Serum alpha-fetoprotein levels in neonatal cholestasis

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Alpha-fetoprotein (AFP) is used as a tumor marker for hepatocellular carcinoma, hepatoblastoma and germ cell tumors. It may also be elevated in infants with some hepatobiliary disorders. The mechanism of AFP elevation in neonatal cholestasis is not known. We retrospectively evaluated serum AFP levels in 53 infants with neonatal cholestasis. Thirty patients (56.6%) had elevated AFP, and the ratio of patients with elevated AFP was significantly high in both the metabolic diseases and idiopathic neonatal hepatitis groups (p=0.021). Serum aspartate aminotransferase (AST) levels increased significantly in patients with elevated AFP (p=0.004). Steatosis was the distinctive histopathological finding of the patients with high AFP. The patients with steatosis had significantly higher standard deviation (SD) score of AFP than the patients without steatosis (p=0.001). We have shown AFP elevation in neonatal cholestasis due to metabolic disorders and idiopathic neonatal hepatitis and its association with steatosis and AST elevation.

Key words: neonatal cholestasis, alpha-fetoprotein elevation, steatosis, aspartate aminotransferase elevation.

Alpha-fetoprotein (AFP) is a glycoprotein produced by the fetal liver and yolk sac in the developing fetus. The fetal liver becomes the main source of AFP as the yolk sac degenerates in the second trimester. AFP levels are usually high at birth and decrease to adult levels within the first year of life. AFP is used as a tumor marker for hepatocellular carcinoma, hepatoblastoma and germ cell tumors[1,2]. However, elevated AFP has also been associated with nonneoplastic liver diseases such as acute and chronic viral hepatitis[3-5]. In infancy, it may also be elevated in some cholestatic disorders like neonatal hepatitis and extrahepatic biliary atresia[6-8] and various genetic and metabolic diseases including ataxia-telangiectasia, hereditary tyrosinemia, cystic fibrosis, and congenital hypothyroidism[1,9]. The cause of AFP elevation in nonneoplastic liver disorders is not elucidated. We evaluated serum AFP levels and their clinical significance in patients with neonatal cholestasis due to heterogeneous etiologies.

Material and Methods

We retrospectively investigated the data of 53 infants with neonatal cholestasis admitted to Hacettepe University, Pediatric Gastroenterology Division, between 2000 and 2010. Neonatal cholestasis was described as conjugated bilirubin concentration >1.0 mg/dl if the total serum bilirubin is <5.0 mg/dl or >20% of the total serum bilirubin if the total serum bilirubin is >5.0 mg/dl within the first three months of life. Age at diagnosis, sex, medical history, and laboratory findings (serum levels of albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), international normalized ratio (INR), and conjugated and total bilirubin) were evaluated. A detailed etiologic work-up including viral studies, metabolic screening (urine and serum amino acids, urine reducing substance, urine organic acids), sweat test, $alpha_1$-antitrypsin level, thyroid function tests, abdominal ultrasonography, and bone marrow aspirate (when indicated) was performed for the differential diagnosis of neonatal cholestasis.

Liver biopsy was obtained from 42 patients. The specific diagnosis was established according to clinical, laboratory and histopathological findings.

Serum AFP levels were measured by chemiluminescent microparticle immunoassay method (Architect, Abbott) in all patients at the time of the initial examination. The AFP level was expressed as a standard deviation (SD) score to adjust for age differences[8,10,11]. An elevated AFP value was defined as a SD score ≥2.0 [SD score = (observed log AFP value – expected log AFP value)/SD(=0.381)].

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) 16.0. The χ^2 test was used for comparison of categorical variables. Continuous variables were compared using Mann-Whitney U test or Kruskal-Wallis test as appropriate. Spearman's rank correlation test was applied to correlate continuous variables. Results were expressed with mean ± SD and median (min-max), and $p<0.05$ was considered as significant.

