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

BY

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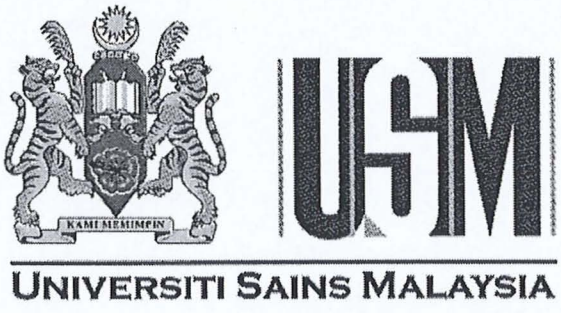

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ABSTRAK

Objektif : Untuk mengesan kes-kes konjunktivitis virus yang disebabkan oleh virus herpes simplex di Hospital Universiti Sains Malaysia, dengan menggunakan teknik 'polymerase chain reaction'.

Bentuk Kajian: Kajian berasaskan hospital secara 'kajian rentang'.

Kaedah Kajian : Kajian dijalankan di klinik mata, HUSM selama 1 tahun di mana seramai 70 pesakit telah dikaji. Gejala-gejala pesakit yang mengidapi konjunktivitis virus telah dikenalpasti dan kikisan pada lapisan konjunktiva dilakukan pada fornix sebelah atas dan sebelah bawah mata yang bermasalah, dengan menggunakan swab yang steril. Analisa PCR telah dilakukan pada kikisan konjunktiva tersebut. Kaedah PCR dijalankan dengan menggunakan primer untuk HSV yang didapati dari kit yang dipasarkan secara komersial. Kehadiran sasaran produk amplifikasi ditentukan oleh gel agarose. Ini menandakan samada konjunktivitis virus adalah disebabkan oleh jangkitan HSV atau tidak, seterusnya menunjukkan prevalens konjunktivitis virus yang disebabkan oleh jangkitan HSV. Data di dalam kajian ini dianalisakan mengikut analisa deskriptif perisian SPSS.

Keputusan : Kadar prevalens bagi kes-kes konjunktivitis virus di HUSM, yang disebabkan oleh virus herpes simplex, dengan menggunakan kaedah PCR, adalah 17.1% (95% CI = 8.1, 26.0). Majoriti dari pesakit yang menghidapi konjunktivitis virus herpes simplex mengalami konjunktivitis yang sederhana dan kebanyakan kes-kes tersebut

melibatkan keradangan pada kornea. Selain daripada itu, sebahagian besar dari pesakit-pesakit ini juga menunjukkan penglibatan konjunktivitis secara unilateral.

Kesimpulan : Kajian menunjukkan kadar prevalens konjunktivitis virus yang disebabkan oleh jangkitan herpes simplex di HUSM adalah lebih tinggi jika dibandingkan dengan kajian-kajian sebelum ini. Salah satu daripada sebabnya ialah kaedah PCR yang lebih sensitif dan spesifik. Sebahagian daripada gejala-gejala konjunktivitis HSV didapati hampir sama dengan gejala-gejala konjuktivitis adenovirus daripada kumpulan D subgenera. Hanya ada satu gejala yang mungkin dapat membezakan konjunktivitis HSV daripada konjunktivitis adenovirus iaitu dari aspek penglibatan secara unilateral.

ABSTRACT

Objective : To detect the viral conjunctivitis in Hospital Universiti Sains Malaysia caused by herpes simplex virus using a polymerase chain reaction method.

Design : A cross sectional hospital-based study.

Method : A total number of 70 patients with viral conjunctivitis were seen in the ophthalmology clinic, HUSM during one year period. The presenting clinical features were identified and conjunctival scrapping using sterile conjunctival swab was taken from superior and inferior fornices of the affected eye for PCR analysis. The PCR was performed with primers obtained from a commercially available primer kit for HSV. The presence of this amplified target sequence was determined by agarose gel. A positive result reflected the prevalence of viral conjunctivitis caused by HSV. Descriptive analysis was performed using SPSS system.

Results : The prevalence of viral conjunctivitis in HUSM caused by herpes simplex infection, using PCR method, was found to be 17.1 % (95 % CI = 8.1, 26.0). Majority of HSV conjunctivitis patients presented with moderate follicular conjunctivitis with frequent corneal involvement, commonly subepithelial punctuate keratitis. It was also observed that a larger portion of HSV conjunctivitis patients presented with unilateral involvement.

Conclusion : This study showed the prevalence of viral conjunctivitis caused by herpes simplex infection, in HUSM was higher than other reported studies. The most important cause for high prevalence is the PCR method used which is a highly sensitive and specific diagnostic test. Some of the clinical features of HSV conjunctivitis was similar to adenoviral conjunctivitis caused by Group D subgenera. The only possible differentiating feature was the unilaterality which may help to discriminate HSV conjunctivitis from adenoviral conjunctivitis.

1. 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 (Ishii et al,1987). The causative agents of acute conjunctivitis clinically diagnosed as epidemic keratoconjunctivitis (EKC) have recently been established 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. Apart from that, other organisms isolated include Herpes Simplex virus (HSV) in 4.3% and Chlamydia trachomatis in 2.5% (Uchio et al,2000).

Herpesvirus infection is frequently diagnosed in dendritic or geographic corneal ulcers, disciform keratitis, and keratouveitis. The virus is 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 (Harding et al,1987; Belongia et al, 1991).

Due to the limited reliability of clinical diagnosis of adenovirus and particularly, HSV induced keratoconjunctivitis , accurate laboratory investigations for this agent is often

valuable. Furthermore, the availability of appropriate treatment for HSV and together with the potentially serious residual morbidity of these infections clearly justify the need for detection of this virus in cases of keratoconjunctivitis. A study by Uchio et al (2000) has shown that 34.6% of HSV induced conjunctivitis cases were treated with topical steroid and these cases showed serious clinical findings, such as superficial punctuate keratitis or diffuse subepithelial corneal opacities.

There are several problems in the diagnosis of HSV conjunctivitis at the outpatient or even ophthalmological clinics. Firstly, uncommon cases of HSV ocular infection presenting as acute follicular conjunctivitis are clinically indistinguishable from other more likely causes such as adenoviruses. Secondly, even though viral culture remains the 'gold standard' for definite diagnosis of HSV conjunctivitis and allows further characterization of the causative agent, the pitfalls of this conventional technique are many. The sensitive cells for culture isolation of HSV, such as Vero, human embryonic kidney, and primary rabbit kidney, are different from those for adenovirus, HEp-2, HeLa and A549. Therefore, if these cells are not adopted for culture isolation of HSV, it cannot be isolated. In addition, cell culture isolation requires viable organisms necessitating special transport media and prompt transport of specimens from patient to laboratory, as well as it is costly and time consuming.

The emergence of new molecular methods adapted to the field of medical microbiology has recently led to improvement of diagnostic procedures. This highly sensitive and specific method, known as polymerase chain reaction (PCR) was developed to detect a

single copy of a gene. It has been applied in a variety of biomedical research studies, including viral detection in many ocular inflammatory diseases. Owing to the sensitivity and speed of PCR, and the need of small sample volume, the technique has been shown to be of great value and offers great advantage compared to conventional method in the diagnosis of viral infections and thus it is one of the diagnostic method used for the diagnosis of herpesviruses.

A study to determine the sensitivity and specificity of culture and the PCR for detecting HSV-1 in the cornea of patients undergoing penetrating keratoplasty was done by Kaye et al (2000). It was reported that the sensitivity and specificity of PCR for HSV-1 was 82% and 78% respectively whereas the sensitivity and specificity of culture was 2% and 100% respectively. In a study by Kowalski et al (1993), utilizing cell culture as a gold standard test, PCR was shown to be significantly more sensitive (95%) in diagnosing herpetic ocular disease than by clinical examination. Another study performed by Hidalgo et al (1998) in which nested PCR (nPCR) versus viral culture as method and tear film versus corneal scrapings as specimen was evaluated in the diagnosis of herpetic keratoconjunctivitis. Overall there was no significant difference between the detection rate for corneal scrapings (85%) and tear film (75%). In both types of specimens nPCR showed a higher detection rate than viral cultures (corneal scrapings: 87.5% vs 31.25%; tear film: 75% vs 12.5%).

1.1 OBJECTIVES

1.1.1 GENERAL OBJECTIVE

To detect the viral conjunctivitis in HUSM caused by herpes simplex virus using a polymerase chain reaction method

1.1.2 SPECIFIC OBJECTIVE

i. To determine the prevalence of viral conjunctivitis caused by herpes simplex virus in HUSM using a polymerase chain reaction method.

ii. To identify common clinical features of viral conjunctivitis caused by HSV detected by polymerase chain reaction method.

2. BACKGROUND

2.1 VIRAL CONJUNCTIVITIS

Viral infections are a common cause of acute conjunctivitis and a variety of viral antigens have been incriminated, including adenovirus, vaccinia, herpes simplex virus, and herpes zoster virus. Each of these agents have been incriminated to affect the epithelium of both the conjunctiva and the cornea; as a result, epithelial keratitis is a frequent accompaniment of viral conjunctivitis (Diamante & Leibowitz, 1998).

Manifestation of viral conjunctivitis may vary from one disease process to another. However, by and large, patients with viral conjunctivitis complain of a red eye, ocular irritation, foreign body sensation, and watery discharge; photophobia and visual loss usually signal corneal involvement. They can either present unilaterally or bilaterally. History of recent exposure to individual with red eye are frequently reported (Krachmer et al,2000; Diamante & Leibowitz,1998; Catania,1995).

Viral conjunctivitis tends to persist for approximately 2 to 4 weeks and usually has a longer course than most types of acute bacterial conjunctivitis. Commonly, a viral etiology is characterized by conjunctival hyperemia with an acute follicular conjunctival response and preauricular lymphadenopathy (Krachmer et al,2000; Diamante & Leibowitz,1998; Catania,1995)

In general, a viral keratitis is characterized by punctate epithelial erosions that stain prominently with fluorescein and often are accompanied by punctate infiltrates in the epithelium. If the conjunctivitis persists or is severe, there may be a disturbance in the anterior stroma beneath the epithelial abnormalities. The stromal abnormalities may be ephemeral and resolve despite persistent epithelial keratitis, or, as in the case of adenovirus infection, they may persist for a period of years, long after the epithelial changes have resolved. The punctate epithelial changes rarely are sufficiently distinct morphologically to permit identification of a specific type of virus as the etiologic agent (Diamante & Leibowitz et al, 1998).

2.2 HERPES SIMPLEX VIRUS : STRUCTURE

According to the International Committee on Taxonomy of Viruses, HSV is a member of the family *Herpetoviridae* and the genus *Herpesvirus.* Complete virus particles are approximately 180 to 200 nm in diameter and consist of :

1) a cylindrical core structure around which the viral DNA is wound.
2) An icosahedral capsid approximately 85 to 110 nm in diameter.
3) A granular zone or tegument which surrounds the capsid.
4) An envelope, which is derived from the host cell as particles bud from the nuclear membrane.

HSV is relatively sensitive to heat and must be stored at -70C if infectivity is to be preserved for significant length of time. Like other enveloped viruses, HSV is readily

inactivated by lipid solvents such as ether, chloroform and alcohol. The virus is also sensitive to a variety of radiant energy and to many proteolytic enzymes.

Infection by HSV begins with the attachment of the virus to a cellular receptor, which may differ for HSV-1 and HSV-2. The virus penetrates the cell via fusion of the viral envelope with the plasma membrane, followed by transcription of the HSV genome. This will result in production of messenger RNA (mRNA) which eventually lead to the synthesis of viral proteins that takes place in the cytoplasm. The proteins (structural and some non structural) are transported back to the nucleus where the nucleocapsid are assembled (Mattison et al, 1991). Among the nonstructural proteins which are coded for HSV DNA are DNA polymerases and a thymidine kinase. Inhibition of the functions of these enzymes has proven to be an effective method of antiviral chemotherapy in man.

2.3 HERPES SIMPLEX VIRUS : EPIDEMIOLOGY

Herpes simplex virus (HSV) is the commonest infective cause of blindness in many developed countries, where it has been reported with incidence of between 5.9 to 20.7 episodes per 100 000 person-years and this appears to be increasing (Dawson & Togni,1976; Liesegang et al,1989)

Initial ocular HSV episodes included incidences of 54% blepharitis or conjunctivitis, 63% epithelial keratitis, 6% stromal keratitis and 4% intraocular inflammation (iritis and uveitis). Age-adjusted rates by sex were comparable and there was no seasonal trend in

incidence, although rates increased in time.(Liesegang et al, 1989) However, Darourgar et al (1985) reported blepharoconjunctivitis in 93%, dendritic keratitis in 15% and disciform keratitis in 2% of the patients in their series. 7% had conjunctival involvement alone without lid or corneal lesion, and 15% developed chronic blepharoconjunctivitis that persisted occasionally for months.

