

Comparison of nested-polymerase chain reaction and virus culture for the diagnosis of genital herpes simplex virus infection

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ABSTRACT

Introduction: This study was performed to compare nested-polymerase chain reaction (PCR) with viral culture as a diagnostic tool for genital herpes simplex virus (HSV) infection in a sexually transmitted infection (STI) clinic in Singapore.

Methods: 103 consecutive patients presenting with clinical features suggestive of genital herpes were enrolled in the study. Two swabs were obtained from each patient. On one swab, cell culture and typing was performed, and on the second swab, nested-PCR was performed and the infecting viral type was determined by using type-specific primers.

Results: 63 patients (61.2 percent) had a positive PCR test for HSV. Of these, 13 patients (20.6 percent) had HSV type 1 (HSV-1), 50 patients (79.4 percent) had HSV type 2 (HSV-2). HSV-1 and HSV-2 were detected by viral culture in only six patients and 24 patients, respectively. The sensitivity of cell culture compared to PCR was 46.1 percent for HSV-1 infection and 48 percent for HSV-2 infection. PCR further detected an additional 52.4 percent of HSV cases. The specificity of PCR was 100 percent.

Conclusion: Nested-PCR has been shown in this study to be an effective diagnostic and typing method for HSV infection in a STI clinic in Singapore with its higher sensitivity and specificity to routine viral isolation in cell culture.

Keywords: genital herpes, herpes simplex virus, polymerase chain reaction, sexually transmitted infection, virus culture

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INTRODUCTION

Genital herpes is a significant public health disease worldwide. Primary genital herpes occurs from three days to two weeks after exposure to an infected sexual partner.⁽¹⁾ Acute genital herpes presents with a group of

painful vesicles which progress to ulcers over several days. Symptoms and the formation of new lesions continue together with active viral shedding for seven to ten days, followed by crusting of the erosions and complete resolution of symptoms taking up to three weeks. Genital herpes is commonly caused by infection with herpes simplex virus type 2 (HSV-2); however, with the lack of use of condoms for oral sex, herpes simplex virus type 1 (HSV-1) is being increasingly implicated. HSV-1 and HSV-2 are both frequently acquired via contact with infectious secretions present on oral, genital or anal mucosa. Clinically, genital herpes caused by both HSV-1 and HSV-2 infections can present as a vesicle, ulcer or crusted erosion, depending on the duration of the lesion at the time of presentation of the individual to the clinic. HSV-1 infection differs from HSV-2 infection as it is associated with fewer recurrences, less frequent viral shedding and hence a better prognosis. HSV-2 infection can cause frequent relapses in some individuals resulting in significant emotional and psychosexual morbidity. It has also been shown that HSV infection increases the risk of transmission of HIV among infected populations, and that coinfecting individuals are more likely to transmit the infection.^(2,3)

It is important for clinicians to make an accurate diagnosis in genital herpes infection due to the consequent psychological, social and sexual impact on individuals, as well as their sex partners. Virus culture-based isolation and typing of HSV is the currently used "gold standard" diagnostic and typing test for acute genital herpes infections.⁽⁴⁾ However, there is mounting evidence that virus isolation by cell culture is highly insensitive, compared to polymerase chain reaction (PCR) analysis.⁽⁵⁻⁹⁾ This causes a significant number of false-negatives. The sensitivity by virus isolation declines according to the lesion duration at the time of sampling:⁽¹⁰⁾ the isolation rate is higher from vesicular lesions and drops towards the crusted stage of the lesion. In addition, the turnaround time may take up to two weeks for virus isolation, compared to less than 48 hours for PCR analysis. In Singapore, genital herpes is the most common cause of genital ulceration. Over the past ten years from 1996 to 2006, the number of

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Table 1. Comparison of HSV detection and typing results by virus culture and PCR.

Virus culture	PCR			Total
	HSV-1+	HSV-2+	HSV-	
HSV-1+	6	0	0	6
HSV-2+	0	24	0	24
HSV-	7	26	40	73
Total	13	50	40	103

Sensitivity of virus culture: $(6+24) / 103 \times 100\% = 29.1\%$

Sensitivity of PCR: $(13+50) / 103 \times 100\% = 61.2\%$

cases of genital herpes seen per year in the Department of Sexually Transmitted Infection (STI) Control (DSC), National Skin Centre, had increased from 429 cases to 1,152 cases. The majority of patients were males with an incidence ratio of 4:1 male to female patients. We present our evaluation results of a nested-PCR,⁽⁷⁾ which is able to simultaneously detect and type HSV, in the diagnosis of genital herpes.

METHODS

Patients attending the DSC clinic with a history and clinical presentation suggestive of genital herpes were enrolled in the study by the STI clinician in attendance. A total of 103 patients were enrolled, of which 81 were male and 22 were female. There were 63 Chinese, 18 Indians, ten Malays, four Caucasians, two Bangladeshis, two Nepalese, two Indonesians and one Thai patient. The ages of the patients ranged from 18 years to 70 years, with the average age being 35.5 years. Written consent was obtained from each patient.

