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Original paper

Serum level of interleukin-13 receptor alpha 2 in infants with biliary atresia – is it of value?

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Abstract

Aim of the study: We aimed to assess the utility of serum level IL-13Rα2 receptors as a non-invasive marker for early diagnosis of biliary atresia (BA) and selection of BA patients indicated for Kasai portoenterostomy.

Material and methods: The study included 60 infants with neonatal cholestasis in three groups; early BA group (n = 20), delayed BA group (n = 20) and non-BA cholestasis group (n = 20). A fourth group of 20 healthy neonates (n = 20) served as controls. IL-13Rα2 was measured by enzyme-linked immunosorbent assay in all patients and controls.

Results: The mean value of IL-13Rα2 was significantly higher in delayed BA group (11.05 ± 10.9 ng/ml) compared to early BA (0.34 ± 0.37 ng/ml), non-BA (0.54 ± 0.85 ng/ml) and control (0.24-0.2 ng/ml) groups. The levels of serum IL-13Rα2 increase with the severity of the degree of fibrosis. IL-13Rα2 at a cutoff level > 0.782 ng/ml could predict late fibrosis with accuracy of 77.55% (p < 0.0001). IL-13Rα2 could differentiate between preserved and disturbed liver architecture at a cut off value of more than 0.42 ng/ml with an accuracy of 81.6%.

Conclusions: Serum IL-13Rα2 not a diagnostic marker for BA however it could be used as a noninvasive marker for detection of advanced liver fibrosis and presence of disturbed liver architecture that helps in patient selection for undergoing Kasai operation. Serum IL-13Rα2 could be a future therapeutic target for management of BA patients and any fibrotic liver disease.

Key words: cholestasis, liver fibrosis, biliary atresia, interleukin-13Rα2, Kasai portoenterostomy.

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Introduction

Biliary atresia (BA) is a neonatal liver disease rapidly progressing to biliary cirrhosis and death in the first years of life, if left untreated [1]. Understanding the various control mechanisms that suppress or inhibit chronic inflammatory reactions is of utmost importance [2].

Interleukin (IL)-4 and IL-13 are related cytokines which induce both pro- and anti-inflammatory effects depending on the cell type they act upon and the nature of the receptors expressed [3]. An even more critical role for IL-13, revealing it as the key mediator of the fibrotic response, has been suggested [4].

IL-13 signals to cells by binding to a complex receptor system composed of IL-4R and two IL-13 binding proteins, IL-13Rα1 and IL-13Rα2. IL-13Rα2 is a 42-kDa monomeric high affinity IL-13 receptor distinct from the more ubiquitously expressed IL-13Rα1/IL-4Rα receptor complex [5, 6]. It is thought to act primarily as a decoy receptor, sequestering IL-13 from the IL-13Rα1/IL-4Rα complex and thus inhibiting its function [4, 7].

There is evidence that IL-13Rα2 may contribute to IL-13-induced TGFβ1-dependent fibrosis [6].

Increased serum levels of IL-13Rα2 have been detected in both humans and mice in other TH_2-dominant immune responses [8, 9]. However, in humans, its functions, expression regulation with the physiological consequences and its role in IL-4/IL-13 transduction are poorly understood and debatable and remain to be determined [4, 10]. We aimed to investigate the serum IL-13Rα2 in infants with BA and other neonatal cholestasis, in relation to liver fibrosis, and its utility as a non-invasive marker for selection of BA patients indicated for Kasai portoenterostomy.

Material and methods

Study population

This prospective study included 60 infants with neonatal cholestasis in whom liver biopsy was indicated for etiological diagnosis. They were divided into three groups: group 1 – BA group (n = 20) all of whom underwent Kasai hepatoportoenterostomy and were followed up for three months post-operatively; group 2 – neglected BA group (n = 20) comprising BA patients with a delayed diagnosis (parental reluctance for referrals, inadequate follow-up of neonatal jaundice and misdiagnosis) who lost the chance for corrective surgery; and group 3 – non-BA cholestasis group (n = 20) with cholestasis due to causes other than BA. Group 4 comprised 20 apparently healthy infants, age and sex matched, enrolled as a control group. The BA group was further divided 3 months postoperatively according to total bilirubin level into successful outcome (total bilirubin < 2 mg/dl) and failed outcome (total bilirubin ≥ 2 mg/dl) [11]. All patients were recruited from the outpatient and inpatient clinics of the Pediatric Hepatology Department, National Liver Institute, Menoufia University, Egypt. A fourth group with healthy neonates (n = 20) was enrolled in the study as controls. Those infants were attending for routine checkup. Written informed consent was obtained from the parents or the legal guardians of the patients and controls before enrollment in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the institution's human research committee. The study was approved by the Research Ethics Committee of the National Liver Institute, Menoufia University, Egypt on 3/5/2015.