Liesegang and coworkers reported that recurrence rates assessed by life–table methods after the first episode were 9.6% at 1 year, 22.9% at 2 years and 63.2% at 20 years. Recurrence rates appeared to rise following repeated episodes. Ocular herpes simplex affecting both eyes at the same time and/or different episodes were seen in 11.9% of cases. One fifth of patients had lid involvement alone, while a third had solely superficial corneal involvement (Liesegang, 1989). Study by Wishart et al (1984) reported that conjunctivitis with lid lesions was the most common form of recurrence of ocular herpes simplex i.e 83% of recurrences; acute follicular conjunctivitis without lid lesions was also more frequent (17.0 %) than dendritic keratitis (9.0 %) as a form of recurrence. There is evidence from other studies that herpes simplex virus may constitute up to 23% of acute conjunctivitis presenting at ophthalmology outpatients clinics and may frequently present without corneal or lid lesions.

Humans are the only natural reservoir of herpes simplex virus. Sources of infection are by direct contact with infected lesions, salivary droplets from children and adults with active disease (cold sores) and via the saliva or fomites of asymptomatic, virus shedding carriers. Iatrogenic sources of infection are the physician's unwashed hands and

contaminated Schiøtz or applanation tonometer head. HSV is viable for up to 2 hours on a dry tonometer head and up to 8 hours on one kept moist. Swabbing the tonometer with 70% isopropyl alcohol is 100% effective in killing the virus, and this should be done between patients along with hand washing with a soapy solution (Ventura et al, 1987).

By the age of 5 years, 60% of all children have been infected with type 1 (oral) HSV, usually through the mouth or nares, with only about 6% developing clinically apparent primary disease. Less than 1% of primary HSV presents as overt ocular disease. The oral and nasal portals of entry allow the virus access to the trigeminal ganglion, which also innervates the eye. The vast majority of first ocular (not primary) or recurrent ocular herpes infections are due to reactivation of latent trigeminal ganglion virus with subsequent appearance of the virus in the eye alone or associated with eruption of cold sores around the mouth or nose.

2.4 HERPES SIMPLEX VIRUS (HSV) CONJUNCTIVITIS

Herpes simplex virus (HSV) conjunctivitis can either occur in primary ocular herpes simplex that is an acute first HSV infection of the nonimmune host, or recurrent ocular herpes simplex whereby the ocular infection occurs after reactivation of the latent virus in the ophthalmic division of the trigeminal ganglion.

HSV conjunctivitis is typically characterized by follicular conjunctivitis which may be seen with or without, ipsilateral preauricular lymphadenopathy. It can also occurs without involvement of other ocular structures such as the eyelids or cornea.

Lid lesion, when present, exhibits an erythematous base and may ulcerate with a honey-yellow crusty border. Patients usually complain of an acute onset of tearing, burning, foreign body sensation and photophobia as well as blurring of vision if epithelial keratitis also develops (Leibowitz & Koleini, 1998). The bulbar and palpaberal conjunctiva are usually diffusely hypereamic. Rarely a pseudomembrane will form which is another potential similarity to adenoviral conjunctivitis, and there may be some atypical superficial punctate keratitis or more severe corneal disease.

Because HSV and adenoviral conjunctivitis are often indistinguishable in the absence of corneal or lid involvement, unilaterality is an important clinical sign, with epidemic keratoconjunctivitis (EKC) more commonly becoming bilateral (Diamante & Leibowitz , 1998). Uchio et al (2000) reported, from their study, that 87% of HSV conjunctivitis was noted to be unilateral and early corneal lesions and preauricular lympadenopathy were less frequent in HSV conjunctivitis than in adenoviral conjunctivitis.

2.5 BACTERIAL AND OTHER NON-VIRAL CONJUNCTIVITIS

Bacterial conjunctivitis typically presents with acute onset of ocular symptoms which consist of burning sensation, eye irritation and matted eyelids on awakening. Clinical signs include variable degrees of conjunctival injection, papillae, and mucopurulent discharge with no preauricular lympadenopathy except in hyperacute cases. Definitive diagnosis is based on stains and cultures after swabbing the conjunctiva.

The three most common pathogens in bacterial conjunctivitis are *Streptococcus pneumoniae, Haemophilus influenzae* and *Staphylococcus aureus.* Infections with *S.pneumoniae* and *H.influenzae* are more common in children, while *S.aureus* most frequently affects adults. (Jackson, 1993)

Bacterial conjunctivitis is usually treated with a topical broad-spectrum antibiotic solution (e.g.,trimethoprim/polymyxin B, ciprofloxacin, ofloxacin) or an antibiotic ointment (e.g., bacitracin,erythromycin, polymyxin B, ciprofloxacin).

Other causes of conjunctivitis can be noninfectious in origin and allergic conjunctivitis is one of the causes. Allergic conjunctivitis encompasses a spectrum of distinct clinical conditions usually characterized by itching which is painless with no history of exposure. Clinical signs consist of conjunctival hyperemia with chemosis and eyelid swelling but preauricular lympadenopathy is absent. The personal or family history is often positive for other atopic conditions, such as allergic rhinitis, asthma or eczema. Treatment

measures include allergen avoidance, cold compresses, vasoconstrictors, antihistamine drops, topical nonsteroidal anti-inflammatory agents and mast-cell stabilizers.

Toxic conjunctivitis or chemical conjunctivitis is another example of noninfectious conjunctivitis. It is caused by direct contact by with noxious fumes, particles, or chemical fluids. It can result in a red eye with tearing, pain, and sensitivity to light. The severity depends on the type of chemical and the length of time the eye was exposed. Treatment includes removal of toxic substance and institution of preservative-free lubricating tears and ointment.

2.6 HSV CONJUNCTIVITIS : LABORATORY DIAGNOSIS

Diagnosis of ocular viral disease is usually made based on clinical impression only. However, most of the time, difficulty will arise in achieving clinical diagnosis of HSV conjunctivitis whereby it is hard to differentiate between adenovirus or HSV as the causative organism in viral conjunctivitis, based on clinical features alone. Therefore accurate laboratory investigation for these agents in conjuctival swabs is often valuable. Sensitivity, specificity, speed and availability determine the choice of a diagnostic test for HSV infection.

2.6.1 VIRAL CULTURE

The presence of viable virus in a clinical specimen as determined by virus isolation in culture is the most definitive evidence of HSV infection, particularly within the context of identifying a reservoir for virus transmission. Samples of ocular lesions are taken on a swab. For best results, specimens should be inoculated within 1 hour after collection. Otherwise, they should be kept in transport medium and refrigerated or frozen (at -70°C) in order to preserve the specimen. Examples of transport medium include Eagle's minimal essential medium, Dulbecco's minimal essential media and Hanks Balanced Salt Solution. The transport medium is supplemented with fetal bovine serum and also antibiotics.

Primary cells are generally more sensitive than the established lines. The most sensitive are primary rabbit kidney (PRK) and human embryo cells. Commonly used cell lines are human lung fibroblasts i.e. MRC-5 and human lung carcinoma cells i.e. A549 (Carter et al, 1997).

Cytopathic effect (CPE) can be seen within 18 h p.i., but it generally takes 2 to 3 days (depending on the sensitivity of the cells and the amount of virus inoculated). Cultures are routinely kept for 14 days before negative results are reported. HSV causes a characteristic CPE consisting of rounded refractile cells. If hematoxylin-eosin stain is applied, the cells exhibit eosinophilic intranuclear inclusions. Polykaryocytosis depends on the virus strain and the cell type.

Result may be confounded by the presence in the specimens of other viruses that cause a similar CPE, such as adenovirus, a common isolate in ocular specimens. To address this problem, at least two cell types that differ in the ability to support the growth of these viruses should be used routinely for viral culture, for example MRC-5 and A549 cells. HSV grows in MRC-5 cells; adenovirus does not. In A549 cells, adenovirus CPE is first seen on day 4 or 5; that of HSV is seen on days 1 through 3. Ultimately, however, virus identification can be done only using specific antisera in an appropriate immunologic test.

However, the pitfalls of these conventional techniques are numerous. Cell culture isolation requires viable organisms necessitating special transport media and prompt transport of specimens between patient and laboratory. In addition, it is costly as well as time consuming and hence, cause a major limitation when rapid diagnosis is essential or required. Then again, it remains the "gold standard" of herpes simplex laboratory diagnosis, as isolation of an infectious agent is definitive and allows further characterization.

2.6.2 ANTIGEN DETECTION

Because the quantity of virus present in clinical specimens is variable (depending on lesion status and collection procedure), antigen detection may provide an alternative to virus culture which has the ability to identify nonviable virus. The basic principle is that

antigens in the lesion interact with the added HSV antibody, producing complex that may be detected by a variety of methods such as immunofluorescence (IF) or immunoperoxidase (IP) staining, enzyme immunoassay (EIA) (*viz.*, ELISA), or radioimmunoassay (RIA). Recently available monoclonal antibodies to HSV proteins have improved the specificity of these assays and provided the necessary tools for differentiation between the two HSV serotypes.

Antigen detection assays are rapid and relatively inexpensive, but there is still uncertainty as to their sensitivities and specificities. A study by Uchio et al (2000) showed that there was no cases of acute follicular conjunctivitis demonstrating a positive result for HSV by using a direct immunofluorescence test (Microtrak HSV 1/ HSV 2), on conjuctival swab samples. However, the fluorescence antibody (FA) test was positive in all strains isolated by cell culture.

Pramod et al (1998) had carried out a study to assess the diagnostic value of indirect immunofluorescence (IF) procedure in comparison with routine viral culture for the diagnosis of herpes simplex virus induced keratitis, from samples of corneal scrapings. Virus specific antigen was detected by indirect IF in 31.42% of cases and in contrast only 20% of cases had positive viral isolation which sometimes took as long as a week to show a cytopathogenic effect (CPE). Therefore, it was concluded that antigen detection by indirect IF is rapid, specific and sensitive technique for demonstrating HSV-1 antigen in corneal scrapings from Herpes simplex keratitis patients.

For direct examination of clinical specimen, the scrapings are placed in a drop of phosphate-buffered saline (PBS) on slides. After drying, the cells are fixed with acetone, washed with PBS and stained with HSV antibody conjugated to a detector molecule such as fluorescein isothiocyanate (FITC). If monoclonal antibodies are used, fixation may have to be in ethanol or methanol, since some epitomes epitopes are acetone sensitive. Detection of fluorescence indicates HSV presence. The indirect assay uses unconjugated HSV antibody followed by the appropriate FITC-conjugated anti-immunoglobulin.

Lee et al (1990) had compared the HERPCHEK™ ELISA method with virus culture and direct immunofluorescence in the rabbit model of HSV keratitis. It was shown that direct immunofluorescence provided lower sensitivity than the HERPCHEK™ test or virus culture in animals whose disease was untreated.

2.6.3 POLYMERASE CHAIN REACTION

In the history of molecular biology, polymerase chain reaction (PCR) which was originally described by Saiki et al in 1985 (Lohmann et al,2000), is an extremely sensitive method designed to amplify and detect specific or selected target DNA sequences.

Basically, PCR is an in vitro method of the enzymatic synthesis of a specific DNA sequence, using two oligonucleotide primers that hybridize to opposite strands and flank the origin of interest in the target DNA sequence, usually one hundred to several base

pairs (Erlich HA, 1989). This technique has been used primarily in research and clinical studies of genetic, infectious disease, forensic medicine and oncology. Repetitive cycles will synthesise an exponential accumulation of specific fragment and hence the amplified DNA sequence can be detected (Byrne et al, 1995).

