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Journal of Dermatology and Clinical Research

Research Article

Effect of Age on Varicella-Zoster Virus-Specific Cell-Mediated Immunity in Patients with Herpes Zoster Assessed by Skin Tests with Varicella-Zoster Virus Antigen

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Submitted: 22 March 2016

Accepted: 11 April 2016

Published: 13 April 2016

Keywords

- Herpes zoster
- Skin test
- Varicella-zoster virus
- Cell-mediated immunity

Abstract

To characterize varicella-zoster virus (VZV)-specific cell-mediated immunity (CMI) immediately after the onset of herpes zoster (HZ), we administered a VZV skin test antigen to patients within 6 days after HZ onset. We analyzed skin reactions by measuring the longest diameter of erythema and edema. This study included 76 patients at three dermatology clinics. Skin reactions differed in patients who were under 50 years old versus those who were 50 years of age or older; positive edema was seen only in the latter group. Successive skin tests showed that patients under 50 years of age recovered VZV-CMI more quickly than did older patients. These findings indicate that there are both quantitative and qualitative differences in VZV-CMI in patients under 50 years of age and those 50 or older. These differences may explain the higher incidence and greater severity of HZ seen in older adults.

ABBREVIATIONS

HZ: Herpes Zoster; VZV: Varicella-Zoster Virus; CMI: Cell-Mediated Immunity; ELISA: Enzyme-Linked ImmunoSorbent Assay

INTRODUCTION

Herpes zoster (HZ) is a common disease that is usually treated at general dermatology clinics. Mild cases can be treated on an outpatient basis. Severe cases are characterized by varying degrees of exanthema, and can be followed by Ramsay Hunt Syndrome or post-herpetic neuralgia (PHN). Anti-herpes drugs can reduce the severity of HZ when used in the early stages of the disease, although immunodeficiency and other conditions can limit these drugs' efficacy. A herpes zoster vaccine designed to limit the impact of this disease has proven effective [1].

HZ is caused by the reactivation of a latent varicella-zoster virus (VZV) infection established in the dorsal root ganglia during a primary varicella in childhood. The frequency and severity of HZ increases with age, suggesting that the VZV-specific cell-mediated immunity (VZV-CMI) decreases with age [2-7]. To test this hypothesis, the VZV-CMI response can be measured in individuals using methods such as the interferon-γ enzyme-linked immunospot (ELISPOT) assay or a VZV skin test that was developed in Japan [8-11].

In a large-scale epidemiological study of HZ, we previously demonstrated that the VZV skin test can predict the risk of HZ, suggesting that the test reliably measures the actual state of VZV-CMI [12]. In that study, however, the VZV skin test was performed a relatively long time before the onset of HZ, and on subjects over 50 years of age. To understand the factors responsible

for HZ development, it is important to investigate VZV-CMI immediately before or after the onset of the disease. Thus, here we administered the VZV skin test to patients of any age who sought treatment for HZ at one of three private dermatology clinics near Osaka City.

Most studies using the VZV skin test have assessed VZV-CMI by measuring the longest diameter of erythema. In this study, we measured not only erythema but also edema, which is the strongest and most specific reaction to the skin test. When we grouped the HZ patients by age (<50 years and ≥50 years), clear differences of the skin test between the two age groups were found, which could account for age-related differences of VZV-CMI.

MATERIALS AND METHODS

Enrollment of patients with herpes zoster (HZ)

This study was approved by the ethical committee of Hyogo College of Medicine. A total of 172 HZ patients, who were diagnosed clinically, visited one of three private dermatology clinics near Osaka City between April 1, 2009 and March 31, 2010. Although many of them received the VZV skin test at the first visit of the clinics, we used data from 120 patients who were confirmed later by real-time PCR and/or by serological tests. Twenty-three out of 120 patients received the test twice and one three times. The youngest patient was a year old; the oldest was 96.

The VZV-antigen skin test

After obtaining written informed consent, we administered the VZV skin test to all patients with HZ during their visit to the clinic. This skin test uses a viral antigen produced by Biken (Research Foundation for Microbial Diseases of Osaka University, Japan), and is commercially available in Japan. A disposable tuberculin syringe was used to inject 0.1 ml of the skin test antigen intradermally into the patient's forearm, and the longest and shortest diameters of erythema and edema were measured 48 hours later.

The onset of HZ was defined as the first appearance of the rash. Since we wished to investigate VZV-CMI in the early phases of HZ, we used data from skin tests conducted within 6 days of HZ onset. Using this criterion, we analyzed data from 76 patients (36 male and 40 female). Of these, 59 patients were 50 years of age or older (26 male and 33 female) and 17 were under 50 years old (10 male and 7 female).

