DETECTION OF HERPES SIMPLEX INFECTION IN VIRAL CONJUNCTIVITIS USING POLYMERASE CHAIN REACTION – A PILOT STUDY

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SUMMARY

A study was performed to detect the viral conjunctivitis in HUSM caused by HSV using a PCR method. Seventy patients with viral conjunctivitis were examined; with clinical features identified and conjunctival scrapping taken from superior and inferior fornices of affected eye for PCR analysis. PCR was performed with primers obtained from a commercially available primer kit for HSV. The prevalence of viral conjunctivitis in HUSM caused by HSV infection, using PCR method, was 17.1 % (95 % CI = 8.1, 26.0) which was higher than other reported studies. This is mainly due to the method used which is a highly sensitive and specific diagnostic test. Majority of HSV conjunctivitis patients presented with moderate follicular conjunctivitis with frequent corneal involvement which was similar to features of adenoviral conjunctivitis caused by Group D subgenera. The possible differentiating feature from adenoviral conjunctivitis was the unilaterality.

Key words : Viral conjunctivitis ; Polymerase Chain Reaction ; Herpes Simplex Virus ; Conjunctival Scrapping

INTRODUCTION

Conjunctivitis and keratitis are common ocular morbidity seen in general practice and eye units. The most common cause of these diseases is microbial infection which can either be viral, bacterial or parasitic infection.

Viral conjunctivitis in East Asia including Japan, Korea and Taiwan is caused mainly by Adenovirus and has gained recognition as a major international public health problem in these regions.[1] In Japan, adenoviruses are the most prevalent causative agent of viral conjunctivitis and were isolated from 91.2% of cases of clinically diagnosed EKC in which the aetiological agent was determined virologically, followed by Herpes Simplex virus (HSV) in 4.3% and Chlamydia trachomatis in 2.5% of cases.[2] Herpesvirus infection is frequently diagnosed in dendritic or geographic corneal ulcers, disciform keratitis, and keratouveitis and very rarely implicated as a cause of conjunctivitis alone without corneal or lid lesions. However, there have been only few papers describing the epidemiological features of HSV conjunctivitis. [3,4]

Accurate laboratory investigations for HSV is often valuable due to the limited reliability of clinical diagnosis of HSV induced keratoconjunctivitis. The potentially serious residual morbidity of these infections and availability of appropriate treatment for HSV further justify the need for detection of this virus in cases of keratoconjunctivitis There problems in diagnosing HSV conjunctivitis includes uncommon presentation which are clinically indistinguishable from other more likely causes such as adenoviruses and difficulty in isolation of this virus using viral culture as it requires sensitive cells for culture isolation, viable organisms necessitating special transport media and prompt

transport of specimens from patient to laboratory, as well as it is costly and time consuming.

Polymerase chain reaction (PCR) has been shown to be of valuable technique and offers great advantage compared to conventional method in the diagnosis of viral infections in view of its sensitivity and speed as well as and the need of small sample volume. Thus, it is one of the diagnostic method used for the diagnosis of herpesviruses.[5,6,7]

Due to the serious morbidity of this infection, we conducted a study to detect HSV infection in viral conjunctivitis and consequently, determine the prevalence rate of the infection in viral conjunctivitis cases in Hospital University Sains Malaysia. In addition, we would also like to identify the clinical features pertaining to HSV conjunctivitis which might help us in differentiating it from other causes of viral conjunctivitis and hence, assist us in diagnosing and managing these cases.

MATERIALS AND METHODS

This study is a cross sectional study in which approval was obtained from the Research and Ethical Committee, School of Medical Science, University Sains Malaysia.

Clinical Examination

All cases of conjunctivitis were screened at the Ophthalmology and Outpatient Clinics, HUSM between November 2002 to November 2003 by one ophthalmic officer. A clinical history was taken and patients were examined with slitlamp. Attention was paid to the signs in the tarsal and bulbar conjunctivae, fornices and lids. The cornea was examined and stained with fluorescein to detect any epithelial abnormality. Clinical features of the viral conjunctivitis cases were then documented. Based on these, seventy patients were identified to have clinically diagnosed viral conjunctivitis and had consented to be included in this study. Patients were of either sex who were 7 years or older so as to be able to give information regarding symptoms. Cases of clinically diagnosed primary microbial conjunctivitis or non-infective conjunctivitis were excluded from the study. Patients started on systemic or topical antiviral treatment were also excluded.

