The Prognostic Value of Serum Gamma Glutamyltransferase Activity in Chinese Infants with Previously Diagnosed Idiopathic Neonatal Hepatitis

JS Wang, ZL Wang, XH Wang, QR Zhu, S Zheng

Abstract

Background and Aim: The significance of serum gamma glutamyltransferase activity and its dynamics have not been well investigated in Asian patients with idiopathic neonatal hepatitis. This study aimed to explore the significance in Mainland China. Methods: A retrospective analysis was performed in 38 cases with a diagnosis of idiopathic neonatal hepatitis. Their prognosis was compared between low or high gamma glutamyltransferase groups, and the mechanics of biochemical parameters were analysed. Results: Poor prognosis was found in 5 out of 6 patients with low gamma glutamyltransferase, compared to 3 out of 32 patients with high gamma glutamyltransferase (P=0.001). Dynamic analysis found that serum gamma glutamyltransferase levels gradually rose to a peak and then normalised along with a remission of jaundice in patients with good prognosis. However, the serum gamma glutamyltransferase level was consistently low in spite of the fluctuations of bilirubin and aminotransferases level in 5 cases with poor prognosis. The gamma glutamyltransferase level was high at presentation and then decreased along with their clinical deterioration in the other 3 cases with poor prognosis. Conclusions: Gamma glutamyltransferase can be used as a prognostic parameter in Chinese infants with intrahepatic cholestasis. Serum gamma glutamyltransferase being consistently low or decreased along with jaundice persistence or deterioration predicts poor prognosis.

Key words ABCB11 protein, human; Cholestasis, intrahepatic; Gamma glutamyltransferase; Jaundice; Neonates

Introduction

Cholestasis is the commonest presentation of liver disease in infancy and affects about 1 in every 2500-5000 live newborns.1,2 Despite extensive investigations, the aetiology of cholestasis in a significant proportion of newborn babies continues to be unknown. Termed as idiopathic neonatal hepatitis (INH), it can comprise up to 25-30% of cases of neonatal hepatitis syndrome.3-5 Most infants with INH will recover completely, but a small proportion of them may progress to end stage liver diseases (ESLD), such as cirrhosis or liver failure, and eventually lead to death or liver transplantation in the first decade of life. Normal or low level of serum gamma glutamyltranspeptidase (GGT) was first described as a predictor of worse prognosis in patients with INH in 1987.6 The last decade has seen the identification of newer diagnostic entities – progressive familial intrahepatic cholestasis (PFIC) 1 that is caused by mutations in FIC1 gene, PFIC-2 that is caused by mutations of bile salt exporting pump (BSEP) gene, and others, that associated with a disproportionately low serum activity of GGT accounting for some of these patients initially labeled as idiopathic.7-9 Although it was named familial, PFIC-1 or -2
has been diagnosed in many sporadic cases also by genetic studies.\textsuperscript{10,11} The long-term outcome of PFIC is much worse than in other INH children.\textsuperscript{7,8} Recently, it was also reported that the mechanics of serum GGT might predict the severity of liver diseases in INH children with good prognosis.\textsuperscript{12}

There is little information on the significance of serum GGT as a prognostic parameter for infants with intrahepatic cholestasis in Asian\textsuperscript{13,14} nor has it been investigated whether PFIC cases exist in Mainland China. The purpose of this paper is to explore the relationship of the dynamics of serum GGT and prognosis in Chinese infants who were diagnosed with INH in our center from June 2001 to May 2004.

