Benign Jaundice In Healthy Blood Donors In The Kashmir Valley Of Indian Subcontinent

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Abstract

Background: Benign asymptomatic jaundice is frequently encountered in clinical practice. However its prevalence is unknown.

Setting: SheriKashmir Institute of Medical Sciences, a 650-bedded tertiary care hospital in Srinagar, the summer capital of the state of Jammu & Kashmir (India)

Aims: To ascertain the prevalence of Gilbert's syndrome amongst healthy blood donors attending the SKIMS.

Methods: 1000 randomly selected blood donors were screened with assessment of bilirubin. Those with hyperbilirubinemia were further evaluated for a cause of jaundice. A fasting test, liver enzymes, hepatitis viral serology, ultrasound examination was performed in all the hyperbilirubinemic patients.

Results: Thirty (3%) blood donors had evidence of Gilbert's syndrome.All were previously unaware of a hyperbilirubinemic state. Serum bilirubin levels were 2.64 ± 0.86 versus 0.53 ± 0.29 in controls, p=0.000). No other cause of jaundice could be identified upon investigation. Caloric deprivation test resulted in a increase in the serum bilirubin levels, findings consistent with Gilbert's syndrome.

Conclusion: Gilberts syndrome is seen in 3% of healthy voluntary blood donors in Kashmir.

INTRODUCTION

Gilbert's syndrome consists of chronic, mild, unconjugated hyperbilirubinemia in the absence of overt hemolysis or evidence of structural or functional liver disease([1,2]). The elevated serum bilirubin concentration is usually noted first in adolescence, either as an incidental finding or because of a slight yellow discoloration of the sclerae. The serum bilirubin concentration characteristically fluctuates daily, occasionally falling within the normal range but at other times, especially during fasting, physical exercise, stress, intercurrent illness, or menstruation, exceeding the upper limit of normal, sometimes by as much as three to four times. The way in which the diagnostic level is selected is relatively arbitrary, which may account for the wide variation in the reported incidence of Gilbert's syndrome, which ranges from 0.5 to 10 percent in different groups. Because the syndrome is often familial, it is assumed to be inherited, most likely transmitted as an autosomal dominant trait ([3])

Gilbert's syndrome is an entirely benign and clinically inconsequential entity, requiring neither treatment nor long-term medical attention. Its clinical importance lies in the fact that the mild hyperbilirubinemia may be mistaken for a sign of occult, chronic, or progressive liver disease. Since the diagnosis is largely one of exclusion, clinicians sometimes find it difficult to dispel lingering fears of serious liver disease, causing patients unwarranted anxiety. The patients often seek a number of medical consultations and adopt a very strict dietary schedule thinking it to be a prolonged hepatitis like illness severe possible consequences. Icteric plasma of a donated blood may attract discarding of the blood at times. The current study addresses the magnitude of the problem in healthy blood donors of Kashmir valley that would give a fair idea of the frequency of the problem. No studies are available from the Indian subcontinent that address this problem.

MATERIAL AND METHODS

A total of 1000 randomly selected healthy voluntary blood donors, generally relatives of admitted patients or those attending blood donation camps organized by the department of Blood Transfusion & Immuno-Hematology of SheriKashmir Institute of Medical Sciences, Srinagar, Kashmir, a tertiary care cum referral centre, were included in the study. The donors undergo a clinical examination before they are bled. A sample of the blood donated was screened for serum bilirubin levels and all those with raised serum bilirubin were evaluated further. Donors with history of hepatitis, history of any other liver disease, or those with raised liver enzymes were excluded from further study. 3 ml of blood was collected and transported in a dark box to the biochemistry lab for estimation of serum bilirubin. All patients with serum bilirubin of greater than 1.1mg/dl were examined clinically and investigated further. Direct and Indirect bilirubin estimation was done by Vandenbergh reaction and on the basis of the results thereof, the patients were classified as having a predominant unconjugated or conjugated hyperbilirubinemia.

The investigations of the hyperbilirubinemic patients included a complete blood count (Sysmex-SF-3000). Urine examination for bilirubin and urobilinogen was done by dipstick method. Hemolytic profile was performed on every patient which included examination of the peripheral blood film, reticulocyte count, direct and indirect Coomb's test, plasma hemoglobin estimation, sucrose lysis test and G6PD estimation. Serum bilirubin, aspartate and alanine aminotransferases, alakline phosphatase, serum proteins, albumin, lactic dehydrogenase, and other baseline biochemical investigations were performed on a Hitachi 704 multichannel analyzer (Hitachi, Japan). Hepatitis serology consisted of serology for hepatititis A, B, C, D and E viruses. Ultrasound examination of the patients was performed and a coagulogram. A caloric deprivation test was performed in all the patients. Serum bilirubin measurements were done at baseline and after 48 hours of a 300 kcal /day caloric deprivation. Patients with Gilberts syndrome had a 3 to 5-fold exaggeration of the baseline bilirubin level.