Clinical Sampling

Conjunctival swab was obtained by scrapping the superior amd inferior fornices with a sterile swab. Specimen was placed in a viral transport medium (Hanks Balanced Salt Solution), stored in the freezer at 4°C and transferred to the laboratory on the same day in an ice box. In the laboratory, the specimen was stored at - 40°C until processed for PCR.

Polymerase Chain Reaction

For detection of herpes simplex virus DNA by PCR, DNA from samples were extracted using Nucleospin Kit (Clontech, USA). The amount of DNA in the samples was determined using a spectrophotometer (Eppendorf Photometer). PCR was carried out using Herpes Simplex Virus Type 1/2, DNA polymerase, Primer set kit (Cat. No.: SP-10319, Maxim Biotech. Inc.), according to the manufacturer's recommendation.

The master mixture was prepared by adding 250 µl of pre-mixed primer, provided by HSV Type 1/2 , DNA polymerase, primer set kit (Sequences: Alignment on database;- HS1DP & HS2POL, X04771, M16321) to each 750 µl tube of optimized PCR buffer (2 mM MgCl2, 15 mM Tris-HCL, 30 mM KCl, 0.25 µM dNTPs Mix, enhancer & stabilizer – component of HSV Type 1/2 , DNA polymerase, primer set kit). The PCR assay was performed under the following conditions : To 20 µl of Master Mixture, 0.1 µl of Taq DNA polymerase and 5 µl of specimen or control cDNA were added. The final volume was made up to 25 µl with distilled water. Positive and negative controls were included for every set of PCRs as measures to avoid contamination. The positive control consisted of a clone containing PCR fragment which was PCR product derived from HSV Type 1 genomic DNA using HSV- 1012 N/ 1013 N as the primers whereas the negative control consisted of deionised sterile water (both were components of HSV Type 1/2 , DNA polymerase, primer set kit) . PCR was carried out using Eppendorf Mastercycler Gradient with the following temperatures : initial denaturation at 96°C for 1 minute, followed by 35 cycles of DNA denaturation at 94°C for 1 minute, primer annealing at 58°C for 1 minute and primer extension at 72°C for 1 minute. For the

RESULTS

Out of 70 patients clinically diagnosed as having viral conjunctivitis, 12 (17.1%) patients were positive for herpes simplex virus (HSV) by PCR method (Table I). Thus, in this study, the prevalence of herpes simplex infection in viral conjunctivitis in HUSM was found to be 17.1 % (95 % CI = 8.1, 26.0).

In our study, the mean age of patients having HSV conjunctivitis was 35.15 years ± 14.98 and it occured most frequently between 21 to 40 year age group that was 58.3% (Table II) . There was slight female preponderance in cases of HSV conjunctivitis which accounted for 7 (58.3 %) females and 5 (41.7 %) males. The majority of the HSV conjunctivitis cases comprised of Malays (91.7 %), followed by 8.3% of Chinese.

Contact conjunctivitis was noted to be the major source of infection for HSV conjunctivitis in this study, accounting for 66.7 % (8 out of 12 cases). The remaining 4 (33.3%) patients had not been aware of contact with conjunctivitis, respiratory infection (URTI) or urogenital disease. Out of 12 patients of HSV conjunctivitis, 7 (58.3%) had been in close contact with a family member suffering from conjunctivitis. One patient gave history of exposure to another patient having conjunctivitis.

