added as part of therapy.

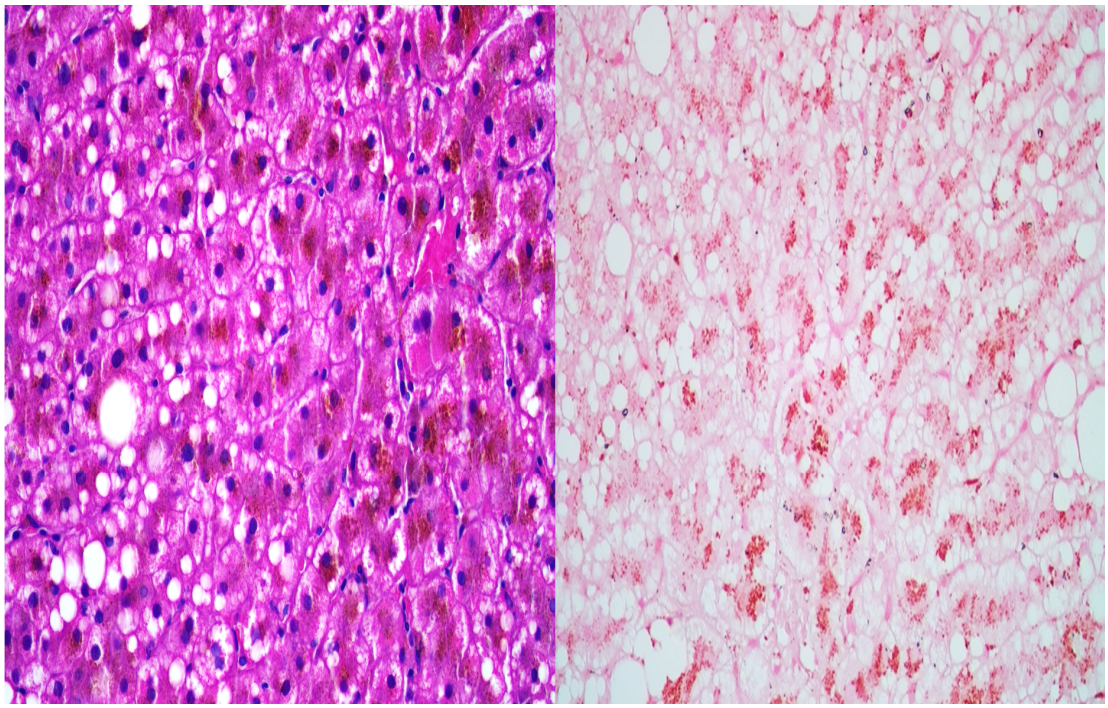

Figure 2. Histopathology of liver biopsy. Hematoxylin-Eosin (HE) staining shows mild steatosis with deposition of brown pigment predominantly in the centrilobular area (A); Fontana-Masson staining shows no black pigment deposition in the hepatocyte (B).

