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Serum ferritin in neonatal cholestasis: A specific and active molecule or a non-specific bystander marker?

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ABSTRACT

Background: Serum ferritin (SF) and consequently hepatic iron have long been considered important in liver fibrosis progression. They have been studied in different liver diseases with no previous reports in neonatal cholestasis (NC). This study aimed to measure SF in different etiologies of NC and investigate its relation to hepatic iron and fibrosis.

Methods: SF was measured in 75 infants, including 50 with NC and 25 with sepsis. SF was compared between these two groups. Biochemical parameters, hepatic iron grades, and liver fibrosis and other histopathological characteristics and correlated with SF were assessed in NC group. Finally, a comparison between intrahepatic cholestasis and obstructive etiology was performed.

Results: SF was elevated in NC (1598 ± 2405 ng/mL) with no significant difference from those with sepsis ($P = 0.445$). NC and sepsis constituted augmenting factors leading to more elevation of SF (2589 ± 3511 ng/mL). SF was significantly correlated with hepatic iron grades ($r = 0.536$, $P < 0.0001$) and a cut-off value of 803.5 ng/mL can predict higher grades (≥ grade 3) of iron deposition with sensitivity of 100%, specificity of 70% and accuracy of 85%. Moreover, SF was significantly higher ($P < 0.0001$) in those with intrahepatic cholestasis (2602 ± 3154 ng/mL) and their prevalent pathological findings of giant cell transformation ($P = 0.009$) and hepatocyte swelling ($P = 0.023$) than those with obstructive etiology (672 ± 566 ng/mL) and their prevalent pathological findings of ductular proliferation ($P = 0.003$) and bile plugs ($P = 0.002$). SF was unrelated to the grade of liver fibrosis ($P = 0.058$).

Conclusions: SF is non-specifically elevated in NC, with positive correlation to hepatic iron grades. SF ≥ 803.5 ng/mL can predict higher grades (≥ grade 3) of hepatic iron. However, an active role of increased SF and hepatic iron in disease progression remains questionable.

Introduction

Serum ferritin (SF) measurement is important for the assessment of common diseases, such as iron-deficiency anemia and iron overload conditions [1]. However, there is evidence that several diseases besides iron overload can be associated with elevated SF. Liver diseases are examples of these clinical conditions, and they were the concerns of many studies of SF in adults [2,3] with few studies concerning childhood liver diseases [4].

The interest of iron studies in liver diseases relies on the presumption that increased SF and hepatic iron are important in the progression of liver fibrosis [5]. Moreover, previous studies demonstrated that depletion of iron in patients with iron overload liver diseases can be promising [6–8]. However, despite these observations, studies assessing the association between SF or hepatic iron and the progression of fibrosis remain controversial [9]. Moreover, SF can be elevated without the presence of increased hepatic iron [10]. Therefore, it remains to be defined when elevated SF could reflect hepatic iron content.

Neonatal cholestasis (NC) constitutes a significant percentage of liver diseases in pediatric age caused by many disorders with different pathological changes. It is usually a serious condition which requires urgent and detailed investigations to achieve a timely and adequate therapy [11], as some of which can progress rapidly to liver failure and death if not managed properly [12]. SF is considered one of the laboratory workup of NC and is recommended as a screening and diagnostic biomarker for some NC etiologies, such as neonatal iron storage disease and hemophagocytic lymphohistiocytosis (HLH) [13]. In spite of the classic clinical presentation in live

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born infants with neonatal hemochromatosis (NH) is liver failure manifesting within hours of birth, a spectrum of illness has been recognized, with some documented cases showing even nearly unaffected liver [14]. Other reported cases presented with cholestasis and severely affected liver function [15].

In our routine workup for NC, many cases with significantly elevated SF were detected that proved later on to be of etiologies other than iron storage disease and HLH. Moreover, it is not yet defined the relation of this elevated SF to hepatic iron in NC and if it is a bystander or an active molecule that may share in the disease progression. This study aimed primarily to investigate SF in different etiologies of NC to elucidate if elevated SF could still be used as a screening or diagnostic tool for specific NC etiologies or it is a non-specific marker; secondly, to study the relation of SF to hepatic iron, histopathological changes specially fibrosis, and other disease parameters.

Methods

Study population

This prospective case-control study included 75 infants; 50 infants with NC (cholestasis group) and 25 infants affected by sepsis without cholestasis (sepsis group) as a control group for SF. They were recruited consecutively from the Department of Pediatric Hepatology, Gastroenterology, and Nutrition, National Liver Institute, Menofiya University within two years duration (January 2015 to December 2016). As ferritin is a known acute phase reactant, it could be elevated non-specifically in different inflammatory conditions [1]. Therefore, sepsis was selected as a control group being one of the commonest causes that lead to elevated acute phase reactants [16], to clarify if SF elevation in NC is due to the cholestatic condition itself or just as an acute phase reactant.