2.6.3.1 CLINICAL SAMPLE

Samples can be obtained from cerebrospinal fluid, aqueous, vitreous humor, blood or tissues. Samples to be tested by PCR for the presence of infectious agent can be frozen because only DNA, and not viable organisms, is required. A temperature of -20°C is acceptable for freezing, but -70°C or -80°C is preferable, especially for longer-term storage. (Byrne et al, 1995)

2.6.3.2 PCR PROTOCOL

The PCR is based on the repetitive cycling of three reactions (Figure 1). Each cycle consists of three steps: denaturation of the target DNA by heating to a high temperature, annealing of oligonucleotide primers to the target DNA, and the synthesis of two new strands of DNA by a DNA polymerase. If a sequence of RNA is to be amplified, a copy of it must first be made (cDNA) by a reverse transcriptase before beginning the PCR. The oligonucleotide primers used average 18 to 25 bases in length and can be easily synthesized with automated equipment. In practice two specific primers are used which flank the region of interest. One primer is complementary to the coding strand and the

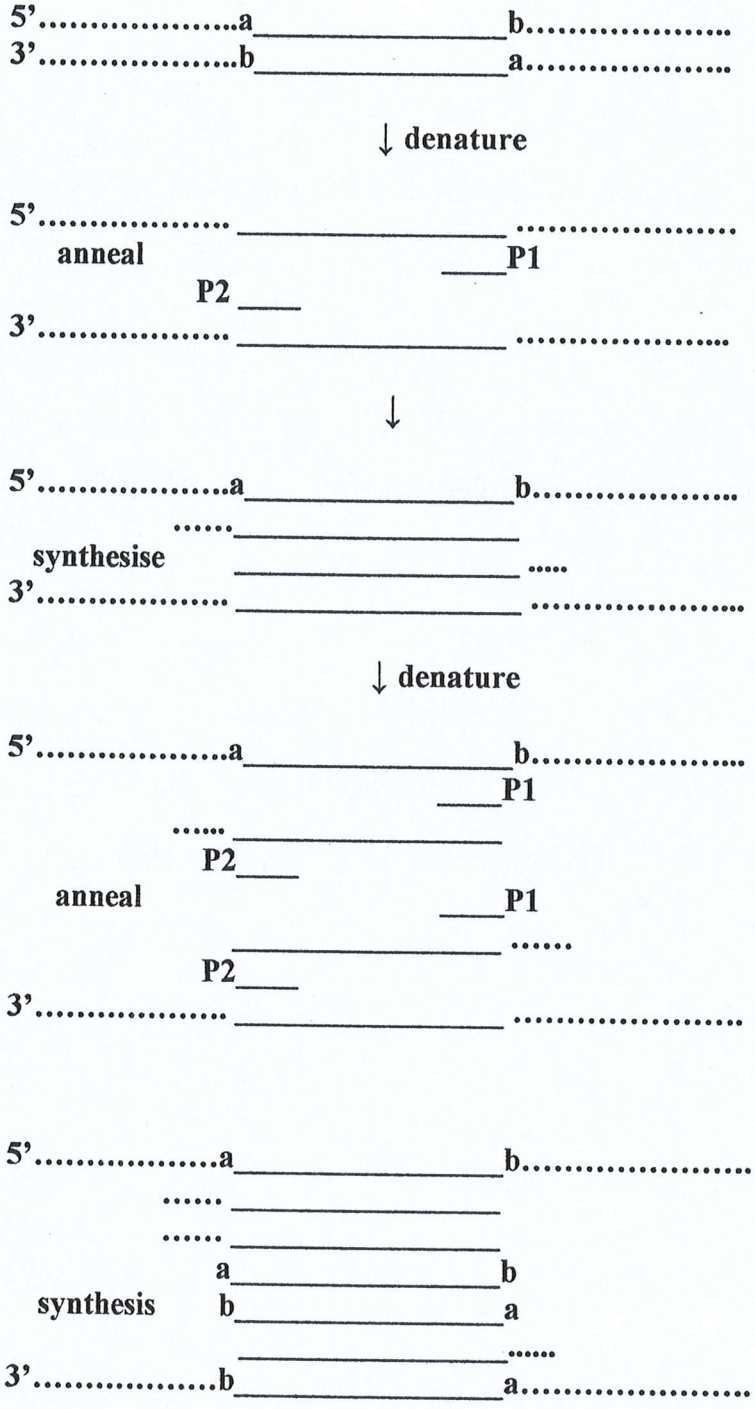


Figure 2.1: Polymerase chain reaction.

ab represents segment of DNA which is amplified. Each cycle consists of denaturation of the DNA, annealing of the primers, P1 and P2 to the DNA and the subsequent synthesis of new strands. Segment ab/ba accumulates at an exponential rate by acting as its own.

2.6.3.2.1 DNA Extraction

In preparation for the PCR procedure, whole genomic DNA must be extracted from the sample (fresh or frozen) to be tested. There are several means to accomplish this. In general, fluids such as cerebrospinal fluid and aqueous or vitreous humor can be heated at 95°C for 30 minutes. Samples such as blood or tissue that contain cells are treated with proteinase K (final concentration, 250µg/ml) / detergent solution for 1 hour at 56°C and then heat denatured at 95°C for 10 minutes. The detergent solubilizes the cell components and the proteinase K digests the proteins. Commercially available products such as Gene Releaser (Bio Ventures Inc., Murfreefboro, Tennesee) can also be used to release genomic DNA. Alternatively, DNA may be purified from the samples to be tested by PCR by using a phenol / chloroform extraction procedure (Byrne et al, 1995)

2.6.3.2.2 PCR Amplification

Amplifications are performed in a small volume, usually 100µL or less. A PCR reaction mix is prepared that contains

1) 10X amplification buffer, standard buffer contains 0.5 mmol/L potassium chloride, 10 mmol/L Tris HCL (pH 8.3), 15 mmol/L magnesium chloride and 0.1% gelatin. The free magnesium concentration is critical for functioning of the DNA polymerase and should be optimized for each primer / target system.

2) DNA polymerase from the bacterium *Thermus acquaticus* (Taq polymerase) survives the repeated high-temperature denaturations in the PCR procedure and is used at a concentration of approximately 2 units, which is depleted after approximately 30 amplifications cycles.

3) Equimolar concentrations of all four deoxynucleotide triphosphate (dNTP) [dNTPs: dATP (2'-deoxyadenosine 5-triphosphate), dCTP (2'-deoxycytidine 5'-triphosphate), dGTP (2'-deoxyguanosine 5'- triphosphate), dTTP (2'-deoxythymidine 5'triphosphate)], 200μmol/ L each.

4) Oligonucleotide primers: 1 μmol/L is usually sufficient for approximately thirty amplification cycles. For target DNA to be amplified by PCR, the nucleotide sequences at either end of the DNA piece must be known. Primers are shortpieces of single-stranded DNA (preferably twenty to twenty-four nucleotides in length) that are complementary to these known sequences. Primers should ideally have a guanine-cytosine content of approximately 50%, should not contain guanine-cytosine-rich stretches, and should not contain any homologous regions between the oligonucleotides that constitute the primer pair. Primer can be made synthetically and are designed to anneal to the end regions of the target DNA to serve as an initiation point for synthesis.

5) The sample to be tested.

6) Sterile distilled water to bring the volume to 100 μL.

Once the water, buffer, deoxyribonucleoside triphosphates (dNTPs), primer pairs, polymerase, and sample are combined in a microcentrifuge tube, the mixture is microcentrifuged to consolidate the reagents and is overlaid with 100 μL mineral oil to prevent evaporation of the mixture during amplification. A positive control and a negative buffer control are included with each reaction. (Byrne et al, 1995)

2.6.3.2.3 Detection Of Amplified Products

Amplified DNA is generally separated and visualized using ethidium bromide / agarose gel electrophoresis. DNA-grade ultrapure agarose, polyacrylamide, or high-density (NuSieve, InterMountain Scientific, bountiful, Utah) gels can be used. Ethidium bromide can be added during preparation of the gel and is used at a final concentration of 0.5 μg/ml, or the gel may be stained after electrophoresis. The samples are mixed with a 10X loading buffer containing glycerol and bromophenol blue and loaded into the wells of the gel. Molecular weight markers are also mixed with loading buffer and run in parallel with the samples. (Byrne et al, 1995)

Electrophoresis is done in Tris-Borate/ethylenediaminetetra-acetic acid (EDTA) buffer until the bromophenol blue dye approaches the bottom of the gel. The amplified DNA fragments then are visualized using a long-wave ultraviolet light. The sizes of the amplified bands are determined by comparing their locations relative to the molecular

2.6.3.3 MINIMIZE RISK OF CONTAMINATION

Because of the extreme sensitivity of the system, it is essential to avoid false-positives, which could occur by cross contamination between samples or by contamination of reagents with amplified products or positive controls. Some general precautions to minimize the risk of contamination include performing the initial processing in a biologic safety hood not used for any other PCR-related procedures. All reagents should be prepared in another biologic safety cabinet, using materials dedicated solely for PCR, and should be aliquoted into sterile tubes before use. Amplified products and procedures for their detection and confirmation should be separated from the other reagents and are preferably handled in another laboratory. (Byrne et al, 1995)

2.6.3.4 CLINICAL INDICATION

PCR is rapidly becoming a major tool in the diagnostic repertoire for infectious diseases, and it promises to play a role in the diagnosis and monitoring of cancer, in clinical genetics, and in forensics (Campbell et al, 1992).

Amplification techniques have been used for the diagnosis of pathogens that are difficult or impossible to grow, such as *Mycobacterium leprae, Borrelia burgdorferi* and *Toxoplasma gondii,* for the rapid identification of slow-growing pathogens such as *Shigella* spp. from a dense background of normal flora. They have been extensively used in viral diagnosis for a wide range of pathogens such as human immunodeficiency virus type 1 (HIV-1), most notably; herpes simplex virus and adenovirus. Nucleic acid amplification has particular value in retroviral studies, since latent, unculturable proviruses can be detected easily. These methods are also useful in epidemiologic analysis, since suitable primers may be used to discriminate between strains of the same organism (Campbell et al,1992).

2.6.3.5 ADVANTAGES OF PCR

The speed, sensitivity and specificity of amplification techniques allow rapid, direct diagnosis of the diseases which formerly could be diagnosed only slowly, indirectly (eg., by serology), at great expense, or not at all (Campbell et al, 1992).

Because amplification methods directly detect minute quantities of pathogen genetic material, they can provide acute-phase diagnosis with high sensitivity without the need to await antibody formation (Campbell et al, 1992)

Amplification methods will detect a pathogen only if nucleic acid from that organism is actually present, so confusion with infections in the distant past is unlikely. It is currently unclear, however, how long DNA or RNA persists after an infection is cleared by host immunity or therapeutic intervention. (Campbell et al, 1992)

It is likely that the species specificity of amplification methods will be used to resolve problems with cross-reactivity in diagnosis of diseases such as spirochetal infection. (Campbell et al, 1992)

2.6.3.6 DISADVANTAGES OF PCR

Serology remains the diagnostic method of choice in conditions such as cysticercosis, in which the infective organism is in a sequestered site, making it difficult to obtain target nucleic acid (Campbell et al, 1992).

Serology may also be used to determine whether a patient has ever been exposed to a pathogen, regardless of whether the infecting organism is actually present, whereas amplification methods require the presence of the organism. (Campbell et al, 1992)

An antibody response also provides information on the pathogenicity or invasiveness of an organism such as *Legionella* sp. In which may be normally present in the environment and thus contaminate clinical specimens. Amplification is unlikely to discriminate between colonization and infection (Campbell et al,1992).

Culture allows for the investigation of special characteristic such as strain pattern or virulence factors which may be difficult or impossible to determine by amplification alone (Campbell et al, 1992)

3. MATERIALS AND METHOD

3.1 RESEARCH STRATEGY

The study design was a cross sectional study.

3.2 POPULATION, SETTING AND TIME

Study population : Patients with conjunctivitis attending Ophthalmology clinic and Outpatient clinic, HUSM

Period of study : November 2002 to November 2003

Place of study : Ophthalmology Clinic, HUSM
Outpatient Clinic, HUSM
Microbiology Lab, HUSM

3.3 SAMPLING METHOD AND SAMPLE SIZE

Sampling of patients according to the non-randomised sampling method whereby all the cases clinically diagnosed as viral conjunctivitis were included in this study.

Sample size was calculated based on a single proportion formula

$$n = \left(\frac{Z\alpha}{\Delta} \right)^2 X P X (1- P)$$

P = HSV conjunctivitis prevalence (4.3 %)

$$= \left(\frac{1.96}{0.05} \right)^2 X 0.043 X (1- 0.043)$$

= 63 ; n= 70

3.4 SELECTION CRITERIA

3.4.1 INCLUSION CITERIA

- All clinically diagnosed viral conjunctivitis patients; as elaborated in the definition of terms; from HUSM.
- Patient or parents consented for conjunctival scraping using conjunctival swab.
- Patient 7 years old or more so as to be able to give information regarding symptoms.

3.4.2 EXCLUSION CRITERIA

- Clinically diagnosed primary microbial conjunctivitis patients.
- Clinically diagnosed non-infective conjunctivitis, eg: chemical related, allergic conjunctivitis.
- Patient started on systemic or topical antiviral treatment.