All HSV conjunctivitis patients presented with foreign body sensation and lacrimation. Other symptoms included eye discharge (9 cases), itchiness (9 cases) photophobia (7 cases) and blurring of vision (6 cases). Majority of HSV conjunctivitis cases were found to be unilateral (9 out of 12 cases; 75.0%). Moderate conjunctivitis was found in 6 patients (50%) whereas mild and severe conjunctivitis were seen in the remaining ones accounting for 3 cases (25%) each. The conjunctival reaction in HSV conjunctivitis was mainly of follicular response which was 50.0% (6 out of 12 cases).

DISCUSSION

Viral conjunctivitis is a common eye problem encountered not only in ophthalmology clinic but in outpatient clinic as well. Various viral aetiologies have been incriminated causing it, including adenovirus, vaccinia, herpes simplex virus and poxvirus, with adenovirus being the most prevalent causative agent.

In this study, the method used was polymerase chain reaction alone. Ideally, there should be a comparison with the present gold standard which is viral culture. There are few reasons why this method had been chosen for this study. Firstly, polymerase chain reaction is a more sensitive and specific method compared to viral culture method. This fact has been established in many studies conducted throughout the world. [5,6,7] The high sensitivity and specificity of PCR in detecting HSV may help us to start antiviral treatment such as acyclovir earlier, and thus, may avoid possible serious complication such as keratitis which can lead to corneal perforation and blindness.

The use of viral culture as a gold standard is not practical in this study. Mainly, it was due to the budget constraint as this method is quite expensive. Secondly, even though viral culture remains the gold standard for definitive isolation and further characterization of the organism, the pitfalls of this method are numerous. Viral isolation requires viable organisms and hence, special transport media as well as prompt transport of specimens from patient to laboratory is necessary [8] and this is difficult to be applied in our clinic setup due to the lack of man power. Thirdly, it is time consuming, insensitive and subjective, along with requirement of highly technically trained personnel.

In our study, it was found that the prevalence rate of herpes simplex infection in viral conjunctivis in HUSM was 17.1% (95% CI =8.1,26.0). However, this is not in

accordance with previous reports in which the prevalence of HSV ocular infection ranges from1.4 to 7%. [2,3,9] The higher prevalence of HSV conjunctivitis in our study might be due to few reasons. First, the prevalence in previous reports of HSV conjunctivitis were referring only to cases with acute follicular conjunctivitis in the absence of corneal or lid signs whereas our study was referring to cases of HSV conjunctivitis which either presented with conjunctivitis alone, blepharoconjunctivitis or keratoconjunctivitis. Therefore, the pick-up rate of HSV infection was more compared to the reported studies.

Furthermore, in our study, the method used in detecting the prevalence of herpes simplex ocular infection was by PCR method which is very sensitive and highly specific as compared to viral culture which was the method used in previous studies in detecting the prevalence of herpes simplex ocular infections. Another explanation that may contribute to the high prevalence rate of HSV conjunctivitis in this study is the fact it was a hospital based study as compared to other studies mentioned, which were epidemiological studies. The results found were actually comparable to one hospital based study done by Wishart et al (1994) showing that 21% of acute conjunctivitis cases found in ophthalmic casualty department were due to HSV infection.[10]

In addition, the small sample size obtained may contribute to the high prevalence rate of HSV conjunctivitis in HUSM. As a result, it may give rise to a seemingly high epidemiological frequency.

This study has shown that the age of the patients with HSV conjunctivitis ranged from 12 to 68 years but the conjunctivitis is more commonly seen among adult age group (21 to 40 years old) accounting for 58.3 % of cases. This result is slightly different from the study done by Uchio et al (2000) whereby most of the cases occurred in 50-59 year

age group. It may be due to the age distribution in these two different places. Kelantan population has 41.5% of total population below 15 years of age whereas Japanese population has a larger size of older population.[11]

In this study, it was impossible to determine whether the infection was primary or recurrent since we did not measure serum antibody level to HSV. However, it has been reported that the reliability of serological tests for the diagnosis of HSV infection is limited.[12,13] Our result, therefore suggest that there is no significance difference in the clinical features of HSV conjunctivitis.

