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Use of bile acids as potential markers of liver dysfunction in humans

A systematic review

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Abstract

Objective: This study aimed to determine the effectiveness of using total, individual serum, or urinary bile acids (BA) as potential markers of liver dysfunction.

Methods: We searched the PubMed and Web of Science databases using the following keywords- "serum bile acids," "liver dysfunction," "liver injury," "liver disease," "traditional liver function tests," "Chronic liver disease," "acute liver injury". The search was complemented by manual screening of the list of references for relevant articles. We selected only English-language manuscripts for adult patients based on predetermined inclusion and exclusion criteria. Animal studies and studies on neonates and children were not included.

Outcome measures: Changes in BA concentrations or ratios at or prior to changes in liver function tests.

Results: A total of 547 studies were identified, of which 28 were included after reading the entire manuscript. These studies included 1630 patients and 836 controls published between 1990 and 2017. The methods used in BA assays varied significantly, and the studies did not agree. on specific individual BA or BA ratios as biomarkers of specific liver injury or dysfunction. Except for the prognostic value of BA in intrahepatic cholestasis of pregnancy (ICP), studies have failed to provide evidence for BA as a liver biomarker.

Conclusions: Despite the research conducted on BA for over 27 years, there are inconsistencies in the reported results and a lack of solid evidence to support the use of individual BA or BA ratios as biomarkers of liver injury. Adequately conducted studies needed to resolve this limitation in the literature.

Abbreviations: BA = bile acids, CA = cholic acid, CDCA = chenodeoxycholic acid, DCA = deoxycholic acid, ICP = intrahepatic cholestasis of pregnancy, LCA = lithocholic acid, LC-MS = liquid chromatography-mass spectrometry, NAFLD = non-alcoholic fatty liver disease, UDCA = ursodeoxycholic acid.

Keywords: bile acid ratios, bile acids, individual serum bile acids, liver dysfunction, liver injury, serum bile acids, urinary bile acids

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Box 1 Strengths and Limitations of this review

- A sizeable comprehensive review of 28 research papers covering the last three decades under different conditions associated with raised bile acids.
- Summary of the available evidence for using bile acids as biomarkers of liver dysfunction or injury.
- Exposed deficiencies in research in this area, particularly concerning the sensitivity and specificity of bile acids and the lack of consistency in the methods used in measurements.
- Highlighted recommendations and priorities for future research

Box 2 New Knowledge learned from this review

- There are inconsistent reporting results and a lack of agreement on specific individual serum bile acids as biomarkers of liver injury/dysfunction.

- There is no substantial evidence to support the use of bile acids as a routine test with liver function tests.

Box 3 Clinical Applications

- There is growing evidence of the use of bile acids as a prognostic test in women with ICP and raised serum bile acids.
- There is weak evidence that bile acids could be added to routine liver function tests in screening workers exposed to certain hepatotoxicants.
- We identified no study covering the use of serum bile acids as a biomarker of non-alcoholic fatty liver disease, and more work is needed in this area.

1. Introduction

In humans, bile acids are synthesized in liver cells as a result of cholesterol metabolism. They comprise a biological pool of 2 main groups: primary bile acids (comprising cholic acid, [CA], and chenodeoxycholic acid [CDCA]) and secondary bile acids (deoxycholic acid, [DCA], and lithocholic acid, [LCA]).[1] Most bile acids (approximately 94%–96%) are recycled in the enterohepatic circulation, and only a small portion (approximately 4%–6%) is synthesized in the liver. Bile acids undergo enterohepatic circulation and are dependent on active transporter systems in the liver and intestine.[2] The portion synthesized in liver cells (hepatocytes) is known as primary bile acids, while secondary bile acids are formed as a result of intestinal bacterial actions on primary bile acids.[3]

Once produced, bile acids are transported across liver hepatocytes and then transported through the canalicular membranes into the bile. The process involves a carrier-mediated transport of bile acids and other solutes, such as glutathione which produces an osmotic gradient for the transcellular and paracellular flow of water into the canaliculi.[4] Bile acids are secreted in the bile into the lumen of the small intestine during contraction of the gallbladder. The majority are reabsorbed at the terminal ileum and transported back to the liver via the portal circulation to add to the bile acid pool; in other words, most bile acids are recycled and reused.[2] In the intestine, bile acids are deconjugated (removal of the glycine or taurine moiety) by the action of intestinal bacteria.[5] In humans, the relative amount of taurine conjugate is dependent on diet and increases in severe cholestasis and liver diseases up to approximately 50%.[6] These changes result in the conversion of CA to DCA and CDCA to LCA; both DCA and LCA are secondary bile acids.[6,7]

In healthy subjects, the normal fasting total serum bile acid concentration is relatively low, in the range of 2 to 10 μmol/L. This is because of the efficiency of the liver in the removal of bile acids from the portal-hepatic circulation. Therefore, bile acid concentration in the peripheral blood is regulated by a balance between 2 physiological processes: intestinal absorption and hepatic elimination of bile acids.[8] However, most of the increase in serum total bile acids could be related to a dysfunction in the sinusoidal hepatocellular uptake of bile acid, impairment of transport of bile acids in hepatocytes, or dysfunction of bile acid efflux at the canalicular domain.[9] Studies have shown that postprandial total serum bile acids are generally higher than the fasting total serum bile acid concentrations. Measurement of both fasting and postprandial concentrations has been proposed to provide additional value in the differential diagnosis of chronic liver dysfunction.[10]

However, serum bile acid concentration is not part of the standard biochemical liver function tests used in clinical practice and may only be ordered in certain centers for the diagnosis of intrahepatic cholestasis of pregnancy (ICP).[11] Standard biochemical liver function tests included serum bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, serum albumin, and international normalized ratio. These tests reflect the different functions of liver-

1. anion excretion (bilirubin),
2. integrity of hepatocytes (transaminases),
3. free bile formation and flow (bilirubin and alkaline phosphatase), and
4. protein synthesis (albumin).[12]

Currently, it is believed that aminotransferase levels could be considered as potential biomarkers of "liver metabolic functioning" rather than the traditional view of being reflective of liver cell damage (hepatocellular integrity). This is because enzymatic activities might reflect key aspects of the pathophysiological functions of hepatocytes.[13] However, the sensitivity and specificity of serum aminotransferases, particularly alanine aminotransferase, for discriminating between those with and without liver disease/dysfunction, depend on the cut-off values chosen to define an abnormal test result.[14,15] A revision of the upper limit of normal transaminases may be recommended, particularly in healthy adult males.[14] However, the revised limits could classify those who were considered normal as abnormal by the recommended revised lower cut-off values, which may increase the absolute number of patients who would require further evaluation without clear clinical benefits or practical outcomes. Other limitations of standard biochemical liver function tests have been discussed in the literature [examples].[16,17,18]

With this information in mind, clinicians and researchers are examining the clinical use of serum bile acid in the diagnosis of liver injury and diseases.

Therefore, this systematic review aimed to determine the diagnostic accuracy of total serum bile acids, individual serum bile acids, bile acid ratios, and urinary bile acid concentrations as potential biomarkers of liver injury or liver dysfunction, and to examine the sensitivity and specificity of bile acid levels compared to those of standard biochemical liver function tests. The review rationales were to assess what serum bile acids can add to the liver function tests or whether any of the bile acid measurements offer a better diagnostic test on its own and address the current gaps observed using standard biochemical liver tests. Although there are several studies on serum bile acids and liver function tests dating back to the 70 second and 80 second of the last century in adults and pediatric patients, it was decided to focus this review on adult patients only and included studies from 1990 to 2017. The reason for not including studies before 1990 is the presence of literature reviews on these years, and we do not want to repeat what we already know from the literature.[17] We also felt that

expanding the scope of the review to cover pediatric and neonatal liver diseases will complicate the scope of this review.

