Comparison of the Sensitivity and Specificity of Tzanck Smear and Immunofluorescence Assay for the Diagnosis of Cutaneous Herpes Simplex Virus and Varicella Zoster Virus Infections in a Real-life Clinical Setting

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ABSTRACT

Objective: This research aims to compare (1) the sensitivity and specificity of Tzanck smear and indirect immunofluorescence assay (IFA) which detect viral antigen for the diagnosis of cutaneous herpes simplex virus (HSV) and varicella zoster virus (VZV) infections; and (2) the detection rates of the tests among various patient groups and lesion morphologies.

Materials and Methods: This retrospective study reviewed 440 and 172 samples from patients with clinically suspicious cutaneous HSV and VZV infections, who underwent both Tzanck smear and IFA, respectively. The gold standard of the study was defined by showing agreement of diagnostic codes between initial and subsequent visits.

Results: For HSV infections, the respective sensitivity and specificity of Tzanck smear were 32.8% and 96.6% whereas those of IFA were 60.7% and 100%. As to VZV infections, the sensitivity and specificity of Tzanck smear were 54.3% and 97.8%, respectively, while the corresponding values of IFA were 71.7% and 100%. According to disease characteristics and lesion morphologies, IFA provided substantially higher ability to detect HSV than the Tzanck smear, especially in patients with immunosuppressed conditions. Tzanck smear and IFA demonstrated no statistically significant difference for early-onset VZV infections (≤ 3 days).

Conclusion: The Tzanck smear and IFA had higher sensitivities for detecting VZV than HSV infections. IFA testing is recommended in patients with immunosuppressed conditions who present with suspected cutaneous HSV infection. Despite the overall sensitivity and specificity of IFA being greater than those of Tzanck smear especially in HSV infections, the latter test is comparable option for early-onset VZV infections.

Keywords: Herpes simplex virus; varicella zoster virus; Tzanck smear; immunofluorescence (Siriraj Med J 2021; 73: 305-311)

INTRODUCTION

Herpes simplex virus (HSV) and varicella zoster virus (VZV) are large, enveloped DNA viruses belonging to the Herpesviridae family.[1] Although cutaneous infections of HSV and VZV are mainly diagnosed by history-taking and clinical characteristics, laboratory examinations are sometimes needed for a definite diagnosis.[2]

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Multiple laboratory options currently available to diagnose HSV and VZV infections can be categorized into four groups: (1) morphological tests, such as the Tzanck smear and tissue histopathology; (2) immunomorphological tests, like immunofluorescence and immunoperoxidase staining; (3) serological methods, for instance, enzyme-linked immunosorbent assay and immunoglobulin M/G titer; and (4) virological testing, for example, viral culture and viral polymerase chain reaction. A viral culture was long considered the gold-standard diagnostic test before the advent of polymerase chain reaction testing.[1,3]

HSV and VZV are more commonly observed as cutaneous infections rather than as infections of internal organs.[1,4] Moreover, their cutaneous symptoms are usually not severe and can be self-limited. Ideally, the chosen diagnostic test for these infections should be easy to perform, give a rapid result, and be inexpensive. In outpatient dermatological settings, the Tzanck smear and immunofluorescence staining are therefore the most frequently ordered tests at our clinic.

Previous research has found that the sensitivity of Tzanck smear ranges from 34% to 78% in detecting HSV, and from 26% to 64% in detecting VZV.[5] However, with a proficient technician, the sensitivity of the test may rise to 80% and its specificity to 90%.[6-8] Even though Tzanck smear is currently considered obsolete in many countries[1], it still has an important role in developing countries. There, the newer testing methodologies are not only often deemed to be too expensive, but also have the drawbacks of slower turnaround times and, sometimes, a lack of availability.

