

Original Article

Detection of streptococcus pyogenes antibodies in acute idiopathic urticaria

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Abstract: It is usually difficult to figure out the exact cause for acute urticaria, so many acute urticaria attacks are thought to be idiopathic. We hypothesized that asymptomatic infection of streptococcus pyogenes, which is one of the most common pathogens in human, might be a primary cause of acute idiopathic urticaria. 72 patients at the first attack of acute idiopathic urticaria (AU), 31 patients who met the first recurrence of chronic idiopathic urticaria (RCU) within three months, and 65 healthy controls were included. Complete blood counts, serum levels of C-reactive protein (CRP) and total IgE were tested in these patients. Serum titers of human group A streptococcus protein (ASP) antibody and human anti-streptolysin O (ASO) antibody (IgM) of all the participants were measured by ELISA. High percentages of AU patients with elevated leukocyte (69.4%) and neutrophil (77.8%) counts were observed. ASP and ASO antibodies were positive in 73.6% and 68.1% AU patients respectively, and serum levels of the two were both significantly higher in AU patients (compared to health controls). Furthermore, elevated leukocyte or/and neutrophil counts were accompanied with positive antibody levels of ASP or/and ASO in AU patients. Although serum levels of ASP and ASO antibodies were also significantly higher in RCU patients compared to health controls, percentages of positive ASP and ASO antibodies in RCU patients were only 35.5% and 32.3% respectively. These results proved that acute urticaria attack accompanied with highly expressed antibodies (ASP and ASO) of streptococcus pyogenes in adult patients. As asymptomatic streptococcus infection accounted for a major cause of acute idiopathic urticaria, investigation of tonsillitis is essential at the early stage of acute urticaria.

Keywords: Acute urticaria, infection, streptococcus pyogenes, ASP, ASO

Introduction

Acute urticaria was caused by allergic reactions to foods, drugs, chemicals, physical stimuli and infections [1, 2]. It is usually difficult to find the incentives of acute urticaria, and 30% to 50% of its attacks were still thought to be idiopathic [1]. It is proved that respiratory tract and other infections (including virus and bacterial infections) are the most common triggers for acute or chronic urticaria in children [1-9]. Little research refers to infection has been done in adult AU patients. As we noticed peripheral leukocytes and neutrophils increased in most adult patients with acute idiopathic urticaria, we supposed an occult or asymptomatic infection leads to urticaria attack in those patients. We hypothesized streptococcus pyogenes, one of the most common pathogens in

human, might be the source of infection in AU patients and tested it in this study.

Materials and methods

Subjects

The Ethics Committee of Hubei provincial hospital of TCM approved this prospective study, and informed consent was obtained from all the participants. The study was carried out from July 2014 to November 2016 on adult subjects at dermatology department in our hospital. 72 patients at the first attack of acute idiopathic urticaria (AU), 31 patients who met the first recurrence of chronic idiopathic urticaria (RCU) within three months, and 65 normal controls were recruited in this study. All the patients also fit the demands as follow: at the first week of

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Table 1. Demographic and clinical characteristics of the study subjects

Characteristics	Healthy controls (n=65)	AU patients (n=72)	RCU patients (n=31)
Age, median (range), years	27.5 (18, 68)	26 (18, 76)	37 (19, 78)
Gender (female/male)	38/27	46/26	16/15
Disease duration, median (range), days	-	3 (0.5, 7)	4 (1, 7)

Table 2. The number and percentage of patients with abnormal results

		AU patients N (%)		RCU patients N (%)	
		Increased	Decreasing	Increased	Decreasing
Complete blood count	Leukocyte	50 (69.4)	0 (0)	15 (48.4)	0 (0)
	Neutrophil	56 (77.8)	0 (0)	21 (67.8)	0 (0)
	Lymphocyte	4 (5.6)	20 (27.8)	0 (0)	8 (25.8)
	Lymphomonocyte	18 (25.0)	5 (6.9)	4 (12.9)	0 (0)
	Eosinophils	0 (0)	49 (68.1)	0 (0)	22 (71.0)
	basophilic leukocyte	0 (0)	0 (0)	0 (0)	0 (0)
	Erythrocyte	7 (9.7)	4 (5.6)	3 (9.7)	3 (9.7)
	Hemoglobin	6 (8.3)	5 (6.9)	4 (12.9)	1 (3.2)
	Platelet	1 (1.4)	1 (1.4)	0 (0)	1 (3.2)
CRP		34 (47.2)	0 (0)	5 (16.1)	0 (0)
IgE		35 (48.6)	0 (0)	9 (29.0)	0 (0)
ASP		53 (73.6)	0 (0)	11 (35.5)	0 (0)
ASO		49 (68.1)	0 (0)	10 (32.3)	0 (0)

the attack; uncertain causes; no systemic glucocorticoids or antibiotics were once taken. Allergic shock was excluded. Diagnostic criteria of acute and chronic urticaria were according to 2013 revision and update of the EAACI/GA2 LEN/EDF/WAO guideline for the definition, classification, diagnosis, and management of urticaria [10]. Demographic and clinical characteristics of all the participants were recorded, and whole blood were collected from each one. Whole blood samples of all the patients were sent for laboratory tests instantly which include complete blood counts, serum levels of CRP and total IgE. 3-4 ml blood samples of all the participants were used to separate serum, and all the serum samples was stored at -70°C for analysis by ELISA.