Bile acids have been measured using several methods, including

1. the enzymatic assay of total bile acids with recombinant 3-alpha-hydroxysteroid dehydrogenases[19] and
2. radioimmunoassay of bile acids to detect conjugated bile acids in the serum, including conjugates of cholic, chenodeoxycholic, deoxycholic, and sulfolithocholic acids. The method showed the absence of a cross-reaction between glycine and taurine conjugates.[20] This method is currently obsolete.
3. High performance liquid chromatography- ultraviolet detection of conjugated bile acids,
4. gas-liquid chromatography for conjugated and individual bile acids,[21] and
5. liquid chromatography mass spectrometry that may come under several names and settings (liquid chromatography-mass spectrometry [LC-MS], high-performance liquid chromatography-mass spectrometry [HPLC-MS], and ultra-performance liquid chromatography-tandem mass spectrometry).[22]

Therefore, the research questions are:

1. Can the measurement of serum bile acids in total or as individual bile acids in serum or urine offer an accurate diagnostic test of liver injury/dysfunction?
2. What are the sensitivity and specificity measures of bile acid concentrations compared to standard biochemical liver function tests?

2. Methods

2.1. Review design

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines.[23] This is a systematic review of the literature; there is no psychological or physical injury to humans or personal information to be revealed. The College of Medicine IRB recommended that the review does not require ethical approval, as per international standards.

2.2. Literature search

We searched the PubMed, EMBASE, and Web of Science databases for bile acid and liver dysfunction or liver injury studies. The search covered studies from 1990 to December 2017. The search was limited to studies in English and was conducted on humans. We searched the databases using the following keywords: "Bile acids," "Bile salts," "Liver dysfunction," "Liver injury," "Liver function tests," "Bilirubin," "Aspartate aminotransferase," "Alanine aminotransferase," "Alkaline phosphatase," "Serum bile salts," "Serum bile acids," "Urine bile salts," and "Urine bile acids." All searches were performed independently by 2 researchers (the first author and a research assistant with a medical background). We performed another search manually by searching the list of references in the identified studies not found in the database search.[24]

We also searched the journals listed by the Journal Citation Reports-2016 of the Web of Science under the category of gastroenterology and hepatology, and the Cochrane Central Register of Controlled Trials (the Cochrane Library) using the same keywords. Both searches were completed in April 2018.

2.3. Criteria for consideration of studies

The following inclusion and exclusion criteria were used. We included studies that reported the use of bile acids as biomarkers for hepatic dysfunction or liver injury. The search was limited to studies in English and conducted on humans. Animal or animal models were not included. Articles that were solely on pediatric patients (age <18 years) were not included. We did not include editorials, commentaries, letters to the editor, conference proceedings, abstracts, or monographs.

2.4. Study selection

Two researchers independently reviewed the titles of articles and the content of the abstracts of all identified citations. Relevant studies have been retrieved and reviewed in detail. Any disagreement on judging the articles was discussed by the 2 evaluators. The full texts of relevant articles were collected, and the contents were examined against the selection criteria selection criteria. The reviewers were not blinded to the authors' names or institutions. Only studies that matched the selection criteria were included.

2.5. Data extraction

Data were independently extracted by 2 evaluators using a predefined extraction form. The following data were abstracted in the form:

1. first author's name,
2. year of publication,
3. aims and research questions,
4. number of patients, number of subjects under control,
5. liver injury investigated,
6. liver biomarkers used in the assessment,
7. type of bile acid analyzed,
8. method used in bile acid analysis,
9. results of bile acids compared to other biomarkers, and
10. the institute, research centre, university, city, and country where the study was conducted.

Details on reported statistical associations and comparison of the results obtained with those obtained using other methods were also evaluated.

2.6. Statistical analysis

Using SPSS software, the agreement between evaluators (measurement of the degree of inter-rater agreement) was calculated using Cohen's kappa coefficient.[25] The sensitivity of serum bile acids describes how well the test can detect the disease or liver dysfunction in patients with the disease. Specificity refers to the percentage of people who test negative for a disease among a group of people. To calculate sensitivity and specificity, we used the following equations:[17]

$$\text{Sensitivity number of true positives} / [\text{true positive} + \text{number of false negatives}$$

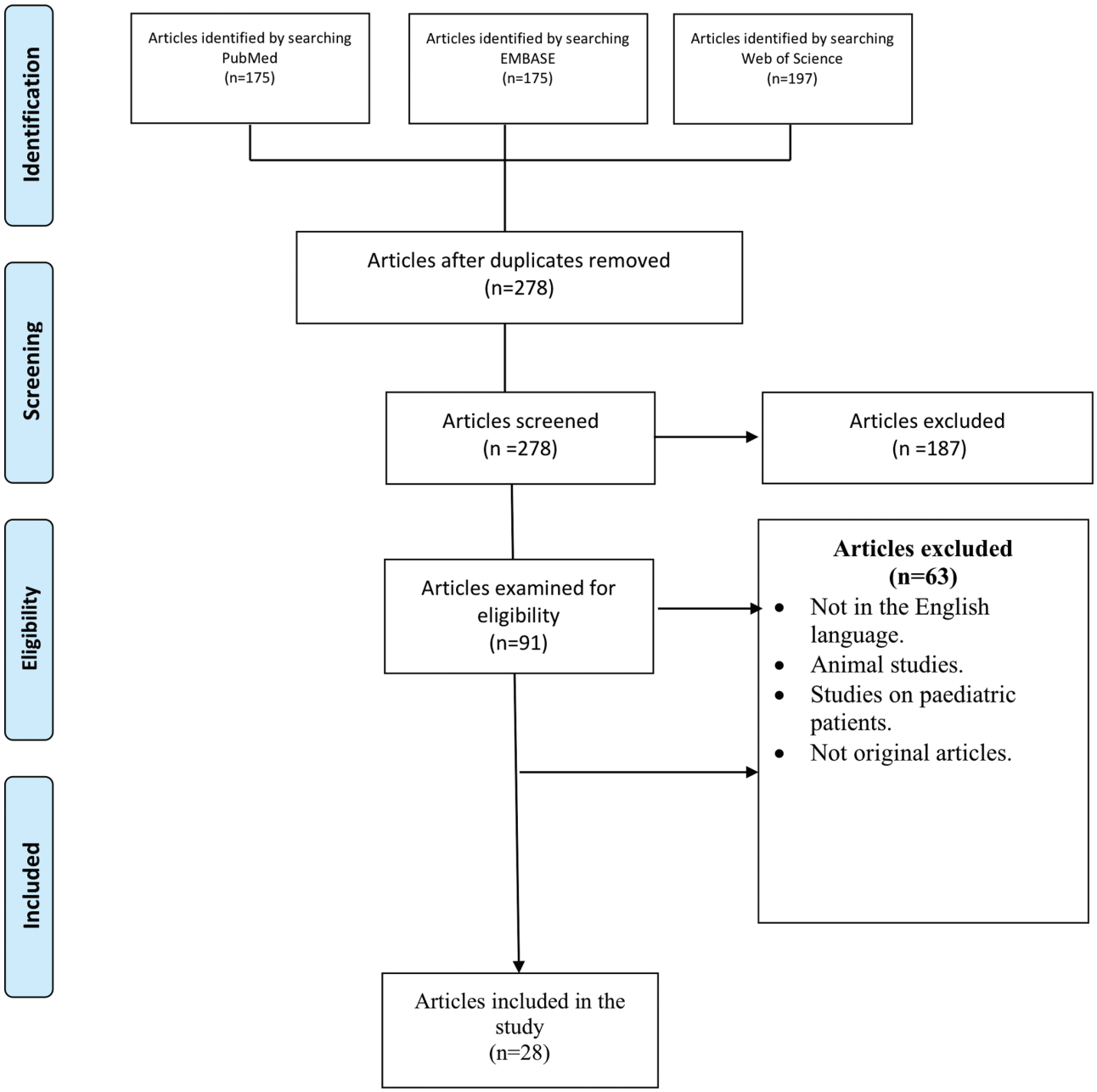

Figure 1. PRISMA flowchart showing articles searched on the use of serum and urinary bile acids in the assessment of liver injury and those finally included in the review.

$$\text{Specificity} = \text{number of true negatives} / [\text{true negative} + \text{number of false positives}].$$

3. Results

3.1. Literature search and selection process

Figure 1 is a flow diagram summarizing the search and selection process for articles in the literature. And 47 potentially relevant publications were identified from the search of the 3 databases. After the removal of duplicates, 278articles remained. Of these, 189 were not relevant to the inclusion criteria. Eighty nine full-text articles were assessed for eligibility. Finally, we identified 26 articles that met our selection criteria and were consistent with the aims of the systematic review.[26–53] The inter-rater agreement between evaluators had a kappa score in the range of 0.85 to 0.89

3.2. Characteristics of studies included in the review

Table 1 summarizes the details of the 8 studies based on the use of total serum bile acids as a liver biomarker.[26–33]Table 2 summarizes 18 studies based on the use of individual serum bile acids and bile acid ratios as liver biomarkers.[34–51]Table 3 summarizes the 2 studies based on the use of urinary bile acids as liver biomarkers.[52–53] A total of 1630 patients and 836 subjects (controls) were included in the study. The number of patients and

Table 1

Studies exploring the use of total serum bile acids as a liver biomarker.