Results

Fifty-three infants with neonatal cholestasis (32 girls, 21 boys) with a mean age of 83.4±63.9 days (range: 1-270 days) at presentation were included in the study. According to their final diagnosis, the patients were classified into five groups as: metabolic diseases (17 patients), idiopathic neonatal hepatitis (14 patients), extrahepatic biliary atresia (9 patients), bile duct paucity (7 patients), and progressive familial intrahepatic cholestasis (PFIC) 1/2 (6 patients). Among the patients in the metabolic diseases group, 9 had a specific diagnosis: 3 galactosemia, 2 neonatal hemochromatosis, 2 Niemann-Pick type C, 1 bile acid synthesis defect, and 1 tyrosinemia type 1. In the remaining 8 patients, clinical, laboratory and/or liver biopsy findings (particularly micro- and macrovesicular steatosis, perisinusoidal and/or portal fibrosis, lack of inflammation, and oncocytic change in some hepatocytes in addition to cholestasis) and positive family history were suggestive for metabolic/mitochondrial hepatopathy, but a specific metabolic defect was not identified with the available investigation methods. Thirteen patients were diagnosed as idiopathic neonatal hepatitis according to their clinical, laboratory and histological findings after excluding other causes of cholestasis. Nine patients had extrahepatic biliary atresia, and the diagnosis was verified at laparotomy. The liver of 7 patients showed bile duct paucity (bile duct to portal tract ratio <0.9), 3 of whom were diagnosed as Alagille syndrome. Six patients had the diagnosis of PFIC 1/2 with normal GGT levels.

Thirty patients (56.6%) had elevated AFP (SD score ≥2.0), and the ratio of patients with elevated AFP was higher in both the metabolic diseases and idiopathic neonatal hepatitis groups than the other groups (p=0.021). There was no significant difference between the idiopathic neonatal hepatitis and metabolic diseases groups in the ratio of patients with elevated AFP (p=0.753) (Table I). The patients with elevated AFP in the metabolic diseases group consisted of 1 patient each with neonatal hemochromatosis, tyrosinemia type 1, and bile acid synthesis defect, 2 patients with

Table I. SD Score of AFP and the Ratio of Patients with Elevated AFP According to Groups

Groups	SD score of AFP median (range)	Patients with elevated AFP (SD score ≥2.0) n (%)
Metabolic diseases (n=17)	4.778 (-2.023-8.928)	13 (76.4)
Idiopathic neonatal hepatitis (n=14)	3.750 (-3.724 -7.417)	10 (71.4)
Biliary atresia (n=9)	1.998 (0.698-10.197)	4 (33.3)
Bile duct paucity (n=7)	1.332 (0.078-6.729)	1 (14.3)
PFIC 1/2 (n=6)	1.809 (-3.673-5.661)	2 (16.6)
Total (n=53)	3.205 (-3.724-10.197)	30 (56.6)
P-value	0.230[a], 0.003[b]	0.021[c]

AFP: Alpha-fetoprotein. PFIC: Progressive familial intrahepatic cholestasis. SD: Standard deviation.
[a] when comparing all groups;
[b] when comparing metabolic diseases and idiopathic neonatal hepatitis groups with the other groups;
[c] the significance results from metabolic diseases and idiopathic neonatal hepatitis groups.

Table II. Biochemical Parameters of Patients with Elevated and Normal AFP Levels

Biochemical parameters	Patients with elevated AFP (n=30) median (range)	Patients with normal AFP (n=23) median (range)	p-value
ALT (IU/L)	130.5 (23-966)	95 (12-374)	0.219
AST (IU/L)	306.5 (59-1062)	165 (20-819)	0,004
GGT (IU/L)	149.5 (42.7-1299)	128 (13.7-2804)	0.451
Total bilirubin (mg/dl)	11.9 (3.4-38.4)	9.6 (3.8-35.5)	0.451
Conjugated bilirubin (mg/dl)	7.6 (2.1-27.4)	6.3 (2.2-23.1)	0.324
Albumin (g/dl)	3.9 (2.4-5.5)	3.8(2.2-4.9)	0.541
INR	1.4 (1.0-4.4)	1.3 (0.9-7.8)	0.882

AFP: Alpha-fetoprotein. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. GGT: γ-Glutamyl transpeptidase. INR: International normalized ratio.