Dacron-tipped swabs were taken in duplicate from every patient in a pre-randomised order. One was sent for routine virus isolation in a tertiary teaching hospital, and the other to dry for DNA extraction in our laboratory. Swabs were sampled from the earliest clinical stage of lesion at presentation. DNA extraction and PCR analysis were performed following the same methodology as previously described.⁽⁷⁾ Briefly, cells were washed off from swabs and pelleted by centrifugation. Cell pellets were digested in digestion buffer. DNA was extracted with phenol-chloroform, precipitated in absolute ethanol and washed according to standard protocol.

PCR products were amplified using a nested protocol with the same thermocycling parameters, except that the amplification cycles were 45 in the first round PCR and 25 in the second round PCR. In the first round PCR, the outer primers TO1 (5'-GGCCAGCAGATCCGCGTCTT-3') and TO2 (5'-GCTGGGGTACAGGCTGGCAA-3') were used, which are common for HSV-1 and -2; in the second round PCR, the inner primers TIA (5'-CTGCCGGACACCCAGGGGCG-3'), TIB1 (5'-

CCCCCCCTCCTCGCGTTCGT-3'), and TIB2 (5'-CGACCTCCTCGCGCTCGTCC-3') were used. TIA is common for HSV-1 and -2, TIB1 and TIB2 are HSV-1 and HSV-2 specific, respectively. The cycling parameters were initial denaturing at 94°C for three minutes, followed by cycles of denaturing at 94°C for 30 seconds, annealing at 65°C for 30 seconds, extension at 72°C for 45 seconds and a ten-minute final extension at 72°C. The PCR reaction mixtures contained 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 0.01% (w/v) gelatin, 200 µM of each dNTP, 0.5 µM each primer, 2.5 U of Taq DNA polymerase (Promega, WI, USA), and 10 µL of extracted DNA in the first round PCR, or 5 µL of the first round PCR products in the second round PCR. PCR products were analysed using agarose gel. To prevent false-positive and false-negative results, in addition to extensive general measures taken in the laboratory setting, sample processing, DNA extraction, reagent preparation, and experimental personnel, a number of positive and negative internal controls were included in each experiment.

RESULTS

We compared the sensitivity and specificity of the nested-HSV PCR with those of virus culture in both detection and typing using genital herpes samples. Of the 103 patients enrolled in the study, 63 (61.2%) had a positive PCR result for HSV, as compared to 30 (29.1%) by virus culture. The nested-PCR was able to detect an additional 33 (52.4%) more HSV infections compared to the virus culture (Table I). Of the 63 PCR HSV-positive cases, 13 (20.6%) had HSV-1 infection and 50 (79.4%) had HSV-2 infection. HSV-1 and HSV-2 were detected by the virus culture in six patients (20.0%) and 24 patients (80.0%), respectively. Therefore, the PCR typing revealed a very similar profile of HSV type distribution to that obtained by the virus culture. The sensitivity of the virus culture as compared to PCR in this study was 46.1% for HSV-1 infection and 48% for HSV-2 infection. The specificity of PCR was 100% as there were no cases with positive virus culture and negative PCR results. The specificity of virus culture versus PCR in genotyping of HSV-1 and -2 was also 100%.

The results of HSV detection by both methods with regard to lesion morphology and duration at the time of presentation to the clinic are shown in Tables II and III, respectively. The detection rates of genital HSV infection by PCR were superior to that of virus culture, irrespective of the lesion morphology or duration at the time of sampling. Both virus culture and PCR had the best performances from vesicle samples, having detected, respectively, 14 (60.9%) and 22 (95.7%) HSV infections out of 23 vesicle samples. In comparison, HSV-positive

rates were significantly reduced in the samples taken from ulcers and crusted erosions by both methods. In terms of lesion duration at the time of sampling, both virus culture and PCR detected higher rates of HSV infections for lesions less than ten days old; while for lesions older than ten days, the HSV detection rates decreased significantly by both methods.

Of the 30 patients diagnosed to have HSV infection using the virus culture technique, 24 (80.0%) patients had first episode genital herpes infection and six (20.0%) patients had recurrent genital herpes infection. Of the 63 HSV PCR-positive patients, 44 patients (69.8%) had first episode genital herpes infection and 19 (30.2%) patients had recurrent genital herpes infection. HSV-1 infection accounted for 25.0% of first episode infections and 5.3% of recurrent infections, while HSV-2 infection accounted for 75.0% of first episode infections and 94.7% of recurrent infections (Table IV).