In terms of immunofluorescence testing, previous studies revealed that the sensitivity and specificity of immunofluorescence staining was greater than those of Tzanck smear, particularly in the case of VZV infections. The sensitivity of immunofluorescence staining in detecting cutaneous HSV infections was found to be around 50% - 100% compared with the viral culture technique, but its sensitivity in detecting VZV infections exceeded that of the viral culture. Moreover, the specificity of immunofluorescence staining was nearly 100%, and it was able to discriminate between the HSV1/2 and VZV pathogens.[2,8]

Earlier studies of the sensitivity and specificity of the Tzanck smear and immunofluorescence testing were usually performed in a small number of patients, and compared with those of the viral culture technique as the gold standard diagnostic modality.[9] It is also noteworthy that few details of the infected patients or the clinical morphologies of their lesions were reported.[2,5,9] Thus, the objective of the current research was twofold. The first aim was to compare the sensitivity and specificity of the Tzanck smear and immunofluorescence assay for the diagnosis of cutaneous HSV and VZV infections in a larger population and in a real-life setting. The secondary aim of this study was to compare the detection rates of two tests among various subgroups of patients, durations, and clinical morphologies of lesions.

MATERIALS AND METHODS

This retrospective research was approved by the Siriraj Institutional Review Board. (Si 333/2020) The study reviewed the samples taken from patients with clinically suspicious cutaneous HSV (ICD-10 B00) and VZV (ICD-10 B01-B02) infections. During 2012-2019, the samples had initially been collected from the Infection Control Clinic of the Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University.

All eligible patients had to undergo both a Tzanck smear and immunofluorescence testing for HSV or VZV. For each patient, demographic data, onset of lesions, morphology of the lesions, suspicious diagnosis, and comorbidities had been collected at their first visit. Tzanck-smear and IFA specimens that were reported as being inadequate for diagnosis were excluded from the study. The sensitivity and specificity of the tests were subsequently analyzed only in clinically confirmed cases. For the purposes of this study, the reference standard for clinically confirmed diagnosis was an agreement of diagnostic codes between the first and subsequent follow-up visits (determined by a dermatologist).

The Tzanck smear was performed by scraping the base of lesions with a blunt scalpel blade and spreading the sample as a thin layer on microscope slides. The slides were then fixed with 100% methyl alcohol for 10 minutes and stained with eosin solution for 20 seconds. After being rinsed with distilled water, the slides were stained with 3% methylene blue for 60 seconds, rinsed with distilled water, and allowed to dry. The slides were subsequently examined under a light microscope. A positive Tzanck smear was defined as the presence of herpetic cytopathic effects, such as the presence of multinucleated giant cells. Throughout the 7-year study period, the Tzanck tests were performed by the same, proficient technician, which obviated inter-rater variability. The typical test turnaround time was 15 minutes.

As to the immunofluorescence staining, our hospital used the technique of an indirect immunofluorescence assay (IFA) with commercial reagent kit containing HSV type 1, 2 antibodies (Bio-Rad Laboratories) and VZV monoclonal antibodies (Merck, Ltd). Specimens scraped from the base of the lesions were fixed in acetone for 15

minutes before adding a primary antibody. The smear was then incubated at 37 degrees Celsius for 30 minutes and rinsed with phosphate-buffered saline; pathogen-specific fluorescein-tagged secondary antibodies were subsequently added, and the mixture was incubated at 37 degrees Celsius for 30 minutes. The smear was examined with an epifluorescence microscope by virology technicians. The test turnaround time was 3 days.

All statistical analyses were undertaken using SPSS Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were presented as numbers or numbers with percentages, while continuous variables were shown as means with standard deviations. The sensitivities and specificities of the two tests were calculated. For the correlation between the tests, Cohen's Kappa coefficient (κ) was reported. A p-value of less than 0.05 was considered to indicate statistical significance.

RESULTS

A total of 440 and 172 specimens from patients with clinically suspicious cutaneous HSV and VZV infections, respectively, were reviewed. The demographic data of the included patients are detailed in Table 1. The mean age of the participants was around 50. The majority of them were tested more than 3 days after onset of the lesions. For cutaneous HSV infections, the main characteristic of the tested lesions was non-vesicle (66.2%) whereas vesicle was the major type of tested lesions in cutaneous VZV infections (70.1%).

TABLE 1. Demographic data and disease characteristics of the included patients.