Etiological diagnosis

After full history taking, thorough clinical examination, routine investigations, and histopathological assessment, the patients were allocated as BA and non-BA. Diagnosis of BA was confirmed by operative cholangiography and/or laparotomy prior to surgery. Routine investigations included total and direct bilirubin, total proteins, albumin, alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma glutamyl transpeptidase, prothrombin time, complete blood count, viral antibodies (immunoglobulin [Ig] M and IgG for rubella, cytomegalovirus, herpes simplex virus type 1 and 2 and hepatitis B virus core), toxoplasma antibodies (both IgM and IgG), hepatitis B surface antigen, ultrasonography (US) and Doppler US, with a set of specific laboratory tests according to the expected etiology.

Liver biopsy

Ultrasonography-guided liver biopsy was conducted for all patients using a Tru-Cut needle (GTA, Quistello, MN, Italy). A core of liver tissue containing at least 5 portal tracts was considered sufficient. Biopsy specimens were fixed in formalin and embedded in paraffin. Five-μm thick sections were cut, mounted on a glass slide and stained with hematoxylin and eosin to evaluate pathological changes, with Mason-Trichrome that stains collagen fibers to assess fibrosis, and with Perls' Prussian blue stain which reveals iron deposits. Portal fibrosis and inflammatory activity were assessed using semi-quantitative histopathological scores as described by Russo *et al.* [11].

Serum IL-13Rα2

Serum samples were collected from all patients and controls in addition to follow-up samples that were collected from BA patients (n = 20) three months after the Kasai operation. Samples were stored in aliquots at –80°C until the time of the assay. Quantitative assessment of human serum IL-13Rα2 levels was performed by enzyme linked immunosorbent assay (ELISA). The RayBio Human IL-13Rα2 ELISA kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human IL-13Rα2 in serum and plasma, according to the manufacturer's instructions. The minimum detectable dose of IL-13Rα2 is typically less than 0.4 ng/ml.

Statistical analysis

Descriptive results were expressed as mean ± standard deviation (SD) or number and percentage. For quantitative data, significance was tested either by the independent sample t-test or Mann-Whitney U-test according to the nature of the data. A paired t-test was used to assess the difference in serum cytokine levels before and after

the Kasai operation. For qualitative and categorical data, significance was tested by the χ^2 test or Fisher's exact test. A correlation was tested by Spearman's test. The diagnostic value of serum IL-13Rα was assessed by calculating the area under the receiver-operating characteristic (ROC) curves. The cutoff value for optimal clinical performance was determined from the ROC curves. The diagnostic performance was measured as sensitivity, positive predictive value (PPV) and negative predictive value (NPV) and expressed as percentages. Results were considered significant if $p < 0.05$. Statistical analysis was performed using SPSS software version 13 (SPSS Inc., Chicago, IL, USA).

Results

Study population's characteristics

The current study included 60 infants divided into the BA group ($n = 20$) with the age range 45-90 days, the neglected BA group ($n = 20$) with the age range 120-210 days, the non-BA group ($n = 20$) with the age range 60-120 days, and the control group with the age range 45-120 days. All groups were sex matched ($p = 0.977$).

Serum levels of gamma glutamyl transpeptidase (GGT) were significantly higher in the BA group than the non-BA group ($p = 0.0001$) and in the delayed BA group than early BA and non BA ($p = 0.0001$) groups. The early BA group had significantly lower serum levels of GGT, lower serum alkaline phosphatase (ALP), shorter prothrombin time (PT) and a higher platelet (PLT) count than the neglected BA group ($p < 0.0001$, $p < 0.002$, $p < 0.038$, $p < 0.007$ respectively). Also, BA patients had significantly higher serum GGT, higher serum ALP and lower PLT than the non-BA group ($p < 0.0001$, 0.002, 0.007 respectively). The neglected BA group had significantly higher serum levels of GGT, ALP, and lower PLT than the non-BA group ($p < 0.0001$, $p < 0.0001$, $p < 0.05$ respectively) (Table 1).