Real-time PCR and gp-ELISA

Blood samples were obtained from all patients with clinical indications of HZ and used for serological tests (mainly gp-ELISA), and vesicular fluid or crust was obtained for real-time PCR. The samples were immediately transported to the surveillance center at Biken, where the tests were conducted. Real-time PCR and gp-ELISA were performed as described previously [12].

Statistics

Differences between groups were assessed by Fisher's exact test.

RESULTS

VZV skin test reactions differed by age

Reactions to the VZV skin test were categorized as no reaction (no measurable longest diameter of both erythema and edema), erythema (measurable longest erythema diameter with no measurable longest edema diameter), or edema (measurable longest edema diameter). Erythema was also present in all patients who developed edema. Patients were divided by age group and then by reaction pattern (Figure 1a). The results showed that HZ tended to affect older patients, with more than three times as many HZ patients who were 50 and older (≥50 years) (n=59) than patients who were under 50 (<50 years) (n=17). We observed a small peak at 20–29 years of age, and a major peak at 60–69 years of age; 38.2% of the 76 patients were 60–69 years of age. Edema occurred only in patients who were 50 or older (Fig. 1b). The rate of erythema was similar in the two age groups, suggesting that some patients 50 years and older developed HZ despite having strong VZV-CMI as indicated positive edema.

Skin test reactions differed according to the interval after HZ onset

To examine changes in VZV-CMI, we compared data from skin tests performed within 6 days of the onset of HZ to those from tests performed 7 or more days after onset. All of the HZ patients under 50 years of age visited a clinic within 28 days of

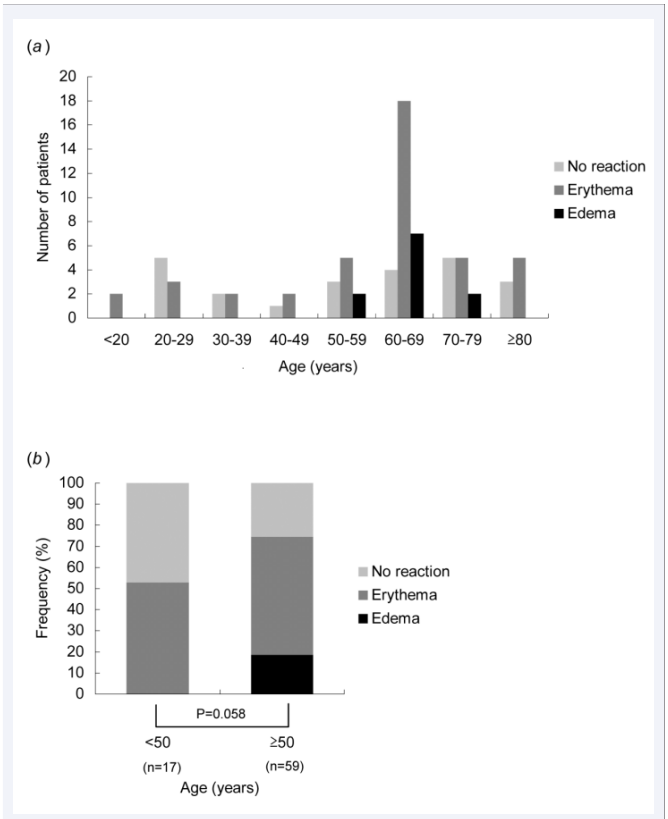

Figure 1 (a) Number of HZ patients divided by age group. Skin reactions were classified as edema, erythema, or no reaction. (b) Frequency of each reaction pattern in patients 50 or more years of age, and in those under 50 years old.

onset (0–6 days, n=17; 7–28 days, n=9) and were given the skin test once except one patient who received it twice (one in 0-6 days and second in 7-28 days) (Figure 2a). Many patients who were 50 years of age or older were given the skin test at the first visit and on at least one subsequent visit. Thus, all the data from tests performed 29–84 days (n=13) and 85 or more days (n=14) after onset were from patients of 50 years or older (Figure 2b).

In patients under 50 years old, there were clear differences in the reactions from tests performed 0–6 days after HZ onset and those performed 7–28 days after onset (Figure 2a). There were no cases of edema in tests performed 0–6 days after onset, while 4 out of 9 patients tested 7–28 days after HZ onset developed edema. These results suggest that patients under 50 regained strong VZV-CMI rapidly after developing HZ. In the group 50 years and older, however, the percentage of patients with edema increased slowly, with a significant increase more than 29–84 days after onset (Figure 2b). These findings together suggest that VZV-CMI may recover more slowly in older adults.

