Sex Differences of the Inflammatory Mediator Level at the Time of Itch Onset in Patients with Chronic Venous Disease

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This study investigated the sex differences of the inflammatory mediator level at the time of itch onset in patients with chronic venous disease (CVD). Twenty-seven CVD patients (nineteen women, eight men) and nine healthy controls (five women, four men) participated. CVD-associated itching was observed in both men and women. Before sclerotherapy, both sexes had elevations in several itch-related mediators. Among these, women had significantly higher tryptase, whereas men had significantly higher β-endorphin and adrenocorticotropic hormone. After sclerotherapy, all levels normalized in both sexes. In this study, itching was increased tryptase in women and increased adrenocorticotropic hormone and β-endorphin in men.

Key words — adrenocorticotropic hormone; β-endorphin; itching; tryptase; chronic venous disease

INTRODUCTION

Chronic venous disease (CVD) is a common disorder of the lower extremities. The prevalence of CVD in the adult population increases with age. In addition, the condition is common among pregnant women and those engaged in standing work. CVD is responsible for a wide range of lower extremity symptoms, including aching, swelling, tickling, and itching. The Edinburgh vein study reported that up to 19% of men and 25.3% of women with the condition suffer from CVD-associated itching, and that the prevalence of itching tends to increase significantly with age in women.

A previous study found that patients with CVD had dry skin compared with healthy individuals. That study also reported that itching before sclerotherapy was related to levels of substance P, histamine, immunoglobulin E (IgE), and tryptase. The mechanisms by which itching is induced can be classified as peripheral versus central. Peripheral itching is mediated through the C fibers, whereas the mechanism of central itching involves the opioid system. Intractable itching that is not responsive to antihistamines can be seen in diseases such as kidney failure and atopic dermatitis. The mechanism of this intractable itching is thought to be reduced itching threshold of the nerve fibers penetrating the epidermis.

An epidemiological investigation of itching and its sex-related differences in patients with CVD has been reported. However, the mechanisms of these sex-related differences in itching remain unclear. Therefore, the aim of this study was to determine sex differences of the inflammatory mediator level at the time of itch onset in patients with CVD.

METHODS

Patients and Control Subjects This study included patients who were diagnosed with CVD and received sclerotherapy at our institution between March 2014 and December 2014. Patient data collected for analysis included age, sex, smoking habits, presence of type II diabetes mellitus or hypertension, family history of CVD, and history of childbirth. CVD was diagnosed with visual inspection and lower extremity ultrasonography. CVD severity was classified according to the Clinical Etiologic Anatomic Pathophysiologic (CEAP) classification, which was created at an international consensus meeting supported by the Vein Forum in the United States (American Venous Forum) in 1994 and subsequently revised. CVD was characterized according to clini-
cal signs: C0 (no clinical signs), C1 (reticular and spider veins), C2s (varicose veins, symptomatic), C2a (varicose veins, asymptomatic), C3 (edema), C4 (skin lesions), C5 (healed ulcer), and C6 (active ulcer). We recruited healthy volunteers with no medical history and no risk factors for CVD as a control group. Written informed consent was obtained from everyone participating in this study. This study was conducted in accordance with the Declaration of Helsinki and its amendments, and was approved by the ethics committee of Mie Heart Center (Approval No.; 2014-07-068).

Study Design For patients who provided consent, we measured the stratum corneum water content, transepidermal water loss (TEWL), and itching severity before and after sclerotherapy. In addition, blood levels of histamine, immunoglobulin A (IgA), IgE, matrix metalloproteinase-9 (MMP-9), tryptase, adrenocorticotropic hormone (ACTH), and β-endorphin were measured as inflammatory mediators before and after sclerotherapy.

Sclerotherapy To coagulate affected veins, 1% polidocanol was injected at the site to be treated, according to a previously described technique.10

Itch Assessment Prior to sclerotherapy and 1 week after the final procedure, patients evaluated itching at their CVD site, and skin measurements and blood samples were taken. A 5-point scale was used to evaluate CVD-site itching, with 0 indicating no itching and 5 indicating the most intense itching possible.11

Blood Sample Collection A 6-mL blood sample was collected from each participant. Plasma was fractionated from collected blood samples by centrifugation at 10000×g for 10 min at 4°C. Supernatant fractions were isolated and stored at −80°C until analysis. After thawing, equal amounts of protein (12.5 μg/lane) were loaded onto 4–12% BIS-TRIS Bolt gels (Life Technologies, Carlsbad) and electrophoresed at 200 V for 30 min. After separation, the proteins were transferred onto a nitrocellulose membrane, using an iBlot western blotting system (Life Technologies), which was subsequently blocked overnight with 5% skim milk at 4°C. After blocking, the membranes were incubated at 25°C for 1 h with primary antibodies for MMP-9 (1:1000; Abnova, Taipei), tryptase (1:500; Santa Cruz Biotechnology Inc., Santa Cruz), and β-actin (1:5000; Sigma, Saint Louis). The immune complexes on the membranes were then visualized using a horseradish peroxidase-conjugated secondary antibody (Dako Cytomation, Glostrup) and ImmunoStar Zeta (Wako Pure Chemical Industries, Ltd., Osaka). Images were acquired with Multi-Gauge software (Fujifilm, Greenwood).