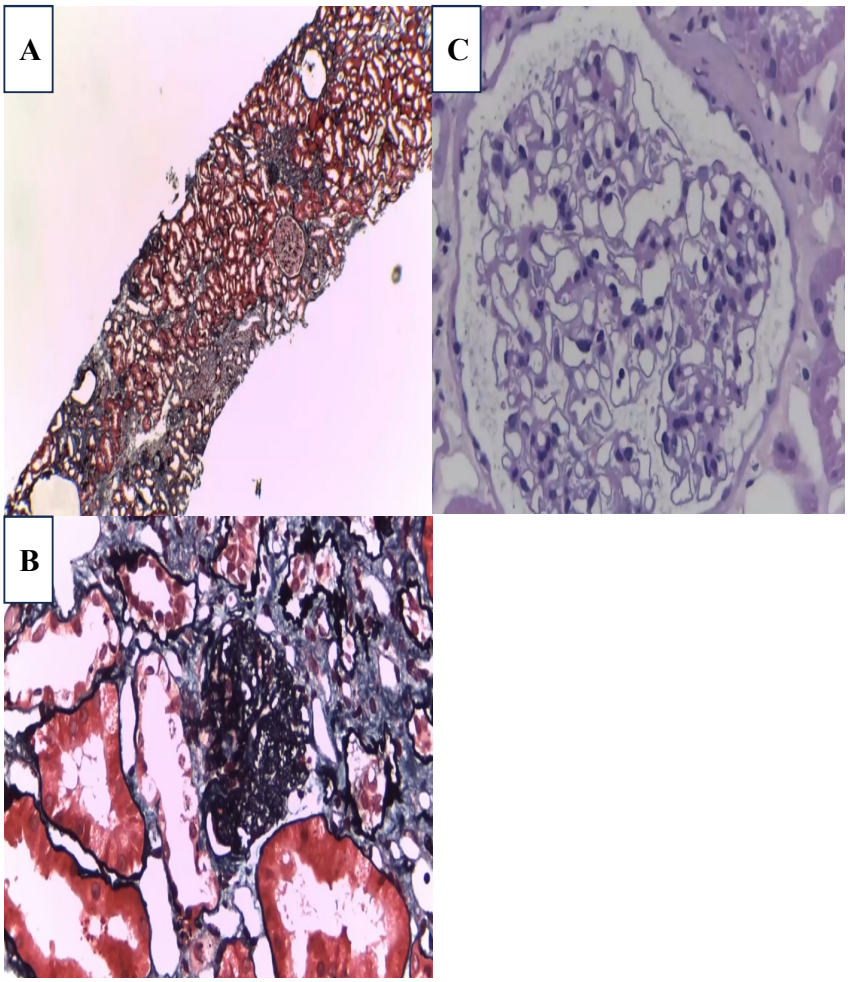

Figure 3. Histopathology of kidney biopsy. Silver trichome staining shows mild atrophy (A); global glomerulosclerosis within 2/13 glomerulus (B); and HE staining shows focal mesangial hypercellularity (C).

In March 2023, the patient returned for regular monitoring. HBV DNA was found undetected. However, total bilirubin was elevated (8.72 mg/dL) with conjugated bilirubin of 7.69 mg/dL and unconjugated bilirubin of 1.03 mg/dL. There was a continuous rise in direct bilirubin within nine months **(Figure 4).** Kidney function test results remained stable. Urinalysis found albumin +1 (25 mg/dL), urobilinogen of +1, and erythrocytes +2 (25µ/µL). Results of the last laboratory test are presented in **Table 4.** The patient was planned for genetic testing due to suspicion of Dubin-Johnson syndrome or Rotor syndrome. Genetic analysis was performed using qPCR to detect a mutation in the ABCC2 gene. Mutation in exon 16 with variant c.2077G>A in homozygous state. This mutation resulted in the substitution of arginine for glycine at codon 693 (p.Gly693Arg).

Currently, the patient is diagnosed with Dubin-Johnson syndrome, chronic hepatitis B, and chronic kidney disease. The patient is planned for continuing antiviral treatment and ursodeoxycholic acid. An angiotensin converting enzyme inhibitor or angiotensin receptor blocker is planned to be given due to the evidence of albuminuria, and regular monitoring of kidney function should be done due to familial risk of kidney failure. Furthermore, this patient also received genetic counselling and education regarding the triggering factors to manage the persistent elevation of conjugated bilirubin.

DISCUSSION

Clinical presentations of this case were mostly asymptomatic. The elevated level of bilirubin was found accidentally during a routine

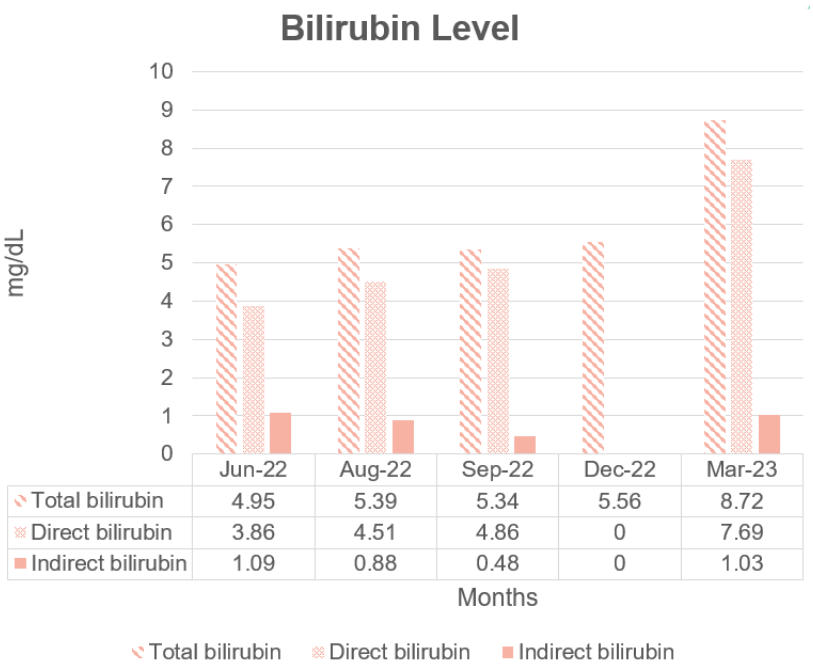

Figure 4. Bilirubin levels of the patient revealed persistent elevation of direct bilirubin.

