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Original Article

Increased expression of TIM-1 predicts the progression of pneumonia in pediatric patients

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Abstract: To investigate the predictive role of the expression of T cell immunoglobulin and mucin domain-containing molecule-1 (TIM-1) in pediatric patients with pneumonia. During May 2014 and May 2016, the study incorporated a total of 166 pediatric patients with pathologically confirmed pneumoniae, and 100 healthy children who did physical examination at the same time during the same hospital Peripheral venous blood samples were collected from each subject. Quantitative real-time polymerase chain reaction (qPCR) assay was applied for the detection of the mRNA expression level of TIM-1 in both groups. Logistic regression analysis was involved to identify the role of TIM1 to the severity of pneumoniae. Expression level of TIM-1 mRNA in the case group was significantly higher than that in the control group ($P < 0.05$). Furthermore, the expression level of TIM-1 mRNA in the severe group was even higher than that in the mild group ($P < 0.05$). Receiver operator characteristic (ROC) curve analysis showed that the area under the ROC curve was 0.832, with corresponding sensitivity and specificity of 80.12% and 70.00%, respectively. Correlation analysis identified positive correlation of TIM-1 levels in the case group, the mild group and the severe group with that in the control group (all $P < 0.05$). Furthermore, the severity of the disease, age distribution and the infection type were all correlated with altered mRNA expression level of TIM-1 (all $P < 0.05$). Univariate and multivariate analyses revealed the important contribution of elevated TIM-1 expression in predicting the severity of pneumoniae (all $P < 0.05$). The mRNA expression of TIM-1 was increased in pediatric pneumonia, and may be correlated with the severity of pneumonia in children. Further exploration is required to elucidate in vivo molecular mechanisms of TIM-1 in regulating the development of pediatric pneumonia, combined with follow-up therapeutic outcomes investigation.

Keywords: TIM-1, pediatric pneumonia, mRNA expression

Introduction

Acute respiratory tract infection, pneumonia in particular, is one of the most common diseases in children correlated with relatively high hospitalization and death rate all over the world, causing a 4.5 million childhood deaths yearly worldwide, most of these death occur in children from developing countries, which is a serious threat to children's health [1, 2]. Mycoplasma pneumonia is one of the most important pathogens of acute respiratory tract infection in children between 5 and 15 years [3], and of infectious pneumonia and other respiratory infections, mainly in the lower respiratory tract infection [4]. Mycoplasma pneumonia, also known as primary atypical pneumonia, is a common type of pneumonia in school aged children and adolescents, and is not uncommon in infants and young children [5]. Mycoplasma pneumonia is a common inflammatory disease, infectious agents (i.e., viruses, mycoplasma bacteria, and other microbes) may be involved in the occurrence of the disease [6]. Annually estimation of the incidence of the disease is about 15.7 cases per 10,000 children, and even higher in children under 2 years of age (over 62.2 per 10,000) [7]. The most common clinical manifestation of mycoplasma pneumoniae infection is respiratory symptoms, including fever, cough and wheezing, etc. [8, 9], which can also cause multiple systemic complications, such as myocarditis, damage to the skin, involving of urinary system, digestive system, and central nervous system [10-12], a few complications also has long duration and cause

severe illness, corresponding prognostic outcome is poor, even endanger life if not treated timely [13].

T helper cell (Th cell) can secrete a variety of cytokines, which is mainly sub-divided into the Th1 and Th2 cells [14], of which the former cell predominantly involves in cellular immunity and delayed hypersensitivity, and the latter one may help B cells differentiate into antibody secreting cells and participate in humoral immune response [15, 16]. Dynamic balance of Th1 and Th2 cells is essential for the maintenance of normal physiological capacity in a manner of mutual antagonism and self-promotion to form a complex and orderly network of cytokines regulating the body's normal immune response [17]. T cell immunoglobulin and mucin domain-containing molecule-1 (TIM-1) is a critical member in the TIM protein family, acting a class of proteins that regulate the activation and tolerance of T cells [18]. It has been reported by previous research that TIM-1 may play a critical role in immune responses that regulate the development of atopic diseases, and this correlation is based on the natural ligand of TIM-1 to modulate T cell differentiation and the development of Th2-driven allergic inflammatory responses [19, 20]. In the present study, we made an assumption that whether the activity of TIM-1 might have a role in the occurrence and development of pediatric pneumoniae, the expression of TIM-1 in pediatric pneumoniae patients was hence investigated to illustrate the predictive role of TIM-1 in pediatric pneumoniae.

