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Measurement of Serum TNF-α as an Accurate Marker in Diagnosing Extrahepatic Biliary Atresia

Original Article

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ABSTRACT

Background:
Most diagnostic tests for biliary atresia (BA) are invasive. This study evaluates the sensitivity and specificity of serum tumor necrosis factor-α (TNF-α) for diagnosing extra hepatic biliary duct atresia (EHBA) in infants.

Materials and Methods:
Infants with cholestasis who were admitted to Children′s Medical Center Hospital were evaluated. A total of 50 infants (20 with EHBA and 30 non-EHBA) were included in this study. We evaluated the definite cause of cholestasis. Intra-operative cholangiography and, if possible, Kasai portoenterostomy were performed in infants with high suspicion for EHBA. Upon admission and prior to surgical intervention, infants had their serum TNF-α levels measured by ELISA. We compared the mean TNF-α level between the two groups, in addition to the sensitivity and specificity of serum TNF-α for diagnosis of EHBA.

Results:
This study enrolled 28 (56%) male and 22 (44%) female infants. Infants' mean age was 2.87 months in the EHBA group and 2.6 months in the non-EHBA group. Sensitivity of serum TNF was 60% and its specificity was 76.66% for predicting EHBA. The positive predictive value was calculated as 63.1%, the negative predictive value was 74.1%, and accuracy was 70%. There was a significantly higher mean serum TNF-α level in the EHBA group (220 pg/ml) compared to the non-EHBA group (79.8 pg/ml; p=0.023). Serum TNF-α cut off point for determining EHBA from non-EHBA was calculated as 80 pg/ml. Mean serum TNF-α in males (171.28 pg/ml) was more than females (90.80 pg/ml).

Conclusion:
There was a significantly higher mean serum TNF-α level in the EHBA group than the non-EHBA group. Serum TNF-α could be a proper test that has comparatively good sensitivity, specificity, positive predictive value and negative predictive value for predicting EHBA.

Keywords: TNF-α; Extrahepatic biliary atresia; Cholestasis; Hepatitis

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INTRODUCTION

Extra hepatic biliary atresia (EHBA) is a progressive disease of infancy characterized by cholestasis, bile duct proliferation, cellular inflammation and fibrosis(1,2). Its frequency ranges from 1/10000-15000 live births(3,4).

The etiology of EHBA is unknown and theories of pathogenesis involve viral infection, immune mediated ductal destruction, and abnormalities in ductal development(5-9). Early diagnosis is particularly essential because prognosis is closely

related with timing of the Kasai portoenterostomy(10). Although different diagnostic methods with various levels of accuracy and reliability are available to establish the diagnosis of EHBA(11), a noninvasive method that clearly identifies the diagnosis does not exist. Thus, there is a real need for sensitive, specific blood tests that have the capability to diagnose EHBA.

Tumor necrosis factor-α (TNF-α) is a cytokine mainly produced by activated macrophages and monocytes. Studies have confirmed an increase in T cells and macrophages that infiltrate the portal tracts in EHBA(12-15). These infiltrating cells secrete Th1 inflammatory cytokines including TNF-α, IFN-γ, IL-2 and IL-12, which further induces Th1 cell-mediated ductal destruction(16).

The objective of this study was to determine if serum TNF-α levels could be a sensitive and specific test for differentiating EHBA from other causes of neonatal cholestasis.

MATERIALS AND METHODS

This cross-sectional study was conducted at a pediatric tertiary-care medical referral center located in Tehran, Iran. In this study all patients with cholestatic jaundice <6 months of age at admission were included. Cholestasis was defined as direct bilirubin serum level >1 mg/dl with total bilirubin under 5 mg/dl, or direct bilirubin serum levels that comprised >20% when total serum bilirubin was >5 mg/dl. We enrolled 50 cholestatic patients (20 EHBA and 30 with non-EHBA cholestasis). Samples of peripheral venous blood were collected from every participant, centrifuged for 15 min, and stored immediately at -80°C for further analysis. Quantitative determination of TNF-α concentrations in serum were performed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (AviBion Kit, Orgenium Inc., Finland).