Materials and Methods

Definition

In this study, conjugated hyperbilirubinaemia was defined as serum total bilirubin (TBil) exceeding 85 \( \mu \text{mol/L} \) with a conjugated fraction of more than 15 percent of the total. Intrahepatic cholestasis of infancy was defined as conjugated hyperbilirubinaemia with elevated blood level of liver enzymes presenting before three months of age where diseases affecting the extrahepatic biliary system, such as biliary atresia, choledochal cyst or tumour, inspissated bile, or haemangioma, etc., had been excluded by imaging of the hepatobiliary system, including ultrasound scan and HIDA scintigraphy in every case and laparotomic cholangiography in selected cases. INH was defined when no specific etiologic factor could be ascertained after a comprehensive conjugated hyperbilirubinaemia work-up. Briefly, the work up include full blood count, Coombs' test, TSH and free T4, fasting cortisol, spine X-ray, ophthalmology exam, blood and urine culture, TORCH screen, syphilis serology, hepatitis A virus IgM, hepatitis B virus surface antigen, hepatitis C virus antibody, hepatitis E antibody. If an unexpected hepatosplenomegaly existed, bone marrow aspiration was done for further exclusion of storage disorders such as Nieman Pick, Gaucher's, or haemophagocytic lymphohistiocytosis. Plasma amino acids or fatty acids abnormalities, and lactate were further done for patients with indications of metabolic diseases. Cytomegalovirus (CMV) infection was not excluded because it is highly prevalent in Chinese infants.\textsuperscript{15,16} CMV infection occurs in more than half of the infants with neonatal hepatitis in Mainland China.\textsuperscript{17,18} It has been reported in Taiwan that those infected with CMV have the same outcome as those without the infection.\textsuperscript{19} The presence of CMV infection does not rule out other causes of intrahepatic cholestasis.\textsuperscript{19,20}

Subjects

Infants who were referred to the Children’s Hospital of Fudan University, a tertiary referral hospital and primary specialist pediatric hospital in eastern China, for investigating conjugated hyperbilirubinaemia under 3 months of age and tentatively diagnosed as INH from June 2001 to May 2004 were enrolled. Infants were excluded if they: 1) had INR >1.2 and could not be fully corrected after vitamin K intravenous injection at presentation; 2) were lost to follow-up before the age of one year, or the follow-up interval between subsequent visits longer than 3 months before the jaundice clearance; 3) accompanied with conditions that may affect jaundice development or resolution before their reference, such as fetal distress or birth asphyxia, intrauterine growth retardation, unconjugated hyperbilirubinaemia requiring phototherapy, polycythaemia, transfusion, operation, glucose-6-phosphate dehydrogenase deficiency, parenteral nutrition, severe congenital abnormalities; 4) received herb medicine or high-dose vitamins before the representation.

Retrospective Analyses of Biochemical Parameters

The medical files of those who satisfied the above inclusion and exclusion criteria were reviewed following the approval of the Institute’s Ethics Review Committee. Gender, birth weight, gestation age, presentation age, age at which conjugated jaundice was noticed, family history of liver disease and the parental consanguinity, initial ultrasound scan results, and the biochemistry parameters were abstracted. Poor prognosis was defined as one of the following: death, liver transplanted or listed for liver transplantation, persistent or recurrence of cholestasis until 1 year of age. The others were classified as good prognosis. CMV infection was defined as either anti-CMV-IgM detected by ELISA and/or CMV-PP65 antigen detected in peripheral blood mononuclear cells by immunofluorescence. Liver function tests and other routine laboratory data were obtained using standard methods, with GGT determined by L-gamma-glutamyl-3-carboxy-4-nitranilide method on HITACHI 7080 Analyzer. The normal upper limits in our hospital for total bilirubin (TBil), alanine aminotransferase (ALT), and GGT were 17 \( \mu \text{mol/L} \), 40 U/L, and 50 U/L, respectively. The prognosis were compared according to their GGT level at presentation or the peak GGT level.
Statistical Analysis

SPSS version 11.0 was used for data entry and analysis. Fisher exact test and Chi-square were used to assess categorical associations. Kolmogorov-Smirnov test was used to test variables distribution. Mann-Whitney U test was used for nonparametric examination or non-normal distribution comparison. The results were presented as mean with standard error. Two-sided P value less than 0.05 was considered statistically significant.

Results

Demographic and Clinical Characteristics

Thirty-eight cases, 34 male and 4 female, were finally included in the study according to the inclusion and exclusion criteria. All except 6 were full-term babies. 84.2% (32/38) of them presented jaundice within 1 month of age, with a mean of 17.26±6.19 days. None of them had a family history of liver disease or parental consanguinity. Eight children (21%), 5 male and 3 female, were defined as poor prognosis. Five were defined because of their death due to progressive liver diseases before 1 year of age and three of persistent jaundice with pruritus and failure to thrive over 1 year of age. All patients with poor prognosis had been born as full-term babies with a birth weight between 3.3 and 3.4 kg. 62.5% (5/8) of them presented with jaundice within 1 month of age, with a mean of 31.08±17.23 days. There was no significant difference of the ultrasound scan results for liver texture, hepatosplenomegaly, and ascites between patients with poor or good prognosis at presentation. The prevalence of CMV positivity was 62.5% (5/8) in patients with poor prognosis compared with 59.4% (19/32) in patients with good prognosis (P=1.000).