3.5 ETHICAL PROBLEM

Conjunctival scrapping using conjunctival swab is not a regular procedure performed in cases of clinically diagnosed viral conjunctivitis. Therefore a written consent was obtained from all patients or parents after explanation (refer to Form A). This study was approved by the Research and Ethical Committee, School of Medical Sciences,USM and also received an IRPA short term grant (304 / PPSP / 6131265) from the Bio-Medical Sciences and Health Committee, Universiti Sains Malaysia, Pulau Pinang on the 11th October 2002.

3.6 DEFINATION OF TERMS

3.6.1 Clinically diagnosed viral conjunctivitis

Clinically diagnosed viral conjunctivitis is defined based on its signs and symptoms. History may include the onset and spread of conjunctivitis, history of any exposure, symptoms of watery discharge and ocular irritation or foreign body sensation, as well as ruling out any noninfective causes. On examination, the conjunctivitis is shown as inflammatory processes involving the conjunctiva characterized by a red eye, visible evidence of dilated conjunctival vessels and the resultant hyperemia with varying degree of conjunctival oedema and mainly conjunctival follicular reaction with presence of serous or mucoid discharge. Patient may also have tender ipsilateral preauricular lympadenopathy or pseudomembrane (Krachmer et al, 2000; Diamante,Leibowitz, 1998). In this study, kerato and blepharoconjunctivitis are included.

3.6.2 Herpes simplex virus (HSV) conjunctivitis

One form of viral conjunctivitis whereby the inflammatory process of the conjunctiva occurs as a result of active herpes simplex virus infection which was detected by polymerase chain reaction (PCR) method and it can occur with or without involvement of other ocular structures such as eyelids or cornea.

3.6.3 Grading of conjunctival injection

The grading of conjunctival injection was as follows :

Grade	Conjunctival injection
Mild	Localize conjunctival hyperemia
Moderate	Diffuse conjunctival hyperemia
Severe	Diffuse conjunctival hyperemia with chemosis and occasionally subconjunctival haemorrhage

(American Academy of Ophthalmology,1982)

3.6.4 Oligonucleotide Primer

Short synthetic DNA molecule used as a probe or a primer of DNA synthesis in laboratory. In this study, commercial primers to detect HSV type1/2 was used (HSV Type 1/2, DNA polymerase, primer set kit; Maxim Biotech).

3.6.5 Polymerase Chain Reaction method positive for herpes simplex virus

Polymerase chain reaction (PCR) is considered positive for herpes simplex virus when there is presence of amplified target sequence (PCR product) which is presented as a band of expected size on agarose gel electrophoresis using commercial primers provided by herpes simplex virus type 1/2, DNA polymerase, primer set kit (Maxim Biotech)

3.7 INSTRUMENTS

3.7.1 Microcentrifuge

Microcentrifuge is a machine which is used to centrifuge the samples or specimens during DNA extraction procedure.

3.7.2 UV spectrophotometer

UV spectrophotometer is a machine which is used to measure DNA concentration and its purity after extraction of the DNA from samples of conjunctival swab.

3.7.3 PCR thermal cycler

PCR thermal cycler is a machine used for amplification in PCR . In this study, Eppendorf Mastercycler Gradient was used.

3.7.4 Agarose gel electrophoresis apparatus

Agarose gel electrophoresis apparatus is an apparatus used to run the PCR product and hence, to analyse the amplified samples.

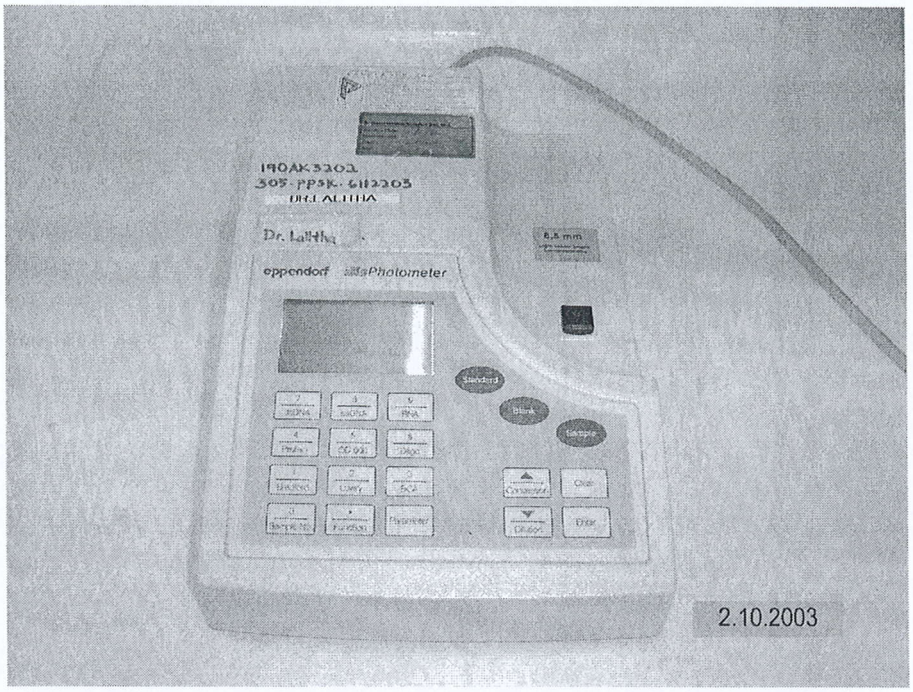


Figure 3.1: UV spectrophotometer

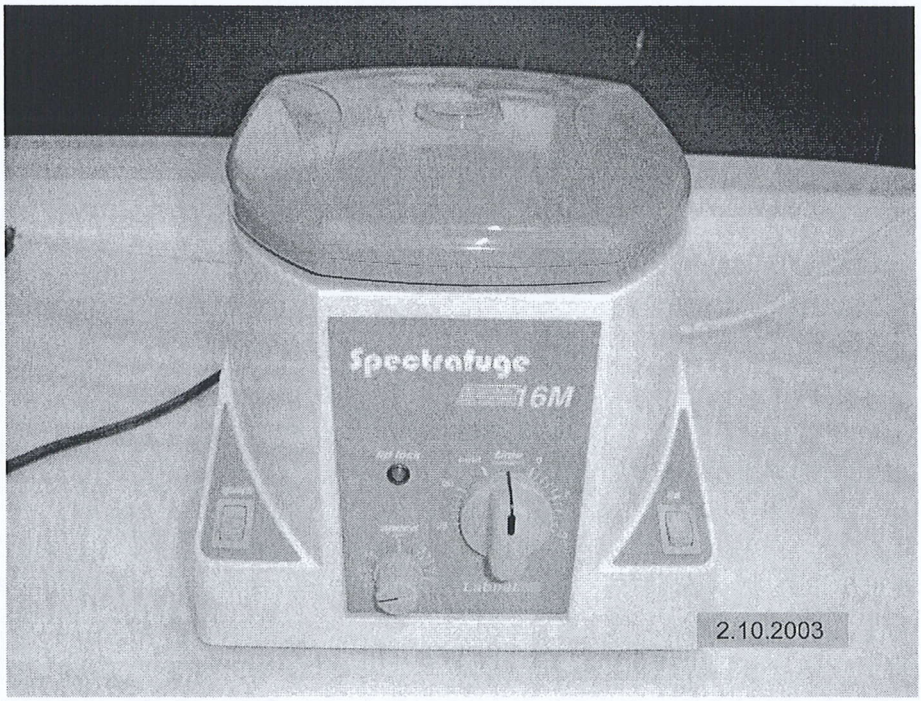


Figure 3.2: Microcentrifuge

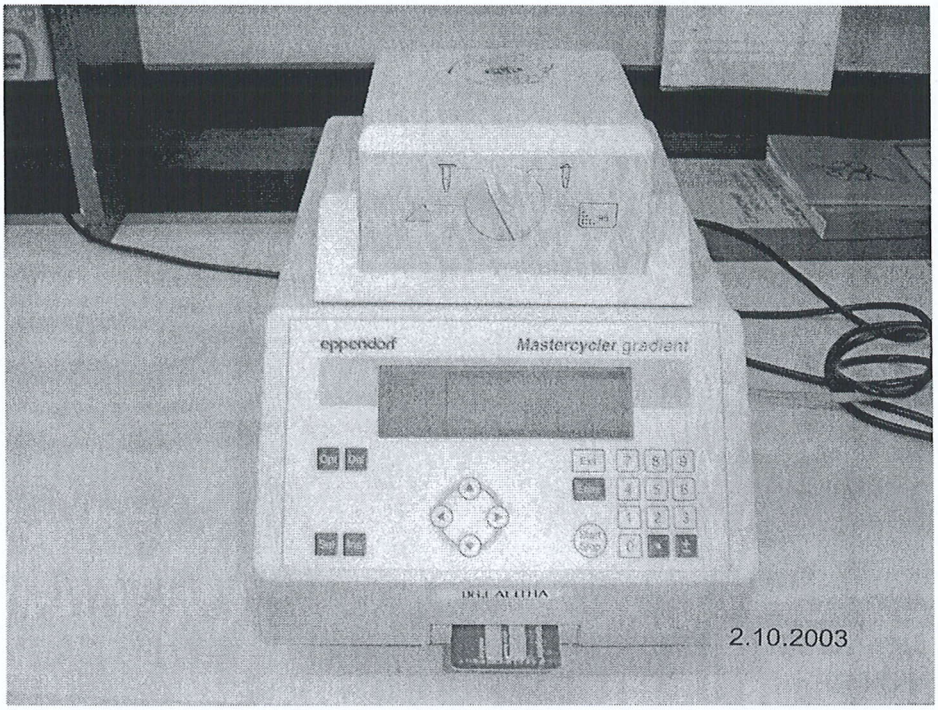


Figure 3.3: PCR thermal cycler

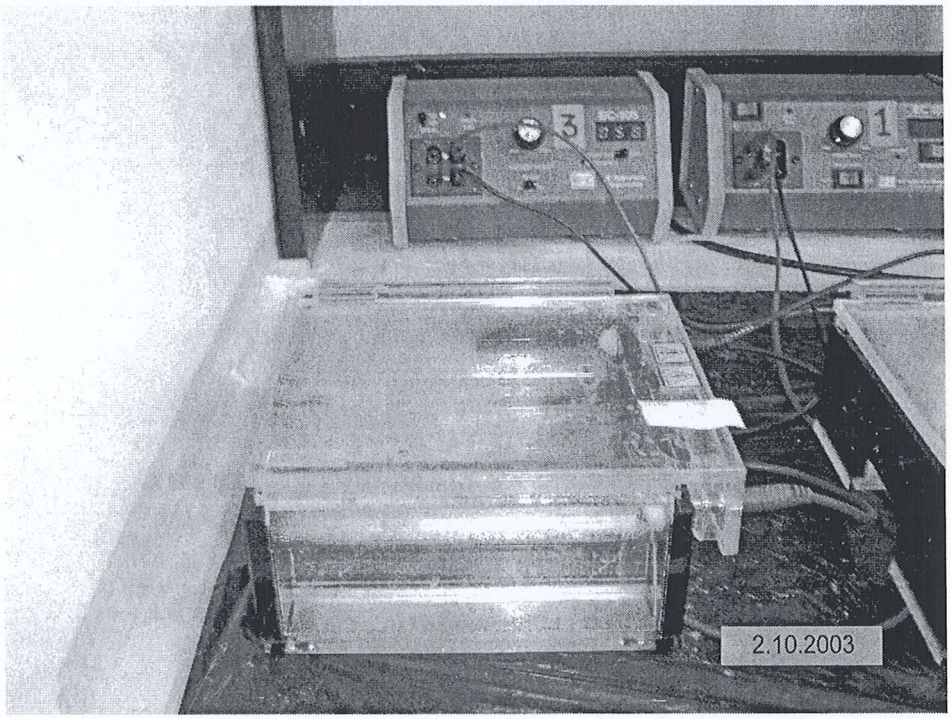


Figure 3.4: Agarose gel electrophoresis apparatus

3.8 METHODS

3.8.1 CLINICAL SAMPLE COLLECTION

All cases of conjunctivitis were screened at the outpatient and ophthalmology clinic between November 2002 and November 2003 and were referred to one ophthalmic medical officer in ophthalmology clinic. To confirm diagnosis, clinical history was again taken and patients were examined under slit-lamp. 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. Based on selection criteria, seventy patients were identified to have clinically diagnosed viral conjunctivitis. Clinical features of the viral conjunctivitis cases were then documented.

