ระดับ soluble tumor necrosis factor receptor type II (sTNFRII) สูง แต่ระดับ soluble FasL (sFasL) ต่ำ ในผู้ป่วยไข้หวัดใหญ่ชนิดเอ (H1N1) 2009 ที่อาการรุนแรง

High plasma levels of soluble tumor necrosis factor receptor type II (sTNFRII) but low plasma levels of soluble FasL (sFasL) in patients with severe pandemic H1N1 2009 influenza infection

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Abstract

Background: Inflammatory mediators play an important role in immune responses to viral infections. Data on the plasma levels of inflammatory mediators in pandemic H1N1 2009 influenza virus-infected Thai patients with severe diseases remain very limited.

Objective: To assess plasma levels of interleukin-1β (IL-1β), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1β, interferon-gamma (IFN-γ), tumor necrosis factor-α (TNF-α), sTNFRII, sFasL and soluble RAGE (sRAGE) in patients with pandemic H1N1 2009 influenza infection.

Methods: Forty-two patients with pandemic H1N1 2009 influenza infection characterized as mild disease (n=21) or severe disease (n=21) were enrolled in this study. The study protocol was approved by the Ethical Committee of the Institute for the Development of Human Research Protection (IHRP), Ministry of Public Health. The study was carried out in Nakhon Ratchasima province during August 2009 to November 2010. Plasma levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, G-CSF, GM-CSF, MCP-1, MIP-1β, IFN-γ and TNF-α were measured using Bio-Plex Pro™ Human Cytokine 17-plex Assay (Bio-Rad). Plasma levels of sTNFRII, sFasL and sRAGE were determined by sandwiched enzyme-linked immunosorbent assay (R&D).

Result: Plasma levels of sTNFRII in pandemic H1N1 2009 influenza-infected patients with severe diseases (median (IQR) (pg/mL); 7,525 (6,470-9,630); n=11) were significantly higher than those with mild diseases (median (IQR) (pg/mL); 3,692 (2,655-4,128); n=21) (p=0.004). In addition, plasma levels of sFasL in pandemic H1N1 2009 influenza virus-infected patients with severe disease (median (IQR) (pg/mL); 39 (10-78); n=21) were significantly lower than those with mild disease (median (IQR) (pg/mL); 10 (10-25); n=11) (p=0.003). However, the plasma levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, G-CSF, GM-CSF, MCP-1, MIP-1β, IFN-γ, TNF-α, and sRAGE did not show any significant difference between the severe pandemic H1N1 2009 influenza virus infection and mild pandemic H1N1 2009 influenza virus infection.

Conclusion: The finding showed that higher plasma sTNFRII and lower plasma sFasL levels in patients with severe pandemic 2009 influenza virus infection might determine dysregulation of host immune response and could be used as prognostic markers of disease severity.

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Keywords: Severe pandemic H1N1 2009 influenza, cytokine, chemokine, sTNFRII, sFasL

บทคัดย่อ

บทนำ: สารสื่อกลางการอักเสบมีบทบาทสำคัญในการตอบสนองสนองทางภูมิคุ้มกันของการติดเชื้อไวรัส การศึกษาก่อนหน้านี้พบว่า ผู้ป่วยไข้หวัดใหญ่ชนิดเอ (H1N1) 2009 ที่อาการรุนแรงมีสารสื่อกลางการอักเสบในระดับสูง แต่อย่างไรก็ตาม ยังมีข้อมูลค่อนข้างจำกัดในผู้ป่วยไทยที่เป็นไข้หวัดใหญ่ชนิดเอ (H1N1) 2009 ที่อาการรุนแรง

วัตถุประสงค์: เพื่อหาระดับ IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, G-CSF, GM-CSF, MCP-1, MIP-1β, IFN-γ, TNF-α, sTNFRII, sFasL และ sRAGE ในพลาสมาของผู้ป่วยไข้หวัดใหญ่ชนิดเอ (H1N1) 2009

