Relation between Autoimmunity Chronic Urticaria and the Levels of Plasma Prothrombin F1+2

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Abstract: A number of recent epidemiologic and experimental studies have enhanced our knowledge of the etiology and pathogenesis of chronic urticaria (CU), a disease that imposes profound impairment on patient’s quality of life. Our study was performed to explore the relation between autoimmunity (CU) and the levels of plasma prothrombin F1+2. Thirty four (CU) patients with positive autologous serum skin test (APST) their F1+2 were measured by ELISA at pre and post therapy. The levels of F1+2 in autoimmunity (CU) patients have been decreased obviously after treatment (3.34±2.87 vs 0.94±0.58, p<0.001). Before treatment, the levels of treatment group was significantly higher than that of control group (3.34±2.87 vs 0.72±0.36, p<0.001). After treatment, they have no significant difference (0.94±0.58 vs 0.72±0.36, p>0.05).

Key words: chronic urticaria, F1+2, Loratadine; dipyridamole

INTRODUCTION
Chronic urticaria (CU) is defined by the almost daily presence of urticaria for at least 6 weeks without an identifiable cause. Symptoms include short-lived wheals, itching, and erythema. CU impedes significantly a patient’s quality of life. (CU) is an extremely distressing and disabling condition for the patient. A recent study has reported that the degree of disability experienced by a (CU) patient (personal, social and occupational) is comparable with that of patients with severe coronary artery disease awaiting surgery[1]. There is little accurate information on the prevalence of the disease, but it is thought to affect 15-23% of the China population and, internationally the numbers are likely to be similar[2,3].

Pathogenic mechanisms of chronic (CU) have not been well understood; however, activation of skin mast cells plays a key role in inflammation associated with the disease. Mast cell activation and release of mediators, particularly histamine, can effect in cutaneous inflammatory processes and accumulation and activation of other cells. Also circulating cells, possibly basophils and platelets, may play a relevant role in urticaria[4,5]. Besides their well-recognized role in hemostasis, platelets are involved in other biologic processes, including inflammation. Possible significance of platelets in urticaria may be related to their proinflammatory properties. During inflammatory and immune response, platelets may be activated and might release inflammatory mediators.

The treatment of CU represents a challenge to the physician because of the incidence of troublesome side effects and the recurrence of urticaria when therapy is stopped[6].

MATERIALS AND METHODS

Thirty healthy individuals (13 males and 17 females) and 34 patients with CU were enrolled in this study which was carried out in Zhongnan Hospital of Wuhan University, Wuhan, China. All these 34 subjects (Male 27, female 37 cases, 18~71 age; 0.5~14 year duration of disease) were Autologous Plasma Skin Test (APST) positive and had a documented history of CU of at least 6 weeks duration and with a frequency of at least three episodes per week in the absence of treatment. Their disease was characterized by continuous or recurrent spontaneous wheals having appeared for more than 6 weeks. Each episode of urticaria was defined as itching papular eruptions of varying size of no more than few hours’ duration

Baseline characteristics of the treatment & control groups were similar; Gender (t =0.55. p> 0.05), age (t=0.74, p> 0.05).

The following patient categories were excluded: Cholinergic urticaria , Drug-induced urticaria , Urticarial Vasculitis , Physical urticaria, pregnant or lactating women, Long-term corticosteroid (in 1 month) and Oral drug (in 1week) used for prevention and
treatment of CU, and any factor that may affect drug absorption, distribution, metabolism and excretion.

At the first visit a complete medical history was taken for each patient. A clinical examination with a standard laboratory tests was also carried out at inclusion and repeated after 1, 2 and 4 weeks of treatment.

**Drug administration:** The treatment group was treated with 5mg twice daily loratadine and 25mg. three times daily dipyridamole for 28 days

Where

**loratadine Tablets:** Brand Names dihengsai Produced by haibin Pharmaceutical Company Ltd of Shenzhen (city in China)

**Dipyridamole Tablets or persantine Tablets:** Brand Names Shuangmidamo Pian Produced by yongda drug manufactory in jiangsu province

**Efficacy criteria:** Patient’s symptoms and signs recorded according to 4 levels as shown in Table 1. Meanwhile, patient's assessment before and after 4 weeks of treatment was done according to integral signs & symptom scores:

\[
\text{Symptom score reduce index (SSRI) = (total score before treatment - total score after treatment) / (total score before treatment)} \tag{1}
\]

The validity of clinical treatment effect according to SSRI:

- Invalid SSRI <0.20,
- Effective: 0.60>SSRI ≥ 0.20,
- Obvious effective: 0.90>SSRI ≥ 0.60,
- Clinical cure: SSRI ≥ 0.90.

