Eur. Cytokine Netw. Vol. 30, n° 1, *March 2019* 34-38

RESEARCH ARTICLE

Elevated cytokine levels associated with acute kidney injury due to wasp sting

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Accepted for publication March 04, 2019

To cite this article: Li F, Liu L, Guo X, Luo Z, Zhang Y, Lu F, Wang G, Chen T, Chen D. Elevated cytokine levels associated with acute kidney injury due to wasp sting. *Eur. Cytokine Netw.* 2019; 30(1): 34-38. doi: 10.1684/ecn.2019.0425

ABSTRACT. *Objective*: **This study mainly to explore the change of serum cytokines in wasp sting patients and the potential correlation between cytokines and acute kidney injury (AKI) due to wasp stings.** *Methods*: **The levels of IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ in 33 wasp sting and 24 healthy people were measured by flow cytometry, the level of IL-17 was detected by enzyme-linked immunosorbent assay and the laboratory examination including inflammatory indicators, muscle enzyme markers, and renal function were detected by automatic biochemical analyzer, blood analyzer, and urine analyzer. The wasp sting patients were divided into AKI ($n = 10$) and non-AKI groups ($n = 23$). The correlation between the levels of serum cytokines and laboratory examination results was analyzed.** *Results*: **The levels of IL-2, IL-6, IL-10, IFN-γ, and IL-17 were statistically increased in wasp sting patients compared with the controls ($P < 0.05$). IL-6, IL-10, and IL-17 levels were markedly increased in the AKI group compared with the non-AKI group ($P < 0.05$). Moreover, compared with non-AKI group, inflammatory markers and muscle enzyme markers were more abnormal in the AKI group. The positive rate of urinary occult blood in the AKI group was higher than that in the non-AKI group. The levels of IL-2, IL-4, IL-6, IFN-γ, and IL-17 correlated positively with white blood cell counts. The levels of IL-2, IL-4, IL-10, IFN-γ, and IL-17 correlated positively with the levels of serum creatinine. The levels of IL-2, IL-4, IL-10, IL-10, and IFN-γ correlated positively with the levels of C-reactive protein. The levels of IL-10, and IFN-γ correlated positively with urinary occult blood.** ***Conclusion:*** **Elevated levels of cytokines in wasp sting patients might be involved in the development and progression of acute kidney injury.**

Key words: acute kidney injury, wasp stings, cytokines

INTRODUCTION

Insect stings and bites are important global public health problems, and wasp sting is not uncommon worldwide [1]. A lot of wasp sting events occurred in China every year, especially in hilly areas. There are about 200 kinds of wasps in China. Unlike bees, the wasp can inflict multiple stings because the stinger has no barbs, which does not get detached when stinging. The clinical manifestations of patients with wasp stings are varied. Local reactions including pain or swelling and mild systemic reactions resulted from single or few wasp stings mostly. It is important to note that multiple wasp stings can lead to severe systemic manifestations or organ damage, such as shock, acute kidney injury (AKI), acute liver failure, rhabdomyolysis, and even multiple organ failure, which may eventually lead to death [2]. Previous studies have shown that the mortality rate of multiple wasp sting is approximately 15-26%. AKI caused by wasp sting is common in clinic and the incidence is about 21%. In wasp sting patients, AKI is usually caused by intravascular hemolysis, rhabdomyolysis, shock, and the direct toxic effects [3]. Recently, studies have demonstrated that immune inflammation is closely related to the occurrence and development of AKI [4]. During AKI, damaged renal tubular epithelium releases pro-inflammatory factors, natural immune effector cells are rapidly recruited to the damaged site to phagocytize and remove antigens. At the same time, inflammatory effector cells are further activated and a large number of inflammatory mediators are released, which augment the inflammation cascade reaction and aggravate the injury of kidney [5]. The alteration of cytokines levels, and whether cytokines involve in the occurrence and development of AKI in patients with wasp stings have not been reported. Our study mainly explored the changes of serum cytokines and the correlation between cytokines and AKI to infer the potential mechanism in patients with wasp stings.