Study (year)[Reference]	Presentation/ Liver injury/ Dysfunction	How confirmed?	No of patients	No of control	What else was measured?	Bile salt results	Other results	Conclusions
Janssen et al (2001)[26]	Patients with acute rejection after liver transplantation.	Clinical presentation and liver biopsy.	22 (patients with acute rejection, Group II)	19 (patients with no rejection after transplantation, Group I).	Serum bilirubin, transaminases and liver biopsy.	Patients in group II (n=22) who showed acute rejection had threefold increases in serum bile acids 3 days prior to biopsy. No changes were noted in Group I. Successful anti-rejection treatment was correlated with significant decrease in serum bile acids.	Serum bilirubin and transaminases did not show significant changes in the acute rejection group or the non-rejection group.	Serum bile acids monitored after Liver transplantation can easily be used to detect acute rejection and at the same time they reflect the success of antirejection therapy.
El Hady et al (2014)[27]	Exposed to solvents	History of exposure	57	59	ALT, AST, GGT, ALP, total and direct bilirubin.	Bile acids significantly elevated in the exposed workers (88%) compared to the controls ($P<.01$).	All other liver function tests exhibited normal mean levels and did not show statistically significant differences between both groups.	Bile acids had significant positive correlation with duration of exposure to organic solvents.
Lalisang (2012)[28]	Patients with severe obstructive jaundice	History, and clinical picture.	21	0	Serum total bilirubin, serum alkaline phosphatase, Pt and APTT, serum transaminases measured before and 7 and 14 days after bile duct decompression.	After decompression, the average serum bile acid decreased significantly ($P<.05$).	After decompression, a significant decrease in serum total bilirubin and serum ALP. Coagulation function and transaminases back to normal limits.	Serum bile acid could be considered to be used as alternative marker to evaluate liver function.
Elsendle et al (2011)[29]	Chronic severe pruritus refractory to treatment.	History and clinical presentation, admission notes, progress notes.	117	50	Liver function tests	Patients with chronic pruritic conditions of unknown origin (PUO) (n=18) showed pathologically high total serum bile acid levels.	Cholestyramine and UDCA were both effective in lowering total SBA and improvement of pruritus.	Total serum bile acids are elevated in a high proportion of patients with PUO.
Nunes de Paiva and Pereira Bastos de Siqueira, (2005)[30]	Exposure to solvents in car repainting shops	History of occupational exposure.	57	51	AST, ALT, total bilirubin, GGT, ALP were determined in the two groups. Urinary hippuric acid was measured in all samples.	SBA was the parameter most frequently altered in exposed workers and showed higher significance between the two groups.	Other tests did not show significant differences between the two groups.	SBA can be considered a sensitive parameter of hepatotoxicity induced by organic solvents than the traditional tests.
Sombattheera et al (2015)[31]	Patients with cholangiocarcinoma without clinical jaundice	Clinical presentation and investigations.	60 (with serum bilirubin <2 mg/dL (LTB) 32 (with serum bilirubin >2 mg/dL (HTB)	115	Serum cholesterol, albumin, ALT, AST and ALP were also measured.	-Total SBA in both LTB and HTB groups of the cholangiocarcinoma patients were significantly higher than that of the healthy controls.	Significant correlation was observed between TSBA and total bilirubin levels in the HTB group of cholangiocarcinoma patients but not in the LTB group of cholangiocarcinoma.	Total SBA may contribute to the diagnosis of cholangiocarcinoma in patients without jaundice.
Shomai et al (2013)[32]	Chronic hepatitis C	Serology, liver biopsy and Fibro test scores	135 90/135 patients, (67%) had nonsevere liver fibrosis and the others 45/135, (33%) had severe fibrosis or cirrhosis	0	AST, blood glucose, cholesterol level.	SBA levels were significantly higher in patients with severe fibrosis as compared to nonsevere fibrosis ($P<.0001$)	The combination of serum bile acids, age, body mass index, serum AST, glucose and cholesterol levels are reliably able to predicts the degree of liver fibrosis	SBA levels may have a clinical role as a simple noninvasive tool to assess the severity of HCV-induced liver disease
Kenyon et al (2001)[33]	Women with obstetric cholestasis	Clinical presentation, pregnancy and pruritus, liver function tests	10 patients	0	ALT, and GGT	Pruritus occurred before any changes in liver function tests including total serum bile acids.	Pregnant women with persistent PUO who have normal liver function tests including TSBA, should undergo repeat testing as they may develop biochemical changes of obstetric cholestasis at later gestation.	SBA is not necessarily an earlier biomarker of obstetric cholestasis.

ALP = alkaline phosphatase, ALT = alanine aminotransferase, APTT = activated partial thromboplastin time, AST = aspartate aminotransferase, GGT = gamma-glutamyltransferase, HTB = high total bilirubin, LTB = low total bilirubin, Pt = prothrombin time, PUO = pruritic conditions of unknown origin, SBA = serum bile acid, TSBA = total serum bile acid, UDCA = ursodeoxycholic acid.

Table 2

Studies exploring the use of individual serum bile acids and bile acid ratios as a liver biomarker.

Study (year)[Reference]	Presentation/ Liver injury/ dysfunction	How confirmed?	No of patients	No of control	What else was measured?	Bile salt results	Other results	Conclusions
Woolbright et al (2014)[34]	Acetaminophen-induced acute liver failure.	Standard clinical criteria, medical history of overdose, detectable serum acetaminophen and ALT levels > 1500 IU/L	Acetaminophen-induced liver injury = 22 Acetaminophen-induced acute liver failure = 62	6	Prothrombin time, serum ALT, ALP, serum bilirubin.	-Serum bile acid levels were elevated 5-80 fold above control values in injured patients on day 1. -GDCA was significantly increased in non-surviving patients compared to survivors.	ALT, total serum bilirubin, prothrombin time were raised. Non were predicting survival in acetaminophen-induced acute liver failure.	GDCA could predict survival in patients with acetaminophen-induced acute liver failure and may serve as prognostic biomarker.
Chen et al (2013)[35]	Intrahepatic cholestasis of pregnancy (ICP)	Standard clinical criteria	Severe ICP = 33 Mild ICP = 28	35	Standard liver function tests	-Patients with severe ICP had significantly higher serum levels of TCDCA, TUDCA, GCA, TCA, and glycochenodeoxycholic acid (GCDCA) than women with mild ICP or a normal pregnancy.	Other tests did not differentiate.	Testing primary bile acids TCA, GCA) in clinical practice may help in management of ICP.
Collazos, (1993)[36]	Chronic active hepatitis and patients with mild liver diseases (Chronic persistant hepatitis, steatosis, and minimal changes).	Standard clinical criteria, liver biopsy assessment.	Chronic active hepatitis = 15 Mild liver diseases = 30	90	Serum ALP, GGT, Albumin, Gammaglobulin.	The levels of glycocholic acid were higher in patients with chronic active hepatitis than those with mild liver disease. -Glycocholic acid appeared to reflect histological severity in noncirrhotic liver disease.	Other tests were not able to predict chronic active hepatitis as compared to mild liver diseases.	The specificity of glycocholic acid was higher in detecting chronic active hepatitis patients.
Neghab et al (1997)[37]	Australian steel industry workers exposed to 1,1,2-trichloro-1,2,2-trifluoroethane (FC 113)	History of occupational exposure.	5-6	7–11	Standard liver function tests and individual serum bile acids.	Some of the subgroups of SBA, and a few of the ISBA were raised in workers exposed to FC 113.	Compared to controls, no indications of changes in standard liver function tests.	Exposure to FC 113 was clearly associated with a significant rise in SBA levels but without showing changes in standard liver tests.
Collazos et al (1993)[38]	Patients with diffuse liver diseases including cirrhosis, chronic active hepatitis, non-cirrhotic livers	Clinical picture, histological evaluation	142	25	Prothrombin activity, serum albumin.	Increased serum levels of glycocholic acid were observed in cirrhotic and non-cirrhotic patients. There were highly significant differences in glycocholic acid levels according to the histological severity of the liver disease.	Discriminant analysis showed that prothrombin activity and albumin were better than glycocholic acid in predicting severity of liver disease.	Fasting glycocholic acid measurement can be helpful in the evaluation and follow-up of liver diseases as a marker of histological severity.
Azer et al (1994)[39]	Patients with graft malfunction or hepatic allograft rejection post orthotopic liver transplantation.	Clinical picture, laboratory investigations and liver biopsies.	Graft rejection = 3 Graft malfunction = 3.	Non- complicated = 3	Standard biochemical liver function tests.	-Patients with biopsy-confirmed graft dysfunction due to rejection or nonrejection causes had significantly higher serum concentrations of glycocholate plus glycochenodeoxycholate and taurocholate/ taurochenodeoxycholate ratios than did noncomplicated grafts. -In acute rejection a significant increase in the concentration of glycodeoxycholate plus deoxycholate and a significant decrease in cholate/chenodeoxycholate ratio were noted compared to that in non-rejection graft malfunction.	The changes in individual SBa antedated any other conventional biochemical parameters by at least 48 hours and were highly sensitive and specific.	Individual SBA measurement and bile acid ratios can detect graft dysfunction at an earlier time than routine biochemical tests, and are sensitive and specific for early detection of graft dysfunction.
Changbumrung (1990)[40]	Patients with cholangiocarcinoma and hepatocellular carcinomas.	Clinical presentation, and investigations.	Cholangiocarcinoma = 25 Hepatocellular carcinoma = 75	21	Conventional liver function tests	Conjugated bile acids and total SBA were elevated in both patient groups when compared with those of controls. -Unconjugated primary bile acids and secondary bile acids were noted in the patient groups; they were not detectable in controls.	Only serum alkaline phosphatase and gamma glutamyl transferase were useful in the assessment.	The sensitivity of tests for cholic acid, chenodeoxycholic acide, alkaline phosphatase and gamma glutamyl transferase were high.
Azer et al (1996)[41]	Patients with chronic cholestatic liver diseases (primary biliary cirrhosis and patients with	Clinical picture, and investigations, Child-Turcotte and Mayo scores.	Primary biliary cirrhosis = 12 Primary sclerosing cholangitis = 6 Over 4 years observations	20	Conventional liver function tests	-Patients with a serum total chenodeoxycholic acid concentration at study entry	None of the other biochemical parameter or clinicopathological scores	Serum chernodeoxycholic acid levels and cholic acid/ chenodeoxycholic acid ratio