Methodology

Serum titers of human group A streptococcus protein(ASP) antibody and human anti-streptolysin O (ASO) antibody (IgM) were measured using ELISA kits (Uscn Life Science Inc, Wuhan, China). All the procedures were taken according to the manufacturers' protocols. 100 µl of working standards or samples were added to

per well of the provided plates coated with purified antibody, then the plates were incubated for 2 hours at 37°C. Removed the liquid of each well, then added 100 µl of Biotin-antibody (1×) to each well. Incubated the plates at 37°C for 1 hour. Removed the liquid, and washed the wells with wash buffer for three times. Added 100 µl of HRP-avidin (1×) to each well, then incubated the plates for 1 hour at 37°C. Wash the plates for five times. Added 90 µl of TMB substrate to each well, and incubated the plates in the dark for 30 minutes at 37°C. Added 50 µl of stop solution to each well to stop the reaction. The absorbance at 450 nm of each well was read using a microplate reader. After subtracting for background optical density (OD), the OD value of 0.3 and 0.4 at 450 nm was taken as the threshold for a positive antibody level of ASP and ASO respectively.

Statistical analysis

Data were shown as percentage or median (range). Differences among groups were determined using the non-parametric Mann-Whitney U test or Kruskal-Wallis H test. $P < 0.05$ was considered to indicate a statistically significant dif-

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Table 3. Comparison of blood tests between AU patients and RCU patients

	Normal range	AU patients	RCU patients	P
Leukocyte (*10 ⁹ /L)	3.5-9.5	12.70 (5.41, 26.77)	9.85 (4.71, 18.42)	0.006
Neutrophil (*10 ⁹ /L)	1.8-6.3	9.03 (2.06, 24.8)	7.61 (2.30, 16.43)	0.021
CRP (mg/L)	0-10	9.5 (0.2, 101.3)	7.7 (0.1, 39.7)	0.024
IgE (IU/ml)	0-87	84.3 (2.4, 2000.0)	118.0 (15.4, 759.0)	0.488
ASP	≤0.03	0.49 (0.09, 0.98)	0.26 (0.06, 0.80)	0.002
ASO	≤0.04	0.54 (0.04, 0.94)	0.32 (0.06, 0.85)	0.001

The differences between the two groups were analyzed using the Mann-Whitney U test. $P < 0.05$ was considered to indicate a statistically significant difference.

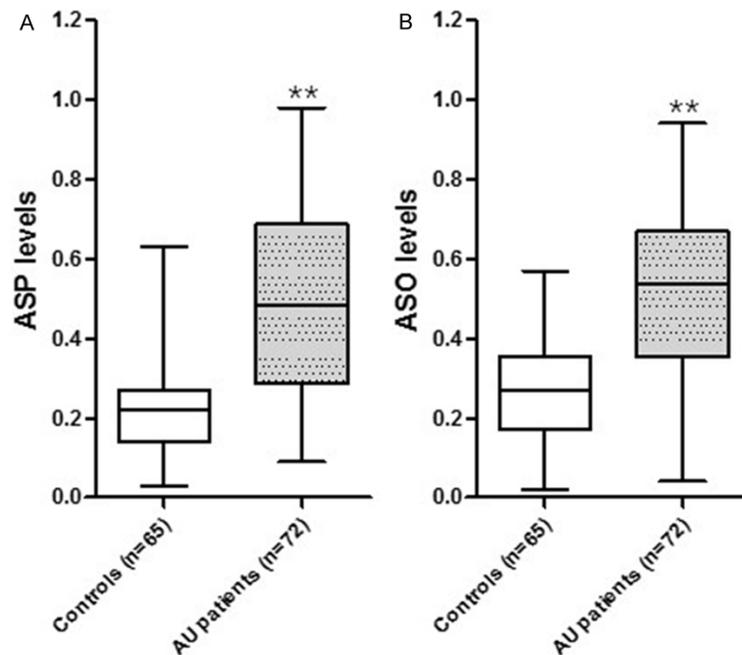


Figure 1. Serum antibody levels of ASP and ASO in AU patients and healthy controls. A. A significant difference in ASP was observed between the two groups; B. A significant difference in ASO was also found between the two groups. The differences between the two groups were analyzed using the Mann-Whitney U test. $P < 0.05$ was considered to indicate a statistically significant difference. $**P < 0.01$ (versus control).

ference. Statistical analyses were performed using SPSS 21.0.