Materials and methods

Study subjects

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. During May 2014 and May 2016, a total of 166 pediatric children with pathologically confirmed pneumoniae and underwent treatment were selected from the pediatric ward. There were 91 males (51.7%) and 75 females (48.3%) aging from 1 to 15 years, with a mean age of (5.82 ± 1.22) years old. The diagnosis of pediatric pneumoniae was in strict accordance with the diagnostic criteria recommended by *Practical Pediatrics* (the 6th edition) [21] and associated with the examination results of X-ray and CT imaging in the lung. Enrolled pediatric patients were all examined with the serum MP-IgM antibody titer of over 20 bu/ml (positive). Meanwhile, pediatric patients were detected with white blood cell > 10×10^9/L or < 4.0×10^9/L. Patients with hospital-acquired pneumonia, previous history of heart, liver and kidney insufficiency, and a recent administration history of drugs with cardiac toxicity, and patients with incomplete clinical data were excluded from the study, meanwhile, extra-pulmonary complications were also diagnosed to exclude the system damage caused by other causes. Included patients were further subdivided into two groups (the severe group, n = 104; and the mild group, n = 62) based on the clinical symptoms and signs. General information (age, gender, and onset), clinical symptoms and signs of each patient were recorded.

In addition, a total of 100 healthy children who did physical examination at the same time during the same hospital were incorporated as the control group, including 62 male cases and 38 female cases, with a mean age of (5.50 ± 1.03) years old ranging from 1 to 15 years old. All normal controls were confirmed without disorders of the heart, liver, lung, kidney and other important organs, and without previous history of any infection lately, both the liver and kidney function test results were normal.

Blood samples collection

Disposable anticoagulant blood collection tube was applied for the extraction and collection of peripheral venous blood samples (4 ml) in each subject in the early morning following overnight fasting. Total RNA of blood cells was extracted from the blood samples and were then preserved at -80°C for quantitative real-time polymerase chain reaction (qPCR) assay.

qPCR assay

Total RNA of blood cells was extracted from half part of the blood samples using the pure blood total RNA extraction kit by Trizol extraction

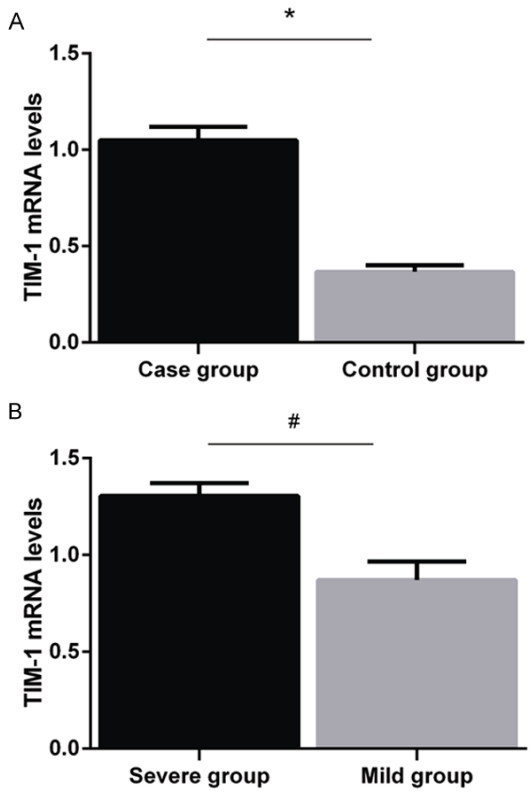

Figure 1. TIM-1 mRNA expression comparison in the case group and the control group (A), as well as between the severe group and the mild group (B) within the case group. Note: *, compared with control group, $P < 0.05$; #, compared with the mild group, $P < 0.05$.