Informed consent was obtained from all the subjects for participation in the study and the study was approved by the institutional postgraduate and ethics committee. Students t-test for paired samples were used for various statistical comparisons. Data have been expressed as mean ± SD and a p-value of <0.05 was considered significant.

RESULTS

Thirty (3%) patients of the 1000 screened blood donors had hyperbilrubinemia (serum bilirubin of 2.64±0.86 versus 0.53±0.29 in controls, p=0.000). The hyperbilirubinemia was predominantly unconjugated (>80% fraction being unconjugated) in all the patients (table 1). Ten of the cases upon questioning gave a history of mild fluctuating jaundice in the past that was never evaluated. None of the cases had a history of transfusions, hepatitis like illnesses or exposure to hepatotoxic agents. Clinical examination was normal in all cases.

Mean levels of AST amongst cases were 31.23±7.61 IU/l (range 11-40) and amongst controls 40.59±15.50 (range 3-91, p=0.001) whereas those of ALT were 31.73±6.86 (range 16-41 IU/l) amongst cases and 39.96±14.23 (range 4-111, p=0.002) among controls. Urobilinogen or bilirubin was not detected in urine. There was no clinical or biochemical evidence of hemolysis with normal levels of LDH, a normal peripheral blood film, normal baseline coagulogram and a negative screen for hemolysis (table 1). Ultrasound scan of the abdomen revealed a normal hepatobiliary system in all cases. All the cases were negative for hepatitis B surface antigen, IgM anti HBc, IgM anti HCV, IgM anti HAV,and IgM anti HEV.

Upon caloric deprivation all the patients exhibited a rise in the bilirubin concentration, the unconjugated fraction exhibiting a 101% rise and the total bilirubin exhibiting a 100% rise. The results have been depicted in table 2.

DISCUSSION

Asymptomatic hyperbilirubinemia was observed in 3% of the cases screened and the diagnosis of Gilbert's syndrome was secured by the absence of any other identifiable causes and a positive caloric deprivation test. This is the first study of this disorder from this part of the globe and the prevalence is similar to the prevalence of the disorder in other geographical regions of the world. A prevalence of 2 to 12% has been reported from various parts of the world depending upon the populations screened and the methods adopted for assessment of the cohorts ([3,4,5,6,7]). A female preponderance in our cases is probably due to the fact that females constituted only a small percentage of the donor population and a coincidental number of cases with jaundice in them. Gilberts syndrome has a male preponderance of 2.7:1

Unconjugated hyperbilirubinemia in Gilbert syndrome has

long been recognized as due to underactivity of the conjugating enzyme system bilirubin-uridine diphosphate glucuronyl transferase (bilirubin-UGT) which is responsible for conjugating bilirubin into bilirubin monoglucuronides and diglucuronides and is located primarily in the endoplasmic reticulum of hepatocytes. Bilirubin-UGT is one of several UGT enzyme isoforms responsible for the conjugation of a wide array of substrates that include carcinogens, drugs, hormones, and neurotransmitters ([1,2,3]).

The diagnosis is generally fairly straight forward and generally diagnostic tests are difficult to justify. Provocation tests consist in fasting, nicotinic acid administration, rifampicin administration, thin layer chromatography ([8]). Polymerase chain reaction can be used to as a novel and rapid method to demonstrate the polymorphism in the TATA box of the UGT1*1 gene using fluorescence resonance energy transfer ([9]).