The rate of affectivity is the combined clinical cure and obvious effect divided by total

**Blood Collection and Analysis:** Blood collection tubes for F1+2 measurements were prepared at the Laboratory for Clinical Biochemistry Research, Department of Pathology, University of Wuhan, and provided to the center. Research personnel participating in blood collection received venipuncture training at the beginning of the study. Data were collected regarding venipuncture quality, including number of needle punctures required, vein collapse, hematoma formation, leakage at site, tourniquet time, total venipuncture time, and venipuncture difficulty. Blood was collected with minimal stasis with a 21-gauge butterfly needle. Blood was drawn into a special coagulation tube designed to avoid in vitro coagulation activation. This tube, at final concentrations, contained 4.5 mmol/L EDTA, 200 KIU/mL aprotinin, and 25 µmol/L d-Phe-Pro-Arg chloromethyl ketone (a potent serine protease inhibitor). [7]. Samples for central analysis were processed within 1 hour of phlebotomy. Tubes were centrifuged at 4°C for 30 000g-minutes. Plasma was aliquoted into color-coded 0.5-mL cryovials (USA Scientific) and frozen at -70° until shipment. Samples were shipped on dry ice via an overnight carrier. F1.2 was measured with an enzyme-linked immunosorbent assay (Behring Inc). The interassay CV was 8%. Prothrombin times were primarily measured locally (70%) with a variety of thromboplastins

**Safety criteria:** Complete medical history and clinical check up was done before treatment, in addition to patient’s blood, urine ALT, urea, creatinine, etc, were inspected. Also analysis done at 7d, 14d, and 28d after the beginning of treatment, effectiveness and adverse effects were observed. Adverse effects were defined as clinical signs or symptoms that appeared or worsened during treatment. Abnormalities in laboratory parameters and vital signs that lead to withdrawal of treatment were also recorded.

**Statistical analysis:** Analyses were performed using SPSS 11.5 software. Descriptive statistics and comparisons between treatment and control groups (t-test) were performed at d₀ and at the last observed value in the study d_end, and on the difference between these two values. Plasma prothrombin fragment F1+2 t-test was used to compare the results of the testing. Comparisons were performed at a significance level of 5%.

**RESULTS**

**Plasma prothrombin fragment F1+2:** The levels of plasma prothrombin F1+2 in autoimmunity (CU) patients have decreased obviously after treatment (3.34±2.87vs 0.94±0.58, p<0.001). Before treatment, the levels of treatment group was higher than that of control group (3.34±2.87vs 0.72±0.36, p<0.001). After treatment, they have no significant difference (0.94±0.58vs 0.72±0.36, p>0.05). Table 4 shows, cu patient plasma F1+2 level has obvious improvement after treatment
Table 1: CU signs and symptoms score

<table>
<thead>
<tr>
<th>Level</th>
<th>Criteria</th>
<th>Zero</th>
<th>One</th>
<th>Two</th>
<th>Three</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter of the largest wheal</td>
<td>No wheal</td>
<td>Less than 1.5 cm</td>
<td>Between 1.5-2.5 cm</td>
<td>More than 2.5 cm</td>
</tr>
<tr>
<td></td>
<td>Number of wheals</td>
<td>No wheal</td>
<td>Less than 10</td>
<td>Between 10-30</td>
<td>More than 30</td>
</tr>
<tr>
<td></td>
<td>Appearance of wheals</td>
<td>No wheal</td>
<td>Just erythema</td>
<td>Erythema and little</td>
<td>Obvious elevated pink or</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>elevated</td>
<td>white pale</td>
</tr>
<tr>
<td></td>
<td>Frequency</td>
<td>No wheal</td>
<td>One or less a day</td>
<td>Two to three times a day</td>
<td>Three or more times a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>day</td>
</tr>
<tr>
<td></td>
<td>Wheals duration</td>
<td>No wheal</td>
<td>Less than one hour</td>
<td>1-3 hour</td>
<td>More than 3 h</td>
</tr>
<tr>
<td></td>
<td>Itching degree</td>
<td>No itching</td>
<td>Light scratch no</td>
<td>Itching with a</td>
<td>Severe itching</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>impact on the daily</td>
<td>heavier impact on daily</td>
<td>disturbing daily life</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lives</td>
<td>life, but patient</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>tolerate it</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Chronic urticari patient F1+2

<table>
<thead>
<tr>
<th>Group</th>
<th>No. cases</th>
<th>Before</th>
<th>After</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>APST+ve</td>
<td>64</td>
<td>3.27±2.74</td>
<td>0.87±0.46</td>
<td>9.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment</td>
<td>34</td>
<td>3.34±2.74</td>
<td>0.94±0.58</td>
<td>6.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>3.19±2.64</td>
<td>1.15±1.12</td>
<td>7.03</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Clinical Effectiveness: After treatment patient were significantly improved, their signs and symptoms scores (X ± s) was significantly decreased than before treatment (before treatment 13.87 ± 2.03. After treatment 3.57 ± 3.63, t=5.74, P <0.001).