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doi: 10.1684/ecn.2019.0425

MATERIALS AND METHODS

Patients

From September 2016 to September 2017, 33 wasp sting patients were selected, including 13 males and 20 females, aged from 20 to 85 years, with an average age of 55.91 ± 15.10 years. Inclusion criteria: a clear history of wasp stings, admission within 6 hours after wasp sting, complete hospitalization records. Exclusive criteria: discharged during treatment; incomplete case data; patients with a history of chronic kidney disease. Twenty-four healthy people who had physical examination in our hospital in the same period were selected as the control group, including 13 males and 11 females, aged from 20 to 65, with an average age of 46.33 ± 15.88 years. AKI was defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) criteria [6]. Increase in SCr by ≥0.3 mg/dL (≥26.5 μmol/L) within 48 hours; or increase in SCr to ≥1.5 times baseline, which is known or presumed to have occurred within the prior seven days; or urine volume <0.5 mL/kg par hour for six hours. The diagnosis of wasp stings was based on clinical history and findings on physical examinations. The wasp sting patients were classified into two groups: AKI group (patients with AKI), non-AKI group (patients without AKI). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee of the People's Hospital of Jianyang (IRB approval number is 2017 [125]) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Methods

Serum cytokine measurement

Blood samples were collected in a sterile manner, 10 mL venous blood was collected, and the sample was sent to the laboratory in an iced container. The supernatant of blood was collected after centrifugation (3,000 rpm × 5 minutes) and placed in -70 °C refrigerator for analyses later. The serum cytokines include IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γwere detected by flow cytometry (Beckman FC 500), a commercial Human Th1/Th2 Cytokine Kit (Becton Dickinson, USA) was used to measure the cytokines mentioned above using the bead array technology as described by the manufacturer. The concentrations of serum IL-17 were measured by ELISA using the Human IL-17 ELISA kits (Elabscience, China), according to the manufacturer's instructions. The detection limits of the IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, and IL-17 are 2.5 pg/mL, 2.5 pg/mL, 2.5 pg/mL, 2.5 pg/mL, 2.5 pg/mL, 2.5 pg/mL, 0.8 pg/mL, respectively.

Laboratory examination

Blood biochemical results were detected by the SIEMENS ADVAI 2400 automatic biochemical analyzer. The complete blood count was analyzed by the Sysmex XS-1000i blood analyzer. Urine samples were analyzed by the Sysmex UF-1000i automatic urine sediment analyzer. All of them were operated according to the manufacturer's instructions.

Statistical analysis

All data were analyzed by SPSS 21.0 software (SPSS Inc., Chicago, IL). The quantitative data of normal distribution were expressed by mean ± standard deviation, and the inter-group comparison was performed by the independent sample t test. The quantified data of non-normal distribution are expressed by four quantiles, and the inter-group comparison was performed by the rank sum test. The qualitative data was expressed by case number (rate), and the inter-group comparison was performed by the χ^2 test. The ranking data were analyzed by the Mann-Whitney test. The correlation analysis was performed by the Spearman test. *P*-values < 0.05 were considered to represent statistically significant differences.

RESULTS

Serum cytokine levels in wasp sting patients and controls

Table 1 showed the cytokine levels of wasp sting patients and controls. The levels of IL-2, IL-6, IL-10, IFN-γ, and IL-17 were statistically increased in wasp sting patients compared with the controls ($P < 0.05$).

Serum cytokine levels in AKI and non-AKI groups

The wasp sting patients were divided into AKI group ($n = 10$) and non-AKI group ($n = 23$). IL-6, IL-10, and IL-17 levels were markedly increased in AKI group compared with non-AKI group ($P < 0.05$), while there were no statistically significant differences in IL-2, IL-4, TNF-α, and IFN-γ levels between AKI group and non-AKI group. The results were shown in *table 2*.

Correlation of laboratory test results and cytokines

The laboratory test results of 33 wasp sting patients were shown in *table 3*. Compared with non-AKI group, inflammatory markers and muscle enzyme markers were more abnormal in AKI group. The positive rate of urinary occult blood in AKI group was

Table 1
The levels of serum cytokines between wasp sting patients and controls.