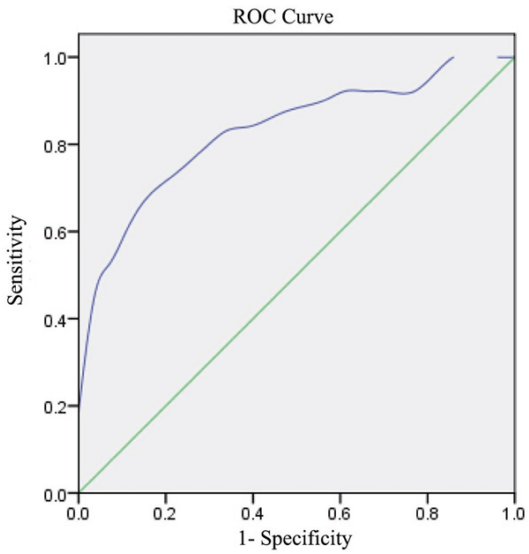

Figure 2. The receiver operator characteristic (ROC) curve analysis for the evaluation of the diagnostic role of TIM-1 between the case group and the control group. Note: The area under the curve was 0.832 (95% CI = 0.784~0.880, $P < 0.001$), with corresponding sensitivity and specificity of 80.12% and 70.00%, respectively; the cutoff value was 0.571, and the positive predictive value and negative predictive value was 74.750% and 74.682%, respectively.

(Invitrogen, Carlsbad, CA, USA) 2 hours after the collection in strictly accordance with the instructions of the instruction. After extraction, total RNA concentration and purity were determined using ultraviolet spectrophotometer (Analytik Jena AG, Germany), prepared samples were then preserved at -80°C for qPCR assay. The primers of PCR were synthesized by Shanghai Sangon Biotechnology Co. (Shanghai, China). The forward primer of TIM-1 was 5'-GAACATAGTCTACTGACGGCCAATAC-3', the reverse primer of TIM-1 was 5'-GAACCTCC-TTTTTGAAGAAATACTTTT-3'. β-actin was used as the internal inference, the forward primer of β-actin (internal inference) was 5'-GGCACC-ACACCTTCTACAATG-3', the reverse primer of β-actin was 5'-TAGCACAGCCCTGGATAGCAAC-3'. All reaction processes were repeated for three times. The amplified fragments were analyzed and recorded after gel electrophoresis. The relative expression level of TIM-1 mRNA was determined by the absorbance ratio of TIM-1 amplified band to β-actin amplified band by using the $2^{-\Delta\Delta Ct}$.

Statistical analysis

The SPSS 17.0 software (SPSS Inc., Chicago, Illinois, USA) was applied for statistical analysis. All measurement data were presented as mean ± standard difference (SD). The means were compared between the two groups by using the *t* test. A Mann-Whitney non-parametric test was performed for the identification of the relationship of the mRNA expression level of TIM-1 with pediatric pneumoniae. Correlative analysis of TIM-1 expression with pediatric pneumonia was performed by applying Spearman correlation analysis. The receiver operator characteristic (ROC) analysis was applied to evaluate the diagnostic ability of TIM-1 for pediatric pneumonia, with the estimation of the area under the ROC curve. Furthermore, univariate and multivariate logistic regression analysis was involved to identify the role of TIM1 to the severity of pediatric pneumonia by controlling for other factors. $P < 0.05$ was considered as statistically significant.

Table 1. The relationship between TIM-1 mRNA expression and clinical parameters in pediatric pneumonia patients

Variables	mRNA levels of TIM-1		*P*
	High levels (n = 102, %)	Low levels (n = 64, %)	
Age			
1~3 years old	58 (56.86)	30 (46.88)	0.211
4~15 years old	44 (43.14)	34 (53.12)	
Gender			
Male	69 (67.65)	22 (34.38)	< 0.001
Female	33 (32.35)	42 (65.62)	
CRP levels			
High level	60 (58.82)	26 (40.63)	0.023
Low level	42 (41.18)	38 (59.37)	
Infection type			
Simple infection	70 (68.63)	20 (31.25)	< 0.001
Mixed infection	32 (31.37)	44 (68.75)	
Severity degree			
Severe	75 (73.53)	27 (42.19)	< 0.001
Mild	27 (26.47)	37 (57.81)	

Note: The cutoff value was 0.651, expression over 0.651 was defined as the high level, and in contrast as the low level.