An informed consent was signed by parents of each infant. This study was approved by the Research Ethics Committee of National Liver Institute, Menofiya University in accordance with the *Declaration of Helsinki*, 1975 and its updates.

Etiological diagnosis

NC was defined in our cases as infants who developed liver disease and conjugated hyperbilirubinemia within the neonatal period [17]. Sepsis was defined as systemic inflammatory response syndrome in the presence of or as a result of infection [18]. Positive blood culture confirmed the presence of infection [19]. Preterm infants, those with recent (within the last three months) blood transfusion, bleeding, or iron intake, and those with comorbid condition, were excluded. Also, infants with NC who were not indicated for liver biopsy or contraindicated were not included in the study.

After complete history taking, full clinical examination, routine investigations (laboratory and abdominal ultrasound), and liver biopsy, infants with NC were categorized [20] as biliary atresia (BA) ($n=26$) and non-BA cholestasis ($n=24$). Diagnosis of BA was confirmed by laparotomy findings with or without intraoperative cholangiography before proceeding to surgery. Those with non-BA cholestasis underwent further investigations for the expected etiology. The diagnoses within the non-BA cholestasis group were progressive familial intrahepatic cholestasis (PFIC) ($n=15$), cytomegalovirus hepatitis ($n=5$), cholestatic sepsis ($n=3$), and galactosemia ($n=1$).

The three cases with the diagnosis of cholestatic sepsis have undergone the complete workup of NC, and sepsis was finally the only defined etiology. Sepsis is a known cause of cholestasis especially in developing countries [21,22]. Usually those who present with NC will be managed without liver biopsy [23]. However, in a minority of cases there is no resolution of NC in spite of infection control. In these conditions, as in our cases, liver biopsy could be accomplished for the exclusion of any other underlying causes; of course after control of the infection. The time point range of liver biopsy in these cases was 3 weeks to 3 months after sepsis. Besides these three cases with cholestatic sepsis there were another 10 cases with sepsis on top of other defined etiologies of non-BA cholestasis. Also, 5 cases with BA had sepsis on top of their original etiology. In summary, sepsis was defined in 18 cases with NC, including 3 cases with sepsis alone, 8 cases with PFIC, 2 cases with congenital cytomegalovirus, and 5 cases with BA.

Measurement of serum ferritin

For all infants, measurement of SF was carried out by automated method using RCHITECT i1000SR immunoassay analyzer (Abbott Park, IL, USA). SF was measured at the timing of liver biopsy to have their values correlated with hepatic iron. Reference range of SF in this age group is 10–95 ng/mL [24].

Ultrasonographic evaluation

Abdominal ultrasound was done using 2–5 MHz curved linear and 4–8 MHz linear transducers (Xario XG; Toshiba, Tokyo, Japan) and color Doppler ultrasound was done using pulse repetition frequency, 1000–1500 Hz; power gain percentage, 80–90%; medium wall filter [25].

Liver biopsy

Liver biopsy was performed for all infants with NC using a Tru-Cut needle with ultrasonic guidance. Liver core biopsies were fixed in formalin, processed, and embedded in paraffin. Sections were cut and stained with hematoxylin-eosin, diastase periodic acid-Schiff, and Orcein for routine histopathological evaluation. Masson's Trichrome and Perls Prussian blue stains were used to assess fibrosis and iron deposits, respectively. Interpretation of the slides was performed blindly by the contributing pathologist (El-Azab DS).

Assessment of the histopathological changes of neonatal cholestasis

All sections were assessed for the presence of: (i) ductular proliferation, (ii) bile plugs, (iii) portal infiltrated with lymphocytes, neutrophils, and esinophils, (iv) giant cell transformation of hepatocytes, (v) hepatocyte swelling, and (vi) cholestatic rosetting. Liver fibrosis was assessed in five grades [26]: grade 0, absent or fibrous expansion of some portal areas; grade 1, fibrous expansion of most portal areas; grade 2, focal porto-portal bridging; grade 3, marked bridging; and grade 4, cirrhosis.

Assessment of hepatic iron

Iron deposition in hepatic tissue was assessed in 5 grades as reported by Paterson and Pietrangelo [27], using an Olympus Microscope (Tokyo, Japan): grade 0, iron granules are absent or barely discernible at magnification power $\times 400$; grade 1, iron granules are barely discerned but confirmed at $\times 200$; grade 2, discrete iron granules are resolved at $\times 100$; grade 3, discrete iron granules are resolved at $\times 20$; grade 4, masses of iron are visible at $\times 4$ or naked eye.

Statistical analysis

Quantitative values were presented in the form of mean $\pm$ standard deviation (SD) and qualitative data as number and percentage.