	Patients ($n = 33$)	Controls ($n = 24$)
IL-2 (pg/mL)	6.96 ± 3.70*	4.65 ± 1.44
IL-4 (pg/mL)	10.20 ± 6.60	7.54 ± 2.62
IL-6 (pg/mL)	46.77 ± 46.11*	23.66 ± 32.30
IL-10 (pg/mL)	65.36 ± 52.07*	8.77 ± 5.30
TNF-α (pg/mL)	8.95 ± 4.68	9.51 ± 3.67
IFN-γ (pg/mL)	24.92 ± 13.61*	16.92 ± 6.10
IL-17 (pg/mL)	20.13 ± 10.59*	9.10 ± 3.07

* Compared with controls, $P < 0.05$.

Table 2
The levels of serum cytokines between AKI and non-AKI groups.

	AKI group (n = 10)	Non-AKI group (n = 23)
IL-2 (pg/mL)	7.59 ± 3.84	6.48 ± 3.61
IL-4 (pg/mL)	10.93 ± 6.42	9.37 ± 6.27
IL-6 (pg/mL)	77.88 ± 60.89*	33.75 ± 31.35
IL-10 (pg/mL)	98.82 ± 69.13*	49.35 ± 35.81
TNF-α (pg/mL)	9.80 ± 6.54	8.65 ± 3.93
IFN-γ (pg/mL)	29.38 ± 19.46	23.31 ± 10.88
IL-17 (pg/mL)	28.36 ± 14.41*	17.17 ± 7.10

* Compared with non-AKI group, $P < 0.05$.

higher than that in non-AKI group as shown in *table 4*. The correlation between serum cytokines and WBC, C-reactive protein (CRP), serum creatinine (SCr), urinary occult blood was shown in *table 5*. The IL-2, IL-4, IL-6, IFN-γ, and IL-17 were positively correlated with WBC, and the same trend as the IL-2, IL-4, IL-10, IFN-γ, and IL-17 correlated with SCr. While the IL-2, IL-4, IL-10, TNF-α, and IFN-γassociated positively with CRP, and the IL-10 and IFN-γshowed the same co-relationship with urinary occult blood.

DISCUSSION

The components of wasp venoms are complex, which contain phospholipase A2, peptides such as melittin, histamine, mast cell degranulating peptide, and others include kinins, apamine [7]. Therefore, the toxins with different biological effects cause a variety of clinical features. Local reactions after wasp sting include pain and swelling, severe systemic complications include AKI, rhabdomyolysis, hemolysis, coagulopathy, liver dysfunction, and even MODS [2].

The increase of WBC and neutrophil in peripheral blood in early stage of disease suggested that immune inflammation plays an important role in wasp sting. Hyaluronidase is the second common allergen in wasp venom, and hydrolyzed hyaluronic acid fragments have strong inflammatory and angiogenic effects [8]. Mast cell degranulation peptide at low concentrations can mediate mast cell degranulation and release large amounts of histamine and cause inflammation [9]. The previous study observed that the levels of some cytokines increased in wasp sting patients; furthermore, cytokine levels were associated with the severity of the disease [10]. Our study demonstrated that the levels of IL-2, IL-6, IL-10, IFN-γ, and IL-17 in wasp sting patients significantly elevated compared with the controls. IL-2 is mainly secreted by T cells, which can stimulate the activation of T cells, promote the proliferation of B cells and NK cells, and induce T cells to secrete IFN-gamma, TNF, CSF, and other cytokines. IL-6 can promote the expression of IL-2r and enhance the mitogenic effect of IL-1 and TNF on TH cells. Toll-like receptors defined by interleukin-6 regulate the production of IL-6 by B cells through innate activation. The combination of IL-6 and IL-1b provides a necessary signal for the production of IL-10 secretory regulatory B cells. IL-10 is an immunoregulatory factor, which can inhibit the secretion of IL-1, IL-6, IL-12, and TNF in dendritic cells and macrophages, and the secretion of IFN-γ and IL-2 in Th1 cells. In addition, it also has an immunostimulatory effect of promoting the proliferation and differentiation of T cells and the release of various cytokines [11]. IFN-γ up-regulates the secretion of IL-2, IL-12, and TNF-α, and inhibits the secretion of IL-10, IL-4, and IL-5 by inhibiting the proliferation of CD4 + Th2 cells. IL-17, as a pro-inflammation cytokine, is secreted by Th17 cells, which is positively regulated by IL-6, TGF-β, while negatively by IFN-γ and IL-4 [12]. Wasp venom promotes mast cell degranulation and leukocyte chemotaxis, activates inflammatory cells, and leads to uncontrolled release of inflammatory mediators [13]. At the same time, endothelial cells and neutrophils activate complement and kinin systems mediated by adhesion molecules, leading to cascade systemic inflammatory response syndrome (SIRS). Our study suggested that IL-2, IL-6, IL-10, IFN-gamma, and IL-17 might be involved in the pathogenesis and progression in wasp sting patients by regulating and activating cytokines.