The gene that expresses bilirubin-UGT has a complex structure and is located on chromosome 2. There are 5 exons, of which exons 2-5 at the 3' end are constant components of all isoforms of UGT, coding for the UDP-glucuronic acid–binding site. Exon 1 encodes for a unique region within each UGT and confers substrate specificity. However, multiple exon 1s (at least 13) exist, and, to complete the gene, one of these exons must be recruited. Exons 1a and 1d encode the variable region for bilirubin UGT1*1 (also known as UGT1A1) and UGT1*2, respectively, with UGT1*1 responsible for virtually all bilirubin conjugation and UGT1*2 playing little, if any, role. Expression of UGT1*1 depends on a promoter region in a 5' position relative to each exon 1 that contains a TATAA box. Impaired bilirubin glucuronidation therefore may result from mutations in exon 1a, its promoter, or the common exons ([8])

A breakthrough in understanding the genetic basis of Gilbert syndrome was achieved in 1995, when abnormalities in the TATAA region of the promoter were identified ([10]). The addition of 2 extra bases (TA) to the TATAA region interferes with binding of the transcription factor IID and results in reduced expression of bilirubin-UGT1 (30% of normal). In the homozygous state, diminished bilirubin glucuronidation is observed, with bile containing an excess of bilirubin monoglucuronide over diglucuronide. Some healthy Asian patients with Gilbert syndrome do not have mutations at the promoter level but are heterozygotes for missense mutations in the coding region. ([11]) In a recent study from Kolkatta, India, among the GS 80% were homozygous for the TA insertion, which was several-fold higher than reports from other ethnic groups. Thus genetic epidemiology of GS is variable across ethnic groups and the epistatic interactions among the UGT1A1 promoter variants modulate bilirubin glucuronidation.([12])

Toxicity of the anti-neoplastic agent Irinotecan has been of a recent concern in patients with Gilbert syndrome. Affected patients have reduced inactivation of the active topoisomerase inhibitor 7-ethyl-10-hydroxycampothecin (SN-38) caused by a mutation in the UDT-1*1 gene promoter. Glucuronidation rates of the active metabolite SN-38 are significantly lower in people who are homozygous and heterozygous for the TA-TATAA variant allele compared with the wild-type genotype (TATAA). Reduced glucuronidation of SN-38 leads to SN-38 toxicity. Preliminary results from clinical trials suggest that screening cancer patients for the UGT1*1 promoter polymorphism may reduce the prevalence of irinotecan toxicity, and, until this evidence is available, caution is warranted before prescribing irinotecan in this subset of patients ([13,14]).

We conclude that Gilbert's syndrome is a frequent clinical entity in our population and few basic investigations should help clinicians arrive at a diagnosis and allay the anxiety of the patients. Also the disorder should be ruled out before putting a patient on antineoplastic regimens containing irinotecan.

Figure 1

Table 1: Showing the various parameters in 30 patients with Gilbert's syndrome

	Parameter	Values (n=30) Mean + SD (range)
1.	Hemoglobin (g/dl)	14.14 ± 1.30 (10.70-15.90)
2.	Leucocyte count (x10^9/l)	7.02 + 1.55 (4.50- 11.0)
3.	Platelets (x10^9/l)	222 ± 56.16 (138-331)
4.	S. Bilirubin (mg/dl)	2.64 ± 0.86 (1.45- 4.20)
5.	Unconjugated bilirubin (mg/dl)	2.12 ± 0.61 (1.35-3.40)
6.	Conjugated bilirubin (mg/dl)	0.50 ± 0.35 (0.08-1.25)
7.	Aspartate aminotransferase (IU/l)	31.23 ± 7.61 (11-40)
8.	Alanine aminotransferase (IU/l)	31.73 ± 6.86 (16-41)
9.	Alakaline phosphatase (IU/l)	225.70 ± 34.87 (138-270)
10.	Lactic dehydrogenase (IU/L)	319.43 ± 32.23 (247-384)
11.	Serum Proteins (g/dl)	7.79 ± 0.74 (6.1-9.4)
12.	Serum albumin (g/dl)	4.62 ± 0.48 (3.2- 5.5)
13.	Reticulocyte count (%)	1.22 ± 0.28 (0.87- 1.9)
14.	Plasma hemoglobin (gm/dl)	3.15 ± 0.52 (2.0-4.0)
15.	Coomb's test (Direct & Indirect)	negative
16.	Sucrose lysis test	negative
17.	G 6PD levels	normal
18.	Prothrombin time (s)	14.20 ± 1.45 (12-17)
19.	APTT (s)	30.56 ± 2.09 (27-35)
20.	INR	1.33 ± 0.12 (1.20- 1.6)

Figure 2

Table 2: Showing the results of the caloric deprivation test.

	Baseline	Postfasting
1. Bilirubin (mg/dl)		
Unconjugated	2.16 + 0.72 (1.35-3.40)	4.27 + 0.99 (2.76 - 6.90)
Total	2.54 + 0.88 (1.45-4.20)	5.28 + 1.00 (3.60-7.10)
	Paired t-test p=0.000	

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