Quantitative analysis of VZV skin tests performed 0–6 days after HZ onset

We next assessed the relationship between age and the

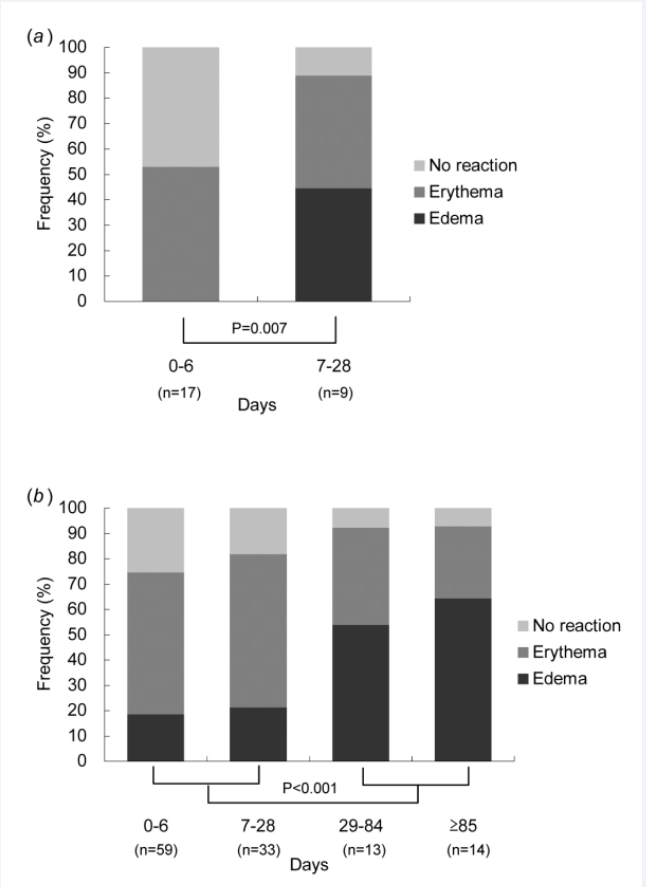

Figure 2 (a) Frequency of each skin test reaction pattern in patients under age 50, in tests performed 0–6 days or 7–28 days after the onset of HZ. (b) Frequency of each skin test reaction pattern in patients 50 years of age or older, in tests performed 0–6 days, 7–28 days, 29–84 days, or more than 85 days after the onset of HZ.

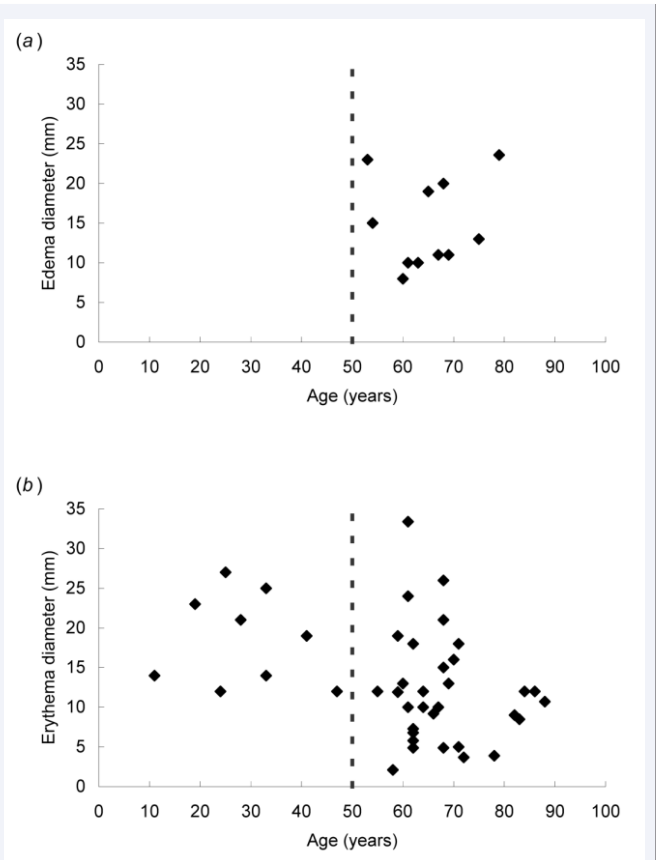

Figure 3 (a) Distribution of the longest diameter of edema according to age. (b) Distribution of the longest diameter of erythema according to age.

longest diameter of edema or erythema (Figure 3). In tests performed within 6 days of HZ onset, edema developed in 11 of the 59 patients who were 50 or older (Figure 3a). The longest edema diameter varied from 8–23.6 mm.