Results

Demographic and clinical characteristics of all the participants

72 AU patients, 31 RCU patients and 65 normal controls were included in this study. **Table 1** shows the demographic and clinical characteristics of the AU patients, RCU patients and healthy controls. No significant difference in age and female-male ratio was found among

the groups. The median age of AU patients was 26 years old, and the median disease period of AU patients was 3 days.

Abnormally elevated leukocytes, neutrophils, CRP, IgE in patients

Abnormalities in blood cell counts and serum levels of CRP and IgE were expressed by percentage in **Table 2**. Elevated leukocyte (69.4%) and neutrophil (77.8%) counts, increased CRP (47.2%) and IgE (48.6%) levels were all quite obvious in AU patients. Although elevated leukocytes (48.4%) and neutrophils (67.8%) were also observed in RCU patients, highly expressed CRP (16.1%), IgE (29.0%) were not remarkable in them. We screened out several items that emerged as the most obvious changes, and compare these between

the two groups (**Table 3**). Leukocyte counts, neutrophil counts, and CRP levels from AU patients were significantly higher than those from RCU patients ($P < 0.05$). Increased leukocytes, neutrophils and CRP are all direct or indirect evidence of infection and inflammatory, which indicated infection is the main predisposing factor of these AU patients.

Increased ASP and ASO antibody levels in AU patients

ASP and ASO antibodies were positive in 73.6% and 68.1% AU patients respectively (**Table 2**).

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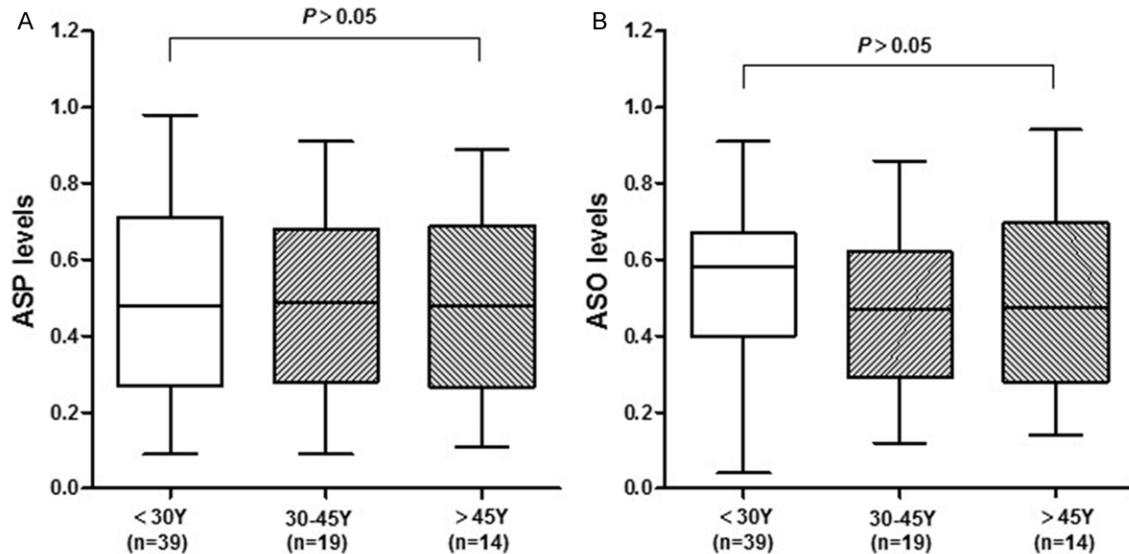


Figure 2. Serum antibody levels of ASP and ASO in AU patients at different ages. A. No significant difference in ASP was observed among groups; B. No significant difference in ASO was found among groups too. The differences among groups were analyzed using the Kruskal-Wallis H test. $P < 0.05$ was considered to indicate a statistically significant difference.

