Tzanck smears in herpes simplex virus infections

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INTRODUCTION

Herpes simplex viruses (HSV) have a worldwide distribution and produce primary, latent, and recurrent infections. Over one-third of the world population are thought to be capable of transmitting the virus during periods of viral shedding 1. All previous studies suggested that presence of HSV in skin lesions of patients could be as a triggering factor for the diseases such as pemphigus which could not be attributed to the suppressive therapy 2. HSV infection is caused by two different virus types (HSV-1 and HSV-2), which can be distinguished clinically rather than via laboratory tests. Both types seem to produce identical patterns of infection. HSV infection has two phases: the primary infection after which the virus becomes established in a nerve ganglion, and the secondary phase which is characterized by recurrent diseases at the same site. A mild uncomplicated form of infection requires no treatment while the severe forms of primary infection, herpetic encephalitis, infection during pregnancy, ocular infection, visceral infection, and infection in immunocompromised hosts (diffuse herpetic infections) are all serious and life-threatening and require early diagnosis and treatment. Clinical diagnosis is usually easy and immediate but doubts may arise in some cases (e.g. Kaposi’s varicelliform eruption, recurrent intraoral herpes simplex vs. miliaria or impetigo) 3.

For some patients, a rapid accurate diagnosis of herpetic infection is a critical part of management and prognosis including patients with primary and secondary immunodeficiency, near term pregnant women, newborns, medical personnel caring for critically ill patients, and those in whom the social

Background: The diagnosis of herpes simplex virus may require virological confirmation. Tzanck smear is an old, rapid, cost effective but nonspecific method that has been recently re-evaluated as a method for the diagnosis of herpes virus infection. This study was conducted to compare Tzanck smear and viral culture in the diagnosis of herpes simplex virus infection in patients clinically suspected to be infected with this virus.

Method: Materials obtained from a fresh vesicle were used to prepare Tzanck smears and viral cultures.

Result: In this study, 40 (71.4%) of the 56 samples were culture positive while Tzanck smears were positive in 36 (64.3%) patients. We found that the sensitivity and specificity of the Tzanck test was 90% and 100% respectively when compared to cell culture. Moreover, the positive predictive value (PPV) and negative predictive value (NPV) of the Tzanck test was 100% and 80%, respectively.

Conclusion: The Tzanck smear has its limitations but is still a suitable rapid, easy, and cost effective diagnostic method for herpes simplex virus infections, especially when viral culture or other virological methods are not available.

Keyword: culture, diagnosis, herpes simplex virus, Tzanck smear
stigma of herpes demands an accurate diagnosis. In all of these situations, the Tzanck test provides a rapid and reliable diagnosis of infection by the herpes virus group.

The sensitivity of all laboratory methods depends on the stage of lesions. Herpes simplex infections may be clarified by any of the several diagnostic procedures. Virus culture which is considered to be the gold standard for the diagnosis of herpes simplex virus requires at least 24-48 hours and therefore does not provide an immediate diagnostic support but can distinguish between HSV-1 and HSV-2. Cytodiagnosis (Tzanck smear) is one of the old, fast, easy, and valuable tests for rapid diagnosis of herpes viruses although it does not differentiate between herpes simplex and varicella viruses. In this study, we evaluated rapid viral diagnosis using the Tzanck smear and compared it with viral culture.

PATIENTS AND METHODS

In this descriptive study, a nine-month trial was conducted to evaluate patients with clinically suspected herpetic skin lesions (with primary or recurrent skin lesions presenting as grouped vesicles on an erythematous base) attending the dermatology clinics of Ghaem and Imam Reza Hospitals. The local ethical committee approved the study. After explaining the procedure to the patients, they signed informed consent forms if they were willing to participate in the study. The viral cultures and Tzanck smears were performed. Exclusion criteria were ulcerative, dry, and erosive lesions, herpetic lesions with superimposed infections, and a history of previous use of topical or systemic antiviral drugs.

The primary efficacy end point was the time to first recurrence of HSV infection, defined as the number of days from randomization until the first onset of lesions. For patients who did not reach this end point, censored event-free times were calculated as to the number of days until the last day when no event was recorded. We selected the statistical sample-size estimation based on the primary efficacy end point described above. Moreover, the sample size of our investigation was based on a study by Oranje et al on the sensitivity and specificity of the Tzanck smear compared to cell culture as a gold standard test. If hazard functions were assumed to be proportional, 56 patients were needed per active-treatment arm with an allowance of 10%, a study power of 80%, and a confidence interval of 95%.

Sampling was performed by an expert. Two samples were taken, one for the viral culture and the other for the Tzanck smear. For the viral culture, vesicles were punctured and the fluid content was collected with a sterile cotton-tipped applicator that was rubbed vigorously on the base of the lesion. The specimen was then inoculated onto the Heila cell media (Pasture Institute, Iran). For Tzanck smear preparation, the intact roof of a fresh vesicle was scraped with a scalpel. The obtained cellular material was then spread as a thin layer onto a microscopic slide, fixed with alcohol, and then stained with hematoxylin-eosin. All of samples for viral cultures and Tzanck smears were assessed by the same virologist and pathologist, respectively.