Of 34 treated cases of (CU) patients, 21 cases clinical cure and 8 obvious effectiveness, effective 5, and the total efficiency is 85.29%. No serious adverse reactions, laboratory examinations before and after the treatment showed no abnormality.

Table 3: Clinical effectiveness at end of treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Cured</th>
<th>obvious effectiveness</th>
<th>Effective</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>34</td>
<td>21</td>
<td>8</td>
<td>5</td>
<td>85.29%</td>
</tr>
</tbody>
</table>

No serious adverse effects: The main adverse reactions in patients account for the mild drowsiness, headache, dizziness, dry mouth, gastrointestinal discomfort, but they are tolerable. Not affect their daily lives and work of withdrawal symptoms. Laboratory examination showed no abnormality in the two groups before and after treatment 4.

DISCUSSION

Autoimmune urticaria represents a relatively recent diagnostic advance, and this condition is now believed to account for as many as 40% of patients with CIU[8]. It is found that circulating autoantibodies of IgG or IgM isotypes to be targeting IgE in a small subset of CIU patients. And more often, these patients had circulating autoantibodies against the IgE receptor. Circulating IgG autoantibodies react with chain of the IgE receptor on dermal mast cells and basophils, prompting release of histamine and other elements that cause urticaria [9,10].

Prothrombin is produced by the hydrolysis of plasma thrombin activation reaction, then signs of coagulation process started over. Under normal circumstances coagulation and fibrinolytic system maintain a balance state. The prothrombin activation fragment F1+2 is an index of in vivo thrombin generation; one molecule of F1+2 is released with the generation of each thrombin molecule[11-14]. Anticoagulation suppresses the F1+2 level in a dose-response fashion[15-17]. It has been demonstrated that CU patients showed high blood coagulation state with F1+2 levels were higher than normal[18]. The mechanism may be: excessive activation of mast cells in patients with (CU). Histamine is released with a wide variety of inflammatory mediators, including cytokines, and then activates plasma prothrombin to thrombin.

Our study confirm this, we found that the pretreatment plasma levels of Prothrombin fragment X ± s
concentrations were significantly elevated in the in 56 consecutive patients with CU when compared to 33 apparently healthy volunteer as control (3.34±2.87 vs 0.72±0.36, p<0.001). Furthermore, after four weeks of therapy with loratadine plus Dipyridamole, there was marked decline in F1+2 (3.34±2.87 vs 0.94±0.58, p<0.001). And when compared to controls there was no significant difference in F1+2 concentrations (0.94±0.58 vs 0.72±0.36, p>0.05).

Blood platelet significance in inflammation is recognized but poorly characterized in urticaria. It is known that platelets are activated during inflammatory processes and are involved in modulating inflammatory and immune response via various mediator releases. Platelets have been described as a source of inflammatory mediators that are implicated in histamine release from basophils\(^{[19,20]}\) and mast cells\(^{[21]}\). Interestingly, data from experimental study prove that PF-4 fragment is able to induce rat mast cells to histamine release in a dose-dependent manner\(^{[21]}\).

Dipyridamoles inhibit phosphodiesterase, this cause relative increase in the concentration of intracellular cAMP. Thereby suppressing the degranulation of mast cells or basophils an active role in the release of subsequently inhibited plasma prothrombin to thrombin in small elements corresponding to reduce F1+2, this agreement with the experimental results of the study\(^{[11]}\).

Moreover, it has been shown that histamine is released by human platelets in response to aggregatory and immunological stimuli. In turn, exogenous histamine potentiates platelet activity measured by the aggregation induced by different agonists, acting through histamine receptors\(^{[22]}\). Meanwhile, mutual dipyridamole can inhibit platelet aggregation and platelet membrane and adenosine diphosphate (ADP), and high concentrations inhibit collagen, thrombin, and epinephrine-induced platelet release reaction. PGF2 also to enhance the synthesis of endogenous anticoagulant effect. Therefore, in patients with prothrombotic CU could be used as indicators for monitoring the severity and efficacy remains to be further studied.

The research also showed that the antihistamine loratadine combined with dipyridamole are effective in the management of patients with CU. Thus, the two can be combined to enhance clinical efficacy in treating (CU) \(\text{(S)} 3.27 \pm 2.74 \text{ vs} 0.87 \pm 0.46; p<0.001\), although the process of using a small number of cases showed mild drowsiness. Gastrointestinal symptoms such as discomfort, but relatively minor and do not affect continue treatment on the clinical use. But observation time is short. Its long-term effects and long-term effects of medication plasma thrombin and the relationship between prothrombotic pending further observations.

The plasma prothrombin F1+2 levels of autoimmunity (CU) elevating may play an important role in the pathogenesis of (CU). These findings provide new insights into the etiology of CU and suggest new therapeutic opportunities for treating this disease.

**CONCLUSION**

These findings provide new insights into the pathogenesis of CU and suggest new therapeutic opportunities for treating this disease.

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**REFERENCES**