Niemann-Pick type C, and 8 patients with metabolic/mitochondrial hepatopathy. There was no radiological finding of liver malignancy in the patient with tyrosinemia type 1 and elevated AFP.

The SD score of AFP did not differ significantly among the patient groups (p=0.230). However, when we combined the metabolic diseases and idiopathic neonatal hepatitis groups in one group and the remaining 3 groups in another group, the difference was significant (p=0.003) (Table I).

Biochemical parameters

Serum AST levels were significantly higher in patients with elevated AFP (p=0.004). Serum ALT, GGT, total and conjugated bilirubin, albumin, and INR levels did not differ between the patients with elevated AFP and normal AFP levels (Table II).

The SD score of AFP was also positively correlated with AST levels (Spearman's rho: 0.372, p=0.006). There were no correlations of SD scores with other biochemical parameters (i.e., ALT, GGT, total and conjugated bilirubin, albumin, and INR).

Histopathological parameters

Histopathological analysis of the 42 patients revealed fibrosis, inflammatory infiltration, giant cell formation, pseudoglandular transformation, and steatosis (Table III). Comparison of these findings showed that steatosis was significantly high in patients with elevated AFP (p=0.001). All patients with steatosis including 6 with metabolic/mitochondrial hepatopathy, 4 with idiopathic neonatal hepatitis, and 1 with bile acid synthesis defect had elevated AFP levels.

The SD score of AFP according to liver biopsy findings was also evaluated. The patients with steatosis had significantly higher SD scores than the patients without steatosis (p=0.001). The SD score of AFP did not show any difference according to the other histopathological parameters (Table IV).

Discussion

Neonatal cholestasis is caused by a heterogeneous group of disorders including structural abnormalities of the biliary tract and infectious, genetic, toxic, and metabolic causes. Idiopathic neonatal hepatitis and biliary atresia have been the most common causes in older

Table III. Liver Biopsy Findings of Patients with Elevated and Normal AFP Levels

Histopathological parameters	Patients with elevated AFP (n=24)	Patients with normal AFP (n=18)	p-value
Fibrosis	23 (95.8%)	16 (88.9%)	0.387
Inflammatory infiltration	22 (91.7%)	14 (77.8%)	0.203
Giant cell formation	21 (87.5%)	11 (61.1%)	0.105
Pseudoglandular transformation	10 (41.7%)	6 (33.3%)	0.582
Steatosis	11 (45.8%)	0 (0%)	0.001

AFP: Alpha-fetoprotein.

Table IV. SD Score of AFP According to Liver Biopsy Findings

Histopathological parameters	SD score of AFP median (range)	P-value
Fibrosis		
Positive (n=39)	3.205 (-3.724-10.197)	0.345
Negative (n=3)	0.245 (-0.644-7.407)	
Inflammatory infiltration		
Positive (n=36)	3.260 (-3.724-8.928)	0.886
Negative (n=6)	1.979 (0.245-10.197)	
Giant cell formation		
Positive (n=32)	1.979 (0.178-8.928)	0.345
Negative (n=10)	3.348 (-3.724-10.197)	
Pseudoglandular transformation		
Positive (n=16)	3.091 (0.178-8.859)	0.623
Negative (n=26)	3.257 (-3.724-10.197)	
Steatosis		
Positive (n=11)	6.569 (3.315-8.928)	0.001
Negative (n=31)	1.986 (-3.724-10.197)	

SD: Standard deviation.

studies. The increased recognition of metabolic and genetic disorders has reduced the number of patients with idiopathic neonatal hepatitis[12]. In a recent study, the etiology of the cholestasis was idiopathic in 25%, metabolic/genetic in 23%, and biliary obstruction in 20%[13]. Similarly, metabolic disorders (32%), idiopathic neonatal hepatitis (26%) and extrahepatic biliary atresia (17%) constituted the majority of our patients.