วัสดุและวิธีการวิจัย: ทำการศึกษาในผู้ป่วยไข้หวัดใหญ่ชนิดเอ (H1N1) 2009 ที่จังหวัดนครราชสีมาในเดือน สิงหาคม 2552-พฤศจิกายน 2553 จำนวน 42 ราย ประกอบด้วย ผู้ป่วยที่อาการไม่รุนแรง 21 ราย ที่อาการรุนแรง 21 ราย ดำเนินการวิเคราะห์ระดับ IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, G-CSF, GM-CSF, MCP-1, MIP-1β, IFN-γ, TNF-α ในพลาสมา โดยใช้วิธี Bio-Plex Pro™ Human Cytokine 17-plex Assay (Bio-Rad) และระดับ sTNFRII, sFasL, sRAGE ในพลาสมา โดยวิธี sandwiched enzyme-linked immunosorbent assay (R&D)

ผลการศึกษา: ระดับ sTNFRII ในพลาสมาจากผู้ป่วยไข้หวัดใหญ่ชนิดเอ (H1N1) 2009 ที่อาการรุนแรง (median (IQR) (pg/mL); 7,525 (6,470-9,630); n = 11) มีค่าสูงกว่าระดับ sTNFRII ในพลาสมาจากผู้ป่วยที่อาการไม่รุนแรงอย่างมีนัยสำคัญทางสถิติ (median (IQR) (pg/mL); 3,692 (2,655-4,128); n = 21) (p=0.0049) นอกจากนั้นระดับ sFasL ในพลาสมาจากผู้ป่วยที่อาการรุนแรง (median (IQR) (pg/mL); 39 (10-78); n = 21) มีค่าต่ำกว่าระดับ sFasL จากผู้ป่วยไข้ที่อาการไม่รุนแรงอย่างมีนัยสำคัญทางสถิติ (median (IQR) (pg/mL); 10 (10-25); n = 11) (p=0.0035) แต่อย่างไรก็ตาม ในการศึกษานี้ไม่พบความแตกต่างของระดับ IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, G-CSF, GM-CSF, IFN-γ, MCP-1, MIP-1β, TNF-α และ sRAGE ในพลาสมาจากผู้ป่วยทั้งสองกลุ่มนี้

สรุปผลการศึกษา: ระดับ sTNFRII ที่เพิ่มสูงขึ้น และ/หรือ ระดับ sFasL ที่ลดต่ำลงในพลาสมาผู้ป่วยไข้หวัดใหญ่ชนิดเอ (H1N1) 2009 ที่อาการรุนแรง อาจบ่งบอกความผิดปกติในการรักษาสมดุลของระบบภูมิคุ้มกัน และอาจจะใช้เป็นตัวบ่งชี้สำหรับการพยากรณ์ความรุนแรงของโรค

วารสารเทคนิคการแพทย์เชียงใหม่ 2558; 48(2): 115-126. Doi: 10.14456/jams.2015.12

คำหัส: ผู้ป่วยไข้หวัดใหญ่ชนิดเอ (H1N1) 2009 ที่อาการรุนแรง ไซโตคัยน์ คีโมคัยน์ sTNFRII, sFasL

Introduction

The pandemic H1N1 2009 (pdmH1N1 2009) influenza A virus has emerged from North America since March 2009. Subsequently, it has rapidly spread to over 214 countries worldwide. Globally over 414,000 cases of the pdmH1N1 2009 virus infections were confirmed in 2010 resulting in at least 18,114 deaths.[1] In Thailand during 2009-2010, the pdmH1N1 2009 virus was responsible for 47,411 laboratory-confirmed cases and 347 deaths.[2] Most of pdmH1N1 2009 virus infections cause mild and self-limited diseases. However, some patients with pdmH1N1 2009 virus infection may develop severe respiratory tract complications and deaths. Previous pathogenesis studies on pdmH1N1 2009 virus infection[3] revealed that virological factors, cytokine storms, and pathological changes contributed to the pathogenesis of pdmH1N1 2009 virus infection.[4]