	HSV N = 440		VZV N = 172	
	N	(%)	N	(%)
Demographic				
Sex, Female	239	(54.3)	103	(59.9)
Age (mean) ± SD	51.4 ± 18.4		56.1 ± 18.2	
Underlying disease				
Hypertension	101	(23.0)	36	(20.9)
Diabetes mellitus	49	(11.1)	20	(11.6)
Autoimmune disease	51	(11.6)	16	(9.3)
Cancer	51	(11.6)	32	(18.6)
HIV (n = 240; n = 62)	45	(18.7)	6	(9.7)
On immunosuppressive drugs	66	(15.0)	26	(15.1)
Disease characteristics				
Onset ≤ 3 days (n = 420; n = 167)	165	(39.3)	75	(44.9)
Taken oral acyclovir before testing	29	(6.6)	21	(12.2)
Site				
Mucosa	233	(53.0)	8	(4.7)
Skin	207	(47.0)	164	(95.3)
Morphology (n = 420; n = 164)				
Vesicle	142	(33.8)	115	(70.1)
Non-vesicle	278	(66.2)	49	(29.9)
Erosion	113	(26.9)	6	(3.7)
Ulcer	105	(25.0)	3	(1.8)
Papule	26	(6.2)	19	(11.6)
Crust	12	(2.9)	12	(7.3)
Erythematous macule	11	(2.6)	8	(4.9)
Verrucous plaque	11	(2.6)	1	(0.6)

Of 440 specimens, 229 (52%) had clinically confirmed diagnosis of HSV, while 127 (73.8%) of 172 specimens had clinically confirmed diagnosis of VZV. Table 2 compares sensitivities, specificities, positive predictive values, and negative predictive values of the Tzanck smear and IFA related to the clinically confirmed cases. For HSV infections, the respective sensitivity and specificity of Tzanck smear were 32.8% and 96.6% whereas those of IFA were 60.7% and 100%. As to VZV infections, the sensitivity and specificity of Tzanck smear were 54.3% and 97.8%, respectively, while the corresponding values of IFA were 71.7% and 100%. The sensitivity and specificity of IFA was substantially higher than those of the Tzanck smear. In addition, the Tzanck smear and IFA showed higher sensitivities in detecting VZV infections than HSV infections. The Kappa agreements between the Tzanck smear and IFA in detecting HSV and VZV infections were moderate, with the values of 0.4 and 0.5, respectively.

A comparison was made on the sensitivity of the Tzanck smear and IFA for cutaneous HSV and VZV infections among various subgroups of patients, durations, and clinical morphologies of lesions.

In cutaneous HSV infections (Table 3), it was found that IFA yielded a greater sensitivity in detecting HSV infections than the Tzanck smear in nearly all subgroups of patients with statistical significance. In terms of disease onset, IFA showed the sensitivity around 60% in both early-onset (≤ 3 days) and late-onset (> 3 days) HSV infections whereas the percentage of HSV detection from the Tzanck smear dropped from 45.2% in early-onset to 24.8% in late-onset HSV infections. Furthermore, the sensitivity rate of Tzanck smear in patients taken oral acyclovir before testing was very low (19%) while IFA in these patients still yielded a sensitivity rate nearly 60%. Clinical morphologies of the lesions also determined the sensitivity rates of both tests. IFA also showed a high sensitivity (around 60%) in detecting HSV in vesicle and non-vesicle lesions. The detection rate of Tzanck smear was only 45.7% in vesicle lesions and very low in non-vesicle lesions (25.4%).

In cutaneous VZV infections (Table 4), even though IFA yielded a greater sensitivity than Tzanck smear but the magnitude of difference was not much as in case of cutaneous HSV infections. Interestingly, the Tzanck smear was not statistically different from the IFA in some situations such as early-onset (≤ 3 days) of infection, non-vesicular lesions and patients who had a history of taking oral acyclovir before testing.