Study (year)[Reference]	Presentation/ Liver injury/ dysfunction	How confirmed?	No of patients	No of control	What else was measured?	Bile salt results	Other results	Conclusions
	primary sclerosing cholangitis)					that exceded 15 mumol/L were 10 times more likely to die or need a liver transplant in the following 4 years than those with chenodeoxycholic acid levels < 15 mumol/L ($P < .05$). -The taurocholic acid/ taurochenodeoxycholic acid ratio fell during progression of primary biliary cirrhosis but rose in temporal relationship with primary sclerosing cholangitis	could similarly discriminate between the two groups at entry. Time-dependent analyses for the cholic acid/chenodeoxycholic acid ratio, serum total bilirubin and albumin concentrations and Child-Turcotte and Mayo scores were able to differentiate between primary sclerosing cholangitis patients who died or were transplanted and those who were not.	in both diseases were independent indices that allowed for the prediction of survival or the need for liver transplantation.
Jurate et al (2017)[42]	Patients with intrahepatic cholestasis of pregnancy (ICP).	Clinical presentation, and investigations	Intrahepatic cholestasis of pregnancy = 61	29	Conventional liver function tests	-In ICP patients, total bile acids levels were higher than in healthy women due to increase in cholic acid and chenodeoxycholic acid. -The CA/CDCA ratio was significantly higher in ICP patients compared to healthy pregnant women -Total SBA, CA, CDCA were of higher sensitivity.	Establishment of lower cut-off values for transaminases activity might only minimally increase the accuracy of diagnosing ICP.	The study did not reveal any single specific and sensitive marker for reliable diagnosis of ICP.
Baumgartner et al (1995)[43]	Patients with graft dysfunction after orthotopic liver transplantation.	Clinical picture, routine serum liver function tests, liver biopsy, and output of biliary lipids over 20–25 days.	12 patients (with different postoperative courses)	0	Routine serum liver function tests	The ratio of TC/GC decreased post transplantation until rejection appeared. At this time serum BA concentration, normalized bile flow, urinary BA excretion and TC/GC-ratio rose; biliary lipid output and bile flow decreased.	The changes occurred in most patients at the same day or 1–3 days earlier than the changes in AST.	Determination of serum bile acids, TC/GC and output of biliary lipids may help in detecting graft dysfunction
Huang et al (2009)[44]	Patients with intrahepatic cholestasis of pregnancy (ICP).	Clinical picture and investigations.	208	0	Routine serum liver function tests	The CA/CDC ratio contributed little to the diagnosis of intrahepatic cholestasis of pregnancy.	Aspartate transaminase and alanine transaminase were elevated.	The study supports the use of total bile acid alone, without analyzing individual bile acid species, for calculation of the bile acid ratio.
Trichet et al (1994)[45]	Patients with alcoholic hepatitis	Clinical picture, investigations, liver biopsy.	36	0	Routine serum liver function tests.	Total SBA were increased in 97% of patients and particularly CA, and CDCA.	-Serum bilirubin levels were increased in only 72% of patients. -The bilirubin level was correlated with the cholestasis score, but not with the alcoholic hepatitis or fibrosis scores.	A significant correlation was noted between the alcoholic hepatitis scores and total SBA, CA and CDCA levels. No significant correlations was found between the alcoholic hepatitis and cholestasis scores.
Trottier et al (2011)[46]	Patients with primary biliary cirrhosis (PBC) and patients with primary sclerosing cholangitis (PSC).	Clinical picture, investigations including ERCP, MRCP.	Primary biliary cirrhosis = 12 Primary sclerosing cholangitis = 6	Control = 30 Control = 30	Routine liver function tests, total cholesterol, high density lipoprotein, low density lipoprotein, triglycerides, body mass index.	- The concentration of total BA, taurine and glycine conjugates of primary bile acids was elevated in both patients with PBC and PSC when compared to non-cholestatic donors. -Samples from PSC patients displayed reduced levels of secondary acids, when compared to non cholestatic and PBC sera. -The ratio of total glycine versus total taurine conjugates was reduced in patients with PBC, but not in PSC.	ALT, AST, ALP, total bilirubin levels were raised in both PBC and PSC.	Circulating bile acids are altered differentially in PBC and PSC patients.
Trottier et al (2011)[47]	Patients with biliary obstruction, before and after biliary stenting.	Clinical presentation and investigations.	17	40	AST, ALT, GGT, and total bilirubin	-Total serum BA concentration increases from 2.7 μM in control to 156.9 μM in untreated patients with biliary stenosis. -Serum TC and GC acids exhibit 304- and 241-fold accumulations in patients with biliary obstruction compared to controls. -The enrichment in CDCA acid species reached a maximum of only 39-fold, while all	AST, ALT, ALP, and GGT were significantly raised in stenosed patients before stenting compared to after stenting.	Biliary obstruction affects differentially the circulating and/or urinary levels of the various bile acids