Cytokines have been shown to play role in both immunoprotection and immunopathology in several viral infections including influenza A.[5-19] Influenza A virus infection can induce robust cytokine production (hypercytokinemia or cytokine storm). This results in hyperactivation of innate immune responses during early stage of infection, and lung tissue damage. *In vitro* experiments demonstrated that influenza A infection excessively stimulated TLR/cytokine signaling, and up-regulated suppressor of cytokine signaling (SOCS)-1 in BEAS-2B human pulmonary epithelial cells.[5] Seasonal influenza A H1N1 (sH1N1) significantly increased gene expression of TLR3, TLR9, IL-6, TNF-α and IFN-β in human pharyngeal epithelial cell line (Hep-2).[6] H5N1 virus induced the expression of CXCL10, IL-6, CCL2, and CCL5 in human lung A549 epithelial cells.[7] Studies in mice models demonstrated pulmonary viral replication, pulmonary pathological lesion, and overwhelmed inflammatory innate immune responses in pdmH1N1 2009 virus-infected susceptible mice.[8-12] In human, Th1 and Th17 hypercytokinemia during early stage of infection were associated with severe disease of influenza infection.[13-17] The elevated plasma levels of IL-17 were observed in influenza A-infected patients with acute lung injury (ALI) and acute respiratory distress syndrome (ARDS)14 whereas the increased plasma levels of IL-1β, IL-6, IL-12 and IFN-γ were observed in influenza A-infected patients with pneumonia.[18,19] The plasma levels of IL-8, IL-9, IL-17, IL-6, TNF-α, IL-15 and IL-12p70 were also significantly increased in patients with severe pandemic influenza infection.[13]

Recent studies demonstrated that TNF and TNF receptor family were not only involved in the immunopathogenesis but also in the control of influenza A infection. TNF had an effect on enhanced recruitment of inflammatory cells and induction of tissue damage in the lungs. These lead to severe respiratory complications such as pneumonia, acute lung injury and acute respiratory distress syndrome. Administration of anti-TNF antibody in mice was shown to decrease pulmonary infiltration, and lung injury. It also prolonged survival of mice with lung disease caused by influenza virus.[20-21] Mice genetically deficient in TNFR I with influenza A virus infection were demonstrated to have the reduction in pulmonary viral infiltration and disease mortality.[22-23] Previous clinical studies showed the increased plasma levels of TNF-α and soluble TNFRI (sTNFRI) in patients with severe and fatal influenza A infection.[13,24] However, little is known about the role of sTNFRII in clinical outcome of pdmH1N1 2009 virus infections.

Receptor for advanced glycation end-products (RAGE), a multi-ligand cell-surface protein and NF-kappa B activator, is involved in inflammatory responses of acute and chronic diseases. The engagement of RAGE with their ligands (such as advanced glycation end products (AGEs), toxic AGEs (TAGE), High-mobility group box-1 (HMGB1), damage-associated molecular patterns (DAMPs)) induces inflammatory responses, resulting in devastating of the diseases. Soluble RAGE (sRAGE) derived from receptor ectodomain shedding, and endogenous secretory (es) RAGE act as decoy receptors to counteract RAGE-mediated pathogenesis. Several studies demonstrated that the decreased level of sRAGE is related with hyperactivation of ligand-AGE axis. The increased HMGB1, and attenuated lung pathology from *Staphylococcus aureus* infection were demonstrated in mice genetically deficient in RAGE.[25] Patients with lung infection had elevated levels of alveolar sRAGE. However, patients with severe lung dysfunction had elevated levels of both plasma and alveolar sRAGE.[26]

The elevated levels of serum sRAGE were also observed in children with severe bronchiolitis.[27] Patients with septic shock showed a positive statistically significant correlation between sRAGE with IL-1α, IL-6, IL-8, IL-10 and interferon-gamma-induced-protein 10 (IP-10), while patients with severe sepsis and septic shock showed positive correlation between plasma sRAGE with IFN-γ.[28]

FasL, belongs to TNF and TNF receptor family, plays a crucial role in participating the Fas-mediated (extrinsic) apoptosis pathway of virus infected cells.[29] Soluble FasL (sFasL), which shed from the membrane-bound molecules by metalloproteinase, acts as a functional regulator of membrane-bound FasL.[30] Evidence for the role of Fas-mediated apoptotic mechanisms in influenza A infection come from the cell culture experiments,[31-32] animal models,[33] and human studies.[34] Influenza A-infected HeLa cells expressed both Fas and FasL on the cell surfaces and induced apoptosis.[31] While A549-infected with pandemic influenza A (H1N1) virus upregulated FasL expression and modulate Fas signaling pathway, thereby enhancing viral replication.[35] Mice infected with the 1918 pandemic H1N1 influenza A virus revealed the expression of FasL/Fas associated genes in the lung is related with the mortality of the infected mammalian host.[36] Inhibition of Fas and FasL signaling also decreased the mortality of mice lethally infected with influenza A virus.[37]

Despite the 2009 H1N1 influenza outbreak in Thailand during 2009-2010 with high disease burden, less is known about the immunopathogenesis of severe pdmH1N1 2009 influenza diseases in this setting. In this study, we investigated the plasma levels of inflammatory markers in patients with pdmH1N1 2009 in*fluenza* infection.