Elevation of AFP in neonatal cholestasis was observed especially in patients with extrahepatic biliary atresia and neonatal hepatitis[6,7]. Some metabolic disorders such as hereditary tyrosinemia[14] and neonatal intrahepatic cholestasis due to citrin deficiency (NICCD)[15], were also identified to be associated with AFP elevation in the cholestatic patients. In addition to extrahepatic biliary atresia and idiopathic neonatal hepatitis, we also showed AFP elevation in various metabolic disorders.

Studies on the diagnostic significance of elevated AFP in neonatal cholestasis have yielded controversial results. High AFP levels were suggested in some studies to favor the diagnosis of neonatal hepatitis rather than biliary atresia[7,16], while others[6,17] suggested that elevated AFP did not distinguish these two disorders. The ratio of our patients with elevated AFP was significantly higher in both metabolic disorders and idiopathic neonatal hepatitis compared to the other groups. The SD score of AFP also tended to be higher in the metabolic diseases and idiopathic neonatal hepatitis group than the others.

Several mechanisms have been postulated in the pathogenesis of AFP elevation in liver diseases. The elevated AFP levels in neonatal hepatitis and extrahepatic biliary atresia may be related to hepatocellular disorganization and possibly reversion of cellular activities to a more primitive state[6]. Giant cell transformation, which develops secondary to parenchymal damage in the infantile liver, was also found to be associated with AFP elevation in neonatal hepatobiliary disorders[6,8]. Virus-induced liver injury may be another mechanism in the pathogenesis of AFP elevations as proposed in acute and chronic viral hepatitis. Hepatic regeneration after parenchymal injury and altered hepatocyte architecture have been suggested to cause AFP elevation in acute viral hepatitis[3,4]. AFP elevation may also be stimulated by loss of normal hepatic architecture due to fibrosis in chronic hepatitis[18]. Histopathological analysis of our patients also demonstrated fibrosis, giant cell transformation, inflammation, and steatosis. However, the patients with elevated AFP had more significant steatosis than the patients with normal AFP (p=0.001). All patients with steatosis had elevated AFP, and SD scores of AFP in these patients were significantly higher than in the patients without steatosis (p=0.001). A relationship between hepatic steatosis and elevated AFP was also observed in other disorders such as nonalcoholic fatty liver disease (NAFLD), chronic hepatitis C (CHC) and NICCD. NAFLD represents as a spectrum ranging from hepatic steatosis

to steatohepatitis. It has been shown that AFP levels rise as the grade of liver steatosis increases in NAFLD patients[19]. Steatosis has also been associated with AFP elevation in patients with CHC[20,21]. Oxidative stress, lipid peroxidation and mitochondrial dysfunction are involved in the development of steatosis in both hepatitis C virus infection[22-24] and NAFLD[25,26]. Similarly, we found metabolic/mitochondrial hepatopathy in approximately half of the patients with steatosis and AFP elevation. The association of steatosis with elevated AFP was also observed in a mitochondrial disorder, NICCD, which is characterized by cholestasis, diffuse fatty liver and multiple aminoacidemia[15,27]. These findings implicate that mitochondrial dysfunction may play a role in AFP elevation by the way of steatosis.

Among the biochemical parameters, only AST levels were significantly high in our patients with elevated AFP. The SD score of AFP was also positively correlated with AST levels. The existence or not of an association between increased AST levels and AFP elevation remains uncertain. Similar to our results, some studies demonstrated that AST elevation was significantly associated with elevated AFP[20,21], but others did not find any association between elevated AFP and AST[5,19].

In conclusion, our study shows that AFP elevation may occur in neonatal cholestasis due to metabolic disorders and idiopathic neonatal hepatitis. Steatosis was the distinctive histopathological finding of the patients with elevated AFP. The role of steatosis and/or increased AST levels in AFP elevation deserves further investigation.

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