Statistical analysis was performed using SPSS software version 20.0 for Windows. The Mann-Whitney U test was used to compare the difference in serum TNF-α concentrations between groups. *p*-values <0.05 were considered to be statistically significant.

RESULT

From 50 study participants, there were 28 (56%) males and 22 (44%) females, which was not statistically significant (Table 1). Mean age in the EHBA group was 2.87 months and 2.6 months in the non-EHBA group.

The mean serum TNF-α level in EHBA patients (220 ng/dl) was markedly elevated compared with to non-EHBA patients. (79.8ng/dl; p=0.023). The sensitivity of serum TNF-α in diagnosing EHBA was 60% and its specificity was 76.6%. The positive predictive value was 63.1% and negative predictive value was 74.1%. EHBA patients had significantly higher αFP than the non-EHBA group. (p=0.0009). Additionally, consanguity of the parents was five times more in the EHBA group compared to the non-EHBA group. AST, ALT, triglyceride, cholesterol, total protein, albumin, total and direct bilirubin, GGT, ALP, PT, PTT, calcium, phosphorus, and CBC levels were not significantly different between groups (Table 2).

Table1: Sex distribution of the patients

	EHBA	Non-EHBA	Total
Male	13	15	28
Female	7	15	22
Total	20	30	50

Table 2: Comparison of different parameters in patients

	EHBA	Non-EHBA	*p*-value
Age(month)	2.87	2.6	0.4
Weight(Kg)	4.8	4.4	0.29
Height(cm)	55.2	54.6	0.62
ALT(U/L)	157.7	133.5	0.49
AST(U/L)	224	259	0.56
TG(mg/dl)	184.5	218	0.32
Chol(mg/dl)	262	184	0.01
Total Protein(g/dl)	5.5	5.15	0.2
Albumin(g/dl)	3.5	3.45	0.77
Total Bilirubin(mg/dl)	10.8	10.2	0.71
Direct Bilirubin(mg/dl)	6.2	5.5	0.34
GGT(IU/L)	818.5	174.3	0.001
ALP(U/L)	1674	1943.3	0.31
PT(sec)	13.85	15.95	0.096
PTT(sec)	36.9	42.3	0.062
Ca(mg/dl)	9.5	9.4	0.58
Phos(mg/dl)	4.7	4.5	0.41
WBC(U/L)	12737	12709	0.98
Hb(g/dl)	10.2	10.3	0.88
αFP(ng/dl)	5289.2	4546.7	0.0009
TNFα(pg/ml)	220	79.8	0.023

DISCUSSION

Biliary atresia (BA) is a devastating, progressive disease. Although the etiology of BA is not exactly known inflammation and immune mediated bile duct destruction may be involved(17). According to the inflammatory component of the disease1 and the principal need for

an early, easy diagnosis of EHBA, we have measured serum TNF-α levels. As in our study, Mack et al. proved that biliary atresia was associated with a large increase in expression of TNF-α at the time of BA diagnosis(16). Ding et al. compared serum TNF-α levels among 42 subjects of infantile hepatitis syndrome which were divided in to two groups of cholestasis and hepatitis. They observed higher serum TNF-α levels in the cholestasis group(18). Nevertheless, none of these studies specified the sensitivity and specificity of serum TNF-α as a marker for early diagnosis of EHBA. In our study we identified 60% sensitivity and 76.6% specificity for serum TNF-α as a new diagnostic method for definitive diagnosis of EHBA which is surgical cholangiography. We recognized that αFP levels were significantly higher in the serum of the EHBA group, which was inconsistent with a study conducted by Uchino et al.(19)

Ultimately, our study suggests that serum TNF-α may be used as a sensitive, specific marker for reliably separating EHBA from non-EHBA cholestatic diseases. Nonetheless, these new data provide a rationale for additional studies to validate and extend our observations with the goal of developing reliable noninvasive diagnostic testing for EHBA.

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