Table 1
Demographic, anthropometric, and laboratory data of the studied groups.

Variables	Cholestasis group ($n=50$)	Sepsis group ($n=25$)	P value
Age (d)	71.0 ± 46.7	79.6 ± 92.1	0.107
Sex (male)	29 (58%)	14 (56%)	0.869
Weight (kg)	4.5 ± 1.3	4.2 ± 2.5	0.087
Length (cm)	57.7 ± 7.0	53.7 ± 10.5	0.073
Head circumference (cm)	37.6 ± 3.0	36.4 ± 6.0	0.169
Total bilirubin (mg/dL)	13.6 ± 5.4	3.8 ± 5.0	**<0.0001**
Direct bilirubin (mg/dL)	9.4 ± 3.9	0.4 ± 0.3	**<0.0001**
Total proteins (g/dL)	5.2 ± 0.8	6.4 ± 1.1	**<0.0001**
Albumin (g/dL)	3.2 ± 0.6	3.8 ± 0.8	**0.001**
ALT (U/L)	139 ± 118	35 ± 19	**<0.0001**
AST (U/L)	264 ± 216	40 ± 19	**<0.0001**
ALP (U/L)	616 ± 382	162 ± 81	**<0.0001**
GGT (U/L)	487 ± 427	114 ± 78	**<0.0001**
PT (s)	12.7 ± 2.1	12.1 ± 0.8	0.510
INR	1.05 ± 0.17	1.05 ± 0.06	0.203
PTT (s)	38.4 ± 6.7	37.3 ± 3.7	0.665
Hemoglobin (g/dL)	9.8 ± 1.8	12.5 ± 2.8	**<0.0001**
WBC ($\times 10^9$/L)	12.8 ± 4.9	14.5 ± 5.7	0.247
Platelet ($\times 10^9$/L)	429 ± 189	234 ± 121	**<0.0001**

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: γ-glutamyltransferase; PT: prothrombin time; INR: international standard ratio; PTT: partial thromboplastin time; WBC: white blood cell.

For quantitative data, statistical significance between two groups was tested by either independent samples t-test or by Mann-Whitney U test according to the nature of the data. Kruskal Wallis test was used to compare among non-parametric data for multiple groups. Chi-square test or Fisher's exact test were used for testing significance between qualitative data. Spearman's test was used for correlations. The cut-off for optimal clinical performance was determined from the receiver operator characteristic (ROC) curve. The diagnostic performance was measured as sensitivity, specificity, and accuracy. A P value < 0.05 was considered significant. Multivariate regression analysis was performed for the predictors of SF level in the two studied groups ($n=75$) and in the cholestasis group ($n=50$). Statistical analysis was carried out using SPSS 13.0 (SPSS Inc, Chicago, IL, USA).

Results

Demographic, anthropometric, and laboratory data of the studied groups

The cholestasis and sepsis groups were matched for age, sex and anthropometric measures. Serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), and platelets were significantly higher in the cholestasis group, while total proteins, albumin, and hemoglobin were significantly lower in the cholestasis group than those in the sepsis group (Table 1).

Serum ferritin in the studied groups

It was found that SF is elevated in the cholestasis (1598 ± 2405 ng/mL) and the sepsis (1175 ± 1478 ng/mL) groups compared to reference range of SF in this age group (10–95 ng/mL) with no statistical difference between the two groups ($P=0.445$) (Fig. 1(A)).

In univariate analysis, NC without sepsis has elevated SF (1041 ± 1220 ng/mL) with no statistical difference from that of the sepsis group ($P=0.872$). When NC and sepsis were present together in the same infant they constituted augmenting factors with higher level (2589 ± 3511 ng/mL) than cholestasis alone ($P=0.019$) (Fig. 1(B)). In multivariate regression analysis, the presence of NC and sepsis together was the only independent predictor of elevated SF ($\beta=1547$, $P=0.013$) (Table 2).

Table 2
Multiple regression analysis for the variables which could predict the level of serum ferritin in the groups studied.

Variables	Coefficient	95% CI		P value
		Lower	Upper	
Cholestasis	−133	−1234	967	0.81
Sepsis	NI	–	–	–
Combined cholestasis and sepsis	1547	332	2762	**0.013**

NI: not included.

Table 3
Multiple regression analysis for the variables which could predict the level of serum ferritin in the neonatal cholestasis group.

Variables	Coefficient	95% CI		P value
		Lower	Upper	
Non-BA cholestasis	1602	255	2947	**0.021**
Sepsis	941	−459	2342	0.183

In univariate analysis, non-BA cholestasis has significantly higher level of SF than BA (2602 ± 3154 vs. 672 ± 566 ng/mL, $P < 0.0001$) (Fig. 1(C)). When omitting associated sepsis from both groups, still non-BA cholestasis has significantly higher level of SF than BA (1835 ± 1709 vs. 625 ± 565 ng/mL, $P=0.007$) (Fig. 1(D)). In multivariate regression analysis, the non-BA cholestasis was the only independent predictor of elevated SF in the NC group ($\beta=1602$, $P=0.021$) (Table 3).