DISCUSSION

The current research demonstrated that both the Tzanck smear and IFA had a higher sensitivity in detecting VZV infections than HSV infections. The comparison of their sensitivities and specificities revealed that the IFA was superior overall to the Tzanck smear, corresponding with earlier findings.[3] The higher sensitivity of IFA was significantly shown in nearly all situations of cutaneous HSV infections.

However, in cutaneous VZV infections, the sensitivity rate of Tzanck smear was not far different from IFA. Our study showed that the Tzanck smear is still comparable to the IFA in some VZV-infection situations, such as the early-onset (≤ 3 days) of infection. This can be explained by the Tzanck smear having a high ability to detect VZV infections[5], as well as by a shorter duration of disease normally resulting in an increase in the sensitivity of the Tzanck smear.[10]

The type of lesions is also an important factor in determining the sensitivity of the two tests. Prior research

TABLE 2. The sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) for the cutaneous HSV and VZV infections.

HSV infection	% Sensitivity	% Specificity	PPV*	NPV**
Tzanck	32.8	98.6	96	57.5
IFA	60.7	100	100	70.1
VZV infection				
Tzanck	54.3	97.8	98.6	43.1
IFA	71.7	100	100	55.6

Abbreviations: *PPV: positive predictive value, **NPV: negative predictive value

TABLE 3. Comparison of the sensitivities of detection of the Tzanck smear and IFA for cutaneous HSV infections.

	Positive Tzanck smears N = 229		Positive IFAs N = 229		P-value
	N	(%)	N	(%)	
Demographics					
Sex F	54/139	(38.8)	88/139	(63.3)	< 0.001
M	21/90	(23.3)	51/90	(56.7)	< 0.001
Age ≤ 60 years	46/145	(31.7)	87/145	(60.0)	< 0.001
Age > 60 years	29/84	(34.5)	52/84	(61.9)	< 0.001
Underlying disease					
Hypertension	15/57	(26.3)	33/57	(57.9)	0.001
Diabetes mellitus	9/28	(32.1)	18/28	(64.3)	0.022
Autoimmune disease	6/25	(24.0)	17/25	(68.0)	0.003
Cancer	10/22	(45.5)	14/22	(63.6)	0.388
HIV	8/30	(26.7)	22/30	(73.3)	0.001
On immunosuppressive drugs	9/32	(28.1)	24/32	(75.0)	< 0.001
Disease characteristics					
Onset ≤ 3 days	42/93	(45.2)	59/93	(63.4)	0.003
Onset > 3 days	31/125	(24.8)	76/125	(60.8)	< 0.001
Taken oral acyclovir before testing	4/21	(19.0)	12/21	(57.1)	0.008
Site					
Mucosa	26/102	(25.5)	56/102	(54.9)	< 0.001
Skin	49/127	(38.6)	83/127	(65.4)	<0.001
Morphology					
Vesicle	46/102	(45.1)	67/102	(65.7)	0.001
Non-vesicle	28/116	(24.1)	65/116	(56.0)	<0.001
Ulcer	12/54	(22.2)	29/54	(53.7)	<0.001
Erosion	8/41	(19.5)	24/41	(58.5)	<0.001
Hypertrophic	4/9	(44.4)	6/9	(66.7)	0.625
Crust	2/6	(33.3)	2/6	(33.3)	1.000
Papule	2/4	(50.0)	2/4	(50.0)	1.000
Erythematous macule	0/2	(0.00)	2/2	(100)	-

TABLE 4. Comparison of the sensitivities of detection of the Tzanck smear and IFA for cutaneous VZV infections.

