Improvement in accuracy of gamma-glutamyl transferase for differential diagnosis of biliary atresia by correlation with age

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In order to determine the accuracy of serum gamma-glutamyl transferase (GGT) as a test for biliary atresia, we reviewed the charts of 29 infants with cholestatic jaundice less than one year of age. All patients underwent liver biopsy or laparotomy with cholangiogram to establish neonatal hepatitis (NH) or extrahepatic biliary atresia (EHBA). We also gathered information from 176 patients from published studies. Sensitivity, specificity, and likelihood ratios (LR) were calculated with 95% confidence interval (95% CI). GGT levels of the EHBA group were higher than those from the NH group. For diagnosis of EHBA at a cut-off level >250 U/L, sensitivity was 83.3% (95% CI, 55.2– 95.3%); specificity, 70.6% (95 CI, 46.9–86.7%); and negative LR, <2.0. When we added data from other studies considering age (<4 weeks, 4−8 weeks, and >8 weeks), GGT performance increased, especially for the first age group: with cut-off of 150 U/L, sensitivity was 91.7%; specificity, 88%; and positive LR, 7.8. Thus, for improving reliability of GGT levels for EHBA diagnosis, they need to be correlated to infant age.

Key words: gamma-glutamyl transferase, biliary atresia, neonatal hepatitis, diagnostic test, jaundice.

Extrahepatic biliary atresia (EHBA) is a disease of unknown etiology with a frequency range from 1/10,000−15,000 live births[1,2]. This disease involves bile duct epithelium, causing cholangitis that may lead to progressive sclerosis, narrowing of the biliary tract, and eventually development of biliary cirrhosis[3]. Because prognosis is closely related with timing of the hepaticoportoenterostomy, early diagnosis is essential. When treatment is delayed, it is likely that infants with EHBA will face irreversible hepatocellular damage and poor long-term survival[4].

Different diagnostic methods are necessary to establish the definitive diagnosis of EHBA, such as blood tests, serologic markers, hepatic ultrasound, scintigraphy and surgery. Each has various levels of accuracy and reliability. Serum activity of gamma-glutamyl transferase (GGT) has been used for diagnosis of EHBA since 1981[5]. Serum GGT activity >300 U/L or a daily increase of 6 U/L has been found diagnostically valuable in patients with EHBA <10 weeks of age[6]. However, serum GGT levels to distinguish EHBA patients from those with intrahepatic disease overlap[6,7]. Thus, different cut-off serum GGT levels have been proposed to improve its accuracy in the diagnosis of EHBA. With cut-off of 150 U/L, sensitivity ranges from 81-100%[5,7-9], whereas specificity ranges from 43-87.5% when the cut-off is >300 U/L[5,9], excluding patients with alpha-antitrypsin deficiency.

Variability in the accuracy of GGT activity at different cut-off levels for diagnosis of EHBA may be associated with the population studied. Studies that established normal serum GGT values in infants showed that GGT activity decreased during

the first months of life; levels up to 200 U/L can be found at birth, and by seven months of age, the infant reaches adult levels[10,11]. Some studies involving patients with biliary atresia included infants <4 weeks of age[6].

In comparison with developed countries, identification of EHBA patients in early stages in developing countries is an issue; many health care facilities lack trained personnel and technology to approach this problem. In our experience, it is usual to identify EHBA infants >8 weeks of age. Given that tests for quantification of serum GGT levels are widely available, we hypothesized that this could be used as screening test for detection of EHBA patients among jaundiced infants. In this study, we determined accuracy of serum GGT levels as a diagnostic test for EHBA considering the age at diagnosis. GGT activity was compared in two groups: EHBA and neonatal hepatitis (NH) patients.

Material and Methods

The study was conducted at the Pediatric Hospital of the National Medical Center of the Mexican Institute of Social Security. This hospital is a tertiary-care medical and surgical referral center located in Mexico City. The study was carried out from January 2000 to August 2002. The hospital Research and Ethics Committees approved the study.

Because jaundiced infants are usually referred to this hospital after six weeks of age, our approach consisted of attempting to rule out EHBA diagnosis. On arrival, all patients are hospitalized and studies aimed at establishing diagnosis (e.g., blood tests, serologic markers, hepatic ultrasound) are performed during the subsequent days.