Study (year)[Reference]	Presentation/ Liver injury/ dysfunction	How confirmed?	No of patients	No of control	What else was measured?	Bile salt results	Other results	Conclusions
						secondary and 6α-hydroxylated species–except TLC acids–were either unchanged or significantly reduced. -Stenting restored almost normal circulating profile and reduced urinary bile acids.		
Dasarathy et al (2011)[48]	Patients with nonalcoholic steatohepatitis (NASH)	Clinical presentation, investigations including liver biopsy.	36	38	Plasma concentration of fatty acids, Beta-hydroxybutyrate, insulin, glucose, leptin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), FGF21, and 8-hydroxydeoxyguanosine.	Fasting plasma bile acids (glycocholate, taurtocholate, and taurochenodeoxycholate) were significantly higher in patients with NASH	Fasting plasma AST, ALT, triglycerides, FGF21, leptin concentrations were higher in patients with NASH.	Elevated bile acid and FGF21 may be responsible for the higher hepatic fatty acid oxidation in NASH.
Tribe et al (2010)[49]	Patients with intrahepatic cholestasis of pregnancy (ICP)	Clinical picture and investigations.	Patients with ICP = 26 Patients with pruritus gravidarum = 43	26	Standard liver function tests.	-ICP was associated with a predominant increase in cholic acid conjugated with taurine and glycine, from 24 weeks of pregnancy. -Bile acid profiles were similar in normal pregnancy (control) and pregnancy associated with pruritus gravidarum.	There was a significant correlation between TUDCA and AST levels.	The bile acid profiles implicated the role of taurine conjugated bile acids in ICP Individual bile acid profiles in pruritus gravidarum is quite distinct from ICP.
Martinefski et al (2012)[50]	Patients with intrahepatic cholestasis of pregnancy (ICP).	Clinical picture and investigations.	32	38	Standard liver function tests	LCA and UDCA/LCA ratio provided information for a more complete and accurate diagnosis and evaluation of ICP than calculation of solely TSBA levels in pregnant women	Serum bilirubin, AST, ALT, and ALP, were significantly raised in patients with ICP and high pruritus score, not when ICP was associated with low pruritus score.	In ICP, individual serum bile acids such as LCA and UDCA/LCA ratio provide more accurate information than total SBA
Mazzella et al (2001)[51]	Patients with intrahepatic cholestasis of pregnancy (ICP).	Clinical picture and investigations.	20 ICP patients treated with UDCA	10 untreated ICP patients.	Liver function tests, measurement of BA in amniotic fluid and umbilical cord serum.	Maternal serum conjugated CA and CDCA levels fell in treated patients and remained unaffected in untreated ones (= not significant). In treated mothers conjugated CA and CDCA in the cord blood were lower compared to those in untreated ones.	Serum AST, ALT, and ALP, were significantly raised in patients with ICP	Treatment with increasing doses of UDCA is more effective in controlling ICP and improving clinical outcomes after delivery.

ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CA = cholic acid, CDCA = chenodeoxycholic acid, ERCP = endoscopic retrograde cholangiopancreatography, GCA = glycocholic acid, GCDCA = glycochenodeoxycholic acid, GDCA = glycodeoxycholic acid, GGT = gamma glutamyltransferase, ICP = intrahepatic cholestasis of pregnancy, ISBA = individual serum bile acids, LCA = lithocholic acid, MRCP = magnetic resonance cholangiopancreatography, NASH = nonalcoholic steatohepatitis, PBC = primary biliary cirrhosis, PSC = primary sclerosing, cholangitis, SBA = serum bile acid, TCA = Taurocholic acid, TCDCA = taurochendeoxycholic acid, TUDCA = tauroursodeoxycholic acid.

Table 3

Studies exploring the use of urinary bile acids as a liver biomarker.

Study (year)[Reference]	Presentation/ Liver injury/dysfunction	How confirmed?	No of patients	No of control	What else was measured?	Bile salt results	Other results	Conclusions
Bathena et al (2015)[52]	Patients with hepatobiliary diseases.	Clinical picture and investigations.	Patients with liver disease =121	90	Standard liver function tests	-The percentage amidation of overall and most individual BAs was higher in patients than controls. -The percentages of primary bile acids (CDCA and CA) were higher in patients. The percentage of secondary bile acids (DCA and LCA) were lower in patients.	Not available	Bile acid indices belonging to percentage amidation and percentage composition were better associated with the severity of the liver disease than the absolute concentrations of individual and total bile acids.
Nanashima et al (2009)[53]	Patients with hepatobiliary diseases who underwent surgical procedures	Clinical picture and investigations	27	0	Standard liver function tests and serum hyaluronic acid level.	-Urinary sulfated BA level increased in patients with cholestasis. -Urinary sulfated BA level tended to be associated with postoperative uncontrolled ascites	-Urinary sulfated BA level was significantly correlated with serum total bile acid, total bilirubin level and serum hyaluronic acid level.	Urinary sulfated BA is a simple and sensitive noninvasive test for cholestasis and is useful to predict postoperative uncontrolled ascites after hepatic resections

CA = cholic acid, CDCA = chenodeoxycholic acid, DCA = deoxycholic acid, LCA = lithocholic acid.

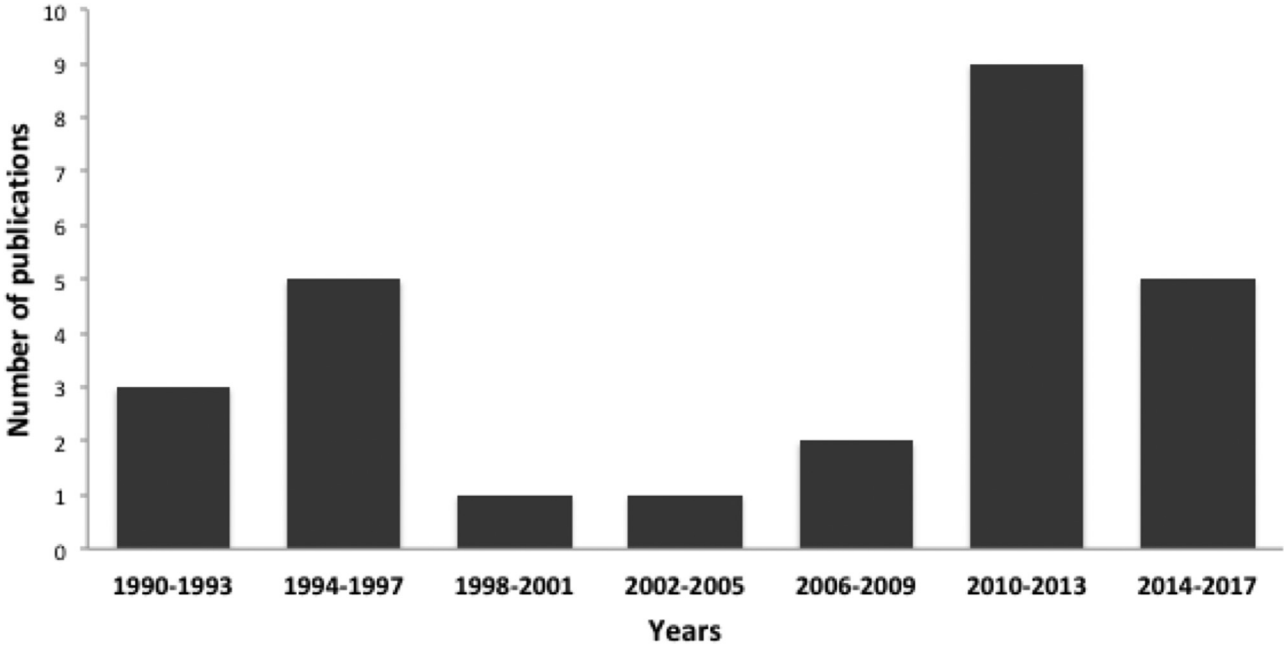

Figure 2. Distribution of publications included in the review over the years (1990–2017).

controls in the studies assessing total serum bile acids were 511and 294, respectively. In the studies assessing individual serum bile acid and bile acid ratios, 971 and 452 patients and controls, respectively. In studies assessing urinary bile acids, the numbers of patients and controls were 148 and 90, respectively.

The studies were conducted between 1990 and December 2017 (over 27 years). Figure 2 shows the distribution and number of studies conducted during these years. It is obvious that there is more interest in this area recently, as 50% of these studies were published after 2010.

3.3. Countries and institutes/universities involved

Geographically, these studies were performed in the United States,[34,44,48,52] Australia,[37,39,41] Spain,[36,38] Germany,[26,43] Canada,[46,47] Thailand,[31,40] Indonesia,[28] Egypt,[27] Austria,[29] Brazil,[30] Israel,[32] China,[35] Lithuania,[42] France,[45] United Kingdom,[49] Argentina,[50] and Japan.[53]

The top universities and research institutes that led such research were the University of Kansas Medical Center, Kansas City, Kansas, University of Arizona, College of Medicine, Arizona; New York University, New York; Cleveland Clinic, Cleveland, Ohio; University of Nebraska Medical Center, Omaha, Nebraska; National Institute of Occupational Health and Safety, Worksafe Australia, Sydney, NSW; University of Sydney, Sydney, NSW; Universitatsklinikum Essen, Hufelandstr. Essen, University of Freiburg; Khon Kaen University, Khon Kaen; Faculty of Tropical Medicine Mahidol University, Bangkok; Sevicio de Medicina Interna, Hospital de Galdakao, Vizcaya; CHUQ Research Center, Faculty of Pharmacy, Laval University, Quebec.