Conjunctival swab was obtained by scrapping the superior and inferior fornices with a sterile swab after instillation of 0.4% guttae Novesine (local anaesthesia) to the affected eye, or more severe eye if both were affected. The scraped specimen was placed in a 1.5 ml microcentrifuge tube containing 1 ml viral transport medium and was stored in the freezer at 4°C. It was then carried 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.

3.8.2 DNA EXTRACTION

DNA extraction was carried out according to the Nucleospin Kit (Clontech, USA). The samples were thawed, homogenized by vortexing and then transferred to a 1.5 ml microcentrifuge tube. The samples were pelleted by centrifugation at 12 000 x g for 5 minutes.

The supernatant was aspirated and each pellet was resuspended in 180 µl of buffer T1 (lysis buffer provided by the Nucleospin Kit). Sample was vortexed to mix. 25 µl of proteinase K was added to each sample and mixture was again vortexed and later, incubated at 56 °C for 3 hours in a shaking waterbath.

After incubation, sample was vortexed and 200 µl buffer B3 (component of Nucleospin Kit) was added. The mixture was mixed vigorously and incubated at 70 °C for 10 minutes.

Subsequently, 210 µl ethanol 95 % was added to the sample and immediately vortexed to avoid precipitation. The sample was then applied to a Nucleospin Tissue column which was placed in a 2 ml collecting tube, followed by centrifugation for 1 minute at 6 000 x g. The flowthrough was discarded and the column was placed back into the collecting tube.

Next, 500 µl of buffer B5 (component of Nucleospin Kit, which was mixed with 95 % ethanol) was added and centrifuged for 1 minute at 6 000 x g with the flowthrough

discarded after spinning. This washing step was repeated and followed with centrifugation at 6 000 x g for 2 minutes. Residual ethanol was removed during this step.

The Nucleospin Tissue Column was then placed into a clean 1.5 microcentrifuge tube. Subsequently, the DNA elution was performed by adding 100 µl prewarmed elution buffer BE (5mM Tris/Cl ; pH 8.5 – component of Nucleospin Kit) which was incubated at 70 °C for 4 minutes, to the Nucleospin Tissue column and centrifuged at 6 000 x g for 1 minute. Finally, the amount of DNA in the sample was determined using a UV spectrophotometry. The extracted DNA was kept frozen at -20 °C until PCR was performed.

3.8.3 PCR PROTOCOLS

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. It was performed in a clean room with pipettes reserved specifically for this purpose.

3.8.3.1 MASTER MIXTURE PREPARATION

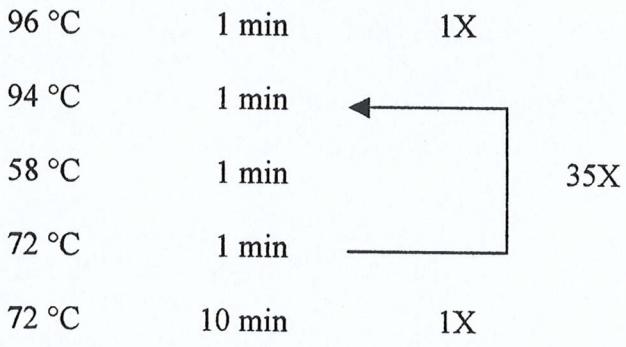
250 µl of pre-mixed primer, provided by HSV Type 1/2 , DNA polymerase, primer set kit (Sequences: Alignment on database;- HS1DP & HS2POL, X04771, M16321) was added to each 750 µl tube of optimized PCR buffer (2 mM MgCl2, 15 mM Tris-HCL, 30 mM KCl, 0.25 µM dNTPs Mix, enhancer & stabilizer – component of HSV Type 1/2 , DNA polymerase, primer set kit). The Master Mixture was then aliquoted and stored at -20 °C.

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 if specimen or control cDNA used was less than 5 µl.

To detect as well as to exclude carryover or contamination of HSV DNA, positive controls and negative controls, respectively, were included for every set of PCRs. 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 (component of Type 1/2 , DNA polymerase, primer set kit) whereas the negative control consisted of deionised sterile water (component of HSV Type 1/2 , DNA polymerase, primer set kit).

3.8.3.2 PCR REACTION PROFILES

PCR was carried out using the following temperatures : initial denaturation at 96°C for 1 minute, followed by 35 cycles of reaction consisting 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 last cycle of PCR, the 72°C step was extended to 10 minutes. Eppendorf Mastercycler Gradient was used for performing the above reaction. In brief, the reaction profiles are shown as below :



3.8.3.3 DETECTION OF PCR PRODUCT

PCR product was run in a horizontal submerged agarose gel. 1.2 % agarose gel was casted on a gel casting tray using 0.5X TBE (0.045M Tris-borate, 0.001M EDTA) with 0.05 µg/ml of ethidium bromide. DNA loading buffer (40% sucrose in 0.5% TBE with 0.25% orange G dye) was mixed with 10 µl of the PCR products which were then loaded on to the gel to undergo electroporesis. The gel was run at 80 volt for an hour at room temperature. Molecular weight marker, 100 bp ladder (provided by the HSV 1/2, DNA polymerase, primer set kit) was used to detect 241 base-pair DNA fragments which were consistent with the size of the target HSV DNA polymerase gene fragment. The PCR products were visualized using UV transilluminator.

3.8.4 FLOW CHART

The methodology of our study was summarized by a flow chart provided, with reference to appendix 3.

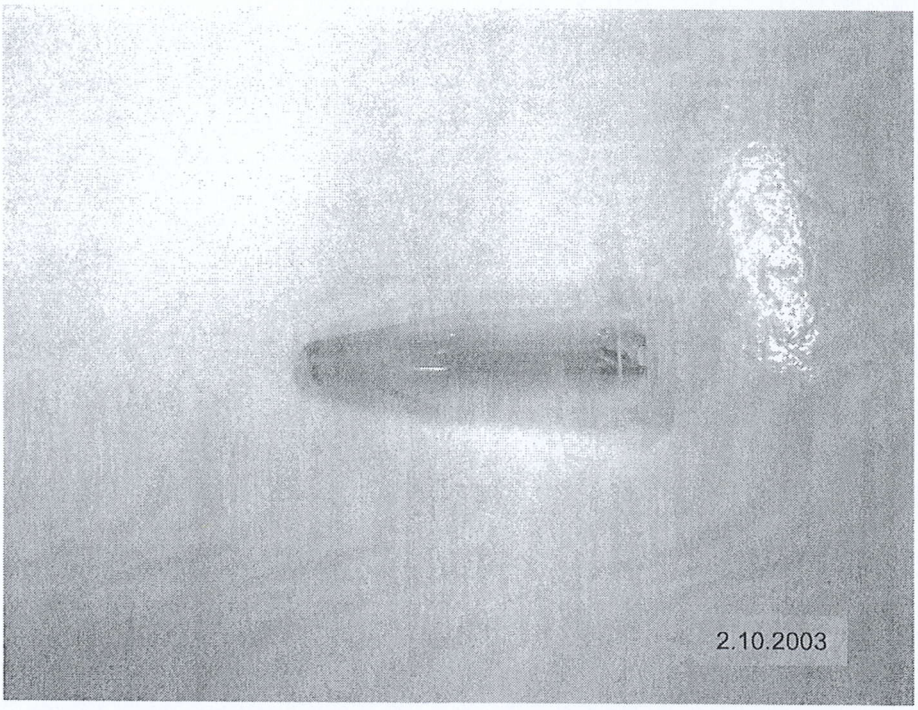


Figure 3.5: Conjunctival scrapping sample in Appendorf tube containing viral transport media

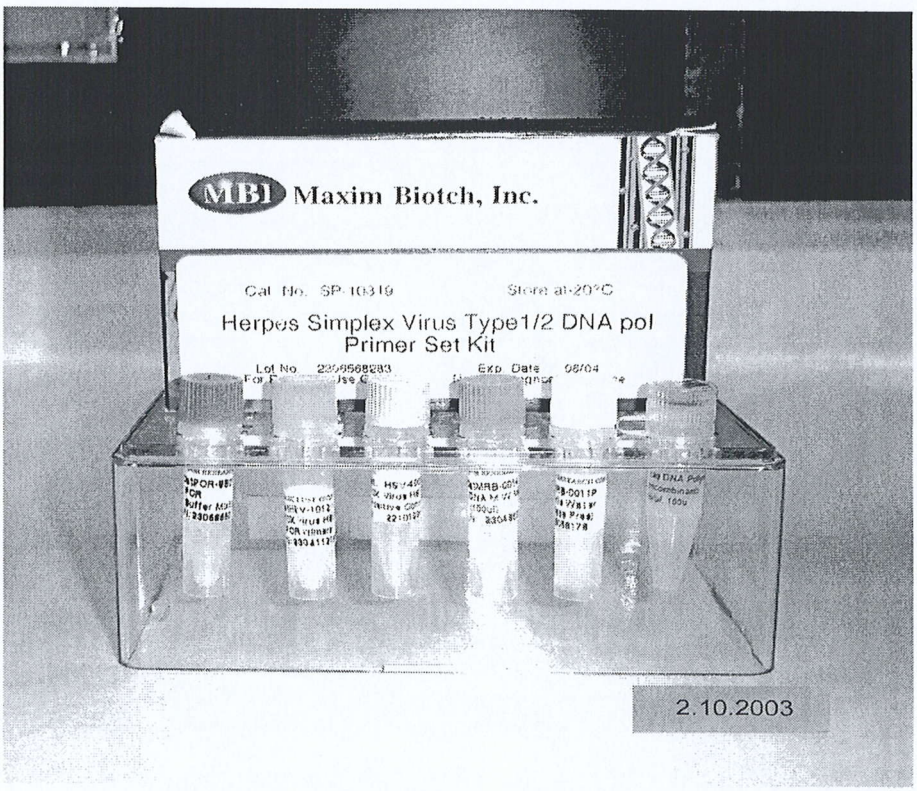


Figure 3.6: Primer set kit

Figure3.7: PCR procedure

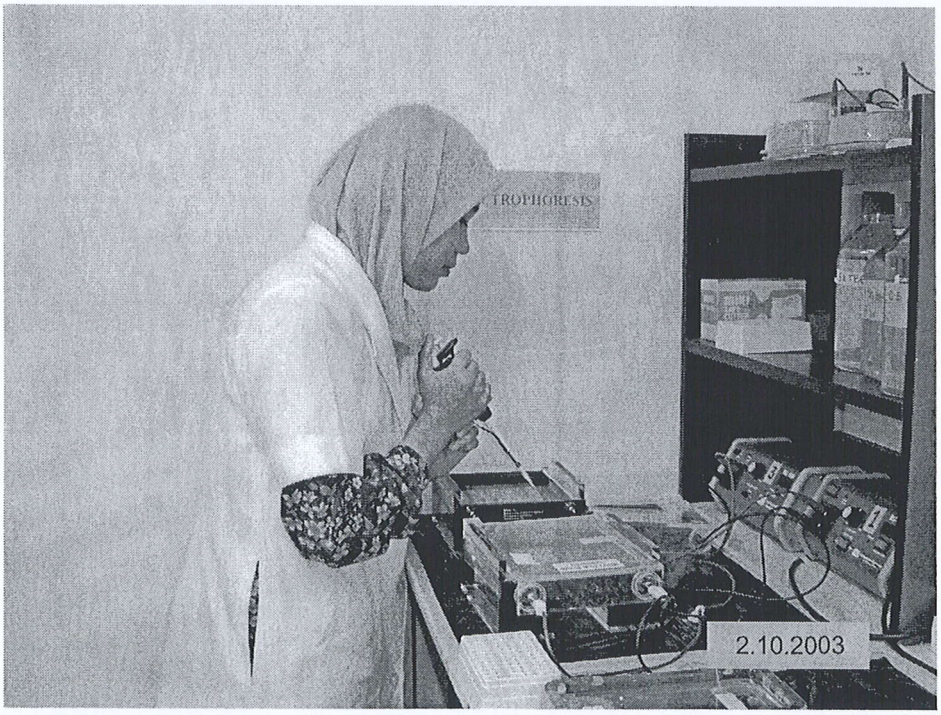


Figure 3.8: Agarose gel electrophoresis procedure

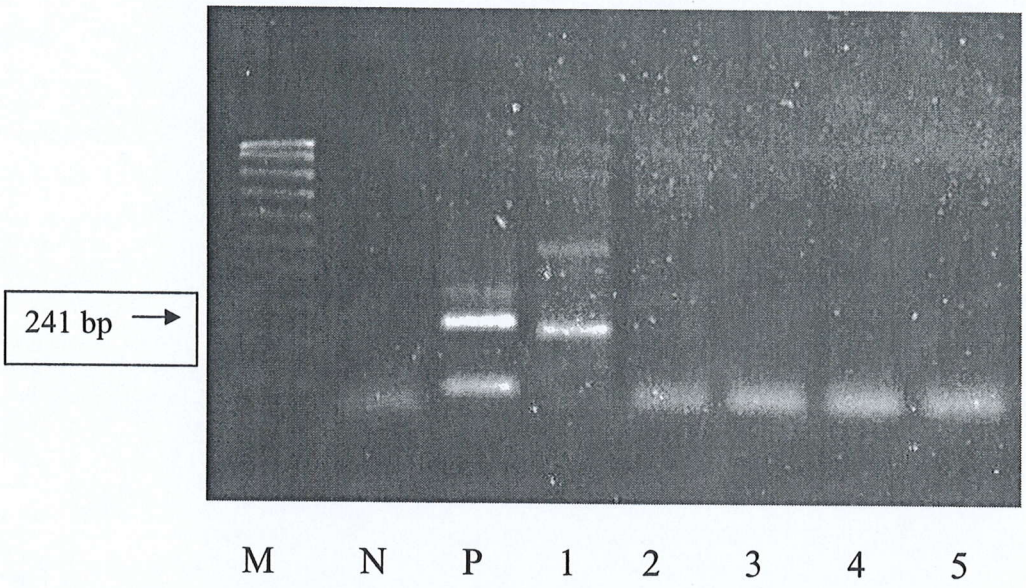


Figure 3.9: Ethidium bromide-stained agarose gel electrophoresis of amplified PCR product of samples