Materials and Methods

Study site

The study subjects were recruited from individuals infected with the pdmH1N1 2009 virus in Nakhon Ratchasima province during August 2009 to November 2010.

Study subjects

Forty two patients diagnosed pdmH1N1 2009 virus infection (age range 21-85 years) with mild (n=21) and severe disease (n=21) were enrolled in this study during September 2010 to October 2010 from the outpatient department and critical care units, Maharaj Hospital, Nakhon Ratsima province, Thailand. Inclusion criteria for the severe influenza group were ≥ 18 years old, both genders, infected with pdmH1N1 2009 influenza virus confirmed by real-time RT-PCR and having pneumonia or acute respiratory distress syndromes. Inclusion criteria for the mild influenza group included ≥ 18 years old, both genders and infected with pdmH1N1 2009 influenza virus confirmed by real-time RT-PCR, having no respiratory insufficiency. Exclusion criteria were < 18 years old, not documented as pdmH1N1 2009 influenza virus infection by real-time RT-PCR, receiving immunosuppressive drugs and refusing to sign the informed consent or cooperate with the specimen collection. The demographic data and clinical characteristics, including age, gender, underlying diseases, symptoms, days since onset, length of stay, death, were recorded. The study protocol was approved by the Ethical Committee of the Institute for the Development of Human Research Protection (IHRP), Ministry of Public Health.

Specimen collection

Throat swabs from patients with pdm H1N1 2009 influenza were collected for diagnosis pdm H1N1 2009 influenza virus infection at the day of enrollment. Blood samples were collected and sera/plasma samples were stored at -20 ºC until used.

Determination of cytokines

Plasma levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p70), IL-13, IL-17, MCP-1, MIP-1β, G-CSF, GM-CSF, IFN-γ, TNF-α were determined using the Bio-Plex Pro™ Human Cytokine 17-plex Assay (Bio-Rad) according to the manufacturer's protocol. Plasma levels of sTNFRII, sFasL, sRAGE were determined by using sandwiched enzyme-linked immunosorbent assay (R&D).

Statistical analyses

Statistical analyses were performed using GraphPad Prism v. 5.0 (GraphPad Softwre, Inc., Sandiego, CA). Differences among patient groups were tested by Chi-square test and/or Mann-Whitney *U* test.

Results

The demographical and clinical characteristics of the study subjects

The demographical and clinical characteristics of the patients are shown (Table 1). Forty two patients with pdm H1N1 2009 influenza virus infection, who developed mild disease (n=21) or severe disease (n=21), were enrolled in this study. The median ages were 33 (range 20 to 85 years) for the mild disease group and 58 (range 16 to 77 years) for the severe disease group. The most common underlying diseases and significantly increased in the severe disease group were type 2 diabetes(33.33%) and chronic obstructive pulmonary disease (COPD) (23.81%). The most common manifestations in both groups were cough (95.24%), fever (57.14%), headache (57.14%), myalgia (54.76%), and sore throat (50%). The symptoms that were significantly present in the severe group were dyspnea (66.67%). The most common complications seen in the severe influenza group included acute respiratory failure (76.19%), pneumonia (71.43%), acute respiratory distress syndrome (52.38%), acute renal failure (28.57%) and death (33.33%). In the severe group, the median of duration of symptom onset and hospital admission were 6 (ranged 2 to 37 days) and the median of length of hospital stay of the severe influenza group were 10.5 days (ranged 4 to 106 days). In the severe group, mortality of 33.3% (7 deaths) was observed.