AKI is closely related to inflammatory immune response. Innate immunity and acquired immunity

Table 3
Laboratory test results of AKI group and non-AKI group.

	AKI group (n = 10)	Non-AKI group (n = 23)	P-value
WBC ($\times 10^9$/L)	18.57 ± 4.05	14.97 ± 5.60	0.047
CRP (mg/L)	11.35(4.15,42.48)	0.67 (0.50,4.25)	<0.01
Blood urea nitrogen (mmol/L)	16.72 ± 12.45	6.71 ± 2.12	<0.01
Serum creatinine (mmol/L)	223.10 ± 186.17	66.88 ± 17.14	<0.01
Creatine Kinase MB (ug/L)	81.95 (23.75, 357.30)	18.00 (6.16, 32.00)	<0.01
Creatine kinase (U/L)	620.50 (225.25, 11,179.25)	277.00 (141.25, 531.50)	0.031
Serum LDH (U/L)	1,921.50 (405.00, 4,023.00)	330.00 (232.25, 539.50)	<0.01
Serum HBDH (U/L)	1,571.50 (278.00, 2,783.75)	218.50 (186.25, 405.75)	<0.01
ALT (U/L)	89.00 (437.50, 39.25)	25.50 (19.00, 57.50)	<0.01
AST (U/L)	275.00 (69.50, 1,866.75)	42.50 (28.00, 82.25)	<0.01

Table 4
The results of urinary occult blood between AKI and non-AKI groups.

Groups	n	BLD (-)	BLD (+)	BLD (2+)	BLD (3+)	Positive rate
AKI	10	1 (10%)	3 (30%)	0 (0)	6 (30%)	90.00%
Non-AKI	23	16 (69.57%)	2 (8.70%)	1 (4.35%)	4 (17.39%)	30.43%

BLD is an indicator of urinary occult blood test, it presented the levels of hemoglobin or myohemoglobin in urine. -: means negative; +: means positive. According to the instruction, the test results are classified into 4 grades, namely, 1+, 2+, 3+. The corresponding urine hemoglobin/myoglobin concentration ranges from 0 to 360 μg/L, 900 to 2,880 μg/L, 2,880 to 7,200 μg/L, and >7,200 μg/L, respectively. In this study, the data of BLD test results from the AKI group and the non-AKI group were analyzed by the Mann-Whitney test, and it showed $U = 45.50$, $P < 0.01$.