Lane M : 100 bp DNA ladder

Lane N : Negative control

Lane P : Positive control

Lane 1-5: Samples from conjunctival scrappings

4. RESULTS

4.1.VIRAL CONJUNCTIVITIS: DEMOGRAPHIC CHARACTERISTICS

Seventy cases of viral conjunctivitis were studied over a period between November 2002 and November 2003. All the cases were from Hospital Universiti Sains Malaysia which were either seen at ophthalmology outpatients or casualty clinics.

The mean age of patients with viral conjunctivitis was 36.6 years ± 17.28. The 21 to 40 year age group comprised the highest (45.7 %) proportion of cases among all age groups.

Table 4.1: Distribution of viral conjunctivitis according to age

Range of age (year)	No of patients (n=70)	Percentage (%)
7 to 20	14	20.0
21 to 40	32	45.7
41 to 60	16	22.9
61 to 80	8	11.4

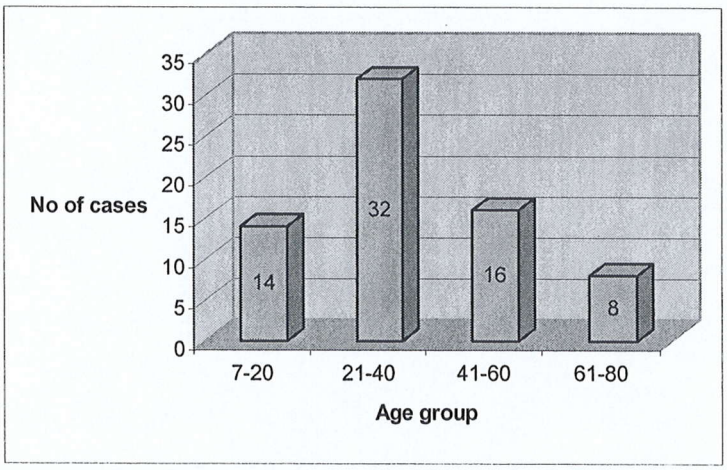


Figure 4.1: Distribution of viral conjunctivitis according to age

Table 4.2 : Distribution of viral conjunctivitis according to sex

Sex	No of patients (n=70)	Percentage (%)
Male	34	48.6
Female	36	51.4

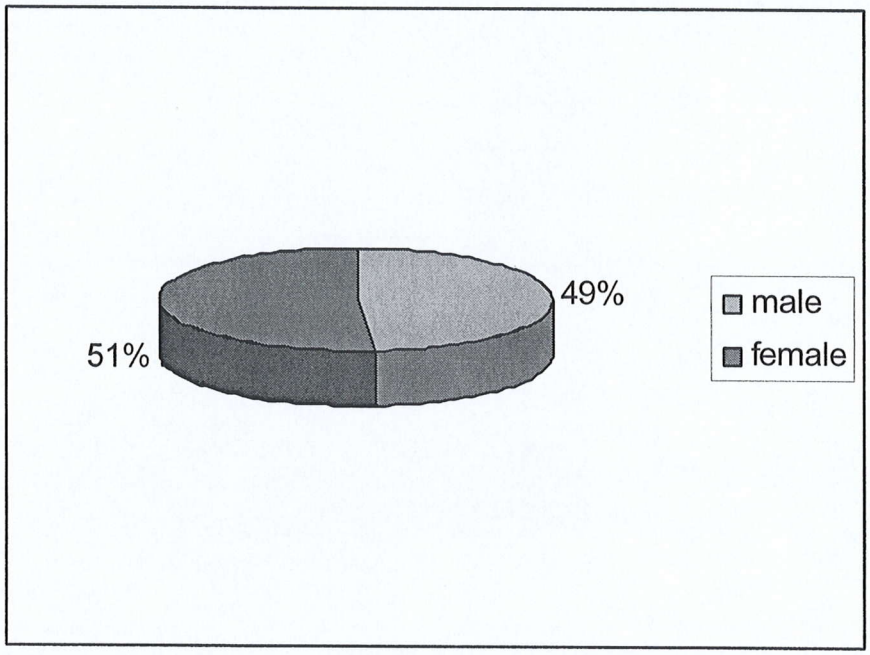


Figure 4.2 : Distribution of viral conjunctivitis according to sex

There was only a slight preponderance for females which accounted for 51.4 % whereby males 48.6 %.

Table 4.3 ; Distribution of viral conjunctivitis according to race

Race	No of patients (n=70)	Percentage (%)
Malay	67	95.7
Chinese	2	2.9
Others	1	1.4

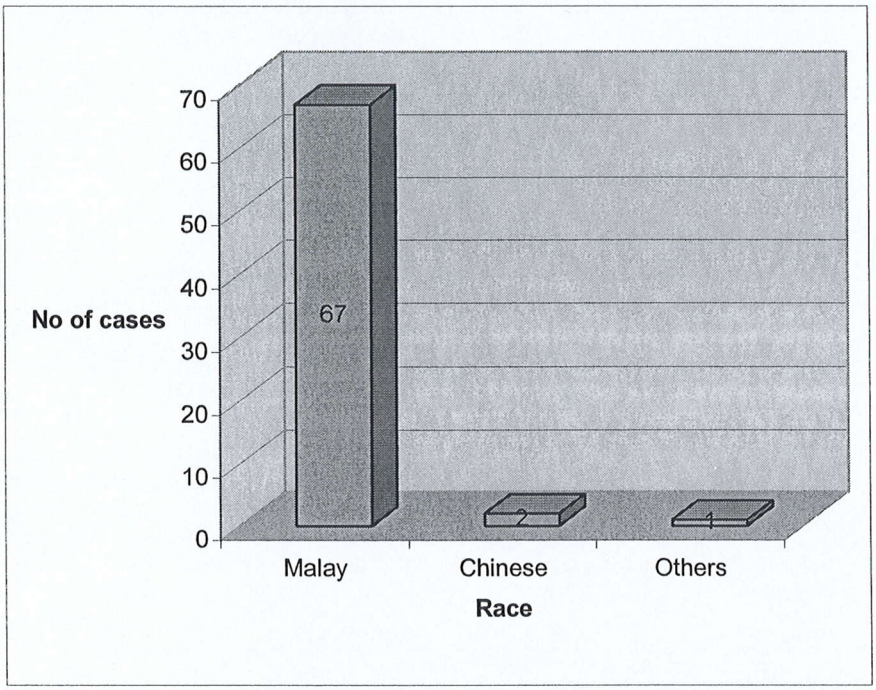


Figure 4.3 : Distribution of viral conjunctivitis according to race

In this study, most of the patients with viral conjunctivitis presented to our eye clinic were Malays (95.7 %) followed by Chinese patients (2.9%) and Eurasian heritage (1.4%).

4.2. HSV CONJUNCTIVITIS : DETECTION OF HSV BY PCR METHOD IN VIRAL CONUNCTIVITIS

Out of 70 patients clinically diagnosed as having viral conjunctivitis, 12 samples (17.1%) from patients were positive for herpes simplex virus (HSV) by PCR method. 58 samples from clinically diagnosed viral conjunctivitis patients were negative by similar method. Therefore, 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).

Table 4.4 : Result of PCR test to detect herpes simplex virus in viral conjunctivitis

PCR test	No of patients (n=70)	Percentage (%)
Positive for HSV	12	17.1
Negative for HSV	58	82.9

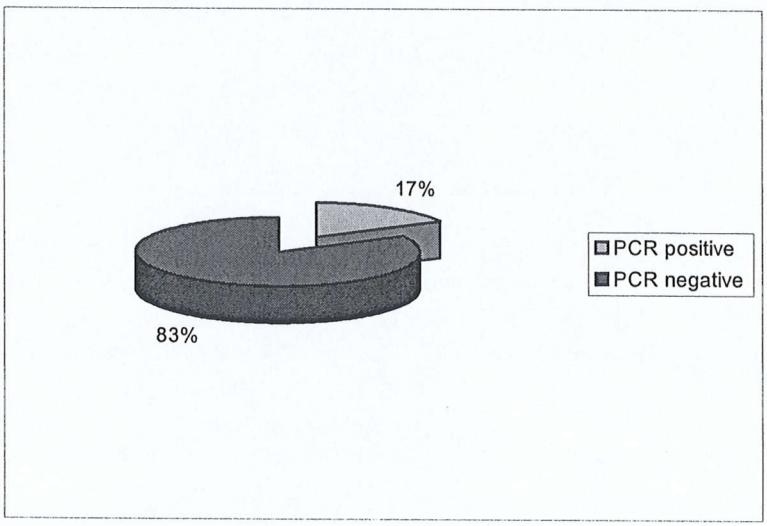


Figure 4.4 : Result of PCR test to detect herpes simplex virus in viral conjunctivitis

4.3. HSV CONJUNCTIVITIS : DEMOGRAPHIC CHARACTERISTICS

The mean age of patients having HSV conjunctivitis was 35.15 years ± 14.98. HSV conjunctivitis most frequently occurred between 21 to 40 year age group (58.3%). The age distribution was similar to that of viral conjunctivitis in general (Table 4.1).

Table 4.5: Distribution of HSV conjunctivitis according to age

Range of age (year)	No of patients (n=12)	Percentage (%)
7 to 20	2	16.7
21 to 40	7	58.3
41 to 60	2	16.7
61 to 80	1	8.3