Results

Baseline information

Age ([5.82 ± 1.22] years old vs. [5.50 ± 1.03] years old) and gender (91 males/75 females vs. 62 male/38 female) distributions of the case group and the control group showed none obvious statistical difference in comparison (both $P > 0.05$). Furthermore, after a subdivision of the case group into the severe group and the mild group, it was counted that there were 102 cases of pediatric children in the severe group, including 58 male cases and 44 female cases, with a mean age of (5.40 ± 1.03) years old; meanwhile, in the 64 cases of the mild group, there were 36 cases of males and 28 cases of females, with a mean age of (5.78 ± 1.35) years old, no statistical differences regarding age or gender were found in the mild group and the severe group. In addition, as for the observation of clinical symptoms and signs, the results showed that there were 78 cases of fever or unchanged body temperature (46.99%), 102 cases with cough (61.45%), 75 cases (45.18%) and 79 cases (47.59%) showing moist and rhonchi rales, respectively, 56 cases accompanied with shortness of breath (33.73%), 40 cases with cyanosis (24.10%), 35 cases indicating flaring of alae nasi (21.08%), 34 cases presenting tri-retraction sign (20.48%), and 30 cases of patients with irritability and lethargy (18.07%).

TIM-1 mRNA expression

In the case group, there were 134 patients indicated high expression of TIM-1, and 32 cases showed low expression; besides, in the control group, there were 30 cases with high expression of TIM-1 and 70 cases with low expression. Expression level of TIM-1 mRNA in the case group and control group was (1.068 ± 0.073) and (0.384 ± 0.035), respectively. Statistical analysis demonstrated significant statistical difference in the expression of TIM-1 mRNA between the case group and control group ($P < 0.05$; **Figure 1**). Meanwhile, as shown in **Figure 2**, ROC curve analysis showed that the area under the ROC curve was 0.832 (95% confidence interval = 0.784~0.880, $P < 0.001$), with corresponding sensitivity and specificity of 80.12% and 70.00%, respectively, indicating relatively high diagnostic value.

Furthermore, the expression level of TIM-1 mRNA in the severe group was even higher than that in the mild group (1.293 ± 0.080 vs. 0.898 ± 0.067, $P < 0.05$), demonstrating that the expression of TIM-1 mRNA might be increased with the elevated severity of pediatric pneumoniae (**Figure 1**). Correlation analysis indicated that there were positive correlation between TIM-1 expression levels in the case group, the mild group and the severe group when compared to the control group ($r = 0.348$, $r = 0.411$ and $r = 0.774$, respectively, all $P < 0.05$).

Correlation of TIM-1 mRNA expression with clinical parameters

There were 102 cases of patients indicating high expression level of TIM-1, and 64 cases of patients showing low expression level of TIM-1. The results showed that the severity of the disease, age distribution and the infection type (simple infection vs. mixed infection) were all correlated with the altered mRNA expression level of TIM-1 (all $P < 0.05$, **Table 1**). However, no statistical difference in gender or CRP levels was detected to be correlated with the increased TIM-1 mRNA expression level (both $P > 0.05$).

Table 2. Univariate analysis for the determination of the role of TIM1 to the severity of pneumonia

Variables	Severity of pneumonia		χ^2	*P*
	Severe (n = 102, %)	Mild (n = 64, %)		
Age				
1~3 years old	62 (60.78)	26 (40.63)	6.416	0.011
4~15 years old	40 (39.22)	38 (59.37)		
Gender				
Male	59 (57.84)	32 (50.00)	1.451	0.228
Female	43 (42.16)	32 (50.00)		
CRP levels				
High level	61 (59.80)	25 (39.06)	6.776	0.009
Low level	41 (40.20)	39 (60.94)		
Infection type				
Simple infection	42 (41.18)	48 (75.00)	18.121	< 0.0001
Mixed infection	60 (58.82)	16 (25.00)		
TIM-1 expression				
High	75 (73.53)	27 (42.19)	16.311	< 0.0001
Low	27 (26.47)	37 (57.81)		

Table 3. Multivariate logistic regression analysis for factors related to the severity of pneumonia

Variables	*β*	SE	Wald	*P*	OR (95% CI)
Age	-1.705	1.211	1.983	0.159	0.182 (0.017-1.951)
CRP levels	1.077	0.601	3.213	0.073	2.936 (0.904-9.536)
Infection type	0.523	0.675	0.600	0.438	1.687 (0.449-6.339)
TIM-1 expression	0.114	0.134	2.455	0.011	2.113 (0.984-2.225)

Note: *β*: regression coefficient; SE: standard error; Wald: test statistics; OR: odds ratio; 95% CI: 95% confidence interval.

Regression analysis

For the further determination of the role of TIM-1 to the severity of pediatric pneumonia, univariate analysis was conducted and revealed that age, CRP levels, infection type and expression of TIM-1 might be associated with the severity of pneumonia (all $P < 0.05$, **Table 2**), which were involved in the univariate logistic analysis model. Multivariate unconditional logistic analysis indicated that after controlling for other factors, were important factors responsible for the severity of pneumonia (all $P < 0.05$, **Table 3**). Other influential factors including did not enter the regression model.