In this retrospective study, all patients with cholestatic jaundice <1 year of age at admission were included. Cholestasis was defined as direct bilirubin serum level >2 mg/dl, or if direct bilirubin serum levels comprised >20% when total serum bilirubin was <10 mg/dl[12]. Two independent reviewers gathered information from charts of all patients included in the study, while a third reviewer resolved inconsistencies. The following information was collected: age at admission, sex, clinical findings (stool and urine color, existence of hepatomegaly and splenomegaly), and laboratory studies (serum bilirubin, aspartate aminotransferase [AST], alanine aminotransferase [ALT], GGT, and alkaline phosphatase levels), as well as results of duodenal tube test and studies aimed at etiologic diagnosis[13], such as hepatic ultrasound and hepatobiliary scintigraphy by technetium 99-m mebrofenin imino diacetate (T99mIDscan). Liver biopsy or laparotomy with cholangiogram findings[12] was considered the gold standard for establishing EHBA diagnosis. Patients who did not meet EHBA criteria were included in the NH group. GGT activity was determined by Flex ® Reagent Cartridge, an adaptation of methodology recommended by the International Federation of Clinical Chemistry[14].

Statistical Analysis

Clinical and diagnostic test characteristics were compared between EHBA and NH patients. Sensitivity, specificity, and likelihood ratios with 95% confidence interval (95% CI) were calculated; best serum GGT activity cut-off level for diagnosis of EHBA was established by receiver operating characteristic (ROC) curves. P value <0.05 was considered statistically significant, and all analyses were performed with the SPSS software version 11.0 (Chicago, IL, USA, 2000).

Results

During the study period, 39 patients fulfilled the selected criteria; 18 comprised the EHBA group and 21 patients the NH group. However, 10 patients were excluded because serum GGT levels were quantified after the surgical procedure. Ages of excluded patients for each group were 40, 60 (3 patients), 90, and 120 days (EHBA group) and 30, 34, 60, and 120 days (NH group), respectively.

Table I shows a comparison of characteristics between the two groups. Overall, clinical findings were similar; however, eight NH patients and no EHBA infants were <60 days of age. With regard to blood tests results, serum GGT and bilirubin levels were significantly higher in the EHBA group than the NH group (p=0.03 and 0.027, respectively). Interestingly, among studies for establishing cholestasis etiology, in all of the 15 patients in whom hepatic T99mIDscan was performed, this study properly identified those patients with or without EHBA. This was not achieved with hepatic ultrasound (Table I).

Table I. Patient Characteristics

	Biliary atresia n=12	Neonatal hepatitis n=17	*P value
Sex, n (%)			
Male	3 (25)	10 (58.8)	
Female	9 (75)	7 (41.2)	0.13
Age (days)§	112.5 (60-240)	70 (14−230)	0.07
Clinical findings			
Jaundice	12 (100)	17 (100)	
Choluria	7 (58.3)	6 (35.3)	
Acholia or hypocholia	9 (75)	9 (52.4)	
Hepatomegaly	9 (75)	9 (52.9)	0.45
Bilirubin (mmol/L)§			
Indirect	0.01 (0-0.05)	0.03 (0-0.12)	0.03
Direct	0.12 (0-20)	0.16 (0-0.47)	0.16
AST (U/L)	184 (16-315)	206 (59-622)	0.37
ALT	145 (10-790)	180 (52-436)	0.80
AP	532 (2-10,344)	314 (14-882)	0.07
GGT	468 (21-1,721)	209 (36-886)	0.027
Positive duodenal tube test	11/12	0/17	0.001
Hepatic ultrasound			
Gallbladder visible	9/11 (81%)	6/16 (37.5%)	0.006
T99mIDscan			
Non-excreting	8/8 (100%)	0/7 (0%)	0.0001

*Fisher's exact test or Mann-Whitney U test. §Median (minimum–maximum).
AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. AP: Alkaline phosphatase.
GGT: Gamma-glutamyl transferase.

Evaluation of serum GGT levels as screening test for EHBA

Figure 1 was constructed with results of serum GGT levels of all included patients considering age at admission. To compare results obtained, we added a plot with normal serum GGT mean values[15]. In general, all included patients had higher serum GGT levels than normal values. Likewise, mean serum levels of EHBA patients were higher than those of NH patients throughout all weeks. A total of 10/12 EHBA patients had serum GGT levels >250 U/L, and the patient with lowest GGT levels was from the EHBA group. As also shown in Figure 1, GGT