Hepatic tissue iron

Different grades of iron deposition were presented in Fig. 1(E) and (F) and Fig. 2(B)–(F). In the cholestasis group, twenty-two cases (44%) showed grade 0, 9 (18%) showed grade 1, 9 (18%) showed grade 2, 2 (4%) showed grade 3, and 8 (16%) showed grade 4. No significant difference was found between NC with sepsis and NC without sepsis regarding the different iron deposition grades ($P=0.075$). Furthermore, iron deposition grades were significantly higher in non-BA cholestasis when compared with those in BA ($P=0.016$). SF at a cut-off value of 803.5 ng/mL can predict higher grades (≥ grade 3) of tissue iron deposition with sensitivity of 100%, specificity of 70% and accuracy of 85% (Fig. 2(A)).

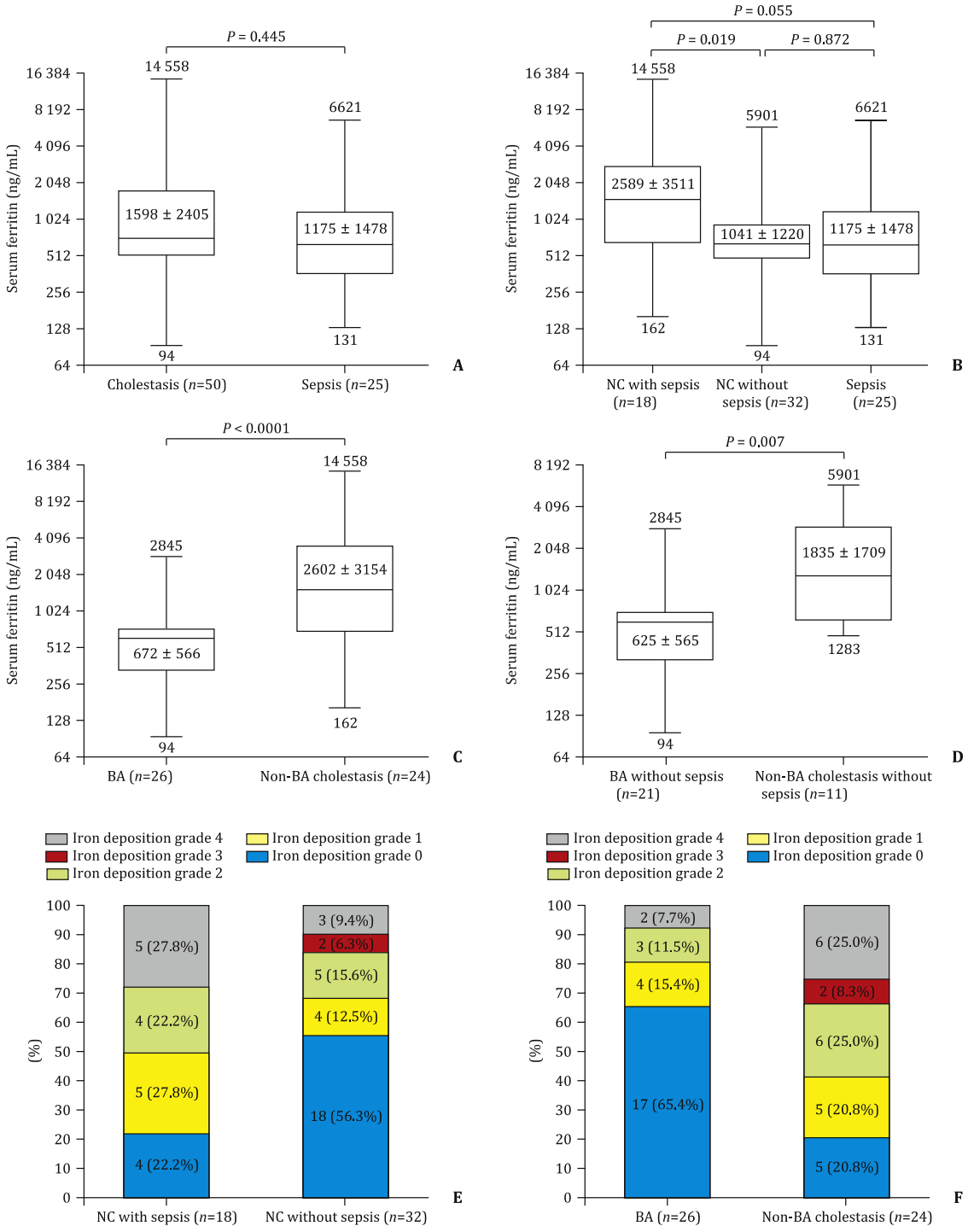

Fig. 1. SF in cholestasis and sepsis groups and hepatic iron grades in cholestatic infants. (**A**) SF is elevated in both cholestasis and sepsis groups without significant difference between them. (**B**) Patients with NC and sepsis had significant higher SF in comparison with NC without sepsis, the SF had no difference between NC patients and septic patients. (**C**) SF in non-BA cholestasis is significantly higher than that in BA. (**D**) When omitting associated sepsis, still SF in non-BA cholestasis is significantly higher than that in BA. (**E**) All the five hepatic tissue iron grades were represented in NC, without statistical difference between those of NC with sepsis and those of NC without sepsis ($P=0.075$). (**F**) Infants with intrahepatic cholestasis (non-BA cholestasis) has higher grades of iron deposition (positive in 79.2%) than infants with BA (positive in 34.6%) ($P=0.016$). SF: serum ferritin; BA: biliary atresia; NC: neonatal cholestasis.

Correlation of serum ferritin and other parameters

SF has a significant positive correlation with hepatic iron deposition grade ($r=0.536$ and $P<0.0001$) while it has a significant negative correlation with age ($r=-0.501$ and $P=0.0002$). However, it has no significant correlations with the hepatocellular and biliary enzymes ($P>0.05$) (Fig. 3).

Histopathological changes and their relation to serum ferritin

Cholestatic rosetting ($n=46$, 92%) followed by bile plugs ($n=28$, 56%), then ductular proliferation ($n=27$, 54%) were the commonest pathological findings detected in the cholestasis group. SF was significantly lower in cases with ductular proliferation and bile plugs (812 ± 805 vs. 2521 ± 3235 ng/mL, $P=0.003$ and