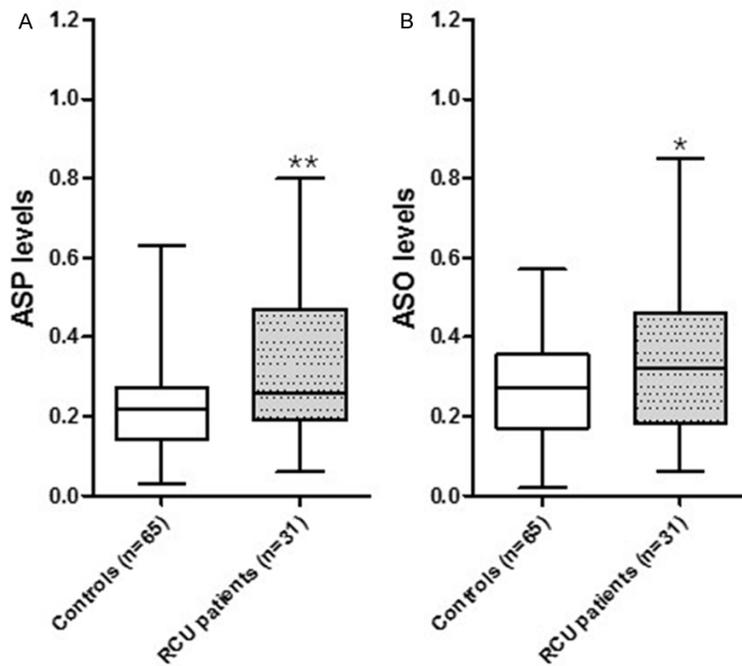


Figure 3. Serum antibody levels of ASP and ASO in RCU patients and healthy controls. A. A significant difference in ASP was observed between the two groups; B. A significant difference in ASO was also found between the two groups. The differences between the two groups were analyzed using the MannWhitney U test. $P < 0.05$ was considered to indicate a statistically significant difference. * $P < 0.05$ (versus control), ** $P < 0.01$ (versus control).

ASP and ASO antibody levels were both significantly higher in AU patients compared to nor-

mal controls and RCU patients (Table 3; Figure 1A, 1B). Further more, ASP and ASO antibody levels of AU patients from different ages were not significantly different (Figure 2A, 2B).

Percentages of positive ASP (35.5%) and ASO (32.3%) antibodies were quite low in RCU patients (Table 2). Although ASP and ASO antibody levels were both significantly higher in RCU patients compared to normal controls, they were still significantly lower than those of AU patients (Figure 3A, 3B; Table 3).

Overlap of increased blood cells and positive streptococcus antibodies in AU patients

There is more evidence that AU came with streptococcus infection. Elevated leukocyte or/and neutrophil counts accompanied with positive anti-

body levels of ASP and ASO were observed in AU patients. The percentages of AU patients

Table 4. AU patients with at least two increased blood indexes

Blood indexes	AU patients n (%)
Leukocyte+ASP	45 (62.5)
Leukocyte+ASO	44 (61.1)
Leukocyte+ASP+ASO	42 (58.3)
Neutrophil+ASP	49 (68.1)
Neutrophil+ASO	47 (65.3)
Neutrophil+ASP+ASO	43 (59.7)
Leukocyte+Neutrophil+ASP+ASO	38 (52.8)

with two or three increased indexes were all quite high (**Table 4**). And the percentage of two increased blood cells associated with two positive antibodies in AU patients was 52.8% (**Table 4**).

Discussion

Acute idiopathic urticaria is more and more common those recent years. Although it is often a self-limiting condition that usually disappears within 2 to 3 weeks, it could be quite severe and may progress to chronic situation in some patients [11]. In most cases, the cause of acute urticaria is ambiguous, and a majority of its attacks were thought to be idiopathic.

In clinical work, we confirmed peripheral leukocyte and neutrophil counts increased in most AU and RCU adult patients with no distinct symptoms of infection. There was a retrospective study of 14 outpatients encouraged streptococcal tonsillitis is the primary cause of chronic urticaria and acute recurrent urticaria [2]. We speculated bacterial infection leads to the start of AU and relapses of RCU, and streptococcal might be the source of infection. In our study, ASP and ASO antibodies were positive in most AU patients, and the two serum markers of streptococcal infection associated with increased peripheral leukocyte and neutrophil counts in those patients. Acute urticaria seems to be more common in young people, so the age group study particularly important. In this study, more than half of the AU patients are under 30 years old, but ASP and ASO antibody levels are not quite different among the three age groups. Although serum ASP and ASO antibody levels were also significantly higher in RCU patients compared to health controls, percentages of positive ASP and ASO antibodies were quite low in RCU patients.

As we proved, there may be an occult or asymptomatic infection of streptococcus at the early stage of acute idiopathic urticaria and triggers acute hypersensitivity. As asymptomatic streptococcus infection accounted for a major cause of acute idiopathic urticaria, investigation of tonsillitis is essential at the early stage of acute urticaria.

Disclosure of conflict of interest

None.

Abbreviations

AU, Acute idiopathic urticaria; RCU, recurrence of chronic idiopathic urticaria; CRP, C-reactive protein; ASP, group A streptococcus protein; ASO, anti-streptolysin O.

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