participate in the immune inflammation of AKI, both of them play positive and negative roles in the process of tissue damage and repair, and the mechanism of regulatory network is complex [4]. In animal experiments, controlling the intensity of inflammation can significantly reduce the degree of renal injury, such as the degree of renal tubular necrosis and the level of serum creatinine [14]. The mechanism of AKI caused by wasp sting is not fully understood. It is believed that intravascular hemolysis, rhabdomyolysis, hypotension, and direct toxicity of the venom components to the renal tubules play an important role in this aspect. However, there are no literatures on the role of immune inflammation in AKI induced by wasp sting. Our study showed that serum levels of IL-6, IL-10, and IL-17 in AKI group were significantly higher compared with non-AKI group, suggesting that these cytokines may be involved in the process of AKI caused by wasp sting. IL-6, as a proinflammatory cytokine, can induce a large number of pro-inflammatory effects leading to tissue damage and mediate inflammation progression in ischemic AKI [15]. IL-17 as an important mediator in the progression of many inflammatory disease, has a proinflammatory effect and plays a role in the activation and maintenance of immune response [16]. In ischemia-reperfusion injury, a large number of IL-17 were released by neutrophils, which could mediate renal inflammation through inducing the release of pro-inflammatory cytokines and chemokines [17]. In our study, IL-10 level in wasp sting patients was markedly elevated compared with the controls. Moreover, the IL-10 level in the AKI group exhibited more increase than that in the non-AKI group. IL-10 is recognized to maintain the balance of cytokines, and has a major inhibitory effect on cytokine synthesis. On the other hand, IL-10 promotes the production of soluble TNF receptor (sTNFR) by monocytes, which can antagonize the tissues damage induced by IL-1 and TNF-α. In our study, the TNF-α levels showed no significant difference between AKI group, non-AKI group, and the controls. We considered that the outcome may be caused by the regulation of IL-10. In an acute ischemia-reperfusion renal injury mice model research, Tregs modulate kidney injury through IL-10-mediated suppression of the innate immune system [18]. Histamine and PLA2 of wasp venom can promote the production of IL-10 by Th2 cells. Our study suggested that IL-10 might decrease the secreting of TNF-α and play a protective role in AKI induced by wasp sting. This conjecture needs further experimental verification. In addition, following the reviewer's suggestion we reviewed the patients' clinical data in the hospital and found that a few wasp sting patients had been performed ESR test, the results in the ESR showed that the difference between AKI and non-AKI groups was obviously significant (AKI group ($n = 4$) *versus* non-AKI group ($n = 8$), 41.25 ± 4.50 mm/h *versus* 29.50 ± 6.52 mm/h, $P < 0.01$, $t = -3.646$). Nevertheless, we do not think it is enough to put the data in the result because of the small sample size. Biochemical characteristics in wasp sting patients were more serious than control, especially in AKI patients. In clinical, dark red urine is usually observed in wasp sting patients before AKI occurs. The positive rate and severity of urinary occult blood in AKI patients was significantly higher than that in non-AKI group. Hemolysis and rhabdomyolysis are generally demonstrated by elevated serum levels of CK and LDH. Many literatures report the increase of CK and LDH in AKI patients induced by wasp sting. It suggests that hemolysis and rhabdomyolysis may be one of the mechanisms of AKI after wasp sting [19, 20]. Similar results had been observed in our study. WBC and CRP, in clinical practice, are wildly used as markers to reflect degree of inflammation. Our present study showed that WBC and CRP increased in wasp sting patients, and also were positively correlated with some

Table 5
Correlation analysis between serum cytokines and laboratory results.

	WBC		SCr		CRP		Urinary occult blood	
	R-value	*P*-value	*R*-value	*P*-value	*R*-value	*P*-value	*R*-value	*P*-value
IL-2	0.431	0.011	0.437	0.010	0.344	0.046	0.259	0.139
IL-4	0.398	0.020	0.400	0.019	0.434	0.010	0.294	0.091
IL-6	0.428	0.013	0.293	0.098	0.153	0.397	0.277	0.118
IL-10	0.267	0.127	0.373	0.030	0.354	0.040	0.436	0.010
TNF-α	0.318	0.067	0.258	0.141	0.510	0.002	0.308	0.076
IFN-γ	0.347	0.044	0.378	0.028	0.608	<0.001	0.404	0.018
IL-17	0.376	0.029	0.492	0.003	0.278	0.111	0.233	0.185

cytokines, suggesting that cytokine levels were correlated with the degree of inflammation. Furthermore, the WBC and CRP in AKI group obviously increased than that in non-AKI group, which demonstrated inflammation reaction may participate in the progression of AKI induced by wasp sting.

Serum creatinine is widely used in the assessment of renal function. The IL-2, IL-4, IL-10, IFN-γ, and IL-17 were positively correlated with serum creatinine in our study; it indicated that the cytokines mentioned above might be involved in the AKI by wasp sting. Besides, IL-4, IL-10, IFN-γ were positively correlated with urinary occult blood, we considered that these cytokines might participate in the progression of AKI through hemolysis or rhabdomyolysis.

Taking together, the present study suggests that serum cytokine levels are elevated in AKI induced by wasp sting, which might thus be involved in the development and progression of this disorder. Nevertheless, understanding of the mechanisms require animal experiments and clinical studies with a larger sample size.

Disclosure. Acknowledgments: This study was supported by the grants from the Scientific Research Project of Sichuan provincial Health and Family Planning Commission (No. 17 PJ065 and No. 18PJ370).

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