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Plasma TGF-β1 Levels Are Elevated in Down Syndrome Infants with Transient Abnormal Myelopoiesis

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Infants with Down syndrome (DS) are at risk of developing a transient myeloproliferative disorder during the neonatal period, known as transient abnormal myelopoiesis (TAM). It is characterized by clonal myeloproliferation and is typically self-limiting. However, TAM can be a life-threatening disorder, when complicated by liver fibrosis. Here, we evaluated cytokine profiles in two male DS infants having TAM with or without liver dysfunction. The first patient, Patient 1, had hyperleukocytosis with cholestatic liver dysfunction, coagulopathy, and increased counts of blasts and was treated with exchange transfusion (ExT) due to the serious general condition. In Patient 1, serum interleukin (IL)-8 and plasma transforming growth factor (TGF)-β1 levels were markedly elevated before ExT (1,518.2 pg/mL and 17,635 pg/mL, respectively). After ExT, serum IL-8 and plasma TGF-β1 levels decreased to 40.7 pg/mL and 6,847 pg/mL, respectively. However, Patient 1 died on day 56 due to cholestatic liver dysfunction; namely, this patient represents fatal TAM. The second patient, Patient 2, had hyperleukocytosis with increased counts of blasts without liver dysfunction and was treated with cytarabine. In Patient 2, plasma TGF-β1 levels, but not plasma IL-8, were elevated (9,068 pg/mL and 28 pg/mL, respectively). Patient 2 was discharged on day 47. In summary, plasma TGF-β1 levels were elevated in the two DS infants with TAM, regardless of the presence or absence of hepatic fibrosis. Importantly, fatal TAM is assoicated with the elevated serum level of IL-8. We thus propose that IL-8 may be involved in the pathogenesis of liver fibrosis.

Keywords: cytokine; Down syndrome; interleukin-8; liver fibrosis; transient abnormal myelopoiesis
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Introduction

Infants with Down syndrome (DS) are at risk of developing a transient myeloproliferative disorder during the neonatal period known as transient abnormal myelopoiesis (TAM) or transient leukemia. TAM is defined as clonal myeloproliferation and characterized by circulating megakaryoblasts in the peripheral blood (Roy et al. 2012). TAM, occurring in approximately 10% neonates with DS (Klusmann et al. 2008; Ishigaki et al. 2011), is usually self-limiting and undergoes spontaneous regression. However, approximately 20%-30% cases are life-threatening due to respiratory distress or liver failure. Previous reports have posited that cytokines, such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, interferon-ɤ, granulocyte macrophage colony stimulating factor (GM-CSF), transforming growth factor (TGF)-β1, and platelet-derived growth factor, contribute to the pathogenesis of TAM (Arai et al. 1999; Shimada et al. 2007; Ogawa et al. 2008; Miyauchi and Kawaguchi 2014). On the other hand, adult patients with cholestatic liver disease have been found to have the highest IL-8 levels of all patients with liver disease (Zimmermann et al. 2011). Thus, IL-8 contributes to acute liver inflammation and fibrosis, and that high serum IL-8 levels may represent a major cause of chronic liver disease.

In the present study, we describe a DS infant with fatal TAM associated with cholestatic liver dysfunction (Patient 1) and another DS infant without cholestatic liver dysfunction (Patient 2). Importantly, high circulating IL-8 and TGF-β1 levels were found in Patient 1.

Patient Presentation

Patient 1

A boy was delivered via cesarean section at 33 weeks' gestation due to late decelerations in fetal heart rate and pericardial effusion, enlarged ventricular volume, and hepatomegaly on fetal echocardiography (birth weight, 1,780 g; Apgar scores, 5 and 7 at 1 and 5 min, respectively). Shortly after birth, endotracheal intubation and ventilation were performed for bradycardia and apnea. On admission, laboratory data demonstrated hyperleukocytosis (50% blasts), anemia, thrombocytosis, hypoalbuminemia, liver dysfunc-

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tion, coagulopathy, and extremely elevated fibrosis markers, including type III procollagen peptide, type IV collagen, and hyaluronic acid (Table 1). Leukocytes expressed CD4, CD7, CD56, CD13, CD33, CD34, and CD41, consistent with TAM. Chromosomal analysis revealed 47,XY,+21. A mutation in exon two of *GATA1* (c.190_197de18) was detected.