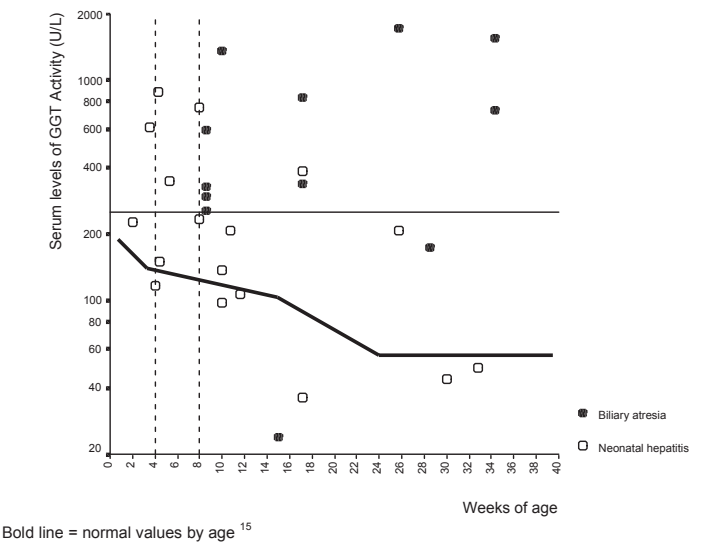


Fig. 1. Gamma glutamyltransferase serum values of extrahepatic biliary atresia (n=12) and neonatal hepatitis patients (n=17).

values in the EHBA group had a tendency to increase according to age; in contrast, GGT values in the NH group had a tendency to decrease.

Sensitivity and specificity results at different serum GGT cut-off levels are displayed in Table IIa. Cut-off level >250 U/L showed the best diagnostic performance: sensitivity was 83.3% (95% CI 55.2–95.3%) and specificity 70.6% (95% CI 46.9–86.7%). Likelihood ratios calculated are shown in Table IIb; in the decision-making process, serum GGT levels <200 U/L appear to be a reliable cut-off point to rule out EHBA diagnosis; however, no cut-off point could help establish EHBA diagnosis with certainty.

MEDLINE from 1966 to 2005 and found only five studies[5,16-19] that reported serum GGT levels by patient. Data from each study were extracted and combined with the information obtained in this study.

A total of 176 patients from six studies were included: 51 infants <4 weeks of age; 64 from 4-8 weeks of age, and 61 patients aged >8 weeks. As shown in Figure 2, across all the weeks, the majority of NH infants had lower serum GGT values than EHBA infants. Between 4 and 8 weeks of age, GGT values overlapped between the two groups. GGT values for infants younger and older than these ages seem to have different patterns: the majority of EHBA infants

Table IIa. Diagnostic Accuracy of Serum GGT Activity at Different Cut-Off Levels

Serum GGT cut-off level	Sensitivity (95% CI)	Specificity (95% CI)
>300 U/L	66.7 (39.1-86.2)	70.6 (46.9-86.7)
>250 U/L	83.3 (55.2-95.3)	70.6 (46.9-86.7)
>200 U/L	83.3 (55.2-95.3)	47.1 (26.2-69.0)
>150 U/L	91.7 (64.6-99.6)	47.1 (26.2-69.0)
>100 U/L	91.7 (64.6-99.6)	23.5 (9.6-47.3)
>50 U/L	91.7 (64.6-99.6)	17.6 (6.2-41.0)

Table IIb. Likelihood Ratios of Serum GGT Activity at Different Cut-Off Levels

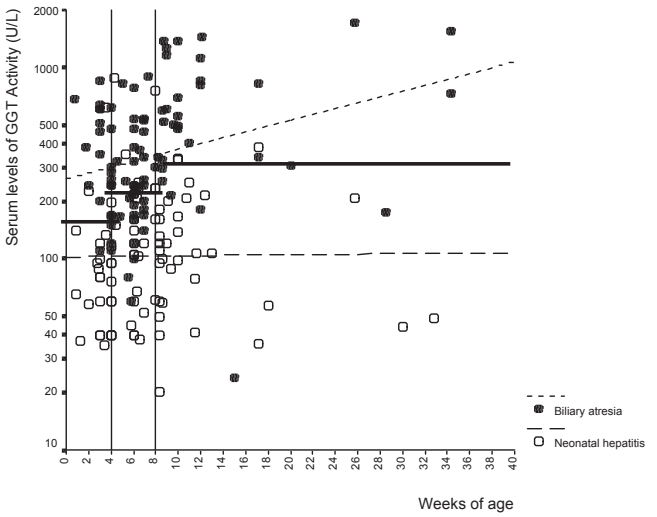
Serum GGT cut-off level	Biliary atresia (n=12)	Neonatal hepatitis (n=17)	Likelihood ratio (95% CI)
>350 U/L	6	5	1.72 (0.7-4.3)
201-350 U/L	4	4	1.4 (0.44-6)
51-200 U/L	1	5	0.27 (0.03-2.1)
<50 U/L	1	3	0.47 (0.05-4)