Statistical Analyses All data are presented as the mean±S.D. The analyses of variance (ANOVA) were performed using the ANOVA procedure. Tukey-Kramer test was used to compare the means between groups. Differences were considered statistically significant at a p-value of <0.05.

RESULTS

Background of Patients and Control Subjects This study included nineteen female patients (mean age, 66.7±7.3 years) and eight male patients (mean age, 58.2±14.6 years). Four patients were smokers (three men, one woman), two patients had type 2 diabetes (one man, one woman), and four patients had high blood pressure (one man, three women). None of the patients had a family history of CVD. All of the women in the study had a history of childbirth. The control group consisted of four men and five women (mean age, 41.0±6.9 years). None of the control...
group participants had smoking habits, hypertension, medical history, or a family history of type II diabetes. All women in the control group had a history of childbirth (Table 1). According to CEAP classification, six patients (22%) with palpable cutaneous vein expansion of 3 mm or more were class C2s, defined as symptomatic varicose veins, and fifteen patients (55.5%) were C3, defined as edema without skin lesions. Five patients (18.5%) were classified as C4 (severe skin lesions excluding ulcers). None of the patients were classified as C5 (history of ulcers) or C6 (active ulcer).

The prevalence of itching was among female patients (14 of 19 patients; 74%) and among male patients (four of eight patients; 50%). The average itching score before sclerotherapy was 1.25 points in men and 1.47 points in women.

For the skin measurement parameters (water content in the stratum corneum and TEWL), there were no significant differences between men and women before sclerotherapy.

**Biochemical Markers of Itching** We measured biochemical markers to examine the mechanism of sex differences in itching. Levels of substance P, histamine, IgA, and IgE were higher before sclerotherapy than after treatment. There were no significant differences in these parameters between men and women (Table 2).

Next, we measured sex differences in MMP-9. MMP-9 dropped to the same level as controls for both men and women. Sex differences were not observed either before or after sclerotherapy (Fig. 1).

In addition, we measured tryptase as a marker of itching. In men, blood levels of tryptase were the same as in controls before treatment. Tryptase levels were clearly higher among women patients than among controls before sclerotherapy. After sclerotherapy, tryptase levels in women patients fell to almost the same level as those in controls (Fig. 2).

Knowing that CVD is related to stress, we measured ACTH levels. The ACTH level in the blood of men before sclerotherapy was higher than after sclerotherapy. In women, there was no difference in ACTH levels before versus after sclerotherapy (Fig. 3).

**DISCUSSION**

In this study, men and women with CVD had changes in several parameters related to itching. Before sclerotherapy, both men and women had low water content in the stratum corneum, high TEWL,

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Table 1. Patient Characteristics according to Sex

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Number of patients</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>Age (years; mean ± S.D.)</td>
<td>58.3 ± 14.6</td>
<td>66.7 ± 7.3</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>3 (37.5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus, n (%)</td>
<td>1 (12.5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>1 (12.5)</td>
<td>3 (15.8)</td>
</tr>
<tr>
<td>Family history of varicosis, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Number of births &gt;1, n (%)</td>
<td>0 (0)</td>
<td>19 (100)</td>
</tr>
</tbody>
</table>

Table 2. Examination Findings according to Sex, before and after Sclerotherapy

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itch score</td>
<td>Male: 1.25 ± 1.75</td>
<td>Female: 0.37 ± 0.74</td>
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<tr>
<td></td>
<td>Female: 1.47 ± 1.12</td>
<td>0.10 ± 0.31</td>
</tr>
<tr>
<td>Corneometer value (μs)</td>
<td>Male: 25.13 ± 17.33</td>
<td>35.62 ± 13.44</td>
</tr>
<tr>
<td></td>
<td>Female: 27.84 ± 10.80</td>
<td>38.63 ± 13.14</td>
</tr>
<tr>
<td>Transepidermal water loss (g/m²/h)</td>
<td>Male: 16.04 ± 9.82</td>
<td>13.04 ± 9.17</td>
</tr>
<tr>
<td></td>
<td>Female: 12.29 ± 8.35</td>
<td>8.40 ± 2.75</td>
</tr>
<tr>
<td>Histamine (nm)</td>
<td>Male: 66.97 ± 17.24</td>
<td>7.63 ± 6.57</td>
</tr>
<tr>
<td></td>
<td>Female: 68.82 ± 13.81</td>
<td>11.14 ± 6.04</td>
</tr>
<tr>
<td>Substance P (pg/mL)</td>
<td>Male: 20.26 ± 7.91</td>
<td>4.39 ± 1.99</td>
</tr>
<tr>
<td></td>
<td>Female: 16.49 ± 8.67</td>
<td>4.61 ± 5.28</td>
</tr>
<tr>
<td>IgA (ng/mL)</td>
<td>Male: 68.00 ± 2.71</td>
<td>58.54 ± 3.33</td>
</tr>
<tr>
<td></td>
<td>Female: 68.93 ± 2.99</td>
<td>62.35 ± 4.91</td>
</tr>
<tr>
<td>IgE (ng/mL)</td>
<td>Male: 25.48 ± 6.19</td>
<td>11.50 ± 7.74</td>
</tr>
<tr>
<td></td>
<td>Female: 21.17 ± 4.74</td>
<td>14.05 ± 3.59</td>
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</tbody>
</table>
and elevated blood levels of histamine, substance P, IgE, and MMP-9. However, women had higher blood tryptase levels than men before sclerotherapy, whereas men had higher levels of ACTH and β-endorphin. In this study, there were many patients who complained of itching, four (44%) male patients and 14 (74%) female patients. It has been reported that lower-extremity varices are more common among women than among men. This study included more female patients than male patients because fewer male patients than female patients were treated with sclerotherapy at our institution during the study period. Therefore, we analyzed all patients treated at our hospital, although the number of men and women included and the number who complained of itching were different.