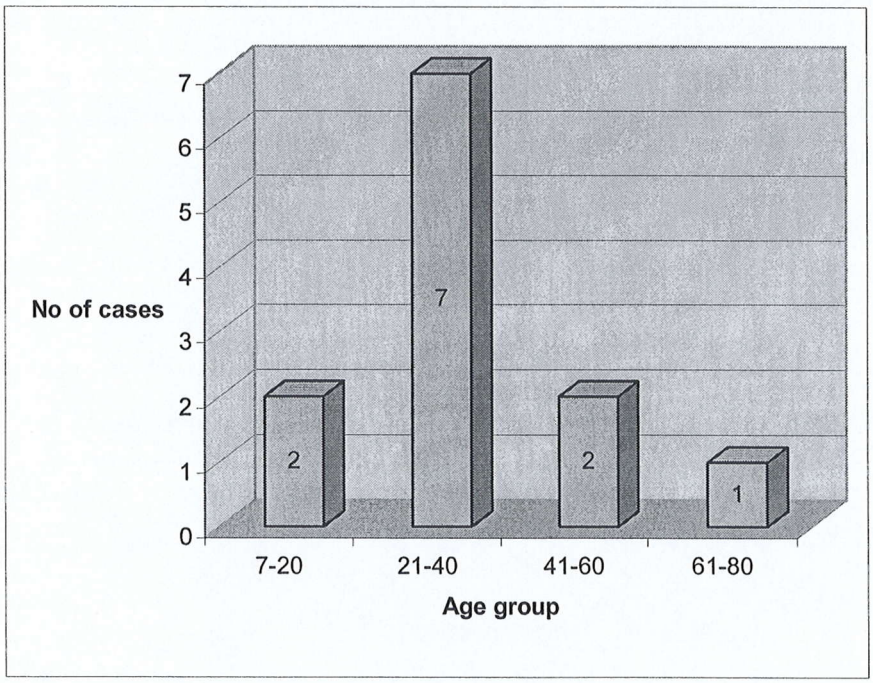


Figure 4.5 : Distribution of HSV conjunctivitis according to age

Table 4.6 : Distribution of HSV conjunctivitis according to sex

Sex	No patients (n=12)	Percentage (%)
Male	5	41.7
Female	7	58.3

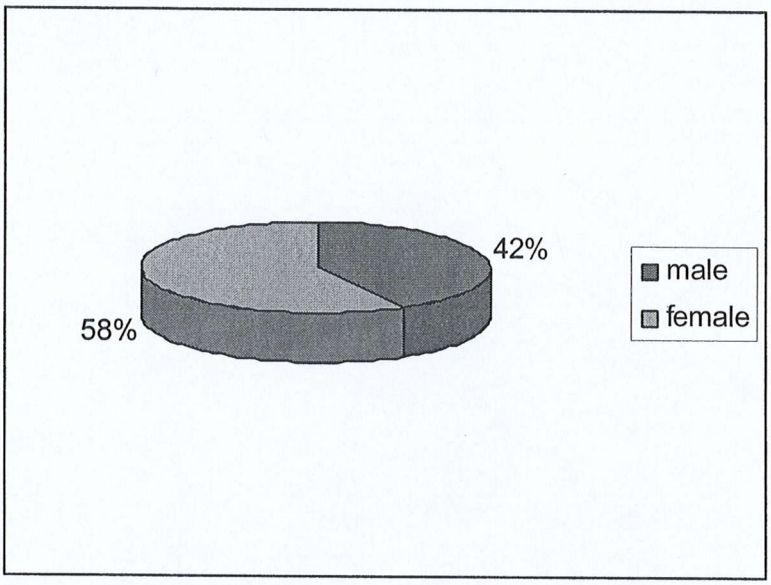


Figure 4.6 : Distribution of viral conjunctivitis according to sex

A female preponderance was found in cases of HSV conjunctivitis presented to the ophthalmology clinic, HUSM with 58.3 % females and 41.7 % males.

Table 4.7 : Gender and age distribution of HSV conjunctivitis

		Range of age (years)			
		0 to 20	21 to 40	41 to 60	61 to 80
Gender	Male	1	3	1	0
	Female	1	4	1	1

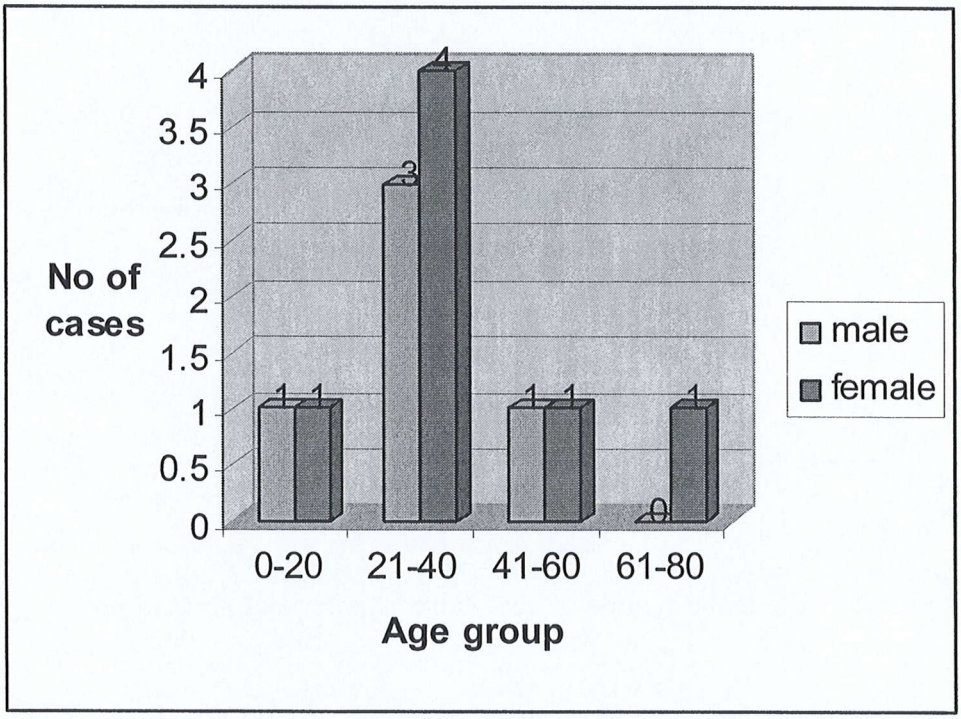


Figure 4.7 : Gender and age distribution of HSV conjunctivitis

A female preponderance of 1.5:1 was noted in patients with HSV conjunctivitis over the age of 20 but no difference was found in those under 20 years of age.

Table 4.8 ; Distribution of HSV conjunctivitis according to race

Race	No of patients (n=12)	Percentage (%)
Malay	11	91.7
Chinese	1	8.3

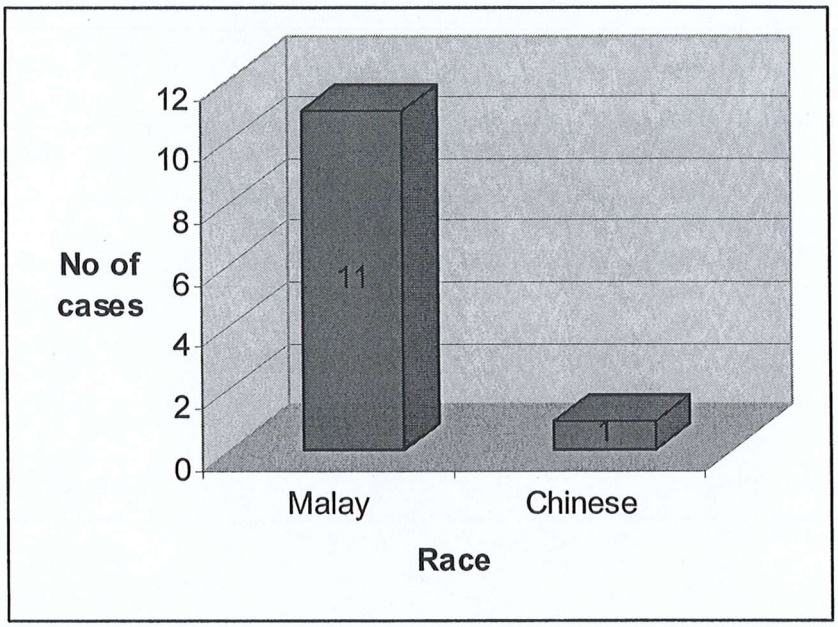


Figure 4.8 : Distribution of HSV conjunctivitis according to race

The majority of HSV conjunctivitis cases comprised of Malays (91.7 %) as for the racial distribution in Kelantan population was similar to the distribution seen in viral conjunctivitis.

4.4. HSV CONJUNCTIVITIS : CLINICAL FEATURES

4.4.1. SOURCE OF INFECTION

The source of infection in HSV and nonHSV conjuntivitis are listed in Table 4.9.

Table 4.9 : Frequency of source of infection for HSV and NonHSV conjunctivitis

	HSV conjunctivitis	NonHSV conjunctivitis
	n=12	n=58
Source of infection	No of patients (%)	No of patients (%)
Contact conjunctivitis	8 (66.7)	23 (39.7)
URTI	0 (0.0)	2 (3.4)
Unknown	4 (33.3)	25 (43.1)
Others	0 (0.0)	8 (13.8)

Contact conjunctivitis was the major source of infection for HSV conjunctivitis, 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. In nonHSV conjunctivitis, the source of infection was not known in majority of cases (43.1%). This was followed by contact conjunctivitis (39.7%) and URTI (3.4%).Other sources of infection (13.8%) were alleged ocular foreign body (5 cases), improper handling of contact lenses (2 cases) and blistering skin lesion (1 case).

Table 4.10 : Frequency of contact person for contact conjunctivitis in HSV and NonHSV conjunctivitis

Contact person	HSV conjunctivitis n=12 No of patients (%)	NonHSV conjunctivitis n=58 No of patients (%)
Family member	7 (58.3)	16 (27.6)
Friends	0 (0.0)	5 (8.6)
Neighbour	0 (0.0)	1 (1.7)
Others	1 (8.3)	1 (1.7)

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. In nonHSV conjunctivitis, majority of cases (27%) had contracted the disease from family members whereas 5 cases (8.6%) from friends, 1 case from a neighbour and another from a patient at a health center.

4.4.2. SYMPTOMS AND SIGNS

The presenting symptoms and signs of HSVand nonHSV conjunctivitis are listed in Table 4.11 to Table 4.12.

Table 4.11 : Presenting symptoms of HSV and NonHSV conjunctivitis

Symptoms	HSV conjunctivitis n=12 No of patients (%)	NonHSV conjunctivitis n=58 No of patients (%)
Laterality		
Unilateral	9 (75.0)	23 (39.7)
Bilateral	3 (25.0)	35 (60.3)
Foreign body sensation	12 (100.0)	57 (98.3)
Lacrimation	12 (100.0)	57 (98.3)
Eye discharge	9 (75.0)	40 (69.0)
Itchiness	9 (75.0)	32 (55.2)
Photophobia	7 (58.3)	35 (60.3)
Blurred vision	6 (50.0)	29 (50.0)

Foreign body sensation and lacrimation were the main symptoms that were present in both HSV and nonHSV conjunctivitis patients. The other common features found in both groups were presence of eye discharge, photophobia and blurring of vision.

However, majority of HSV conjunctivitis cases were unilateral (9 out of 12 cases; 75.0%) whereas majority of nonHSV conjunctivitis cases were bilateral (35 out of 58 cases; 60.3%).

Table 4.12 : Presenting signs of HSV and Non HSV conjunctivitis

Signs	HSV conjunctivitis n=12 No of patients (%)	NonHSV conjunctivitis n=58 No of patients (%)
Conjunctival injection		
Mild	3 (25.0)	28 (48.3)
Moderate	6 (50.0)	24 (41.4)
Severe	3 (25.0)	6 (10.3)
Conjunctival reaction		
Follicular	6 (50.0)	28 (48.3)
Papillary	5 (41.7)	20 (34.5)
Mixed	1 (8.3)	10 (17.2)
Conjunctival haemorrhage	0 (0.0)	3 (5.2)
Eye discharge (mucoid)	8 (66.7)	37 (63.8)
Lid involvement	9 (75.0)	32 (55.1)
Swelling	9 (75.0)	30 (51.7)
Blisters	0 (0.0)	2 (3.4)
Corneal involvement	7(58.3)	31(53.4)
Punctate keratitis	5 (41.7)	18 (31.0)
Dendritic keratitis	1 (8.3)	1(1.7)
Disciform keratitis	1 (8.3)	9 (15.5)
Nummular keratitis	0 (0.0)	10 (17.2)
Pseudomembrane	3 (25.0)	5 (8.6)
Preauricular lympadenopathy	1 (8.3)	4 (6.9)

In both HSV conjunctivitis and nonHSV conjunctivitis, the conjunctival reaction was mainly of follicular response which was 50.0% (6 out of 12 cases) and 48.3% (28 out of 58 cases), respectively. Mucoid eye discharge was also a common presentation in both the groups, comprising of 66.7% and 63.8%, accordingly. The other common feature noted in this study was the corneal involvement which occurred in 7 (58.3 %) out of 12 patients with HSV conjunctivitis and 31 (53.4%) out of 58 patients of nonHSV conjunctivitis. In both the groups, the corneal involvement were mainly of punctate type. However, in nonHSV conjunctivitis, there were 7 patients presented with more than one type of keratitis. Preauricular lymphadenopathy was elicited in HSV as well as nonHSV conjunctivitis which accounted for 8.3% (1 of 12 cases) and 6.9% (4 of 58 cases), respectively.

Regarding conjunctival injection, majority of HSV conjunctivitis patients presented with moderate conjunctivitis comprising of 50% (6 of 12) of cases whereas in nonHSV conjunctivitis, most of them presented as mild conjunctivitis accounting for 48.3% (28 of 58 cases). Lid involvement was seen more in HSV conjunctivitis than nonHSV conjunctivitis which was 75.0% and 55.4%, respectively. Nevertheless, no herpetic vesicles was seen in patients with HSV conjunctivitis. Thirty (51.7%) cases of nonHSV conjunctivitis presented with lid swelling and 2 (3.4%) cases had blistering lesion over the eyelids which followed the dermatome of ophthalmic division of trigeminal nerve. Pseudomembrane formation was seen quite frequently in HSV conjunctivitis (25.0%).

