

## GALLSTONE DISEASE WITH UNCONJUGATED HYPERBILIRUBINEMIA: CLINICAL APPROACH TO GILBERT'S SYNDROME

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### Abstract

*Gallstones are the commonest ailment affecting the hepato-biliary system. Associated jaundice is usually direct, commonly due to biliary obstructive lesions. Unconjugated hyperbilirubinemia with cholelithiasis is commonly seen with hemolytic disease. In the absence of hemolysis or systemic causes, congenital causes prevail, commonest of which is Gilbert's Syndrome. This study aims to ascertain a clinical approach to the patient of gallstones with Gilbert's syndrome. This is retrospective study of 58 patients with gallstone associated unconjugated hyperbilirubinemia, who underwent surgery over a two-year period. Patients underwent repeat blood investigations and ultrasound to confirm the diagnosis. Obstructive biliary pathology was ruled out by MRCP images; EUS added if indicated. The remaining patients underwent genetic test for Gilbert's syndrome – namely UGT1A1 gene assessment by PCR. All patients underwent laparoscopic cholecystectomy as routine; with addition of intra-operative liver biopsy. Sixteen of the 58 patients were short-listed to be high risk factors for harboring Gilbert's syndrome after ruling out other systemic causes. On gene study, 14 patients tested positive for UGT1A1 gene, hence Gilbert's syndrome. The other two were kept on follow up for jaundice recurrence in future. The management algorithm is depicted as flowchart. Gilbert's syndrome can be identified in select "high-risk" individuals presenting with gallstone disease. Genetic testing is gold standard, and helps in effective management and better patient counselling.*

**Keywords:** *cholelithiasis, indirect, congenital hyperbilirubinemia, cholecystectomy, jaundice*

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### Introduction

Cholelithiasis is a common surgical problem affecting adults around the world, with laparoscopic cholecystectomy as the standard accepted treatment. The association of hyperbilirubinemia along with cholelithiasis requires further evaluation and management. Conjugated or direct hyperbilirubinemia is

usually due to obstruction in the biliary tree by stone or sludge; which requires endoscopic intervention prior to surgery. Unconjugated or indirect hyperbilirubinemia maybe due to hemolysis, heart disease, pregnancy, estrogen replacement therapy, medications, total parenteral nutrition (TPN), rapid weight loss, hematological, gastrointestinal and hepatobiliary disorders and various infections [1]. In addition,

this may be congenital in origin, the commonest etiology being Gilbert syndrome (GS) [2].

Gilbert syndrome is an autosomal dominant genetic disorder that results from increased serum unconjugated bilirubin level in absence of liver disease, hepatitis or overt hemolysis [3]. Patient presents with intermittent jaundice & no abnormal physical finding other than icterus [2]. The cause of indirect hyperbilirubinemia is reduction in hepatic glucuronidation activity. There exists a genetic basis that explains the association between Gilbert's syndrome and cholelithiasis [4]. The bilirubin monoconjugates seen in circulation in patients with GS may play a role in pigment stone formation.

The aim of the present study is to define the clinical approach in patients of gallstone disease (GSD) with unconjugated hyperbilirubinemia; and to ascertain the diagnosis of Gilbert's syndrome. The study also aims to look back on any identifiable risk factor that may contribute to the diagnosis, and the precautions to be taken.

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## Materials and Methods

### *Study Design and Patients*

This is a retrospective review of prospectively maintained data of patients operated in the department of surgical gastroenterology at a tertiary level referral institute in Northern India. The duration of study was about two years, between Jan-2017 and Dec-2018. A total of 58 patients (37 female and 21 male) of GSD with associated unconjugated hyperbilirubinemia were included in the analysis. Patients with identifiable pathology causing obstructive jaundice associated with cholelithiasis were excluded.

### *Pre-operative routine investigations*

Most of these patients were referred to our out-patient department with liver function tests and ultrasound of abdomen done elsewhere. History and physical examination findings were recorded, including associated predisposing factors like hemolytic disorders, liver diseases, Crohn's disease, artificial heart valves, ileal resection, TPN, rapid weight loss, estrogen replacement therapy, or positive family history.

Liver function tests were always repeated, and a finding of unconjugated hyperbilirubinemia

was regarded as an indication for further work-up. Other hematological and biochemical tests were recorded. All the patients underwent a repeat institutional ultrasound of whole abdomen and Magnetic Resonance Cholangiopancreatography (MRCP) as a routine protocol to rule out any occult biliary tract obstruction as the cause of hyperbilirubinemia in GSD.