As shown in Table 2, patients with CVD had dry skin. However, sex differences in itching scores were not observed. It has been reported that MMP-9 and substance P are associated with itching. In this study, MMP-9 and substance P levels were higher in patients with CVD than in healthy controls, but there were no sex differences in these levels (Fig. 1). Histamine is well known to induce itching. Histamine and tryptase bind to the receptors of nerve endings, sending a signal to the brain and inducing itching. However, the level of tryptase in men in this study was the same as in controls before and after sclerotherapy (Fig. 2). In contrast, women with
CVD had a significantly higher tryptase level than the control group before sclerotherapy (Fig. 2). Tryptase levels after sclerotherapy were lower than before sclerotherapy. These findings indicate that histamine and tryptase may play important roles in the development of itching in women with CVD. Histamine increases the release of substance P, which is a factor associated with peripheral itching in the skin. Also, tryptase degrades skin collagen, leading to drying of the skin, which induces itching. In men, histamine was elevated before sclerotherapy, but men had no increase in tryptase. The mechanism by which tryptase was elevated before sclerotherapy, but men had no increase in tryptase. The mechanism by which tryptase is increased in women but not in men is not yet clear and needs further investigation. Furthermore, there is a report that tryptase has no sex difference in healthy people. Likewise, there was no sex difference in the control group of this study. However, the expression of MMP-9 has sex difference on healthy person (control group), and it is reported that men is higher than women. In this study, there was no significant difference between men and women in the control group. The reason for this is that, it is possible that the background of the men and women who are control of this study is different. Although it considered that we arranged the background on men and women in this study, we were not able to arrange strictly. In addition, the amount of MMP-9 in the blood has been measured by ELISA method in other reports, however, in this study, we measured by western blotting method. Therefore, it is thought that the difference in a measuring method has influenced.

CVD is associated with stress, with involvement of the proopiomelanocortin (POMC) system. We measured the levels of ACTH and β-endorphin, which are closely related to itching. The levels of ACTH and β-endorphin were higher in both men and women before sclerotherapy than after treatment (Figs. 3 and 4). β-Endorphin is derived from POMC. In response to stress, corticotropin releasing factor is secreted from the hypothalamus. This results in POMC secretion from the anterior pituitary, which produces ACTH and β-endorphin at a ratio of 1:1. In general, men have greater biochemical changes than women in response to stress. Because CVD causes stress, ACTH and β-endorphin could be increased more in men with the condition than in women. In the present study, β-endorphin levels were clearly higher than ACTH levels in men (Figs. 3 and 4). This difference may be attributed to the fact that β-endorphin is secreted from cells of the immune system, such as macrophages and neutrophils. Inflammation in patients with CVD induces infiltration of immune cells. The increase in these cells results in increased secretion of β-endorphin. In addition, β-endorphin is elevated in patients with hypertrophic scars and is reported to play an important role in the process of itching. In dialysis patients, there is a strong relationship between β-endorphin and itching. It has been reported that β-endorphin induces itch by attaching to the μ-opioid receptor. In patients with atopic dermatitis, itching frequently results from dry skin. A positive correlation has been reported between itching intensity and β-endorphin, serum IgE, and TEWL. Generally, β-endorphin levels are higher in women than in men. In adult asthma patients, the reported levels of β-endorphin are higher in women than in men. Surprisingly, in patients with CVD in this study, the level of β-endorphin was higher in men than in women (Fig. 4). This finding might be explained by the relationship between β-endorphin levels and CD4+ T cells. In the present study, men had higher numbers of CD4+ T cells than women (data not shown). This higher number of CD4+ T cells might have promoted elevation of β-endorphin. However, the reason for the increased number of CD4+ T cells in the pathogenesis of CVD in men is not clear and needs further study. Our results suggest that itching in male patients with CVD was induced by ACTH and β-endorphin.

CONCLUSIONS

Based on our findings, there are differences in the mechanisms by which CVD-related itching is induced in men versus women. In women, increased levels of tryptase induced itching. In men, increased β-endorphin levels induced itching. We do not fully understand the detailed mechanisms of CVD-associated itching; however, our findings could be helpful in the treatment of patients with CVD.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES


2) Beebe-Dimmer J. L., Pfeifer J. R., Engle J.