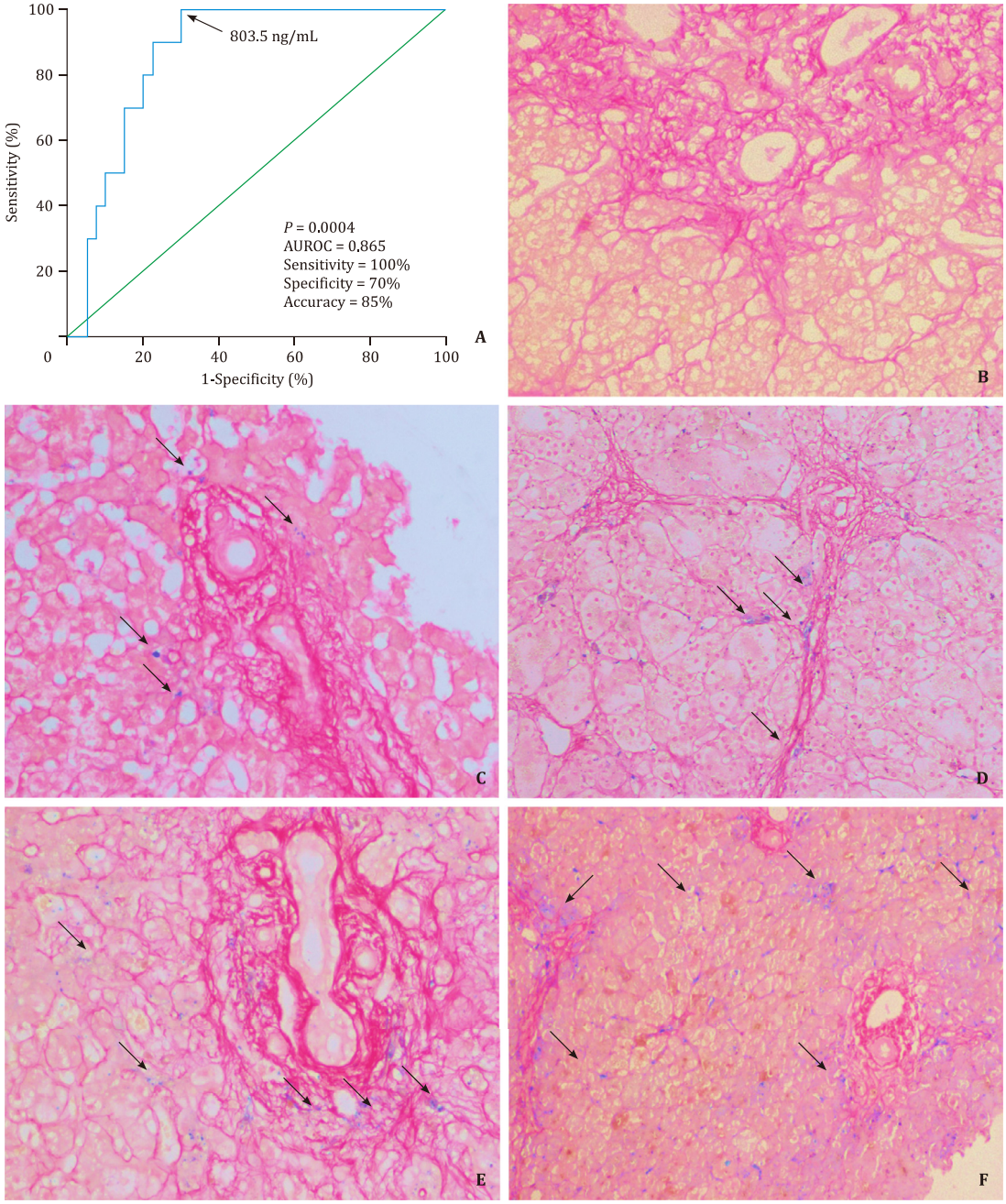

Fig. 2. Hepatic iron grades and clinical performance of serum ferritin for detection of high grades of hepatic tissue iron. (**A**) Serum ferritin at a cut-off value of 803.5 ng/mL can detect high grades of hepatic tissue iron with 100% of sensitivity, 70% of specificity, and 85% of accuracy. Hepatic iron is represented in the 5 grades from 0–4. (**B**) Grade 0, iron not seen at magnification power × 400. (**C**) Grade 1, iron granules barely discerned but confirmed at × 200. (**D**) Grade 2, discrete iron granules resolved at × 100. (**E**) Grade 3, discrete iron granules resolved at × 20. (**F**) Grade 4, masses of iron visible at × 4. Iron granules appear blue colored and referred to by arrows.

908 ± 1197 vs. 2477 ± 3194 ng/mL, $P = 0.002$, respectively, Table 4) while higher in those with giant cell transformation and hepatocyte swelling (2273 ± 3335 vs. 1219 ± 1621 ng/mL, $P = 0.009$ and 2446 ± 3318 vs. 932 ± 941 ng/mL, $P = 0.023$, respectively, Table 4). There was no significant difference of SF values in relation to different liver fibrosis grades and the other pathological findings (Table 4).

Discussion

NC constitutes a significant morbidity burden for the affected babies and their parents [12]. Although SF was investigated in many diseases [4,28,29], it was not studied in NC. Moreover, SF has long been considered as a screening tool for NH [14,30] and HLH [31,32]. Both diseases can present with a spectrum ranging from severe form with neonatal liver failure [33,34] to mild cases with NC [14,35]. It is debatable if SF could be a reliable marker for these etiologies. Moreover, little is known about SF value in NC disorders.

In the present study, we found that SF was elevated in NC group. The etiology of the studied NC cases comprised BA, PFIC, and others with no case of either NH or HLH. Moreover, the mean value of SF in the studied NC infants (1598 ± 2405 ng/mL) was higher than the SF levels suggested for NH (> 800 ng/mL) [34,36] or HLH (≥ 500 ng/mL) [37] by the diagnostic groups. This result suggests that relying on SF as a screening or a diagnostic investigation for both etiologies, when presenting with NC, could be misleading. Of note that all included cases in the present study were