Evaluation of serum GGT as diagnostic test considering different studies

At this point, results obtained only added a little additional information of the usefulness of serum GGT values for diagnosis of biliary atresia. When reviewing results, two findings led us to believe that it was worthwhile to perform further analyses considering age: none of the EHBA patients was <8 weeks of age, and serum GGT levels demonstrated opposite tendencies between EHBA and NH groups. However, as mentioned in previously published reports, our study failed to include a large number of patients that would allow carrying out further analyses. Thus, to increase the sample size, we carried out a review of the literature of published studies. We searched in

<4 weeks of age had GGT values >100 U/L, while EHBA infants aged >8 weeks had GGT values >200 U/L.

Three ROC curves were plotted according to age (Fig. 3); the best cut-off level achieved for infants <4 weeks of age was 150 U/L. At this cut-off GGT level, sensitivity was 87.5% (95% CI, 69–95%) and specificity 88% (95% CI, 69–96%). Among infants between 4 and 8 weeks of age, the best cut-off level was 250 U/L with sensitivity of 51% (95% CI, 36–67%) and specificity of 85% (95% CI, 66–94%); in infants >8 weeks of age with cut-off level of 300 U/L, sensitivity was 90% (95% CI, 75–96%) and specificity was 90% (95% CI, 76–97%). Considering these cut-off GGT levels, likelihood ratios calculated were



* Data obtained from Wright et al.[5], Vazquez et al.[19], Platt et al.[16], Tazawa et al.[17,18], and Rendon-Macias et al. (current study).

Fig. 2. Gamma glutamyltransferase serum values of 176 infants with extrahepatic biliary atresia or neonatal hepatitis*.

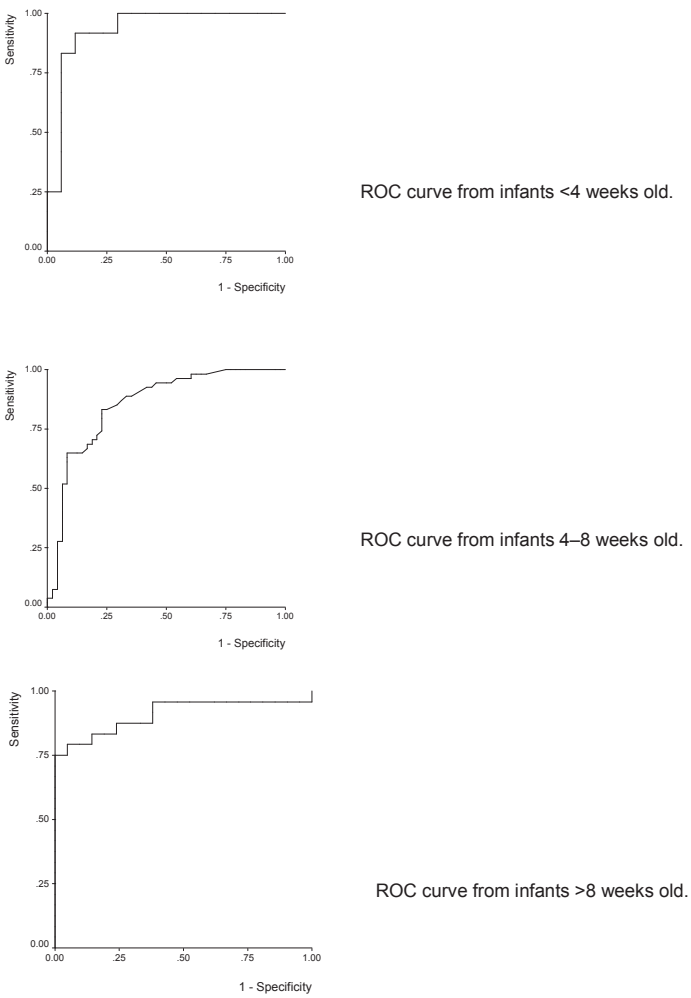


Fig. 3. GGT ROC curves for the diagnosis of atresia biliar according to infants age.

7.8 (95% CI, 2.1–28.9), 7.0 (95% CI, 2.6–18.4), and 5.8 (95% CI, 2.0–16.8) for the three age groups, respectively.

Discussion

Differential diagnosis in infant cholestatic jaundice continues to be a challenge for pediatricians and surgeons. One of the principal challenges is reaching early EHBA diagnosis, which leads to the decision of performing a curative surgical procedure. Similar to other studies carried out in developing countries[5,12], detection of EHBA in early stages was not common at our pediatric hospital. No patient in the study was referred prior to 60 days of age.