Table 1. Laboratory characteristics of the studied patients

	Naïve BA, $n = 20$	Delayed diagnosed BA, $n = 20$	Non-BA cholestasis, $n = 20$	P1	P2	P3
Total bilirubin (mg/dl)						
Range	6.18-24.80	5.60-25.00	4.40-21.00			
Mean ± SD	13.30 ± 4.65	12.71 ± 4.55	13.36 ± 8.71	0.617	0.543	0.665
Total protein (g/dl)						
Range	4.10-6.30	4.10-6.70	4.10-6.30			
Mean ± SD	5.21 ± 0.61	5.48 ± 0.78	5.13 ± 0.61	0.144	0.673	0.261
Albumin (g/dl)						
Range	3.20-4.8	3.10-4.30	3.50-4.50			
Mean ± SD	3.83 ± 0.542	3.28 ± 0.61	3.41 ± 0.50	0.15	0.114	0.15
Prothrombin time (seconds)						
Range	10.80-19.60	11.00-18.90	10.30-34.00			
Mean ± SD	13.06 ± 2.62	14.27 ± 2.30	14.24 ± 6.05	0.048*	0.616	0.038*
Hb (g/dl)						
Range	7.30-12.00	8.20-11.30	5.60-13.00	0.655	0.159	0.167
Mean ± SD	9.11 ± 1.37	9.60 ± 0.74	9.82 ± 1.96			
WBCs × (10^3/μl)						
Range	3.60-18.80	8.40-23.00	3.60-24.00	0.607	0.194	0.147
Mean ± SD	10.8 ± 4.9	14.34 ± 4.64	13.27 ± 5.15			
PLT × (10^3/μl)						
Range	144-744	222-500	163-694	0.213	0.05*	0.007*
Mean ± SD	482 ± 211	286 ± 115	360 ± 164			
IL-13Rα2 (ng/ml)						
Range	0.07-1.81	0.24-29.8	0.08-4.04	< 0.0001	0.273	< 0.0001
Mean ± SD	0.34 ± 0.37	11.05 ± 10.9	0.56 ± 0.18			

P1 – significance between naïve BA and delayed diagnosed BA group, P2 – significance between naïve BA and cholestasis group, P3 – significance between delayed diagnosed and cholestasis group

Histopathological findings

The occurrence of higher grades of portal fibrosis were significantly higher in the neglected BA group (58.8% had focal porto-portal bridging, 41.2% had marked bridging). 85% of BA patients had no fibrosis and 15% had fibrosis. The entire non-BA group (100%) did not reach porto-portal bridging (mild fibrous expansion), $p < 0.0001$. Patients with neglected BA had statistically significantly higher disturbed liver architecture, ductular proliferation and bile plugs ($p < 0.0001$, $p < 0.0001$, $p = 0.008$, respectively). The number of giant cells was significantly higher in the non-BA group than other groups ($p = 0.036$).

Comparison of preoperative serum IL-13Rα2 in the studied groups

Serum levels of IL-13Rα2 were significantly higher in the neglected BA group (11.05 ± 10.9 ng/ml) than in the early BA (0.34 ± 0.37 ng/ml), non-BA (0.54 ± 0.85 ng/ml) and control group (0.24-0.2 ng/ml) ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$ respectively). Levels were higher in both BA and non-BA groups than the control group ($p < 0.0001$), while no significant difference between BA and the non-BA groups was detected ($p = 0.273$).

Clinical performance of preoperative serum IL-13Rα2 in predicting fibrosis in BA patients and non-BA patients

The levels of serum IL-13Rα2 increase with the severity of fibrosis. IL-13Rα2 at a cutoff level > 0.782 ng/ml could predict late fibrosis with 92.3% sensitivity, 60.9% specificity, 72.7% PPV, 87.5% NPV and accuracy of 77.55% ($p < 0.0001$). IL-13Rα2 could differentiate between preserved and disturbed liver architecture at a cut-off value of more than 0.42 with 84.6% specificity, 78.3% sensitivity, PPV 81.8%, NPV 81.5%, and an accuracy of 81.6%, with higher levels in the latter (Fig. 1).

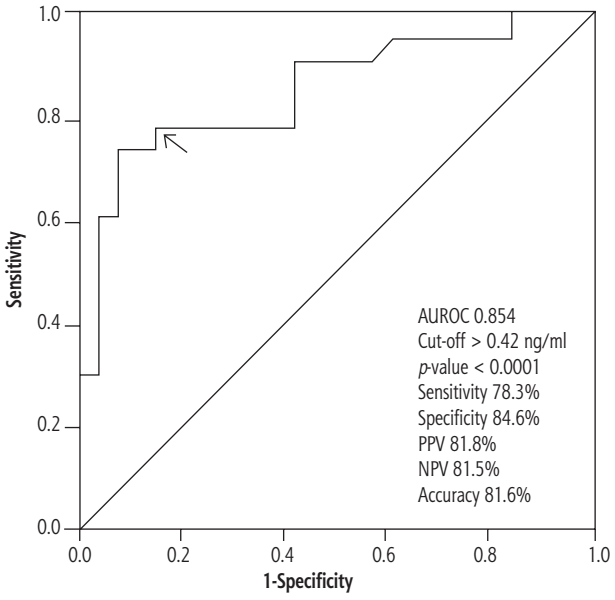

Fig. 1. IL-13Rα2 could differentiate between preserved and disturbed liver architecture at a cut-off value of more than 0.42 with 84.6% specificity, 78.3% sensitivity, PPV 81.8%, NPV 81.5%, and an accuracy of 81.6% with higher levels in the latter

Correlation of IL-13Rα2 with the studied parameters in all individuals

Serum levels of IL-13Rα2 were statistically significantly correlated with age, white blood cells (WBCs), and ultrasound spleen length ($p < 0.0001$, $p = 0.006$, $p = 0.009$ respectively). On the other hand there was no significant correlation as regards other parameters ($p > 0.05$).