Table 1 Demographic and clinical characteristics of patients

Characteristics	All patients with pdm H1N1 2009 influenza infection (n=42)	Patients with pdm H1N1 2009 influenza infection		*p*-value
		Mild Influenza (n=21)	Severe influenza (n=21)	
Age (years)	40(16 - 85)	33 (20 - 85)	58 (16 - 77)	0.007
Gender ratio (M/F)	22/20	8/13	14/7	0.0638
Underlying diseases				
COPD	5/42	0/21	5/21	0.0172
Type 2 Diabetes	8/42	1/21	7/21	0.0184
Hypertension	9/42	2/21	7/21	0.0601
Chronic lung disease	2/42	0/21	2/21	0.1473
Presenting symptoms				
Fever > 38°C	24/42	10/21	16/21	0.0566
Rhinorrhea	22/42	17/21	5/21	0.0002
Sore throat	21/42	17/21	4/21	< 0.0001
Cough	40/42	21/21	19/21	0.1473
Dyspnea	17/42	3/21	14/21	0.0005
Myalgia	23/42	19/21	4/21	< 0.0001
Headache	24/42	19/21	5/21	< 0.0001

Statistical significance was determined by Chi-square test ($p<0.05$)

Table 1 Demographic and clinical characteristics of patients. (continued)

Characteristics	All patients with pdm H1N1 2009 influenza infection (n=42)	Patients with pdm H1N1 2009 influenza infection		*p*-value
		Mild Influenza (n=21)	Severe influenza (n=21)	
Complications				
Bronchitis	3/42	3/21	0/21	0.0794
Acute respiratory failure	15/42	0/21	16/21	< 0.0001
Pneumonia	17/42	2/21	15/21	< 0.0001
ARDS	11/42	0/21	11/21	0.0001
Acute renal failure	6/42	0/21	6/21	0.0082
Days since onset (day); median (range)	2 (2-38)	4 (0-5)	6 (2-37)	0.0016
Length of stay (day); median (range)	7 (0-106)	0 (0-6)	10.5 (4-106)	< 0.0001

Statistical significance was determined by Chi-square test (p<0.05)

Plasma cytokines/chemokines and inflammatory mediators in patients with pdm H1N1 2009 influenza virus infection

In order to determine the plasma cytokines/ chemokines and inflammatory mediators in severe and mild patients with pdm H1N1 2009 influenza virus infection, plasma levels of sTNFRII in patients with severe pandemic H1N1 2009 influenza (median (IQR) (pg/mL); 7,525 (6,470-9,630); n=11) were significantly higher than those with mild disease (median (IQR) (pg/mL); 3,692 (2,655-4,128); n=21) (p=0.0049) (Table 2, Figure 1). While, plasma levels of sFasL in patients with mild pdmH1N1 2009 influenza (median (IQR) (pg/mL); 39(10-78); n=21) were significantly higher than those with severe disease (median (IQR) (pg/mL); 10(10-25); n=11) (p=0.0035) (Table 2, Figure 1). However, levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p70), IL-13, IL-17, G-CSF, GM-CSF, IFN-γ, MCP-1, MIP-1β, TNF-α and sRAGE did not show any significant difference among these patients.

Table 2. Plasma levels of IFN-γ, TNF-α, IL-17, IL-6, sRAGE, sFasL and sTNFRII at the day of enrollment of patients with pandemic H1N1 2009 influenza

Cytokine	Patients with pandemic 2009 H1N1 influenza		*p*-value
	Mild disease	Severe disease	
IFN-γ			
Median (pg/mL)	369.5	350	
(IQR)	(175.1 - 561.4)	(232 - 507.7)	0.8785
Sample size	21	7	

Statistical significance was determined by The Mann-Whitney U test (p<0.05). IQR, interquartile range

Table 2. Plasma levels of IFN-γ, TNF-α, IL-17, IL-6, sRAGE, sFasL and sTNFRII at the day of enrollment of patients with pandemic H1N1 2009 influenza. (continued)

Cytokine	Patients with pandemic 2009 H1N1 influenza		*p*-value
	Mild disease	**Severe disease**	
TNF-α			
Median (pg/mL)	79.09	45.99	
(IQR)	(33.43 - 128.3)	(30.51 - 67.97)	0.3359
Sample size	21	6	
IL-17			
Median (pg/mL)	23.72	21.19	
(IQR)	(9.69-50.54)	(7.18-26.37)	0.4825
Sample size	20	6	
IL-6			
Median (pg/mL)	14.93	33.13	
(IQR)	(7.11-33.49)	(9.24-14.3)	0.1392
Sample size	21	11	
sRAGE			
Median (pg/mL)	922	1,436	
(IQR)	(614.3-1406)	(447-9,519)	0.4633
Sample size	21	11	
sFasL			
Median (pg/mL)	**39**	10	
(IQR)	**(10-17)**	(10-25)	**0.0035**
Sample size	**21**	11	
sTNFRII			
Median (pg/mL)	3,692	**7,525**	
(IQR)	(2,655-4,128)	**(6,470-9,630)**	**0.0049**
Sample size	21	**11**	