3.4. Liver injuries studied

The liver injuries included in these studies may be summarized as follows: first, studies involving total serum bile acids as a liver biomarker (Table 1): acute graft rejection after liver transplantation,[26] exposure to solvents,[27,30] severe obstructive jaundice,[28] chronic severe pruritus refractory to treatment,[29] cholangiocarcinoma,[31] ICP,[33] and chronic hepatitis C.[32] Second, studies involving individual serum bile acids and bile acid ratios as liver biomarkers (Table 2): acetaminophen-induced acute liver failure,[34] ICP,[35,42,44,49,50,51] chronic active hepatitis,[36] occupational exposure to chemicals,[37] chronic liver disease,[38] graft malfunction or hepatic allograft rejection after orthotopic liver transplantation,[39,43] cholangiocarcinoma and hepatocellular carcinoma,[40] chronic cholestatic liver disease,[41,46] alcoholic hepatitis,[45] biliary obstruction,[47] and non-alcoholic steatohepatitis.[48] Third, studies involving urinary bile acids as a liver biomarker (Table 3): hepatobiliary disease[52] and hepatobiliary disease in patients who underwent surgical procedures.[53]

3.5. Methods used in bile acid assay

The methods used in bile acid assay may be summarized as follows (Table 4): First, studies involving total serum bile acids as a liver biomarker: total bile acids assay kits and enzymatic colorimetric determination[27,28,29,30,31] and enzymatic photometric method.[26] Second, studies involving individual serum bile acids and bile acid ratios as liver biomarkers: ultra-performance liquid chromatography,[34] high-performance liquid chromatography-tandem mass spectrometry,[35] radioimmunotherapy using commercially available kits,[36,38,44] high-performance liquid chromatography,[37,39,41,43,45] gas-liquid chromatography,[42] LC-MS/MS system with an electrospray interface,[46] high-performance liquid chromatography with an MS/MS system with an electrospray interface,[47,48,49] capillary electrophoresis, and cyclodextrin-modified micellar electrokinetic chromatography.[50]Third, studies involving urinary bile acids as liver biomarkers: LC-MS/MS system with an electrospray interface,[52] and a commercially available kit utilising direct enzymatic assay.[53]

Table 4

Summarizes research questions, methods used in bile acid assays, and institute, country of first authors.

Study (year)[Reference]	Study goal/Research question	Method used in bile salt assay	Bile acid sensitivity/Specificity	Institute/University (Country)
Janssen et al (2001)[26]	Investigate the use of serum bile acids in the diagnosis of acute rejection after liver transplantation.	Bile acids were determined using an enzymatic photometrical method.	Serum bile acids had a sensitivity of 86% and specificity of 100%	Universitätsklinikum Essen, Hufelandstr. Essen, (Germany).
El Hady et al (2014)[27]	Assess the impact of occupational exposure to mixture of organic solvents on liver function tests.	Serum bile acids were measured using enzymatic colorimetric determination	The optimal sensitivity of SBA was 83.3% and specificity was 60.2%	Banha Faculty of Medicine, Banha (Egypt)
Lalisang (2012)[28]	Assess SBA as a marker for liver function test as compared to the conventional liver function tests	Automated clinical chemistry analyzer (ACA) TRX 7010 was used to measure serum bile acids.	Not available.	University of Indonesia-Cipto Mangunkusumo Hospital, Jakarta (Indonesia).
Elsendle et al (2011)[29]	Investigate the role of elevated TSBA levels in patients with pruritus of unknown origin (PUO).	Total serum bile acid concentrations were determined using quantitative enzymatic spectralphotometric test according to the manufacturer's instructions (Trinity Biotech, Bray, Ireland).	Not available.	Medical University Innsbruck, Innsbruck (Austria).
Nunes de Paiva and Pereira Bastos de Siqueira, (2005)[30]	Evaluate total SBA in car painters exposed to organic solvents and to compare the levels with classic liver function tests.	An enzymatic method using the Sigma Diagnostic Kit used.	Bile acid showed the highest sensitivity in detecting liver injury (X^2, $P = .0024$).	Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo (Brazil).
Sombattheera et al (2015)[31]	Determine the feasibility of using the total serum bile acid level as an aid for the diagnosis of cholangiocarcinoma in patients without jaundice.	Total serum bile acids was determined using the Total Bile Acids Assay kit (Diazyme Laboratories, Poway, CA) based on the enzyme cycling method.	The cut-off value of total SBA was determined for the low total bilirubin group of cholangiocarcinoma patients was 6.05 μmol/L with the sensitivity and specificity of 46.7% and 84.4%, respectively. When the total SBA and alkaline phosphatase were combined, the specificity increased to 72.9% compared with total SBA alone (67.4%) or alkaline phosphatase alone (43.5%).	Faculty of Associated Medical Sciences, Faculty of Medicine, Khon Kaen University, Khon Kaen, (Thailand).
Shomai et al (2013)[32]	Assess whether SBA levels are elevated in non-cholestatic chronic liver diseases, and whether they correlate with disease severity	Total SBAs were quantified using the Total Bile Acids Assay Kit (Diazyme Laboratories, CA 92064, USA).	The specificity of the SBA-based model (65%) was only moderately higher than the SBAs alone (63%). The SBA-based model had a sensitivity of 93%.	Tel Aviv University, Tel Aviv (Israel)
Woolbright et al (2014)[34]	Determine whether individual bile acid levels could determine outcome in patients with Acetaminophen-induced acute liver failure.	Bile acids were measured using Acquity ultra- performance liquid chromatograph (UPLC) equipped with a Waters Acquity BEH C18.	Not measured.	Department of Internal Medicine, University of Kansas Medical Center, Kansas City, Kansas (USA) Department of Medicine, and Center for Toxicology and

(*continued*)

Table 4
(continued).

Study (year)[Reference]	Study goal/Research question	Method used in bile salt assay	Bile acid sensitivity/Specificity	Institute/University (Country)
				Pharmacology Education and Research, University of Arizona College of Medicine, Phoenix, Arizona (USA)
Chen et al (2013)[35]	Identify possible biomarkers for the clinical grading of intrahepatic cholestasis of pregnancy (ICP) through serum bile acid profiling in women with ICP.	Bile acids were measured using highperformance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS)	Not measured	Key Laboratory of Laboratory Medical Diagnostics, Ministry of Education, Chongqing Medical University, Chongqing, (China).
Collazos (1993)[36]	Determine whether GCA can identify the severity of various noncirrhotic liver diseases.	GCA was measured by radioimmunotherapy assay using a commercially available kit (Abbott Laboratories, North Chicago, Illinois).	The specificity of glycocholic acid was high in the detection of chronic active hepatitis patients at different cut-off levels.	Servicio de Medicina Interna, Hospital de Galdakao, Vizcaya (Spain).
Neghab et al (1997)[37]	Examine the hepatic effects of occupational exposure to 1,1,2-trichloro-1,2,2-trifluoroethane (FC 113) using conventional liver function tests and serum bile acids.). Also gather further data to support or refute the contention that serum bile acid levels could provide a sensitive biological marker of exposure to these solvents.	Bile salts were measured using high performance liquid chromatography.	Not measured	Toxicology Unit, National Institute of Occupational Health and Safety Worksafe Australia, Sydney, NSW, (Australia).
Collazos et al (1993)[38]	Determine the clinical value of measuring the glycocholic acid in patients with benign diffuse liver diseases.	GCA was measured by radioimmunotherapy assay using a commercially available kit (Abbott Laboratories, North Chicago, Illinois).	Not measured	Servicio de Medicina Interna, Hospital de Galdakao, Vizcaya (Spain).
Azer et al (1994)[39]	Determine whether changes in individual SBA levels after engraftment are sensitive, specific and reliable indicators of graft function and whether these changes can antedate other biochemical indicators of hepatic allograft rejection.	Bile acids were measured using high performance liquid chromatography.	The sensitivity and specificity of bile acids were in the range of 95–100%.	University of Sydney, National Institute of Occupational Health & Safety, NSW, Sydney (Australia).
Changbumrung (1990)[40]	Determine the changes in bile acids in patients with cholangiocarcinoma and hepatocellular carcinoma.	Bile acids were measured using high performance liquid chromatography.	Not measured	Faculty of Tropical Medicine, Mahidol University, Bangkok, (Thailand).
Azer et al (1996)[41]	Determine the value of serum bile acids in predicting the course of		Not measured	University of Sydney, National Institute of Occupational

(*continued*)

Table 4
(continued).