### *Specific genetic testing*

The patient with unconjugated hyperbilirubinemia (Indirect Bilirubin <5mg/dL; Direct bilirubin < 20% of Total Bilirubin) in whom obstructive pathology, underlying liver disease and hemolysis were ruled out; were subjected to genetic testing of UDP Glucuronyl Transferase Family 1 Member A1 (UGT<sub>1A1</sub>) gene by polymerase chain reaction (PCR). The presence of two polymorphism in UGT<sub>1A1</sub> gene, namely G71R in exon 1 and A(TA)<sub>7</sub>TAA in the TATA box of promoter region were considered responsible for Gilbert's syndrome. PCR by direct DNA sequencing is currently the gold standard diagnostic test for Gilbert's syndrome.

### *Surgery*

All patients were taken up for routine laparoscopic cholecystectomy, along with an intra-operative liver biopsy. Conventional four-port approach utilizing energy devices such as monopolar diathermy and ultrasonic Harmonic® scalpel shears (Ethicon Inc., Johnson & Johnson, Somerville, NJ, USA). Cystic duct and artery were ligated with plastic Hem-o-Lok® clips (Weck Closure Systems, Research Triangle Park, NC, USA). Drains were not routinely inserted. Post-operatively, liver function tests were repeated on day one. In the absence of any obvious surgical complications, patients were usually discharged by first or second post-operative day; and followed up on out-patient basis after 10 days.

### *Ethical clearance*

Being an observational retrospective study, clearance from the institutional ethical committee was not considered mandatory. Since there was no experimental intervention involved, and the genetic testing was previously established, the study was conducted sans any ethical clearance. All patients consented to testing and surgery.

## Results

Among 58 individuals of cholelithiasis with unconjugated hyperbilirubinemia on initial presentation, three patients were excluded as they had normal LFT on repeat investigations. After ruling out other causes of unconjugated hyperbilirubinemia, 16 individuals suspected of Gilbert's syndrome underwent UGT<sub>1A1</sub> gene PCR testing for confirmation.

Fourteen of these patients, 12 males & 2 females (M:F ratio 6:1), were found to be UGT<sub>1A1</sub> gene positive. Mean ( $\pm$ SD) age of presentation was 26.58 $\pm$ 4.7 years and 27.5 $\pm$ 5.2 years for males and females respectively. The other two patients were further evaluated with Endoscopic Ultrasound (EUS) and Fibroscan, and kept on regular follow up.

All patients underwent laparoscopic cholecystectomy. Prior to surgery, patients were counselled regarding the nature of jaundice; chance of recurrent or persistent jaundice post-surgery, due to Gilbert's syndrome. Intra-operative findings of all patients were unremarkable; no obvious difficulty, aberrant anatomy or adverse events noted. Two patients had thick walled gallbladder (wall thickness >4 mm), which appeared inflammatory.

On final histopathology, gallbladder specimen in all patients were unremarkable. Majority of liver biopsy (n=11/14) were reported normal; with the remaining three showing evidence of hypertrophy of agranular endoplasmic reticulum.

The immediate post-operative course was unremarkable in most patients. Two patients had recurrent jaundice on first post-operative day, which was unconjugated in nature. All patients discharged home by second post-operative day, and followed up after 10 days. On follow-up, none of the patients were symptomatic, and showed excellent post-operative recovery. They were all back to their daily activities without much hindrance.

## Discussions

Gilbert's syndrome is a genetic condition characterized by mild unconjugated hyperbilirubinemia with recurrent episodic exacerbations. It is not an uncommon condition with a worldwide prevalence rate between 4%

and 16% [5]; with about 7.4% prevalence in northern India [6]. In a recent report, the incidence of cholelithiasis in GS was determined to be 8.9%, with a slight male preponderance (9.0% vs 7.1%) [7].

The genetic basis for pathogenesis of GS is based on mutation in the promoter region of UGT<sub>1A1</sub> gene, resulting in increased bilirubin monoglucuronide excretion in bile [4],[8]-[12]. Two polymorphisms in UGT<sub>1A1</sub>; namely G71 R exon and A(TA)<sub>7</sub>TAA in the TATA box of promoter region are most prevalent, and responsible for development of GS [8]-[10]. The length of TA repeats in the promoter region is a modulator of enzyme activity; the greater the number of repeats, higher the serum bilirubin levels and greater the association with cholelithiasis [11],[12]. Studies show increased risk for development of cholelithiasis in patients with coinheritance of GS [13],[14]. Persistently elevated bilirubin levels are by itself a high-risk factor for black pigment stone formation [15]. The increased biliary output of bilirubin monoglucuronides seen in GS, along with reduced enterohepatic circulation of bile salts might present additional risk factor for cholelithiasis [16].