Two different forms of biliary atresia have been postulated. The first is a fetal or embryonic feature present in 10-25% of patients, i.e., absence of a jaundice-free interval: infants are jaundiced from birth. The second feature, comprising the majority of patients, is the postnatal form of biliary atresia. These infants usually appear well during the first weeks of age and become clinically jaundiced at the age of 3-6 weeks[3]. This observation can partially explain the delay for referral of these infants to specialized centers. Another issue in developing countries is lack of imaging methods necessary for differential diagnosis in primary-care facilities. Additionally, procedures intended to establish definitive diagnosis (e.g., duodenal tube test, hepatic ultrasound, hepatobiliary scintigraphy, and laparotomy) at referral centers usually require a few weeks before they can be performed. Bearing all these problems in mind, in addition to the fact that to date no single test can discriminate NH from biliary atresia, we sought to determine whether the usefulness of serum GGT activity could be improved, considering that this test is widely available. Several studies have found high sensitivity of GGT activity with a cut-off level of 150 U/L for establishing EHBA diagnosis[12]; however, this cut-off level has low specificity. Thus, it is difficult to rule out NH, and a number of patients (up to 30%) may be submitted to unnecessary surgical procedures.

Age appears to influence GGT level patterns in normal and cholestatic infants[15]. NH infants show gradual decreases in GGT levels as a possible progressive response to ductal inflammation caused by hepatopathy[20], while among EHBA patients, the tendency is toward an increase in GGT levels due to progressive obstruction. Histopathologic studies have revealed that in biliary atresia, bile duct characteristics progressively change; increasing levels of this enzyme could be an expression of chronic inflammatory effects in obstructed channels. Azar et al.[20] described four EHBA patients followed-up by biopsy prior to reaching the age of six weeks; one patient had low GGT levels until the age of seven weeks and levels showed a progressive increase, at which time atresic disease was more evident.

In this study, we showed how GGT levels provide a more reliable screening test for EHBA diagnosis when considering the age of jaundiced infants. This was possible only after we assembled data of previous studies with those of the 29 patients described in this report. The majority of reports have included a limited number of patients; by increasing the sample size, we could analyze a wider spectrum of patients, especially infants <2 months of age[5,16-19]. In order to improve accuracy and considering the results obtained, it would be necessary to correlate age with serum GGT levels as part of the process for differential diagnosis of EHBA. However, reliability of GGT values is lower among infants aged between 4 and 8 weeks. As shown in Figure 3, performance of this diagnostic test is better among children <4 weeks and >8 weeks of age.

Although a cut-off level >150 U/L has high sensitivity and specificity among infants aged <4 weeks, we consider that levels >200 U/L could be the criterion for referral to specialized health care centers, based on serum GGT levels of normal infants, reported as up to 200 U/L[4]. GGT levels >300 U/L appear useful to identify EHBA patients among children >8 weeks of age, as recommended by other authors[1]. At this age, this cut-off level can help accelerate the surgical decision, although it is well known that patient prognosis is not as favorable as in younger infants. The low GGT accuracy in the group of jaundiced infants between 4 and 8 weeks of age has generated more controversy; serum GGT levels widely differ and overlap in biliary atresia and NH patients[10]. This phenomenon has led investigators to consider different cut-off levels. This age period is crucial for the surgical decision; thus, considering that quantification of GGT levels does not

appear useful, jaundiced infants at these ages must undergo a thorough study (e.g. hepatic ultrasound, scintigraphy, etc.) when GGT levels are ≥100 U/L, before physicians can make the therapeutic decision. At this level, the sensitivity is high enough to detect almost all EHBA patients, but its specificity is very low. Although several patients will be misdiagnosed with this cut-off level, it is necessary to rule out the possibility of EHBA.

It is important to mention that GGT levels can only be considered as a test that can help in identifying those patients with EHBA; definitive diagnosis is established only after several studies have been performed. The findings in this study could be used as criteria for referral to tertiary medical care centers in those centers lacking the required technology to differentiate between EHBA and NH among jaundiced infants.

In conclusion, to increase serum GGT accuracy for differential diagnosis of cholestatic jaundice, the values should be correlated to infant age. For identifying EHBA patients among infants <4 weeks of age, the best GGT cut-off level is >150 U/L; in infants between 4 and 8 weeks of age, the cut-off level is >250 U/L, while it is >300 U/L for patients >8 weeks of age.

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