At 7 h of life, exchange transfusion (ExT) was performed. Immediately after transfusion, laboratory data demonstrated a decreased leukocyte count of 17,900/μL (54% blasts) and improved liver function with the following serum values: aspartate aminotransferase, 27 IU/L; alanine aminotransferase, 21 IU/L; and lactate dehydrogenase, 1,350 IU/L. On day 2, laboratory data demonstrated severe liver dysfunction, renal dysfunction, hypoalbuminemia, and coagulopathy (Table 1). Second ExT was performed for multiple organ failure, after which liver dysfunction improved. On day 10, serum bilirubin levels became elevated (total bilirubin (T-Bil), 11.4 mg/dL; direct bilirubin (D-Bil), 1.9 mg/dL). Accordingly, ursodeoxycholic acid was administered to improve bile flow and drainage. On day 27, blasts were absent from peripheral blood. However, pathological jaundice persisted with peak T-Bil and D-Bil levels of 23.7 and 17.9 mg/dL, respectively. The patient died on day 56 due to cholestatic liver dysfunction.

Patient 2

A boy was delivered via cesarean section at 36 weeks' gestation due to maternal complications with uterine myoma. Pregnancy and prenatal laboratory screening tests

Table 1. Laboratory results on day 0 and day 2 in Patient 1 and on day 0 in Patient 2.

	Patient 1		Patient 2
	Day 0	Day 2	Day 0
WBC (/μL)	60,700	17,600	96,800
Blast (%)	50	-	69
Hb (g/dL)	8.6	13.6	17.5
Plt (/μL)	988,000	167,00	520,000
TP (g/dL)	3.3	3.3	4.7
Alb (g/dL)	1.9	1.8	3.0
AST (IU/L)	58	918	53
ALT (IU/L)	58	190	25
LDH (IU/L)	3,640	3,476	1,384
T-Bil (mg/dL)	2.2	7.6	5.8
D-Bil (mg/dL)	0.3	0.4	0.2
BUN (mg/dL)	10	20	14
Cr (mg/dL)	0.57	1.21	0.92
Type III procollagen peptide (U/mL)	47	-	18
Type IV collagen (ng/mL)	145.3	-	22.7
Hyaluronic acid (ng/mL)	438.3	-	218.8
PT-INR	2.93	2.53	1.57
APTT (s)	86.2	64.9	39.2
Fibrinogen (mg/dL)	< 30	76	117
AT-III (%)	8	34	32
D-dimer (μg/mL)	1.0	1.0	0.9

WBC, white blood cells; Hb, hemoglobin; Plt, platelet; TP, total protein; Alb, serum albumin level; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; T-Bil, total bilirubin; D-Bil, direct bilirubin; BUN, blood urea nitrogen; Cr, creatinine; PT-INR, prothrombin time/international normalized ratio; APTT, activated partial thromboplastin time; AT, antithrombin.

Table 2. Cytokine profile results before and after ExT in Patient 1 and on admission in Patient 2 and cut-off levels of each cytokine.

		Patient 1		Patient 2
	Cut-off	Before ExT	After ExT	
Serum				
IL-1β (pg/mL)	< 4.3	6	1.7	
IL-6 (pg/mL)	< 2.2	233.9	148.3	
GM-CSF (pg/mL)	< 8.3	19.9	5.4	
TNF- α (pg/mL)	< 3.2	< 3.2	< 3.2	
IL-8 (pg/mL)	< 5.2	1518.2	40.7	
Plasma				
IL-8 (pg/mL)	< 8			28
TGF-β1 (pg/mL)	< 31.2	17,635	6,847	9,068

IL, interleukin; GM-CSF, granulocyte macrophage colony-stimulating factor; TNF, tumor necrosis factor; TGF, Transforming growth factor; ExT, exchange transfusion.