Discussion

Type 2 cytokine responses, described as anti-inflammatory mediators, are critically involved in tissue repair. Others clearly demonstrated many of them as proinflammatory, with unidentified mechanisms that regulate beneficial regeneration versus pathological fibrosis [12]. A more critical role for IL-13 had been suggested as a key mediator of the fibrotic response in many chronic infectious diseases through the induction of a receptor formerly considered to function only as a decoy receptor [13-15].

As far as we know, this is the first study on serum IL-13Rα2 in BA in humans. In the current study, serum IL-13Rα2 was detected in all the studied groups, with the highest levels among the delayed diagnosed BA. It was found that the cell surface IL-13Rα2 can be induced in response to high concentrations of IL-4 or IL-13 and regulated by tumor necrosis factor [6]. Evidence suggests the existence of an intracellular pool of receptors capable of rapidly populating the cell surface in response to inducing agents [16]. Enzymatic cleavage from the cell surface may increase the soluble form of IL-13Rα2, thus increasing its level [17]. The high level of IL-13Rα2 could be attributed to marked elevation in its gene and protein expression as it had been reported after infection by *Schistosoma mansoni* in both humans and mice [18]. Consistently with our results, Hussein *et al.* reported the serum level of IL-13Rα2 in atopic asthma, allergic rhinitis, and atopic dermatitis respectively [19]. The impact of the disease itself and age group may explain the higher level in neglected BA than the results Hussein *et al.*

The control group had a minimal level of IL-13Rα2. In agreement with us, Hussein *et al.* (2011) Khodoun *et al.* and Chen *et al.* (2009) did not detect signifi-

cant levels of IL-13Rα2 in the control groups [19-21]. However, the control group had a significantly lower level than the newly diagnosed BA and the cholestatic groups, which may be attributed to the presence of different grades of liver fibrosis in newly diagnosed BA and the cholestatic groups. Figueiredo *et al.* suggested that an increase in IL-13 early in the fibrogenesis process saturates the receptor and makes IL-13 freely available in the microenvironment [22].

Assessing IL-13 production and its receptors' association in different degrees of liver fibrosis remains to be answered. On the other hand, serum IL-13Rα2 level could not differentiate between early diagnosed BA and non-BA as a cause of cholestasis. This comparable level of IL-13Rα2 may indicate that it is not disease specific.

A significant positive correlation between the level of IL-13Rα2 and age was detected in this study. Prolonged exposure to proinflammatory and Th2 cytokines (TNF-α and IL-13) in prolonged inflammation may be the main factor. Leukocytosis was positively correlated with serum level of IL-13Rα2. IL-13 upregulates cysteinyl leukotrienes (cysLTs), which in turn increases the recruitment of leukocytes and their biosynthesis of cysLTs [23].

IL-13Rα2 expression is most closely correlated, but not limited to, expression of mesenchymal signature genes [24, 25]. IL-13 directly induces expression of collagen I and other critical fibrosis-associated genes, e.g., α-smooth muscle actin and connective tissue growth factor, in hepatic stellate cells. IL-13 is known as a potent inducer of matrix metalloproteinases-9 and cathepsin-based proteolytic pathways which stimulate cleavage of latency-associated peptide and thus activate TGF-β [9]. Also, direct cleavage by MMP-8 contributes to the solubilization of IL-13Rα2 and increases its level. Others found that prevention of IL-13Rα2 expression reduced production of TGF-β_1 and marked downregulation of collagen, and regression of fibrosis [6].

In this study, the levels of serum IL-13Rα2 increased with the severity of fibrosis. Higher grades of fibrosis in neglected BA could be an additional factor explaining the highest IL-13Rα2 levels. IL-13Rα2 had a very good accuracy in differentiating patients with preserved and those with disturbed liver architecture, with higher levels in the latter. Thus we can select patients indicated for the Kasai operation.

Conclusions

Serum IL-13Rα2 not a diagnostic marker for BA, but it could be used as a noninvasive marker for detection of advanced liver fibrosis and presence of disturbed liver architecture, which helps in patient selection for undergoing the Kasai operation. Serum IL-13Rα2 could be a future therapeutic target for management of BA patients and any fibrotic liver disease.

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Disclosure

Authors report no conflict of interest.

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