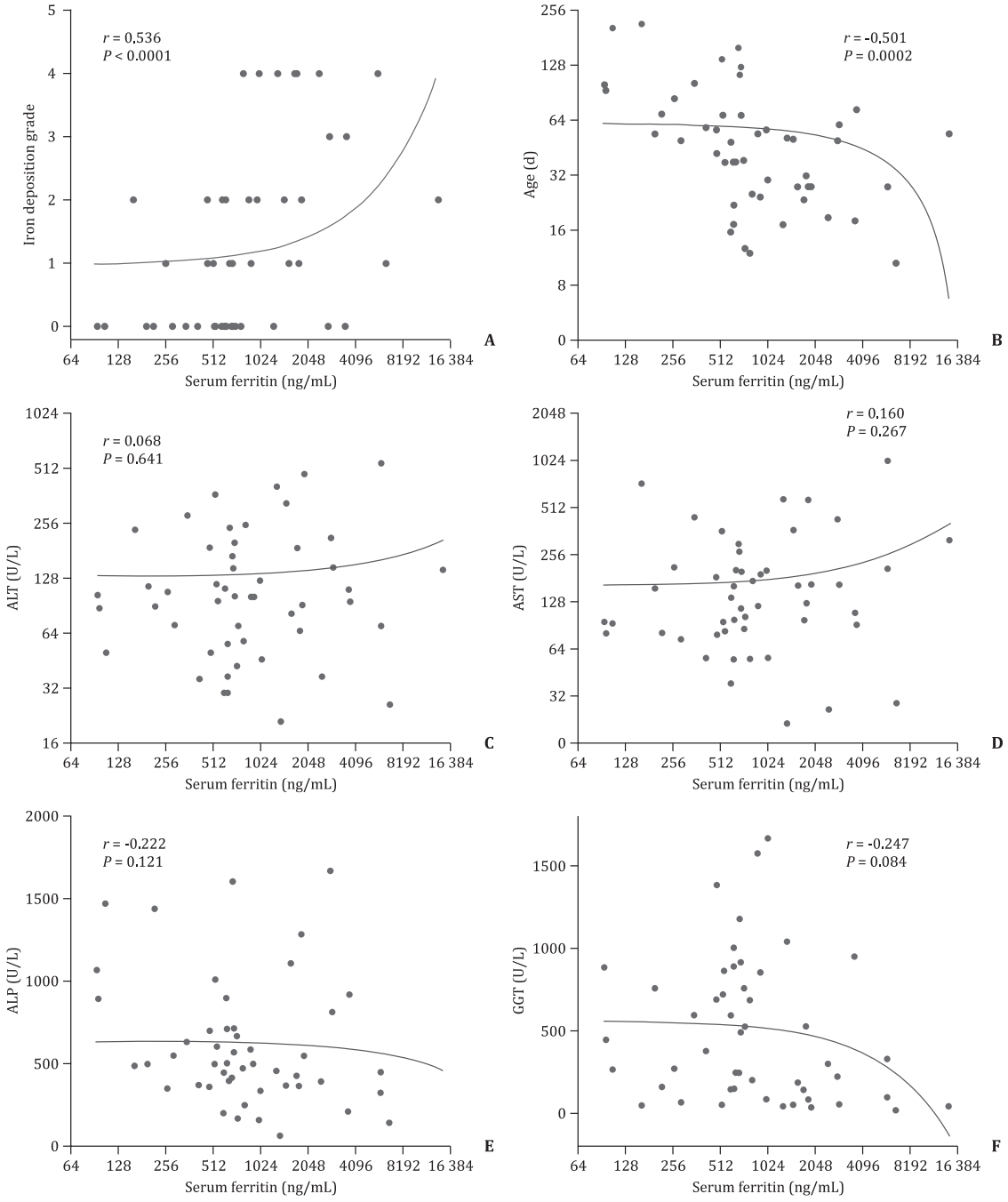

Fig. 3. Correlation of serum ferritin with hepatic iron grade, age, and hepatocellular and biliary enzymes. Serum ferritin has positive correlation with hepatic tissue iron grade (**A**), and significantly negatively correlation with age (**B**). However, serum ferritin is not significantly correlated with ALT (**C**), AST (**D**), ALP (**E**), and GGT (**F**). ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: γ-glutamyltransferase.

without severe synthetic dysfunction and with no liver failure; that is by definition the SF analysis is skewed towards milder phenotypes.

According to the previous data a question is raised; why SF is elevated in cholestatic conditions? Is it just an acute phase reactant secondary to the hepatic inflammatory process or it is related to cholestasis itself?

Sepsis is a known condition that causes release of acute phase reactants; one of which is SF [16]. In the present study, SF in NC group was compared with that in a sex and age matched sepsis group. In univariate analysis, results showed higher SF in NC group than in the sepsis group, but with no significant difference. Moreover, it was found that SF is significantly higher in NC when accompanied with sepsis than in NC without sepsis, and borderline higher than in the sepsis group. Furthermore, there was no significant difference between the NC without sepsis and sepsis groups. In other words, either cholestasis or sepsis alone can