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Molecular mechanisms underlying fibrosis and elastin destruction in childhood interstitial lung diseases

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ABSTRACT

Objective: This study aimed to evaluate fibrosis and elastin destruction in childhood interstitial lung disease (chILD) patients.
Methods: Sixty patients and twenty healthy children were recruited. On admission, evaluation of chILD severity was made using Fan chILD score. Participants provided urine and blood samples. Plasma levels of transforming growth factor (TGF)-β_1, connective tissue growth factor (CCN2), soluble factor related apoptosis (sFas) and long non-coding RNAs and urinary levels of desmosine/urinary creatinine (UDes/UCr) were measured.
Results: In patients, clinical findings were crackles (100.00%), tachypnea (65.00%), cardiomegaly (45.00%), digital clubbing (43.30%), cough (33.00%), cyanosis (26.70%), hepatomegaly (28.30%) and wheezes (23.30%). Categorizing of the patients with Fan chILD clinical score revealed that most patients 33.30% scored (3, symptomatic with abnormal saturation/cyanosis during exercise) then 28.30% scored (5, symptomatic with clinical and echocardiographic features of pulmonary hypertension), 18.30% scored (2, symptomatic with normal room air saturations), 15.00% scored (1, asymptomatic) and 5.00% scored (4, symptomatic with abnormal room air saturation/cyanosis at rest). TGF-β_1, CCN2, sFas, lncrRNA-2700086A05Rik relative gene expression and UDes/UCr levels were higher in patients than controls ($P = 0.002$, $P = 0.001$, $P = 0.001$, $P = 0.001$, $P = 0.001$, respectively). In patients, significant positive correlations were found between TGF-β_1 and CCN2, sFas, UDes/UCr; between CCN2 and both sFas and UDes/UCr; between UDes/UCr and sFas. Morbidity and mortality rates were 46.70% and 10.00%, respectively.
Conclusion: Markers of fibrosis (TGF-β_1, sFas, CCN2) and elastin destruction (UDes/UCr) were increased in chILD especially in patients with long disease duration. So blockage of their pathways signals may offer novel therapeutic targets.

1. Introduction

Childhood interstitial lung diseases (chILDs) represent a rare heterogeneous group of clinical entities with diffuse lung involvement. These disorders involve the interstitium as well as the distal airspaces that result in restrictive lung physiology and significant impairment of gas exchange. Three studies reported the frequency of chILD in general populations [1–3]. Incidence estimates varied from 0.13 cases/100,000 children <17 years of age/year in Germany [3] to 0.36 cases/100,000 in immune–competent children <17 years of age in the United Kingdom and Ireland [1] to 10.8–16.2 cases/100,000 children <15 years of age/year in Denmark [2]. Five hospital–based studies of children [4–8] indicated that between 1.3

Abbreviations: ARDS, acute respiratory distress syndrome; AECs, alveolar epithelial cells; BMI, body mass index; CXR, chest X-ray; chILD, childhood interstitial lung disease; CPI, chronic pneumonitis of infancy; CCN2, connective tissue growth factor; CT, critical threshold; ECM, extracellular matrix; DIP, desquamative interstitial pneumonitis; DAD, diffuse alveolar damage; EMT, epithelial-mesenchymal transition; ERS, European Respiratory Society; HRCT, high-resolution computed tomography; lncRNAs, long non-coding RNAs; LIP, lymphocytic interstitial pneumonia; NSIP, idiopathic interstitial pneumonias; IPF, interstitial pulmonary fibrosis; OP, organizing pneumonia; PAP, pulmonary alveolar proteinosis; sFas, soluble factor related apoptosis; TGF-β_1, transforming growth factor-β_1; UDes/UCr, urinary levels of desmosine/urinary creatinine; UIP, usual interstitial pneumonia.

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to 5.2 new cases of chILD present to pediatric or referral hospitals each year.

There are many classifications of chILD depending on the etiology, histopathological changes of the lung and age of the patients. The European Respiratory Society (ERS) Taskforce defined subgroups of chILD according to underlying etiology and histopathology and related it to the clinical-radiologic-pathologic diagnosis [9]. The ERS divided the diagnoses made clinically into four categories: (1) diffuse lung parenchymal disease of unknown association (drug reaction, aspiration, connective tissue disorders, infection, environmental disorders); (2) idiopathic interstitial pneumonias (NSIP), cellular/fibrotic, desquamative interstitial pneumonitis (DIP), lymphocytic interstitial pneumonia (LIP), diffuse alveolar damage (DAD)/acute interstitial pneumonia, organizing pneumonia (OP), usual interstitial pneumonia (UIP) to include familial cryptogenic fibrosing alveolitis, and chronic pneumonitis of infancy (CPI); (3) other forms of interstitial pneumonia to include lymphangioleiomyomatosis, Langerhans cell granulomatosis, pulmonary alveolar proteinosis (PAP), sarcoidosis, eosinophilic pneumonia, idiopathic/infantile pulmonary hemosiderosis; and (4) congenital disorders (DIP and LIP), lipoid pneumonia, nonspecific interstitial pneumonia/UIP, and surfactant deficiencies). A more organized classification scheme for chILD was recently published by the chILD Research Network. The children classification scheme is divided into three categories depending mainly on the age group: (1) disorders more prevalent in infancy (diffuse developmental disorders, growth abnormalities, specific conditions of undefined etiology, surfactant dysfunction mutations, and related disorders), (2) disorders not specific to infancy (disorders of the normal host, disorders related to systemic disease processes, disorders of the immunocompromised host and disorders masquerading as interstitial disease) and (3) unclassified (end-stage disease, nondiagnostic biopsies, and those with inadequate material) [10]. An advantage of chILD Research Network classification strategy is that the first category recognizes some disorders present largely in infancy, but may also develop later in childhood and adulthood, whereas the second category acknowledges that infants can develop conditions that are more common in older children and adults.