Discussion

Our present study evaluated the activity of TIM-1 in pediatric pneumonia in an in vivo experiment. As mentioned above, TIM-1 is a critical member found in recent decades belonging to the TIM family [19]. TIM-1 may be specifically expressed on the surface of Th2 cells, and subsequently stimulate the proliferation and cytokines secretion of Th2 cells by binding with its ligand, suggesting that TIM-1 may possess the function of regulating Th2-mediated immune response [22]. Th2 cells can contribute to the production of cytokines such as IL-4, IL-5 and IL-10, predominant by the role of anti-inflammation, thereby promote the production of antibodies and mediate the response of humoral immune [23]. By applying qPCR assay, corresponding detection results revealed that the mRNA expression level of TIM-1 in pediatric children with pneumonia was significantly higher than that of the healthy controls. The results might indicated that TIM-1 might have a role during the process of mycoplasma pneumoniae infection. At the same time, ROC curve analysis showed that the area under the ROC curve was 0.832, with corresponding sensitivity and specificity of 80.12% and 70.00%, respectively, indicating relatively high diagnostic value of TIM-1 in the diagnosis and prediction of pediatric pneumonia. In fact, the process of pneumonia in children not only depends on the characteristics of pathogens and the scope of the disease, but also relies on the body's immune system. In the acute phase of pneumonia, the cell immunity function is low, manifested as the decreased expression of T cells, T lymphocyte transformation rate, and reduced phagocytic activity of leukocytes [24, 25]. Combined with the positive results of correlation analysis, we could speculated that high expression of TIM-1 might predict the occurrence of pediatric pneumonia, and might also predict the severity of the disease which we

were explored in the following regression analysis. In theory, TIM-1 is a characterized molecule expressed on the activated surface of Th2 cells, the increased expression of such molecule suggested that in the acute phase of mycoplasma pneumoniae infection, the cellular response of Th1 cells was inhibited, and the Th2 cells immune response was highlighted [26].

In recent decades, along with the in-depth research on T cell subsets, TIM-1 has been proved to regulate the balance of Th1/Th2 and the immune response of Treg cells in various diseases, such as asthma and systemic lupus erythematosus [27, 28]. By blocking the TIM-1 signaling pathway to promote the immune tolerance of mice, Ding et al. discovered in their study that TIM-1 expression in mice was observed in B cells mainly to exert the immune function, and up-regulating the expression of IL-4 and IL-10 to promote the immune response of Th2 cells [18]. Not only that, as for allergic asthma predominant by immune response of Th2 cells, TIM-1 could activate the expression of nuclear transcript or activator protein-1 separated from activated T cells to produce costimulatory signals; besides, TIM-1 could combined with TIM-3 to affect T cells mediated immune response to regulate the expression of Th2 cells [29, 30]. Subsequent correlation analysis indicated that patients with mixed infection indicated a much higher expression level of TIM-1 than those with single infection, which also highlight the diagnostic role of TIM-1 in pediatric pneumoniae. Meanwhile, the expression level of TIM-1 mRNA in the severe group was detected to be much higher than that in the mild group, demonstrating that TIM-1 mRNA level might be increased with the elevated severity of pediatric pneumoniae, which revealed that TIM-1 might be strongly promoted to interfere the progression of pneumonia. More importantly, univariate and multivariate analyses results suggested the role of TIM-1 expression in predicting the severity of pediatric pneumoniae, which in turn confirmed the above hypothetical discussion and mechanism explanation.

In conclusion, evidence from our present study showed that the mRNA expression of TIM-1 was increased in pediatric pneumonia, and may be further correlated with the severity of pneumonia in children. Further investigations are required to elucidate the molecular mechanisms of TIM-1 in regulating the development of pediatric pneumonia, combined with follow-up therapeutic outcomes investigation. Taken together, TIM-1 expression may serve as a biomarker for pediatric pneumonia.

Disclosure of conflict of interest

None.

Authors' contribution

Wan-Wen Liu writing of the manuscript. Wan-Wen Liu and Yong-Feng Liang: analysis and interpretation of the results. Wan-Wen Liu: Design of the study. Wan-Wen Liu: carried out the experiments. All authors reviewed the manuscript.

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