Study (year)[Reference]	Study goal/Research question	Method used in bile salt assay	Bile acid sensitivity/Specificity	Institute/University (Country)
	chronic cholestatic liver diseases (primary biliary cirrhosis, and primary sclerosing cholangitis).	Bile acids were measured using high performance liquid chromatography.		Health & Safety, NSW, Sydney (Australia).
Jurate et al (2017)[42]	Assess the sensitivity and specificity of laboratory tests used for diagnosis of intrahepatic cholestasis of pregnancy including serum bile acids.	Bile acids were determined using gas-liquid chromatography.	Total serum bile acids has a sensitivity of 94% and specificity of 63%; Cholic acid has a sensitivity of 96%, and specificity of 63%; Chenodeoxycholic acid has a sensitivity of 88%, and specificity of 59%.	Department of Gastroenterology, Lithuanian University of Health Sciences, Kaunas, (Lithuania).
Baumgartner et al (1995)[43]	Determine the usefulness of monitoring serum bile acids and biliary lipids after OLT with respect to early recognition of graft dysfunction.	Bile acids were measured using high performance liquid chromatography.	Bile acids were more sensitive and specific than standard liver function tests.	Department of Surgery, University of Freiburg, (Germany).
Huang et al (2009)[44]	Determine if the bile acid ratio of cholic acid to chenodeoxycholic acid (CA:CDCA) is an important component for diagnosis of intrahepatic cholestasis of pregnancy (ICP).	Bile acids were measured using one commercial laboratory (Quest Diagnostics Nichols Institute, San Juan Capistrano, CA).	The authors used test-positive and test-negative instead of sensitivity and specificity because there is no generally accepted criteria to diagnosis ICP.	Department of Obstetrics and Gynecology, New York University, New York, (The United States).
Trinchet et al (1994)[45]	Assay serum bile acids in patients with alcoholic hepatitis and to assess the relationship between these parameters, the usual liver tests and the histological features of alcoholic hepatitis.	Serum bile acids were measured using high performance liquid chromatography (HPLC).	Not measured	INSERM Unit-21, Villejuif, (France).
Trottier et al (2011)[46]	Compares serum bile acid levels in patients with primary biliary cirrhosis and primary sclerosing cholangitis.	Bile acids were measured using a LC–MS/MS system with an electrospray interface.	Not measured	Laboratory of Molecular Pharmacology, CHUQ Research Center and the Faculty of Pharmacy, Laval University, Québec, (Canada).
Trottier et al. (2011)[47]	Investigates how biliary obstruction and restoration of bile flow interferes with urinary and circulating levels of 17 common bile acids.	Bile acids were measured using a HPLC-MS/MS system with an electrospray interface,	Not measured.	Centre Hospitalier Universitaire de Québec Research Center and the Faculty of Pharmacy, Laval University, Québec, (Canada).
Dasarathy et al (2011)[48]	Determine the changes in serum bile acids in patients with nonalcoholic steatohepatitis (NASH) and possible causes for increased hepatic fatty acid oxidation	Plasma bile acids were quantified in NASH and in controls using liquid chromatography mass spectrometry/mass spectrometry	Not measured	Department of Gastroenterology, Cleveland Clinic, Cleveland, Ohio, (The United States).
Tribe et al (2010)[49]	Determine the temporal changes in bile acids in normal pregnancy	Bile acids were determined using an HPLC-MS system with electrospray	Not determined	Division of Reproduction and Endocrinology, King's College

(*continued*)

Table 4
(continued).

Study (year)[Reference]	Study goal/Research question	Method used in bile salt assay	Bile acid sensitivity/Specificity	Institute/University (Country)
	and in pregnancies complicated with intrahepatic cholestasis of pregnancy and pruritus gravidarum.	interface using a novel method that allowed the evaluation of 15 bile acids.		London, London, (The United Kingdom).
Martinefski et al (2012)[50]	Determine better markers, including serum bile acids, in ICP for a precise diagnosis and parameters associated with severity of symptoms.	Bile acids were determined by capillary electrophoresis and were separated using cyclodextrin-modified micellar electrokinetic chromatography.	The highest sensitivity (84%) was achieved by determining LCA level, and the highest specificity (100%) from calculating UDCA/LCA ratio, and the highest accuracy (91.5%) if both LCA and UDCA/LCA were both considered.	Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, (Argentina).
Bathena et al (2015)[52]	Compare the urinary BA profiles between healthy subjects and patients with hepatobiliary diseases.	Urinary bile acids were quantified by LC-MS/MS	Depending on the cutoff value of BA concentration, the % amidation UDCA had a sensitivity in the range of 90%–93%, and specificity in the range of 70%–88%. The total CDCA had a sensitivity in the range of 70%–77% and specificity in the range of 70%–84%. The CA had a sensitivity in the range of 70–77% and specificity in the range of 70%–82%. The % sulfated DCA: sulfated BA had a sensitivity in the range of 70–81% and specificity in the range of 70–83%.	College of Pharmacy, Department of Internal Medicine, College of Medicine University of Nebraska Medical Center, Omaha, Nebraska (The United States)
Nanashima et al (2009)[53]	Clarify the clinical significances of urinary bile acid concentrations in liver diseases in adults.	Urinary bile acids were measured by a commercially available kit "UBASTEC by Marukin Bio, Inc, Kyoto, Japan," utilizing direct enzymatic assay.	Not determined	Department of Translational Medical Sciences Nagasaki University Graduate School of Biomedical Sciences, 1 Sakamoto, Nagasaki, (Japan)

DCA = deoxycholic acid, GCA = glycocholic acid, HPLC = high performance liquid chromatography, LCA = lithocholic acid, SBA = serum bile acid, TSBA = total serum bile acid, UDCA = ursodeoxycholic acid.

3.6. The sensitivity and specificity of bile acid concentrations

The sensitivity and specificity of bile acid concentrations may be summarized as follows (Table 4): First, studies involving total serum bile acid concentrations as a liver biomarker: There was variability in these scores, which can be summarized as follows: Bile acids had a sensitivity of 86% and specificity of 100%,[26] sensitivity of 83.3%, and specificity of 60.2%;[27] bile acids had the highest sensitivity compared to other liver function tests,[30] with a sensitivity of 46.7% and specificity of 84.4%,[31] sensitivity of 93%, and specificity of 63% to 65%.[32] Second, studies involving individual serum bile acids and bile acid ratios as liver biomarkers: Not all studies reported the sensitivity or specificity of individual serum bile acids. A study reported that the specificity of glycocholic acid was high in the detection of chronic active hepatitis at different cut-off levels,[36] In determining the use of individual serum bile acids to assess graft function after orthotopic liver transplantation, the sensitivity and specificity of bile acids were in the range of 95% to 100%, none of the liver biochemical parameters such as serum bilirubin, alkaline phosphatase, transaminases, or gamma-glutamyltransferase were able to show such a level of sensitivity or specificity,[39,43] In ICP, serum cholic acid had a sensitivity of 96% and a specificity of 63%, and chenodeoxycholic acid had a sensitivity of 88% and specificity of 59%.[42] In another study of patients with ICP, the highest sensitivity (84%) was achieved by determining the lithocholic acid (LCA) level, and the highest specificity (100%) was achieved by calculating the ursodeoxycholic acid (UDCA)/LCA ratio, and the highest accuracy was 91.5% if both LCA and UDCA/LCA were achieved.[50] Third, studies involving urinary bile acids as liver biomarkers: the percentage amidation UDCA had sensitivity in the range of 90% to 93% and specificity in the range of 70%–88%. The total CDCA had a sensitivity in the range of 70% to 77% and specificity in the range of 70% to 84%. The CA had a sensitivity in the range of 70% to 77% and specificity in the range of 70% to 82%.[52]

4. Discussion

This systematic review examined whether bile acid concentration could be used as a biomarker of liver injury. It also examines the sensitivity and specificity of bile acids (BA) concentrations compared with changes in traditional liver function tests. The review included a total of 28 studies covering total serum bile acids, individual serum BAs, bile acid ratios, and urinary BAs. The review shows that there has been significant interest in this area from over 17 countries over the last 3 decades and is dedicated to a range of liver disorders.