Table 4. The result of the laboratory test performed in March 2023

Laboratory Test	Results	References
Total bilirubin	8.72 mg/dL	<1.3
Conjugated bilirubin	7.69 mg/dL	<0.5
Unconjugated bilirubin	1.03 mg/dL	
AST	19 U/L	0-37
ALT	14 U/L	0-50
GGT	17 U/L	<66
BUN	24 mg/dL	12.8-42.8
Creatinine	1.09 mg/dL	0.67 – 1.17
eGFR	77 ml/min/1.73 m^2	

medical checkup, and the coexistence of chronic hepatitis B might exacerbate the level of bilirubin in this patient. The presence of moderate fibrosis (10 kPa) might be due to chronic hepatitis B infection.[6] The mild stricture found during MRCP could not explain the persistent increase in total bilirubin level due to minimal proximal duct dilation, and the stricture was mild.[7]

The liver biopsy of this patient showed histopathological features of centrilobular coarse granular brown pigment that can be found in DJS. However, Fontana-Masson staining did not stain the granules black. MRP2 immunohistochemistry can be helpful to identify an absence of MRP2 staining of the canalicular membrane, but it is not currently available in our center. Hunter and Masuda had reported that concomitant infection of hepatitis B could affect the pigment deposition within hepatocytes in DJS.[8,9] The patient described by Masuda had a liver biopsy performed three months after the onset of hepatitis and had no typical pigmentation of DJS.[8,9] Another report by Varma et al. found sufficient pigmentation within one year after the diagnosis of hepatitis B, although the pigment had not yet become coarse as commonly found in DJS.[8,10] The inflammation and necrosis of hepatocytes might lead to the release of the pigment, which is then taken by macrophages or Kupffer cells by macrophages, thus reducing the deposition of pigment found in hepatocytes.[10]

The variant mutation of Gly693Arg in exon 16 of the ABCC2 gene is a missense mutation that causes the substitution of arginine for glycine

at position 693. The sequence of amino acids 693 of MRP2 was conserved among species. An in vitro study on the mutation p.Gly693Arg demonstrated decreased expression of MRP2 in the p.Gly693Arg mutant, attributed to higher degradation of mutant MRP2. MRP2 from p.Gly693Arg is also predominantly located in the cytoplasm rather than the cell surface. On further testing, MRP2 from p.Gly693Arg also demonstrated decreased activity of organic anion transport compared to wild-type MRP2. Therefore, the p.Gly693Arg mutant resulted in downregulation, mislocalization, and decreased organic anion transport activity. A recent report by Wu et al. found that the p.G693R mutation was observed in 2 out of 7 patients, which might indicate the possibility of hotspot mutation of the p.G693R in the Chinese population with DJS.[11] In our patient, genetic analysis showed homozygous mutations of p.Gly693Arg, which has never been reported before.

It is currently unknown whether the presence of kidney failure has any correlation with the genetic origin of the disease (**Figure 5)**. MRP2 is a multiple organic anion transporter found in the liver and kidney. Mutation of MRP2 causes impaired transport of organic anions, such as sulfate, glucuronide, and GSH. MRP2 is also responsible for the elimination of endogenous metabolites like conjugated bilirubin.[4,12,13] In the kidney, MRP2 was found in the apical membrane, and the expression of MRP2 was shown to be increased in cases of chronic renal failure.[14] Although the reduction in kidney function could potentially be associated with a defect in MRP2, there is currently no strong scientific evidence demonstrating a causative relation of DJS with chronic kidney disease. In this study, there is little evidence to suggest that DJS is responsible for explaining the etiology of chronic kidney disease. Therefore, DJS should not be stipulated as the sole perpetrator of the declining kidney function in this patient. Another possible cause of the declining kidney function is hepatitis B virus-associated nephropathy, which typically shows membranous nephropathy.[15] However, biopsy of the kidney did not show immune complex deposition that is usually found in hepatitis B virus-associated nephropathy.[16] Furthermore, even after the treatment of chronic hepatitis B, there were no signs of improvement in the kidney function. Therefore, HBV-associated nephropathy is ruled out as the potential cause of kidney impairment. Another potential etiology that may cause the declining kidney function is polyarteritis nodosa (PAN), a necrotizing inflammation of medium vessels, with or without glomerulonephritis, which can be secondary to hepatitis B.[17] The histopathology of PAN should demonstrate vascular inflammation in medium-sized or small arteries.[17] However, vascular inflammation was not identified from the renal biopsy of this patient. This might be because the biopsy was performed blindly without knowing the affected area of the kidney. Therefore, renal visceral angiography should be performed when a histologic diagnosis of vasculitis cannot be achieved.[17] Familial traits of the kidney failure might also have been caused by a different disease unrelated to the current diagnoses.