RESULTS

Of 74 patients clinically suspected to have HSV infection, 18 patients whose lesions were only ulcers or crusts were excluded from the study. A total of 56 patients were enrolled. The baseline demographic data of the enrolled subjects is summarized in Table 1. They were 27.16 ±15.73 years old. HSV infections were more common in women than men. Lip and the perioral area were the most common sites of infection. The results showed that of 56 patients, the Tzanck smear and cell culture were both positive in 36 (64.3%) patients. Furthermore, 40 patients (71.4 %) had positive cell cultures of whom 36 (90%) had positive Tzanck smears, showing that the Tzanck smear was negative in 4 (10%) patients with positive cell cultures. In 16 (80%) patients with negative Tzanck smears, cell culture was also negative and cell cultures were positive.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24 (42.9)</td>
</tr>
<tr>
<td>Female</td>
<td>32 (57.1)</td>
</tr>
<tr>
<td>Site</td>
<td></td>
</tr>
<tr>
<td>Lip and perioral</td>
<td>39 (69.6)</td>
</tr>
<tr>
<td>Genital area</td>
<td>7 (12.5)</td>
</tr>
<tr>
<td>Cheek</td>
<td>4 (7.1)</td>
</tr>
<tr>
<td>Oral mucosa</td>
<td>2 (3.6)</td>
</tr>
<tr>
<td>Other site</td>
<td>4 (7.1)</td>
</tr>
</tbody>
</table>

Table 1. Patients’ demographics
positive only in 4 (20%) of these patients.

In our study, the sensitivity and specificity of the Tzanck test, when compared to the cell culture as a gold standard for the diagnosis of herpes infection, was 90% and 100%, respectively. Moreover, the positive (PPV) and negative predictive value (NPV) of the Tzanck test was 100% and 80%, respectively.

DISCUSSION

Cytology was first used in cutaneous disorders by Tzanck in 1947. Since then, cytology has been used for diagnosis of various dermatoses including infections (mainly HSV and VZV infections and leishmaniasis), vesiculobullous disorders (pemphigus), genodermatoses (Hailey-Hailey disease), tumors (basal cell carcinoma, squamous cell carcinoma, Paget disease and melanoma), and granulomatous disorders. A definite diagnosis of any HSV infection is based on laboratory methods including viral culture, cytologic detection, histopathologic studies, polymerase chain reaction, serology, western blot assay, and immune fluorescent or immuno peroxidase techniques. Viral culture is considered the gold standard for the diagnosis of herpes simplex virus but it does not provide an immediate diagnostic support and requires one to five days. The detection of herpes virus DNA by polymerase chain reaction (PCR) is another method that can yield confirmatory results within a few hours. PCR has proved to be at least comparable with the virus culture, if not superior to it, and can identify the virus and the type at other sites. Western blot assay is also very sensitive and specific, but it is only available for research purposes. Other procedures such as skin biopsy, electron microscopy, immune fluorescent or immune-peroxidase chain reaction, and serology can be used to clarify herpes simplex infections. However, these methods are expensive and time taking and not available everywhere. The Tzanck smear is an old diagnostic procedure for the diagnosis of herpes infections. It looks for giant cells and inclusion bodies. It is important to chose the earliest vesicle for cytological smear preparation. Cells from the base of the vesicle will offer the best opportunity to detect the virus. The smears are positive in approximately 75% of virus cultures. However, this test is not specific because the lesions of herpes simplex, herpes zoster, and varicella have an identical appearance. Nonetheless, this method is very rapid and simple and available everywhere. In our study, 71.4% of the samples had positive virus cultures that was similar to the results of a study by as Nahass et al, and Tzanck smears were positive in 90% of the patients who had positive virus cultures, which was similar to a report by Salmon.

A previous study by Oranje et al on 76 patients showed that the sensitivity and specificity of the Tzanck smear in patients with clinical herpetic infections was more than 80% and 90%, respectively. However, we found higher sensitivity and specificity, i.e. 90% and 100% respectively. All previous studies emphasize that the Tzanck smear is simple, inexpensive, easy to perform, rapid, and does not require a specialized laboratory, but experience and correct technique of sampling are necessary.

Nowadays, only a small minority of dermatologists use the Tzanck smear in their practice. The Tzanck smear has of course its limitations and is by no means a substitute for other diagnostic procedures such as culture. In general, our results showed the value of cell culture for herpetic lesions and also demonstrated that the Tzanck smear is suitable for the diagnosis of herpes simplex virus infections when culture or other methods are not available, especially in developing countries because cytodiagnosis is a simple, rapid, inexpensive, and reliable diagnostic tool in experienced hands that should be reintroduced.

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