Several studies had shown that there was no sex difference in primary HSV ocular infection. [9,14] . However, in recurrent HSV ocular infection, studies by Wilhelmus et al (1981) and Wishart et al (1987) demonstrated that incidence was higher in male compared to female aged over 25 and 15, respectively.[15, 16.]

Our study has shown that there is female preponderance in cases of HSV conjunctivitis accounting for 58.3% females and 41.7% males but in term of cases, the distribution is almost equal where 7 cases are females and 5 cases were males. As this study consists of both primary and recurrent HSV ocular infections, the result obtained cannot be compared with other series and hence, sex difference cannot be elicited. However this is not significant considering sample size was very small and thus, not reflecting the true picture.

Based on ethnic group, majority of HSV conjunctivitis cases in this study comprised of Malay, again reflecting the racial distribution in Kelantan population.[11] Apart from that, there was no significance of this observation given the small number of cases involved.

With regards to the source of infection in this study, the major source of infection for HSV conjunctivitis was contact conjunctivitis in which 7 had been in close contact with family members and one contracted it from other patients. This finding is similar to the pattern seen in the study of HSV conjunctivitis by Uchio et al (2000), the largest proportion of patients had been in close contact with a friend or colleague suffering from conjunctivitis.[2]

Humans are the sole natural hosts of HSV. The virus can be transmitted by direct contact with infected cutaneous lesions, secretions of infected mucosa, salivary droplets from children and adults with active disease (cold sores), and the saliva or fomites of asymptomatic, virus-shedding carriers.[17] A study by Darougar et al, demonstrated that the source of infection identified in 24% of cases were contact with patients with an active skin HSV infection or patients having HSV lesions on their own lips, nose or face.[9] Spread via droplets is postulated, but not well documented.[18] These various sources of infection could probably be elicited in our study with a larger sample size.

Regarding the clinical presentation of HSV conjunctivitis in our study, the symptoms were mainly of eye irritation described as foreign body sensation and lacrimation, as well as mucoid eye discharge, followed by photophobia and blurred vision which were less frequent. Majority of HSV conjunctivitis patients showed moderate donjunctivitis which accounted for 50% of cases with the conjunctival reaction seen was mainly of follicular type. This finding corresponds well to the fact that one of the main causes for follicular reaction is viral infection.[19]

As for keratitis associated with conjunctivitis in this study, generally the corneal involvement was seen frequently in the HSV conjunctivitis, commonly of subepithelial

punctuate type. Apart from that, it was noted in our study that all four cases of HSV conjunctivitis treated by topical antibiotic with steroid showed corneal involvement (3 cases of punctate keratitis and a case of dendritic ulcer). Nevertheless, these findings could not really differentiate whether the corneal involvement was due to the treatment or they were just part of the natural course of HSV ocular infection.

Topical steroid can be associated with punctate keratitis which may be attributed to the preservatives, or the mechanical effects of aggregates of steroid particles in suspension producing a mechanical epithelial keratitis.[20] The use of topical steroid has been associated with prolongation of infectious epithelial ulceration and with an increase in the size of these ulcers. Progression from dendritic to geographical ulceration and, probably, the risk of developing stromal inflammation later, are enhanced by the use of steroids, particularly when used during the stage of active viral replication in the epithelium.[21]

Though, looking at our study the corneal involvement in HSV conjunctivitis occurred quite frequently (58.3%), we are still unable to say whether it is a part of the natural course of disease or as a complication of steroid therapy as this was a cross-sectional study.

Herpes simplex virus should always be considered in the differential diagnosis of acute or subacute follicular conjunctivitis.[18] Acute follicular conjunctivitis in HSV ocular infection can occur in both primary and recurrent HSV ocular infection, with or without lid or corneal involvement.[9, 16] Parallel to these observations, most of the patients with HSV conjunctivitis in this study presented with acute follicular conjunctivitis.

The clinical features seen in our study was similar to the ones seen in adenoviral conjunctivitis type 8 and 19 from group D subgenera which consisted of moderate to severe conjunctivitis with commonly subepithelial punctate lesion.[22] The degree of moderate to severe follicular conjunctivitis of HSV infection with less frequent preauricular lympadenopathy were also comparable to the study done by Uchio et al (2000) but their study noted that early corneal lesions was less frequent.[2] These mixed and dissimilar findings may be due to the small sample size of our study in comparison with other published studies.