Association of Serum GGT Activity and Prognosis

A significantly worse prognosis was seen in patients with low GGT than those with high GGT when they were divided by the usually used normal upper limit (50 U/L) not only at presentation, but the peak level at follow-up as well (Table 1). When the patients were divided by GGT level at 100 U/L (usually normal upper limit × 2), the differences was significant with the peak level at follow-up, but just marginly significant at presentation (Table 1).

Dynamics of Biochemical Parameters

The dynamic changes of TBil, ALT and GGT levels were studied in our patients. It was found that the dynamics of GGT were quite different from that of TBil or ALT. The GGT level peaked as the TBil and ALT levels were falling in all patients with good prognosis (Figure 1), except one whose GGT level was consistently low (if jaundice occurs in future waiting follow-up). However, in patients with poor prognosis, there were two types of GGT dynamics. Type 1 was seen in 5 cases, in whom bilirubin and ALT levels exhibited significant fluctuations while GGT activity were consistently low during the follow-up period (Figure 2). Two of them died before the age of 1 year, one died at the age of 4.5 years from ESLD, two others were still alive with obvious growth retardation, and persistent cholestasis with deterioration during infections until this paper submitted; Type 2 dynamics were seen in 3 patients, in whom the GGT level was elevated at the outset of the disease, but declined to nearly normal with deterioration of clinical conditions. Figure 3 showed a case in whom GGT rose to about 400 U/L with jaundice declining at first, but then GGT decreased to less than 50 U/L while ALT level increased and jaundice deteriorated. The patient eventually died from liver failure at the age of 8 months. In

<table>
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<th>GGT level at presentation (U/L)</th>
<th>Peak GGT level at follow up (U/L)</th>
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<td>≤50</td>
<td>&gt;50</td>
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<td>C1</td>
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<td>C2</td>
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†GGT: Gamma glutamyltransferase; ‡The cases who did not have any events that were defined as cases with poor prognosis; §The cases who satisfied one of the following: death, liver transplanted or listed for liver transplantation, persistent or recurrence of cholestasis until 1 year of age.

Table 1 The association between serum GGT† activity and prognosis in infants with tentative diagnosis idiopathic neonatal hepatitis
Figure 1  Typical biochemistry dynamic profile of patients with good prognosis. This graph is representative of the relationship between bilirubin, ALT and GGT levels. GGT levels rise as bilirubin and AST levels fall. This particular patient who is randomly selected from cases with good prognosis presented on day 7 and jaundice was resolved by day 127.

Figure 2  Typical biochemistry dynamic profile of patients with poor prognosis (type 1). This graph is representative of the relationship between bilirubin, ALT and GGT levels. Bilirubin and ALT levels exhibited significant fluctuations while GGT activity were consistently low during the follow-up period. This particular patient who is randomly selected from the type 1 patients presented on day 53 and died from gastroenteric bleeding on the age of 4.5 years.
the other 2 patients who died from liver failure before 1 year of age as well, the serum GGT levels had increased to about twofold of the upper normal limit, but then declined quickly and maintained at about 50 U/L until death while the jaundice aggravated and aminotransferase level increased.

**Discussion**

Neonatal cholestasis is the commonest cause of hospitalisation to pediatric liver services in Mainland China.\(^4\)Although an increasing range of specific disorders have been described as causing neonatal cholestasis, INH is still a common tentative diagnosis.\(^4,5\) Most cases with INH were able to clear their jaundice completely before 1 year of age, and their long-term prognosis was generally benign. However, a small proportion of the patients may present persistent or recurrent jaundice, and die from liver failure or need liver transplantation in their early age. This study is the first to show that serum GGT activities, especially combined with its dynamics, might serve as an efficient predictor of prognosis in Chinese infants with intrahepatic cholestasis.