cause elevated SF. However, when present together, they have a synergetic effect for the elevation of SF level. In multivariate regression analysis the combined cholestasis and sepsis was the only significant independent predictor for SF.

These results give an impression that SF elevation in NC is nonspecific to cholestasis itself, but rather an acute phase reactant that is related to the associated inflammatory process. Serum bile acids

Table 4
Relation of serum ferritin to different pathological changes in neonatal cholestasis patients ($n = 50$).

Pathological changes	n	Serum ferritin (ng/mL)	P value
Ductular proliferation			**0.003**
Present	27	812 ± 805	
Absent	23	2521 ± 3235	
Bile plugs			**0.002**
Present	28	908 ± 1197	
Absent	22	2477 ± 3194	
Cellular infiltrate			0.562
Present	12	1346 ± 1072	
Absent	38	1678 ± 2700	
Giant cell transformation of hepatocytes			**0.009**
Present	18	2273 ± 3335	
Absent	32	1219 ± 1621	
Hepatocyte swelling			**0.023**
Present	22	2446 ± 3318	
Absent	28	932 ± 941	
Cholestatic rosetting			0.411
Present	46	1255 ± 1343	
Absent	4	5544 ± 6695	
Grades of fibrosis			0.058
0	13	2899 ± 3925	
1	11	2085 ± 2149	
2	7	1132 ± 877	
3	18	594 ± 378	
4	1	669	

which are elevated in NC and participate in the ongoing inflammatory process [38,39] may have a role in this elevation of SF. Allen et al. [40] reported that bile acids increase the levels of numerous cytokines including IL-1B and IL-10. Both cytokines stimulate hyperferritinemia [41,42].