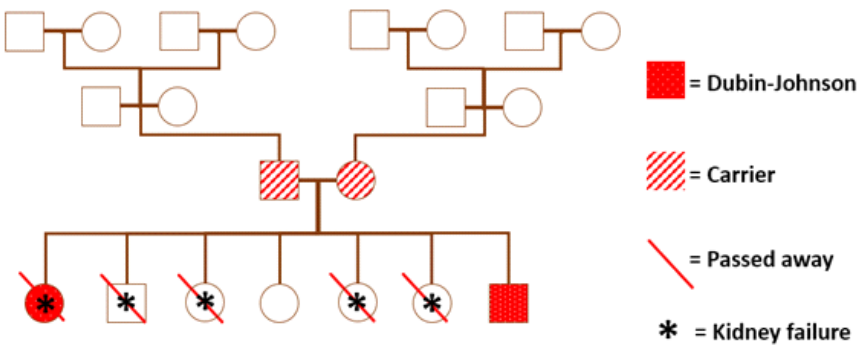

Figure 5. Pedigree of the patient shows possible autosomal recessive inheritance of Dubin-Johnson syndrome.

DJS is a benign condition. It commonly does not show any symptoms, and it does not contribute to the progression of cirrhosis or chronic liver disease. Therefore, DJS typically does not require any specific medical treatment. In case of an elevated level of conjugated bilirubin, which causes symptoms, pharmacological therapy should be considered. Phenobarbital has been studied to be able to stimulate the gene of UGT1A1 and reduce the levels of bilirubin by 25 %.[18] Khan et al. showed 57% and 67% reduction in serum levels of conjugated and total bilirubin in patients with DJS after administration of phenobarbital.[19] The use of ursodeoxycholic acid and rifampicin could also provide some benefits in reducing serum bilirubin in DJS.[20] Ursodeoxycholic acid has the properties to improve bile flow and hepatoprotective effects.[21] However, in this case, the administration of ursodeoxycholic acid did not have any effect on the levels of bilirubin.

CONCLUSION

Dubin-Johnson syndrome is commonly present with no symptoms. Therefore, a thorough examination of clinical history, physical examination, and laboratory tests should be performed to identify Dubin-Johnson syndrome. Persistent elevation of direct bilirubin should be checked and regularly monitored to find other possible causes of hyperbilirubinemia. Absence of typical stain within hepatocytes of DJS might be due to reactivation of hepatitis B, causing loss of bile deposition due to the immune response. Management of the etiology of hyperbilirubinemia, such as hepatitis B, should be the priority to normalize the level of bilirubin.

AUTHOR'S CONTRIBUTION

The study conception and design, data collection, and analysis were performed by Juferdy Kurniawan. Juferdy Kurniawan was responsible for visualization, investigation, original draft preparation, and writing, including reviewing and editing.

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CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

ETHICAL STATEMENT

All procedures performed in the study involving human participants were in accordance with the ethical standards of the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No.KET-951/UN2.F1/ETIK/PPM.00.02/2022, Protocol Number 22-09-1078) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all patients before their involvement in the study.

ABBREVIATIONS

ANA: antinuclear antibody; ABCC2: ATP-binding cassette subfamily C member 2; DJS: Dubin-Johnson syndrome; eGFR: estimated glomerular filtration rate; GSH: glutathione; HBV DNA: hepatitis B virus DNA; MRI: magnetic resonance imaging; MRCP: magnetic resonance cholangiopancreatography; MRP2: multidrug resistance-associated protein 2; PAN: polyarteritis nodosa; UGT1A1: uridine diphosphate glucuronosyltransferase 1A1.

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