An important feature of the pathologic process in ILD is an altered communication between epithelial and mesenchymal pulmonary components. Prolonged denudation of the basement membrane after injury contributes to altered interactions between alveolar epithelial (AECs) and mesenchymal cells, resulting in profound cell functions modifications with the production of many polypeptide mediators [11]. Transforming growth factor (TGF)-β_1 and Fas/FasL system play important roles in ILD's pathology including early lung injury and later tissue repair. In ILD, increased apoptosis with upregulation of Fas-FasL molecules demonstrated in AECsII, meanwhile fibroblasts/myofibroblasts isolated from lungs showed decreased apoptosis [12]. Connective tissue growth factor-2 (CCN2) has been reported as a factor in extracellular matrix production and profibrotic activity mediated by TGF-β_1. CCN2 was useful in diagnosing or predicting disease progression in some fibrotic diseases as scleroderma, progressive glomerulosclerosis and end-stage renal disease [13]. Elastin is a major component of extracellular matrix (ECM) of the lung. Lung injury that involves ECM catabolism is associated with elastin destruction and release of quantifiable desmosine cross-links. Urinary desmosine (UDes) concentration has been identified as a potential surrogate marker of active lung injury [14]. Long non-coding RNAs (lncRNAs) are functional RNA molecules that are not translated into proteins. lncRNAs modulate gene transcription, RNA splicing, protein translation and regulation of various physiological pathways [15]. Dysregulation of lncRNAs appears to be a primary feature of many complex human diseases, including leukaemia [16], colon cancer [17], prostate cancer [18], breast cancer [19], hepatocellular carcinoma [16], psoriasis [20], ischemic heart disease [21], Alzheimer's disease [22] and spinocerebellar ataxia type 8 [23]. Sun and his colleagues [24] reported that differentially expressed lncRNAs are implicated in cell differentiation, epithelium morphogenesis and wound healing and pathways closely associated with epithelial-mesenchymal transition (EMT). Furthermore, they identified the evolutionally conserved target genes of two up-regulated lncRNAs, uc.77, and 2700086A05Rik, as Zeb2 and Hoxa3, respectively, both of which are important modulators of EMT. Collectively, their results uncovered a crucial role of lncRNA in the regulation of EMT during lung fibrosis which is an important step in the pathophysiology of chILD and provide potential avenues for the discovery of novel molecular markers and therapeutic targets for idiopathic pulmonary fibrosis. There is a limited study on lncRNA expression during interstitial lung diseases and lung fibrosis.

This hospital based study aimed to evaluate lung fibrosis by measuring plasma levels of TGF-β_1, CCN2, sFas, and lncrRNA-2700086A05Rik and lung elastin destruction by measuring UDes/UCr in chILD patients. This study also aimed to identify possible candidate biomarkers and pathways that might offer therapeutic targets changing the natural course of this disease.

2. Materials and methods

2.1. Patients

This hospital based study conducted on 60 chILD patients recruited from Pulmonology Unit at the Assiut University Children Hospital, Assiut, Egypt, over a 3-yr period (January 2012 to January 2015). They were 36 boys and 24 girls, with their ages ranging from 0.50 to 16 years (mean ± SD, 6.54 ± 4.62 years). Twenty apparently healthy children and adolescents (13 boys and 7 girls), with their ages ranging from 1.50 to 17 years (mean ± SD, 8.68 ± 4.24 years) were selected from the outpatient's clinic served as control group. This study was approved by the Ethical Committee of Faculty of Medicine, Assuit University Childen Hospital according to latest revision of *Declarationof Helsinki* (2013) and informed consent was obtained from participant's parent/legal guardian.

ChILD diagnostic criteria were persistence of at least three of following for more than one month: (1) respiratory symptoms (e.g., cough, rapid and/or difficult breathing, or exercise intolerance); (2) respiratory signs (e.g., resting tachypnea, adventitious sounds, retractions, digital clubbing, failure to thrive, or respiratory failure); (3) hypoxemia; (4) diffuse abnormalities on chest X-ray (CXR) or high-resolution computed tomography (HRCT) [10] or proven to have chILD by lung biopsy. Patients were excluded from this study if they had bronchopulmonary dysplasia, congenital heart disease, primary or acquired immunodeficiency, primary autoimmune disorder, cystic fibrosis or pulmonary tuberculosis.

In all participants, height, weight, and calculated body mass index (BMI) were measured and recorded. ChILD patients were undergoing a comprehensive history, questionnaire including family history of similar lung diseases, occupational history, and animal contact history as well as disease duration. Initial pulmonary evaluation included biochemical investigations, chest roentgenography, and cardiac evaluation were done. On admission, Fan chILD clinical score for each patient was calculated [25]. The patients with chILD were scored from 1 to 5 based on increasing severity of illness; score (1): asymptomatic; score (2): symptomatic with normal room air saturations; score (3): symptomatic with abnormal saturation/cyanosis during exercise; score (4): symptomatic with abnormal room air saturation/cyanosis at rest; score (5): symptomatic with clinical and echocardiographic features of pulmonary