NC infants have different etiologies of cholestasis with different pathogenesis that can broadly be categorized into obstructive (biliary) cholestasis and intrahepatic or hepatocellular cholestasis (non-obstructive) [12,43]. Regarding these two main NC categories, intrahepatic causes (non-BA cholestasis) were found, in univariate analysis, to have significantly higher SF level than BA (obstructive). Moreover, with elimination of sepsis, as a comorbidity factor, intrahepatic causes of cholestasis still have significantly higher SF than BA, and it was a significant independent predictor of SF in multivariate regression analysis. Both categories are different in their etiopathogenesis and their main cellular targets; intrahepatic cholestasis is more concerned with hepatocellular damage [44].

It is not known if this different elevation of SF is a result or a cause of the pathologic process in either categories, or a combination of both. On one hand, elevated SF could be attributed to the more hepatocellular damage and release of iron from these damaged cells in the intrahepatic category. On the other hand, elevated SF and the consequent more deposition of hepatic iron could participate in the pathogenesis of intrahepatic cholestasis. Explanation of this dilemma could be achieved by knowledge of the source of this elevated SF.

In the present study, we did not find the correlation between SF and the biochemical markers of either hepatocellular damage (ALT and AST) or biliary damage (ALP and GGT). Furthermore, SF had significantly higher levels in those with the pathological findings of giant cells and hepatocyte swelling; parameters that are statistically higher in intrahepatic cholestasis. While, SF was significantly lower in those with ductular proliferation and bile plugs; parameters that are statistically higher in obstructive cholestasis (BA).

Although SF was correlated with giant cell and hepatocyte swelling, it was not correlated with the biochemical parameters of hepatocellular damage expressed by ALT and AST. Many studies showed the absence of correlation of the degree of inflammatory activity in some liver diseases and the hepatocellular enzymes [45,46]. This could be explained by the degeneration pattern of cellular injury rather than necrosis and cell membrane rupture. Moreover, the small number of the studied group together with the expected ALT and AST changes over time could share in the explanation.

SF was found to be positively correlated with iron deposition in hepatic tissue in our study. Ferrara et al. [47] found similar results in adults having chronic hepatitis C. Moreover, we found that SF, at a cut-off 803.5 ng/mL, can predict higher grades (≥ grade 3) of hepatic iron with a sensitivity of 100% and a specificity of 70%, irrespective to the etiology of the NC.

To know the relation of different etiologies to the grade of hepatic tissue iron, we compared both categories of NC and found that, hepatic iron was significantly higher in intrahepatic cholestasis (non-BA cholestasis) than in BA. Moreover, hepatic iron had no significant difference between NC with sepsis and NC without sepsis; a result that suggest a relation of hepatic iron to cholestasis itself rather than sepsis.

The significant difference of SF and hepatic iron store between the two cholestatic categories may suggest a differential role in pathological progression, as this deposited iron can lead to cellular damage by its known oxidant effect [5,8,48,49]. However, the insignificant relation of SF to liver fibrosis, stands against this concept. Despite the claim that increased hepatic iron store is determinant for fibrosis progression [5,48], other studies [9], like the present one, reported that SF and the grade of hepatic iron were unrelated to the grade of fibrosis.

In conclusion, SF in NC is a non-specific bystander marker. It is elevated in NC as an acute phase reactant. However, it can significantly differentiate intrahepatic category from those with obstructive etiology. Sepsis constitutes an augmenting factor for elevated SF. Therefore, any case of NC with elevated SF should be screened for sepsis and managed properly. Although SF can predict higher hepatic iron grades, which is known in previous studies to have a damaging oxidative stress on hepatocytes, an active role of ferritin in NC pathological progression is still debatable.

Contributors

BBE, KHA, AHT, EADS, ANM, and SAM participated in the study concept and design. BBE, KHA, AHT, ANM, and SAM participated in recruitment of patients, clinical management and follow-up, and contributed to data acquisition. EADS performed the histopathological examination. SAM performed statistical analysis and designed the figures. BBE, KHA, ANM, and SAM performed data interpretation. BBE, KHA, AHT, ANM, and SAM wrote the manuscript. All the authors reviewed the manuscript and finally approved it for submission. SAM is the guarantor.

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Ethical approval

This study was approved by the Research Ethics Committee of the National Liver Institute, Menofiya University and in accordance with the *Declaration of Helsinki*.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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