In this study, it had shown that 75% of HSV conjunctivitis patients presented with unilateral involvement. This is consistent with several studies indicating that HSV ocular infection is unilateral in majority of cases (about 80% to 90%) whereas bilateral disease is unusual and occurs in about 2% of patients in separate studies.[2,15,23] Although the low occurrence of bilateral illness may help to discriminate HSV conjunctivitis from adenoviral conjunctivitis, it seems difficult to differentiate them clinically, especially in the early clinical stage.

Currently diagnosis of infectious disease is carried out by routine microscopy, culture and serological methods, which takes around 1-3 days and even up to 2 weeks to a month. For serious life threatening and vision threatening, infections and pathogens that are difficult to culture, immediate diagnosis will have a great impact in appropriate treatment and proper clinical management. This is rightly so in cases of HSV ocular infections. In addition to the availability of treatment which are used not only to treat but preventing complications, the higher prevalence rate of HSV conjunctivitis in this study may warrant the need for diagnostic testing.

In this genomic era with the availability of microbial genome sequences it is possible to carry out molecular DNA based diagnostic tests for almost all infections and this includes HSV infections. Having said that, although this prevalence study was not validated by comparison against an established gold standard which is the viral culture but in ideal situation, the use of PCR as a diagnostic test might be very useful.

Polymerase chain reaction (PCR) has been a proven model for rapid diagnosis. Apart from having higher sensitivity compared to conventional methods, the other potential advantages are the same day diagnosis (2 to 4 hours result) as well as the same day identification of pathogen. Nonetheless, due to its sensitivity, false positive result may be found as a result of contamination of samples and this can be avoided by meticulous use of preventive measures. The specificity of primers is typically analyzed by evaluating the production of the target fragment in relation to other products by gel electrophoresis. In our study, we had used a commercially available primer kit which is Herpes Simplex Virus Type 1/2, DNA polymerase, Primer set kit (Cat. No.: SP-10319, Maxim Biotech. Inc.).

Furthermore, nowadays, the PCR test is becoming less expensive than before. The expenditure of a laboratory setup for PCR is actually more or less similar to other laboratory set up, particularly the viral laboratory, ranging around RM 300 000 to RM 350 000 and thus, the application of PCR as a diagnostic test may actually be cost effective.

Once again, owing to its high sensitivity and specificity as well as other promising advantages, the PCR may one day be the 'gold standard' of diagnostic test in replacement of the conventional method, with special regard to viral isolation which obviously has a

CONCLUSION.

This study showed the prevalence of viral conjunctivitis caused by herpes simplex infection, in HUSM was higher than other reported studies. This could be due to the method used which was PCR method; as well as the type of study carried out which was a hospital based study as compared to other studies with lower prevalence rate of HSV conjunctivitis which were of epidemiological based studies.

One of the limitations in this study is a lack of comparison with other method such as viral culture which remains "the gold standard" at present. It could not be done in this study due to the time and budget constraints. In terms of clinical features, unilaterality with presence of moderate follicular conjunctivitis are highly suggestive of HSV conjunctivitis and may help to distinguished it from adenoviral conjunctivitis. Even though certain results are comparable to other studies but their significance could not be elicited due to a small sample size which was another limiting factor. This problem is also applied in analyzing the demographic characteristics of HSV conjunctivitis.

Finally, we recommend further study with larger sample size needed to determine the true prevalence of HSV conjunctivitis and its clinical features as well as to identify the demographic pattern which may contribute as risk factors for HSV conjunctivitis. This study should be done with comparison with the current gold standard that is the viral culture and preferably, multicentered, especially if time is the major constraint in getting a larger sample size. This study should also be a prospective type as information regarding the natural course of the disease can then be obtained and hence developing strategies to bring the disease under control, along with preventing complications.

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