	Positive Tzanck smears N = 127		Positive IFAs N = 127		P-value
	N	(%)	N	(%)	
Demographics					
Sex F	43/76	(56.6)	56/76	(73.7)	0.011
M	25/51	(49.0)	35/51	(68.0)	0.021
Age ≤ 60 years	40/69	(58.0)	50/69	(72.5)	0.031
Age > 60 years	28/58	(48.3)	41/58	(70.7)	0.007
Underlying disease					
Hypertension	16/31	(51.6)	25/31	(80.6)	0.012
Diabetes mellitus	10/16	(62.5)	14/16	(87.5)	0.125
Autoimmune disease	7/12	(58.3)	11/12	(91.7)	0.125
Cancer	10/21	(47.6)	14/21	(66.7)	0.344
HIV	3/5	(60.0)	4/5	(80.0)	1.000
On immunosuppressive drug	15/19	(78.9)	15/19	(78.9)	1.000
Disease characteristics					
Onset ≤ 3 days	40/58	(69.0)	47/58	(81.0)	0.092
Onset > 3 days	27/67	(40.3)	43/67	(64.2)	0.002
Taken oral acyclovir before testing	6/20	(30.0)	10/20	(50.0)	0.344
Site					
Mucosa	1/3	(33.3)	3/3	(100)	–
Skin	67/124	(54.0)	88/124	(71.0)	0.001
Morphology					
Vesicle	55/95	(57.9)	75/95	(78.9)	<0.001
Non-vesicle	8/26	(30.8)	13/26	(50.0)	0.180
Crust	4/10	(40.0)	7/10	(70.0)	0.250
Papule	2/9	(22.2)	3/9	(33.3)	1.000
Erosion	1/3	(33.3)	0/3	(00.0)	-
Erythema	1/2	(50.0)	1/2	(50.0)	1.000
Ulcer	0/2	(00.0)	2/2	(100)	-

has found that vesicles and blisters generally yield higher sensitivities of detection than other types of lesions with these two tests[2,10]; the present study had a similar finding. However, our work determined that there was no statistical difference in the sensitivities of detection of the Tzanck smear and IFA for non-vesicular lesions of VZV. It is possible that the non-vesicular lesions which were usually in the late stage of infection might have a low number of virus and was therefore comparable difficult for both tests to yield the positive result[1], or the number of specimens enrolled in the non-vesicular-VZV group might not be enough to provide a statistically significant difference.

Furthermore, in terms of underlying disease of the patients, the sensitivity of IFA in cutaneous HSV infections was prominently higher with statistical significance compared to Tzanck smear particularly in patients with immunosuppressive conditions including HIV infection and taking immunosuppressive agents. The detection ability of HSV by Tzanck smear in these patients was around 30% which was substantially lower than IFA (above 70%). IFA testing in suspected cutaneous HSV patients with immunosuppressed conditions is recommended. Whether the underlying disease would affect the yield of Tzanck smear or IFA test in cutaneous VZV infections was difficult to conclude. As the majority of underlying diseases or comorbidity subgroups in cutaneous VZV infections contained a small number of patients.

There are some limitations in this study. The reference standard for confirmed cases used in this study was a clinical diagnosis by dermatologists on two separate occasions, rather than a standard laboratory investigation like viral culture or polymerase chain reaction testing. The explanation is that this was a retrospective study conducted at a dermatology outpatient clinic in a developing country and in a real-life clinical setting, where dermatologists need to make prompt diagnosis without the ready utilization of sophisticated laboratory testing. For example, the use of viral culture tends to be avoided because specimens need to be promptly transported on ice to a laboratory, refrigerated-culture media are required, and long turnaround times are involved. Polymerase chain reaction testing, generally recognized as the platinum standard for VZV and HSV infections, has a higher sensitivity and specificity than any other test. Nevertheless, its relatively high cost and limited accessibility are problematic for developing countries.

In addition, the number of patients with morphology of vesicle were substantially higher in VZV (70.1%) than HSV (33.8%). This might affect the overall sensitivity of both tests and was another limitation of our study. However, focusing in subgroup analysis based on morphology of the lesions, Tzanck smear and IFA still yielded higher sensitivity in VZV than HSV in either vesicle or non-vesicle subgroup.

In conclusion, this study revealed the sensitivity and specificity of the Tzanck smear and IFA which could be used as a benchmark in a real-life setting. The tests had a higher sensitivity in detecting VZV infections than HSV infections. Even though IFA had an overall higher sensitivity and specificity than the Tzanck smear, the Tzanck smear is a comparable option to IFA for early-onset VZV infections. This information is valuable, especially in an outpatient dermatologic clinic, where prompt diagnosis of HSV and VZV infections is required.

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