Statistical significance was determined by The Mann-Whitney U test ($p<0.05$). IQR, interquartile range

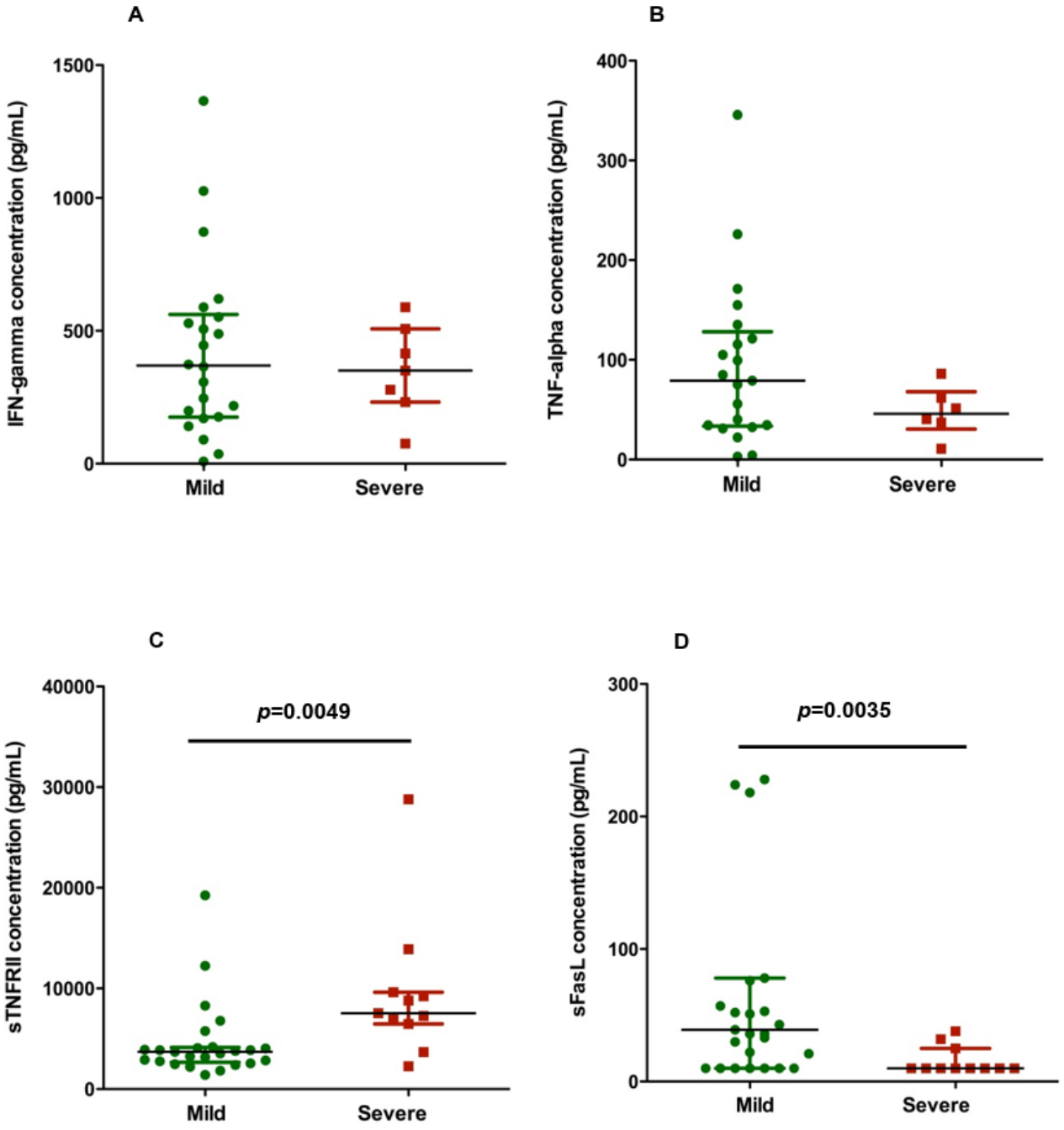

Figure 1 *Plasma levels of IFN-γ, TNF-α, sTNFRII and sFasL at the day of enrollment in patients with mild and severe pandemic H1N1 2009 influenza. The Mann-Whitney U test was used to compare the levels: (A) IFN-γ, (B) TNF-α, (C) sTNFRII, (D) sFasL. Data presented as median, interquartile range (IQR). Mild, Mild pandemic H1N1 2009 influenza; Severe, Severe pandemic H1N1 2009 influenza.*