DISCUSSION

Nested-PCR has been shown in this study to be a potential diagnostic tool for HSV infection in a STI clinic in Singapore. The results indicate a higher sensitivity of PCR to routine viral isolation in cell culture, regardless of the morphology or duration of the lesion at presentation. In this study of 103 patients with a clinical diagnosis of genital HSV infection, in which 63 patients had a positive PCR test for HSV, only 30 patients tested HSV-positive by virus culture. There were no patients with positive viral culture and negative PCR results, indicating 100% specificity of the nested-PCR technique. PCR was able to further detect an additional 52.4% of HSV infections which were not picked up by the virus culture technique. This represents a significant number of patients in whom the diagnosis of HSV infection would have been missed by the technique of virus culture. As a diagnosis of genital herpes has a major psychosocial and sexual impact on patients and their sexual partners, resulting in substantial morbidity and transmission potential, it is important that a diagnostic method demonstrates a high sensitivity and specificity for HSV. The technique of nested-PCR that we used in this study appears to fulfill this requirement.

Our results also show that PCR is a superior detection method to virus culture at all clinical stages and duration of lesions. Previous studies have shown that for virus culture, the rate of virus isolation decreases towards the older crusted stage. This was shown in our study. Although PCR showed the same detection pattern as that of virus culture, it was able to further detect at least 50% more genital HSV infected patients who presented with ulcers and crusted erosions, and were missed by the virus culture technique. Interestingly, of the 23 patients presenting

Table II. HSV detection results by virus culture and PCR based on lesion morphology.

Lesion morphology	No. patients	No. HSV-positive patients (%)	
		By virus culture	By PCR
Vesicle	23	14 (60.9)	22 (95.7)
Ulcer	44	8 (18.2)	25 (56.8)
Crusted erosion	36	8 (22.2)	16 (44.4)

Table III. HSV detection results by virus culture and PCR based on lesion duration.

Lesion duration (day)	No. patients	No. HSV-positive patients (%)	
		By virus culture	By PCR
1-5	56	19 (33.9)	41 (73.2)
6-10	25	8 (32.0)	15 (60.0)
>10	22	3 (13.6)	7 (31.8)

Table IV. Distribution of HSV-1 and -2 in first episode and recurrent infections by PCR typing.

	No. HSV-1 (%)	No. HSV-2 (%)	Total
First episode	11 (25.0)	33 (75.0)	44
Recurrent	1 (5.3)	18 (94.7)	19

with genital vesicles, as many as 22 were HSV-positive by PCR. This may suggest that genital vesicles are more likely associated with HSV infections.

Although both HSV-1 and HSV-2 are transmitted sexually, they differ in terms of risk factors for acquisition and frequency of recurrences. Thus, HSV typing is not only important from an epidemiological point of view, but also for accurate counselling of infected persons.^(8,11) Among the HSV-positive patients detected by virus culture, 20.0% demonstrated HSV-1 infection and 80.0% HSV-2 infection. Among the HSV-positive patients detected by the nested-PCR technique, 20.6% demonstrated HSV-1 infection and 79.4% HSV-2 infection. Both detection methods reflect a very similar prevalence for the HSV subtypes causing genital herpes in our population in Singapore; approximately four out of five infections of genital herpes in this study were due to HSV-2 infection.

In looking at our HSV PCR typing results, HSV-1 infection accounted for 25.0% of first episode infections and only 5.3% of recurrent infections, while HSV-2 infection accounted for 75.0% of first episode and 94.7% of recurrent infections. This is consistent with the findings in a retrospective study on genital herpes done at our STI clinic over a one-year period in 2001,⁽¹²⁾ as well as a study on patients with recurrent genital herpes in a STI clinic in Sweden⁽¹³⁾ which found 6% of recurrent infections to be due to HSV-1 infection and 94% due to HSV-2 infection. These results also support that genital HSV-1 infection is associated with fewer recurrences, and has a better

prognosis than genital HSV-2 infection.⁽¹⁴⁾ 40 (38.8%) patients were not detected to have HSV genital herpes by the laboratory techniques although they were suspected clinically. Of these 40 patients, four (10.0%) patients were found to have primary syphilis infection, and five (12.5%) patients gave a history of a traumatic episode causing the genital ulceration.

Chancroid is very uncommon in Singapore now, and cultures taken from those patients with ulcers suspected to be due to chancroid, were found to be negative. A recent study using a multiplex PCR method found *Varicella zoster* virus in nearly 3% of specimens taken from patients with presumed genital herpes infection.⁽¹⁵⁾ As such, clinicians should maintain a wider spectrum of differential diagnoses when faced with a clinical presentation of genital vesicles, ulcers or crusted erosions, especially for the last two presentations. Furthermore, of the remaining undiagnosed patients, 23 (74.2%) patients had older lesions at the time of presentation and this may explain the lack of detection in some of these patients.

In conclusion, our findings from this evaluation study suggest that the nested-PCR is able to diagnose genital HSV infection,⁽⁷⁾ and simultaneously provide typing information with excellent sensitivity and specificity. We hope that this study will help to advance efforts to develop commercially viable and cost-effective PCR testing for the diagnosis and management of genital HSV infection.

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