were unremarkable (birth weight, 2,838 g; Apgar scores, 8 and 9 at 1 and 5 min, respectively). On day 1, he was transferred to our neonatal intensive care unit due to clinical features of DS and hyperleukocytosis. Supplemental oxygen administration was required due to mild respiratory failure. On admission, laboratory data demonstrated hyperleukocytosis (69% blasts) and thrombocytosis (Table 1). Leukocytes expressed CD4, CD7, CD16, CD56, CD13, CD33, CD34, and CD41, consistent with TAM. Chromosomal analysis revealed 47,XY,+21. A mutation in exon of *GATA1* (c.219A>G) was detected. As the leukocyte count increased to 244,000/μL by day 12, a single course of low-dose cytarabine (Ara-C, 1 mg/kg once daily) was intravenously administered for 7 consecutive days. On day 18, the leukocyte count decreased to 7,900/μL. On day 32, blasts disappeared from the peripheral blood. The patient was discharged on day 47.

Cytokine Profiles

We investigated cytokine profiles before and after ExT in Patient 1 and on admission in Patient 2 (Table 2). In Patient 1, six serum cytokines were measured: IL-1β, IL-6, GM-CSF, TNF-α, and IL-8 using LUMINEX® (Thermo Fisher Scientific Inc., Massachusetts, United States) as well as TGF-β1 using Quantikine® human TGF-β1 (Funakoshi Co., Ltd., Tokyo, Japan). In Patient 2, plasma cytokines, IL-8 and TGF-β1, were measured by IL-8 EASIA (FUJIREBIO Inc., Tokyo, Japan) and Quantikine® human TGF-β1 (Funakoshi Co., Ltd., Tokyo, Japan), respectively. In Patient 1, IL-8 and TGF-β1 levels were markedly elevated before ExT. After ExT, IL-8 and TGF-β1 levels decreased to 40.7 pg/mL and 6,847 pg/mL, respectively. In Patient 2, TGF-β1 but not IL-8 levels were markedly elevated.

Discussion

TAM is characterized by several hematological abnormalities, including leukocytosis, abnormal coagulation or disseminated intravascular coagulation (DIC), and increased peripheral blood blasts, usually with megakaryoblastic morphology. Moreover, the majority of cases of TAM have acquired mutation(s) in exon 2 or exon 3 of the *GATA1* gene on chromosome X (Roy et al. 2012). Thus, *GATA1* mutation analysis should be performed on peripheral blood cells to confirm the diagnosis in patients with suspected TAM or on the basis of hematological findings. Therefore, we performed *GATA1* mutation analysis and demonstrated acquired exonal mutations of the *GATA1* gene in both patients.

TAM most commonly presents with hepatomegaly, splenomegaly, jaundice, and pleural/pericardial effusions. Less common presentations include liver fibrosis, ascites, and renal failure (Roy et al. 2012). Patient 1 demonstrated hepatosplenomegaly and pericardial effusion. Liver biopsy was not performed; however, we presumed Patient 1 was complicated by liver fibrosis, according to the clinical course and laboratory data demonstrating markedly elevated fibrosis markers. Liver fibrosis is life-threatening and often fatal. However, precise frequency of liver fibrosis is unknown, and the detailed clinical characteristics of TAM with liver disease remain unclear. D-Bil is a reliable TAM marker, with peak D-Bil levels > 5 mg/dL being associated with liver disease and early mortality (Park et al. 2014). A peak D-Bil level of 17.9 mg/dL was observed in Patient 1. Accordingly, Patient 1 may have been complicated by liver fibrosis that ultimately became fatal. No evidence of hepatomegaly was observed in Patient 2, and liver function tests were normal.

The majority of neonates with TAM do not require treatment as the clinical and laboratory abnormalities spontaneously resolve within 3-6 months of birth. Mortality risk factors include liver fibrosis, preterm delivery, hyperleukocytosis (white blood cells > 100 × 10^3/μL), liver or renal dysfunction, hydrops fetalis, multiple effusions, massive organomegaly causing respiratory compromise, DIC, and failure to clear peripheral blast cells (Hoskote et al. 2002; Hojo et al. 2007; Roy et al. 2012; Park et al. 2014). Further, fatal TAM is reportedly associated with hepatomegaly and

elevated liver enzyme levels (Hojo et al. 2007). Patient 1 was a preterm infant with liver failure, renal failure, and DIC. Accordingly, we considered Patient 1 to be representative of fatal TAM.