Discussion

In this study, we presented the cytokine, chemokine and plasma sTNFRII and sFasL responses of patients with pdmH1N1 2009 virus infection. The result demonstrated that significantly higher plasma levels of sTNFRII were found in patients with severe pdmH1N1 2009 influenza compared to those in patients with mild disease. The finding was consistent with the previous study showing that signaling via TNF receptor enhanced influenza disease severity[23] and human studies in HIV,[38] adenovirus,[39] RSV,39 coronavirus[40] and dengue[41] infections that demonstrated elevated serum levels of sTNFRII were associated with severe clinical outcomes, supporting the important role of receptors and ligands of the TNF family in determining influenza disease severity. In addition, in this study, significantly decreased in plasma levels of sFasL were observed in patients with severe disease compared to those of mild disease. Comparable to previous in *vitro* study in the influenza-infected HeLa cells[31] and influenza-infected human mononuclear cells[42] that upregulation of Fas (CD95), FasL (CD95L) and apoptosis were observed during influenza infection. The mechanism underlying the contribution of elevated plasma sFasL in mild influenza clinical outcomes could be possibly involved with manipulation of Fas-mediated apoptosis signaling by high amount of plasma sFasL antagonizing the effect of membrane-bound Fas during acute influenza infection[43-44] and then protect the apoptosis. Moreover, in this study, there were high percentages of patients aged ≥50 years in the severe group. However, stratified analysis according to age group could not be performed due to the small sample size of patients aged ≥50 years. It is likely that elderly patients with severe influenza may have an increase in apoptosis and impair immune responses to influenza virus infection, leading to disease severity.[45]

Soluble RAGE, a decoy receptor for several ligands including advanced glycation end products (AGEs), toxic AGEs (TAGE), High-mobility group box-1 (HMGB1), damage-associated molecular patterns (DAMPs), has been reported as a severity predictor of community acquired pneumonia46. In this study, there was no significant difference of sRAGE in patients with severe influenza compared to those of mild influenza.

Hypercytokinemia has been reported in immunphathogenesis of severe influenza. Previous studies reported an elevated T-helper 17 (IL-8, IL-9, IL-17, IL-6) and T-helper 1 (TNF-α, IL-15, IL-12p70)[15] and immunosuppressory cytokines (IL-10 and IL-1ra)[47] in adults with severe pdmH1N1 2009 influenza. Anotherr group in Taiwan demonstrated increased plasma levels of IL-1β, IL-6, IL-12, and IFN-γ in pediatric patients with influenza A H1N1 virus infection with pulmonary complications.[19] In this study, no significant differences of plasma levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p70), IL-13, IL-17, G-CSF, GM-CSF, IFN-γ, TNF-α were observed between the mild and severe disease groups. The results were inconsistent with the previous studies in younger adults from Spain[13] and in children from China[14,16], Taiwan[19], Japan[48] that demonstrated Th1 and Th17 hypercytokinemia in patients with severe influenza. A possible underlying cause of this controversy may be related to the age-related decrease in immune response following influenza infection.[45] Other possibilities may be due to the transient, temporal and spatial nature of cytokine production and the short plasma half-life of the cytokine/chemokine. In addition, the difference of study design and study subjects, mixed phenotypes of severe influenza, patients with variable symptom onset, the small number of patients with severe disease, the course of disease at the sample collection and the pharmaceutical/ non-pharmaceutical interventions may be the limitation and confounding factors in this study. Further investigation in larger and well-defined cohort is recommended to explore the role of cytokine/chemokine, inflammatory mediators in influenza patients with severe respiratory complications.

Conclusion

The present results revealed that increased plasma levels of sTNFRII and decreased sFasL in patients with severe 2009 pandemic influenza might determine dysregulation of immune responses, such as an excessive activation of the role receptors and ligands of the TNF family, modulation of Fas/FasL, and could predict the clinical outcomes and disease severity. Further studies in cellular, molecular and genetic basis

underlying the dysregulation of cytokine pathway and related processes during influenza infection may provide insight into the development of novel therapeutics strategies against severe influenza.

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