The distribution of the longest diameter of erythema differed between patients under 50 and those 50 years and older (Figure 3b). Although the rate of erythema between the two groups did not differ greatly (Figure 1b), other differences were observed. In patients 50 years and older, the erythema varied in size, from 2.1 mm to 33.4 mm in diameter, while those in patients under 50 was more than 12 mm. The average size of erythema in the older group was 12.3 mm, while that in patients under 50 years was 18.6 mm.

DISCUSSION

There is consensus that the incidence of HZ increases with age, particularly in people over 50 years old [2,4,13]. Although everyone who is infected with VZV in childhood preserves latent VZV in the dorsal root ganglia, not everyone develops HZ. Extensive immunological studies of VZV-specific immunity in adults have shown that lack of VZV-CMI, but not a VZV-specific antibody, is responsible for HZ [6,14-16].In the present study, we injected VZV skin test antigen into patients in the early stages of HZ, to examine how decreased VZV-CMI contributes to the development of HZ.

It should be noted that reaction patterns of the skin test using VZV antigen was different from those using purified protein derivative (PPD) in tuberculin test which categorized as induration, palpability and erythema [17,18]. The strongest reaction of the former test is edema which may correspond to palpability of tuberculin test. This is supported by our earlier study that, in more than 5,000 of individuals who were injected VZV antigen intradermally, no one developed induration [12]. These differences of reaction pattern in the two tests might reflect different pathogenesis of each original disease.

The age distribution of HZ cases in our study (Figure 1a) is in harmony with a report that the incidence of HZ increases rapidly after 50 years of age, with the highest incidence in Japan at ages 60–79 [4]. When patients in our study were grouped by age (<50 years and ≥50 years), the most notable difference was that edema was observed only in patients 50 or more years of age (Figure 1b). However, the positive rate of edema in patients 50 years and older was 18.6%. This figure was quite low compared to the 69.6% of healthy subjects in our previous study who developed edema after the VZV skin test [12]. Although the skin test was performed after the onset of HZ in the current study, these results seem to suggest that VZV-CMI in HZ patients decreased considerably just before the onset of the disease, especially in patients under 50 years of age.

The severity of HZ also increases with age, as shown by the greater frequency and duration of PHN with age and the increase in complications with age [19-21]. This seems to agree with our results (Figure 2) in that a late recovery of edema frequency in HZ patients over 50 years of age compared to those under 50 years of age. Our quantitative analysis of skin test reactions also revealed some age-related differences (Figure 3). Although the rate of edema was small, edema occurred only in patients 50 or more years of age, and the longest diameter of edema was relatively large (Figure 3a). For erythema, we observed a reverse trend, in which the longest diameter of erythema was larger in patients who were under 50 years old than in those 50 or older (Figure 3b). These observations suggest that there are both quantitative and qualitative differences between the VZV-CMI in patients in the two age groups.

Most recently, VZV skin test antigen supplied from Biken was used to investigate T cell phenotype which affects immune responses at the site of skin challenge [22,23]. They demonstrated that VZV-specific CD4 T cells and CD4 regulatory T cells (Tregs) accumulate in parallel after VZV challenge in the skin of healthy individuals [22]. When subjects were grouped as young (<40 years old) and old (>60 years old), there were significantly increased numbers of Tregs in the skin of old compared with young individuals, which result in weak skin reactions in older subjects after intradermal challenge with VZV antigen [23]. These observations might also provide evidences that the incidence and severity of HZ increase with age.

CONCLUSION

VZV-CMI in HZ patients assessed by the skin test with VZV-skin test antigen demonstrated age-related differences in magnitude and recovery speed after onset of the disease. To consider preventive and therapeutic measures against HZ, the result will provide useful ideas in that incidence and severity of HZ increase with age.

ACKNOWLEDGEMENTS

We thank F. Ohnishi, T. Nozaki, and K. Matsuda for their support in data management and analysis. We also thank K. Maeda for the laboratory examination of patient samples.

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Cite this article

Okuno F, Minami M, Masuda R, Okuno T, Okuno Y (2016) Effect of Age on Varicella-Zoster Virus-Specific Cell-Mediated Immunity in Patients with Herpes Zoster Assessed by Skin Tests with Varicella-Zoster Virus Antigen. J Dermatolog Clin Res 4(1): 1065.