While a few studies support the use of bile acid measurements as a potential biomarker in ICP, and in occupational exposure to hepatotoxicants, the value of elevated serum bile acids as a diagnostic criterion has been questioned and not confirmed.[54] Studies covering ICP have recently been reviewed in a Cochrane study.[54] In our review, we included studies examining the use of bile acids as a biomarker of liver function in these patients, and we believe that the Cochrane study is an additional resource for those interested in further information. On the other hand, Ovedia et al (2019) recently quantified the adverse perinatal effects of ICP in women with increased serum BA concentrations. Their work showed that the risk of stillbirth increased in these women when the serum BA concentration was $\geq 100\,\mu mol/L$. Therefore, repeated measurements of serum BA are recommended for these women until delivery. This study highlights the significance of BA as a predictive test for ICP.[55]

The findings from this systematic review show no consistency and agreement on a specific individual bile acid to be recommended in the majority of liver injuries/disorders included in this review. This inconsistency might be related to several factors, including differences in the methods used in bile acid assays, the severity and stage of liver disease, and the type of individual or total BA used in the study. In addition, not all studies included liver histological examination to assess the nature and severity of the lesion and compare these parameters with serum bile acid levels.

Furthermore, not all studies examined the sensitivity and specificity of bile acids at the time of the study to identify the severity of liver damage from bile acid levels. These limitations in the current literature raise several questions.

First, why do bile acid measurements seem promising as a test for ICP or exposure to certain chemicals? ICP occurs in women with increased susceptibility to cholestasis caused by higher oestrogen levels and other hormonal changes. The disease is characterized by maternal itching, raised serum BA, and is more likely to occur in the third trimester or with multiple pregnancies, which have higher hormone levels than the first trimester or singleton pregnancy.[56] Symptoms and biochemical changes usually resolve postpartum. Currently, there is evidence suggesting that increased susceptibility to the disease in specific populations is related to one or more genetic variants.[57] Therefore, more studies are needed to address the current limitations of the literature.

Second, what are the determinants of serum and urinary bile acids that could affect their sensitivity and specificity as biomarkers of liver injury? Theoretically, in healthy individuals, the determinants of serum bile acids are related to bile acid conjugation, bile acid absorption, and hepatic clearance. The evidence demonstrated that acute and chronic interruption of enterohepatic circulation causes a significant decrease in the basal levels of cholyl conjugates.[58] In healthy individuals, the majority of bile acids are recycled in the enterohepatic circulation, and only a small portion (about 4%–6%) is synthesized in the liver. Considering the above evidence, the authors concluded that the determinant of serum bile acids in healthy men is the rate of intestinal absorption.[58] This factor is not the primary determinant when there is interference with hepatic uptake, transport, or efflux of bile acids, as is the case with ICP or exposure to certain chemicals.

Third, what is the future of bile acid research in this area? Based on what was discussed earlier, it appears that BA could be a promising biomarker in the case of hepatotoxicants that interfere with hepatic bile acid transport. In addition, the kinetics of primary bile acids are affected, as in patients after orthotopic liver transplantation.[59] Assessing the histopathological changes together with liver function tests may provide a better understanding of the changes in bile acids and their significance. In addition, evaluating graft malfunction after orthotopic liver transplantation by determining bile acid concentrations seems promising and needs further evaluation by involving a large number of patients and comparing findings with simultaneous changes in serum bile acids. Studying the kinetics of primary bile acids in these patients (2 males and 4 females) 6 to 20 months after transplantation who were treated with cyclosporin A showed that cholic acid and chenodeoxycholic acid were

simultaneously determined after oral administration of radioactive bile acids [24–13C]-CA and [24–13C]-CDCA, based on isotope dilution. When these patients were compared with control (10 healthy individuals), pool sizes, fractional turnover rates, and synthesis rates of both primary bile acids, CA and CDCA, were not significantly different from control subjects.[59]

This review has several strengths: first, the search was carried out on 3 search databases commonly used by researchers, and we used search words reflective of the review aims. To maximize the search yield, we manually searched the list of references of the identified articles and reviews. Second, we explored the journals listed by the Journal Citation Reports-2016 of the Web of Science and Cochrane Central Register of Controlled Trials (Cochrane Library). Third, the evaluation of the studies was carried out independently by 2 evaluators, and the Cohen kappa score for the inter-rater agreement was satisfactory. However, this review was not without limitations; only studies in the English language were considered, raising the possibility that other potential studies on the topic in others were not included.

Therefore, despite our meticulous search protocol, we might have missed the inclusion of a few studies. This review presents a valuable resource for gastroenterologists, hepatologists, physicians, and internists. It is also useful to researchers in this area as well as trainees in this field. Twenty eight studies could be an ideal reading material for researchers in this field to identify gaps in these studies and what should be addressed in future research.

Considering the outcomes outlined in this review, there are several recommendations concerning future directions in the research on bile acids. These can be summarized as follows:

First, there is a need for multicenter research in which a large number of patients are recruited in controlled studies. In these studies, total serum bile acids, individual serum bile acids, and urinary bile acid concentrations may be compared with the results of standard liver function tests. There is also a need to explore any correlation between histopathological changes in the liver (the gold standard of liver injury measurement) and bile acid concentrations. Studies with such a design will accurately estimate the sensitivity and specificity of bile acids and compare the findings with those obtained from standard liver function test results.[59]

Second, the determination of BA and BA profiles has been used in screening for liver disease in spray workers and ICP; the value of BA concentration in the diagnosis has been questioned and not confirmed. It might have prognostic value in women with ICP when BA concentrations are increased. No studies have addressed the use of bile acids in patients with severe chronic liver disease, predicting liver failure, or the need for liver transplantation. In addition, in patients with cirrhosis and significant fibrosis when the liver transaminases are within the normal range, exploring the use of bile acids and bile acid profiles in these patients.[60,61]

Third, nonalcoholic fatty liver disease (NAFLD) causes significant changes in the liver. Metabolic risk factors, including obesity, type-2 diabetes mellitus, dyslipidemia, and metabolic syndrome, have been linked with the pathogenesis of NAFLD. Currently, NAFLD is progressively becoming the most common cause of chronic liver disease in Western countries. Most studies in the literature exploring NAFLD and bile acids have focused on the role of bile acids in the pathophysiology, pathogenesis, and metabolic aspects of bile acids. In addition, changes in circulating BA, bile acids, and inflammatory mediators have been addressed.[62] They do not discuss the relationship between BA concentrations as a Predictor of liver function or severity/stage of NAFLD Further studies are recommended to assess the potential of BA as a biomarker for NAFLD.

5. Conclusions

This review reflects research over the last 3 decades exploring the use of bile acids and bile acid profiles in assessing liver dysfunction or injury and the sensitivity and specificity of bile acid concentrations. The question that may be raised is, what can we learn from this review? This analysis highlighted several deficiencies in the literature in this area. Not all studies have examined the sensitivity and specificity of bile acids. In addition, this review shows inconsistencies in the reported results and the lack of agreement on specific individual bile acid biomarkers of liver injury and liver dysfunction. While these differences might be related, in part, to differences in the methods used in bile acid assays, several studies were based on a small number of patients. However, weak evidence could add bile acids to routine liver function tests in ICP and exposure to certain hepatotoxicants. We identified no studies on the use of BA as a biomarker of NAFLD. However, there is growing evidence of the use of BA as a prognostic test in women with ICP and increased serum BA. Therefore, there is a need for multicenter studies and controlled studies with a larger number of patients to compare bile acid concentrations with liver function test results and the severity of changes in liver histopathology.

Author contributions

Conceptualization: Samy A Azer.
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Funding acquisition: Samy A Azer.
Investigation: Samy A Azer.
Methodology: Samy A Azer, Rana Hasanato.
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Software: Samy A Azer, Rana Hasanato.
Supervision: Samy A Azer.
Validation: Samy A Azer.
Writing – original draft: Samy A Azer, Rana Hasanato.
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