Severely affected neonates may benefit from chemotherapy (Dormann et al. 2004; Koga et al. 2012). However, organs and tissues are especially vulnerable to antimitotic therapies, and the immature status of the kidneys and liver leads to the uncertain activity of agents (Gale et al. 1982). We supposed that multiple organ failure may potentiate this side effect, so we did not use chemotherapy in Patient 1 due to the patient's serious general condition. Furthermore, ExT is effective for improving hyperleukocytosis, respiration, and organ failure in neonates with TAM (Koga et al. 2012; Hayasaka et al. 2015). We therefore treated Patient 1 with ExT. After two rounds of ExT, liver function improved and blasts disappeared from the peripheral blood. However, the patient subsequently died of cholestatic liver dysfunction.

Cytokines, such as IL-1β, TNF-α, interferon-γ, GM-CSF, TGF-β1, and platelet-derived growth factor, have been posited to cause TAM (Arai et al. 1999; Shimada et al. 2007; Ogawa et al. 2008; Miyauchi and Kawaguchi 2014). TGF-β1 is a likely candidate involved in liver fibrogenesis of TAM (Arai et al. 1999). In Patient 1 and 2, plasma TGF-β1 levels were similarly markedly elevated. Furthermore, serum IL-8 levels were markedly elevated in Patient 1. Oxidative stress is important in DS pathology, and reactive oxygen species (ROS) levels are important for IL-8 expression (Busciglio and Yankner 1995; Ko et al. 2014). Neutrophils are effector cells that kill bacteria or destroy affected tissues, predominantly through ROS production. These activities are regulated by chemokines, including IL-8 (Kobayashi 2008). Adult patients with cholestatic liver diseases had the highest IL-8 levels of all patients with liver diseases (Zimmermann et al. 2011). They suggested that IL-8 contributes to acute liver inflammation and fibrosis, and high IL-8 levels represent a major cause of chronic liver disease. Furthermore, the hepatic expression of IL-8 had high sensitivity for biliary atresia at diagnosis (Bessho et al. 2014). In Patient 1, elevated blood TGF-β1 and IL-8 levels may have contributed to the severe liver dysfunction observed. A previous study reported markedly elevated IL-7, IL-8, and TGF-β1 levels in TAM with hepatic fibrosis (Sugiura et al. 2010). Before ExT, serum IL-7, IL-8, and TGF-β1 levels were 1,210.1 pg/mL, 534.2 pg/mL, and 129,000 pg/mL decreasing to 279.3 pg/mL, 166.9 pg/mL, and 53,600 pg/mL, respectively, after ExT. Elevated plasma TGF-β1 levels were observed in the two patients in the present study, regardless of the presence or absence of hepatic fibrosis. Serum cytokine profiles were comprehensively measured using LUMINEX® in Patient 1 because the patient's general condition was very serious. In Patient 2, serum was not preserved because the general condition was not bad. Therefore, plasma IL-8 levels were measured in Patient 2 and compared with serum IL-8 levels in Patient 1. In the present patients, the different IL-8 levels between the two patients may have been due to technical aspects, such as differences in the materials and/or methods used. However, serum IL-8 levels were markedly elevated in Patient 1 compared with previously reported patients with hepatic fibrosis. To our knowledge, this is the first report showing that IL-8 is associated with TAM with liver fibrosis. Our findings demonstrate that IL-8 may have a role in the pathophysiology of fatal TAM with liver fibrosis. We speculate that overexpression of IL-8 in the presence of TGF-β1 may be associated with an increased risk of hepatic fibrosis. Our findings demonstrate that IL-8 contributes to the establishment of a pro-fibrogenic hepatic microenvironment in patients with DS.

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Conflict of Interest

The authors declare no conflict of interest.

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