Symptomatic gallstone disease is a common occurrence among adult obese women, especially in northern India [17],[18]. However, increased prevalence of GS with GSD was found among middle aged male patients in our study. This is in accordance with literature, which supports GS as predominantly affecting middle aged males [7],[19]. When GSD is associated with hyperbilirubinemia, they usually require further evaluation with MRCP or EUS to rule out choledocholithiasis, malignancy or underlying liver disease. Unconjugated hyperbilirubinemia in an otherwise normal individual poses a challenge in evaluation and management.

According to the Genetic Testing Registry data provided to the National Centre for Biotechnology Information (NCBI), the genetic testing of GS by PCR with the UGT<sub>1A1</sub> gene has 99-100% sensitivity and 100% accuracy and precision. However, the limited availability and high cost of this investigation limits its use in routine cases. Moreover, the diagnosis of GS would be of academic interest and would not alter the management protocol in the patient who presented for symptomatic gallstone disease.

However, it would allow us to sound the patient regarding the genetic condition, its propensity to cause clinically apparent jaundice occasionally, including in the immediate post-operative period. In our study, patients who were strongly suspected as GS underwent genetic testing, which helped to avoid unnecessary and repeated investigation in the peri-operative period. As evidenced by our study, neither routine Fibroscan nor liver biopsy contributed to the diagnoses and management of this condition.

Our study showed the prevalence of UGT<sub>1A1</sub> gene positivity in 14 of 16 patients suspected to harbor GS. Among other factors, we noted lower values of hemoglobin, hematocrit and absolute red blood cell (RBC) count among these patients; which could indicate an underlying occult hemolysis as an added cause of unconjugated hyperbilirubinemia. According to literature, coinheritance of GS with other disease that increase unconjugated hyperbilirubinemia, such as beta-thalassemia and hereditary spherocytosis, have an increased risk factor for development of gallstones [14],[20].

Hence, it is important to be aware of GS in patients presenting as gallstones with unconjugated hyperbilirubinemia. A prudent approach can avoid unnecessary and repeated investigations. Additionally, an informed consent should be obtained for persistent and fluctuating jaundice despite uneventful surgery owing to GS, as it can avoid future confusion. We recommend performing genetic testing for GS on “high-risk” patients suspected to harbor the condition; namely the middle-aged male patient with persistent unconjugated hyperbilirubinemia in whom all other contributory factors have been ruled out (Table 1). This will not only improve the quality of practice and patient care, but reduce health care costs by avoiding unnecessary and invasive investigations.

Gilberts’ patients usually present with jaundice exacerbation following periods of stress or starvation. Hence, it is expected to witness some jaundice in the post-operative period after major abdominal operations under general anesthesia [21]. In our case series, two patients developed recurrent jaundice immediately post-surgery; which resolved spontaneously within a week. The pre-operative counselling helped allaying any fears in the mind of patients, with regards to unsuccessful surgery.

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- Persistent unconjugated hyperbilirubinemia despite repeat testing

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  - Systemic and hemolytic causes of unconjugated hyperbilirubinemia ruled out

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  - MRI/EUS ruled out any biliary obstructive lesions

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  - Middle aged male patients

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  - History of recurrent jaundice, especially provoked by starvation or stress

**Table 1 – “High-risk group” for Genetic Testing for Gilbert’s Syndrome in Cholelithiasis.**

Our study is limited by the retrospective and observational nature of its design. However, considering the relatively uncommon association of Gilbert’s syndrome with cholelithiasis which we encounter in daily practice, designing a prospective or randomized study would not be feasible. Also, we were unable to comment on any long-term follow-up; since once fully recovered, these patients hardly ever returned to the surgical team. Moreover, there was an overlap in patients with biliary obstructive lesions presenting with raised unconjugated fraction. This may be explained by the effect of prolonged cholestasis on hepatocyte function.

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## Conclusion

Gilbert’s syndrome should be considered as a cause of recurrent and persistent unconjugated hyperbilirubinemia in the middle-aged male patient presenting with cholelithiasis. Genetic testing with PCR is diagnostic and allows patient counselling.

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