The normal reference range of GGT is age dependent. It could be quite high in newborns, and then decreased to adult range by about the age of 6 months.\(^4\) It could also be population and methodology dependent. In Mainland China, 50 U/L is widely used as the upper normal limit regardless of age or gender of the patient, because the lack of age specific data. In a study from Taiwan, the upper normal limit of GGT in infants less than 6 months of age is defined as 93 U/L, about as twice as that in adults.\(^4\) In Hong Kong, more than 200 U/L is recommended as the cutoff of GGT in infants less than 2 months of age by the local chemical pathologists. Therefore, both 50 U/L and

![Typical biochemistry dynamic profile of patients with poor prognosis (type 2).](image)

**Figure 3** Typical biochemistry dynamic profile of patients with poor prognosis (type 2). This graph is representative of the relationship between bilirubin, ALT and GGT levels. GGT level elevated at the outset of the disease, then declined to nearly normal with significant fluctuations of bilirubin and ALT during the follow-up period. This particular patient who is randomly selected from the type 2 patients was referred on day 77, in whom GGT rose to about 400 U/L while jaundice declining at first, then GGT normalised while ALT level increased and jaundice deteriorated with eventually died from liver failure at the age of 8 months.

TBil, total bilirubin; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase
100 U/L was used as artificial cutoff values in this study to differentiate the infants with intrahepatic cholestasis into low GGT group or high GGT group. Although statistic significance was achieved as well when either of them was used as a cutoff value to predict the outcome, a value of 50 U/L resulted in a more significant difference, especially when the no more than 50 U/L of GGT level at presentation was used as a parameter of worse prognosis (Table 1). Therefore, it is no doubt that low GGT activity might also be considered as a parameter of worse prognosis in Chinese infants with idiopathic intrahepatic cholestasis though exception exists, and 50 U/L as the upper normal limit seems more appropriate concerning its accuracy in prognosis assessment.

Similar biochemistry dynamic profiles were found in this study in the patients with benign prognosis as previously reported from Europe.12,25 Their GGT level peaked only as bilirubin and ALT levels were falling (Figure 1). Type 1 dynamics (Figure 2) that was seen in the five cases who quite resemble the clinical course of PFIC is understandable that may attribute to FIC1 or BSEP gene mutation. In fact, homozygous A167T and heterozygous Q702X mutations of BSEP gene were found in one case each of them (unpublished data, manuscript in preparation). Therefore, extremely low GGT even in neonatal period may indicate genetic defects of related genes. However, type 2 dynamics (Figure 3) is astonishing that elevated GGT level fell into normal range in the other 3 cases with poor prognosis while the jaundice and clinical conditions deteriorated.

Although GGT has been identified more than 20 years, its role in hepatobiliary system is still not fully clear. Some data suggests that hydrolysis of glutathione rather than transpeptidation may be the true physiologic function of GGT.26 Glutathione is a major determinant of bile salt independent bile flow (BSIF). In humans, canalicular bile is divided relatively evenly between bile salt dependent bile flow and BSIF.27 GGT may reflect the function of bile formation in bile salt dependent and independent pathways in patients with intrahepatic cholestasis.9-11 We hypotheses that the elevation of GGT, accompanying normalisation of bilirubin and aminotransferase level, may represent the recovery of liver function in bile formation either bile salt dependent or independent pathway in children with benign prognosis, but the decline of GGT activity with bilirubin elevation may represent the deterioration of liver function in bile formation due to the failure of bile salt synthesis or secretion, or bile salt independent pathway, usually leading to poor prognosis.

It might be argued that the prognosis in this cohort is unusually poor compared to the common description from many books. The reason may partly be the referral bias, the patients who came from other provinces accounted for about two-thirds of our patients. Those usually are the patients with severe or prolonged jaundice. Also the unusually poor prognosis can also be attributed to our selection criteria. Only patients with serum bilirubin more than 85 μmol/L were enrolled, compared to the usually used definition of more than 20 μmol/L or so. Those may limit the extrapolation of our conclusion to the INH patients with fluctuating GGT and very mild cholestasis.

Apart from the referral bias, other limitations of this study include the retrospective nature and relatively small sample size. However, it still could be inferred that serum GGT activities, especially combined with its dynamics, might serve as an efficient predictor of prognosis in Chinese infants with moderate or severe intrahepatic cholestasis. More attention should be paid to the patients with very low serum GGT activities because of the high incidence of poor prognosis in that kind of patients. The pathophysiology significance of GGT dynamics in infants with intrahepatic cholestasis needs further investigation.

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References