5. 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.

Herpes simplex virus (HSV) is rare cause of conjunctivitis alone without cornea or lid involvement. This is shown in a study by Uchio et al wherein the prevalence of HSV conjunctivitis is very low accounting for 4.3 % of cases (Uchio et al,2000). However, having said that, HSV conjunctivitis may involve into corneal involvement if not properly managed. In fact, HSV is the major cause of corneal blindness in developed countries (Darougar et al, 1985).

Due to the serious morbidity of this infection, we planned to conduct 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. Apart from that, 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.

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 choosen for this study. First and foremost, 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. In a study by Kaye et al (2000), it was shown that the sensitivity and specificity of PCR for HSV-1 were 82% and 78% respectively whereas the sensitivity and specificity of culture were 2% and 100% respectively. Using 2 different types of specimens, Hidalgo et al (1998) showed that nested PCR gave a higher positive rate of HSV than viral cultures, in corneal scrapings being 87.5% vs 31.25% and in tear film being 75% vs 12.5%. 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, particularly in this study. There are few reasons for this method to be inapplicable. Firstly, 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 (Elnifro et al, 1999) 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, seventy cases of viral conjunctivitis were studied over a period of 1 year (November 2002 to November 2003). The samples were collected from patients attending ophthalmology and out patient clinics in Hospital Universiti Sains Malaysia. These cases presented to the clinic with either conjunctivitis alone, keratoconjunctivitis or blepharoconjunctivitis. It was shown that in our study, viral conjunctivitis occurred more in adult population (80%) whereby the highest proportion of cases seen between 21 to 40 years age (45.7%). This result corresponds to a study done by Weiss et al (1993) showing that only 13 % of acute conjunctivitis in childhood were due to viral infection whereas up to 80% of cases were due to bacterial infection. However, Fitch et al (1989) reported that in adult the prevalence of viral and bacterial etiologies of acute conjunctivitis were almost equal, comprising of 36 % and 40 %, respectively.

With respect to the gender distribution, this study showed a slight female preponderance (51.4 % vs 48.4 %). In general, there is no sex predilection in viral conjunctivitis except in certain types of viral etiologies. Although, some studies have shown a slight male preponderance affecting people between ages 20 and 40 years old in adenoviral conjunctivitis and in recurrent herpetic ocular disease (Asbell et al, 1996; Hyndiuk & Glasser, 1996).

In Kelantan, the male to female ratio is almost equal . However, in Kota Bharu district itself, in term of figures, the female population is slightly more compared to the male population (Housing census, 2000). Therefore, this may explain the slight female preponderance of viral conjunctivitis in HUSM found in this study.

The ethnic groups in Malaysia consist of three major races and they are Malay, Chinese and Indian. According to the population distribution and basic demographic characteristics, majority of the population in Kelantan belong to Malays (95.0 %), followed by Chinese (3.8%) and Indians (0.3%) (Housing Census 2000). In this study, majority of viral conjunctivitis patients presenting to the ophthalmology clinic, HUSM were Malays comprising of 95.7 %. Therefore, the result obtained actually reflects the racial distribution in the population.

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% (Uchio et al, 2000; Harding et al,1987; Darougar et al, 1985;) 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. PCR, a nucleic acid amplification procedure is very sensitive and highly specific compared to viral culture, proven by various studies (Kaye et al, 2000; Hidalgo et al, 1998; Kowalski et al, 1993). Owing to this, PCR is recognized

as a potential modern diagnostic tool for detection and identification of certain infectious agents such as adenovirus, HSV and *C trachomatis*. Jackson and coworkers (1996) had developed a multiplex PCR for simultaneous investigation of adenovirus and HSV in ocular swabs and reported that PCR had a considerable potential for the rapid diagnosis of viral eye disease particularly if β-globin primers can be included in the reaction. Another study by Yamamoto et al (1994), concluded that PCR can be a useful diagnostic tool for confirming clinical observation in cases of herpetic keratitis. All in all, this may explain the high prevalence rate of HSV infection in viral conjunctivitis in our study.

In previous studies, the methods used in detecting the prevalence of herpes simplex ocular infections were by HSV isolation via viral culture method which, as mentioned, has a lower sensitivity rate. As a result, it may the cause of low prevalence in those studies.

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.

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, similar to age distribution in viral conjunctivitis (45.7%). 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. However, Uchio et al (2000) reported in his study that the age distribution of HSV conjunctivitis was similar to adenoviral conjunctivitis with 65% occurring in 20-59 year age group. This is similar to our study where our age distribution of HSV conjunctivitis in HUSM occurring in 20-60 years old accounted for 75% (9 out of 12 patients).

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 (Pramod et al, 1997; Field et al, 1993). It also has been reported by many workers from developed countries that circulating antibodies against HSV relatively remain constant, though fluctuations occur occasionally (Wilhelmus et al, 1986)

In previous studies, it has been reported that primary ocular HSV infection presents predominantly in two age groups consisting of children and young adult age group, with equal prevalence. However, Darougar et al (1985) reported in his case series, on 108 patients with primary ocular HSV infection, the proportion of cases in adolescents and young adults has increased from 41 % to 64 % with mean age of 25 years. A study by Liesegang et al (1989) also suggested that primary herpes is now more frequent in adults than previously. This is further supported by a recent study done by Pramod et al (1997) showing that 50% of "first episode"of HSV keratitis were seen in adolescent and young adult whereas only 9.09% constituted children below 5 years of age. Thus, in our study, where the age ranged from 12 to 68 years (both primary as well as recurrent cases) were seen. As a consequence, we could not really differentiate the clinical features of HSV conjunctivitis between primary and recurrent ocular infection.

Several studies had shown that there was no sex difference in primary HSV ocular infection (Darougar et al 1985; Uchio et al,1993). 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). No difference in terms of gender distribution was found in age less than stated. On the contrary, Uchio et al (1993) had found that the recurrence rate was higher in women aged 50 years or younger than in women aged 51 years or older but in general, there was no sex difference in recurrent HSV ocular infection.

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. In addition, a female preponderance of 1.5:1 is noted in patients over the age of 20 years old but no difference is found in those under 20 years of age. 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 (Housing census, 2000). 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.

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 (Nahmias et al, 1973). 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 (Darougar et al, 1985). Spread via droplets is postulated, but not well documented (Verdier & Krachmer,1984). 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 conjunctivitis which accounted for 50% of cases as compared to nonHSV conjunctivitis patients which showed mild conjunctivitis, comprising of 48.3% of cases. In both the groups, 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 (Kanski, 1999).

As for keratitis associated with conjunctivitis in this study, generally the corneal involvement was seen equally and frequently in both the HSV and nonHSV conjunctivitis, which was 58.3% and 53.4%, accordingly. Subepithelial punctuate keratitis was found in both the HSV and nonHSV conjunctivitis (41% and 31%, respectively). Whereas dendritic keratitis was more frequently seen in HSV conjunctivitis

(8.3%) as compared to nummular and disciform keratitis in nonHSV conjunctivitis (17.2% and 15.5%, respectively). The dendritic keratitis which was the least frequent keratitis seen in nonHSV conjunctivitis (1.7%) in this study, was most likely due to herpes zoster.

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 (Palmer ML & Hyndiuk RA, 2000). 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 (Hyndiuk & Glasser, 1996)

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.

Lid involvement was seen more frequently in HSV (75.0%) than nonHSV conjunctivitis (51.7%) in the form of lid swelling, whereas 2 patients (3.4%) had blisters on eyelids in nonHSV conjunctivitis. However, the vesicular blepharitis in these patients actually followed the dermatome of ophthalmic division of trigeminal nerve distribution which made the diagnosis of herpes zoster ophthalmicus the most probable one. In our study, pseudomembrane formation (25.0%) was seen more frequently than preauricular lymphadenopathy (8.3%) in HSV conjunctivitis.

Herpes simplex virus should always be considered in the differential diagnosis of acute or subacute follicular conjunctivitis (Verdier et al, 1984). Acute follicular conjunctivitis in HSV ocular infection can occur in both primary and recurrent HSV ocular infection, with or without lid or corneal involvement (Wishart et al, 1987; Darougar et al, 1985). 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 (Aoki et al, 1982). 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. 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 (Uchio et al, 2000; Bell et al,1982; Wilhelmus et al, 1981). 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

6. CONCLUSION

HSV is the major cause of corneal blindness in developed countries. The documentation of clinical and virological features of this important ocular pathogen, however, is far inadequate in developing and under developed countries. Well documented clinical and virological features are a paramount importance in cases of HSV ocular diseases for planning ophthalmic services, estimating morbidity and social impact of this condition, investigating risk factors and developing strategies to control the disease.

In comparison to other studies, our study showed higher prevalence rate of HSV conjunctivitis in HUSM . This could be due to the method used as well as the type of study carried out. In most studies, viral culture which is less sensitive was used whereas in this study, PCR method which has been shown to have a high sensitivity and specificity was utilized. Because of its exquisite sensitivity, false positive result may be encountered which could give rise to higher prevalence rate in this study but scrupulous technique was applied in this PCR study to prevent contamination of the samples and thus, avoiding false positive result. In addition, this study was a hospital based study as compared to other studies with lower prevalence rate of HSV conjunctivitis which were 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 addition, viral culture needs a technically

trained personnel to ensure viral growth. On the whole, this could perhaps illustrates the superiority of PCR over viral culture.

As for 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. This problem is also applied in analyzing the demographic characteristics of HSV conjunctivitis.

The other limitations in analyzing the clinical features were the use of topical treatment, particularly, topical steroid which may alter the findings in patients with HSV conjunctivitis as well as nonHSV conjunctivitis. This problem was a little bit difficult to overcome since most of the patients in our study were patients whose conjunctivitis was not resolved with prior treatment either from local health clinics or general practitioner.

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. Since our present study is cross-sectional, we could not really study the natural history of HSV ocular infection in our setup. Therefore, in addition to the suggestion mentioned, this study should also be a prospective type as information

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APPENDIX 1

OPTHALMOLOGY DEPARTMENT
SCHOOL OF MEDICINE
UNIVERSITY SAINS MALAYSIA

DATE:

ANALYSIS OF VIRAL CONJUNCTIVITIS CASES TO DETERMINE THE PREVALENCE OF HSV CONJUNCTIVITIS USING PCR

1. BIODATA

Name																					

RN								Age			years			mths			Days

Sex		F / M	Race		M – Malay C – Chinese I – Indian S – Siamese O – Others ; specify _____

2. CLINICAL PRESENTATION

2.1 SOURCES OF INFECTION

CONTACT FROM

CASES	
Conjunctivitis	
URTI	
Urogenital ds	
Unknown	
* Others	

* if others to specify

PERSON	
Family	
Friends	
Colleague	
Neighbour	
Unknown	
* Others	

PLACES	
Home	
Health centre	
School	
Swim. pool	
* Others	

2.2 SYMPTOMS AND SIGNS

SYMPTOMS

duration		
affected eye	RE	LE
foreign body sensation		
lacrimation		
eye discharge		
itchiness		
photophobia		
blurred vision	RE	LE
* others (to specify)		

SIGNS

vision	RE		LE
conjunctival injection	mild	moderate	severe
conjunctival reaction	follicular	papillary	mixed
conjunctival haemorrhage			
Eye discharge	mucoid		Purulent
* others (to specify)			

Involvement of other ocular structure :

Eyelids	RE	LE
Cornea	RE	LE
Types : punctate keratitis		
Dendritic keratitis		
* others (to specify)		
Preauricular lympadenopathy	yes	no
Pseudomembrane	yes	no

APPENDIX 2

BORANG MAKLUMAT DAN KEIZINAN PESAKIT

Kajian Prevalens untuk Mengesan Jangkitan Herpes Simplex Virus dalam Kes-kes Konjuktivitis Virus di HUSM dengan Menggunakan Teknik PCR

Pengenalan

Jikalau anda mengalami jangkitan mata (conjunctivitis), anda dijemput untuk menyertai satu kajian penyelidikan secara sukarela yang melibatkan kehadiran anda ke Klinik Mata HUSM, di mana pemeriksaan mata untuk menentukan samada jangkitan kuman tersebut adalah disebabkan oleh kuman virus atau tidak, dan seterusnya swab pada konjunktiva akan diambil untuk ujian PCR bagi mengesan virus Herpes simplex.

Seramai 100 orang peserta akan menyertai kajian ini, di mana mereka akan dipilih dari Klinik Mata dan Klinik pesakit luar, HUSM mengikut kelayakan penyertaan.

Anda akan dikehendaki datang ke Klinik Mata HUSM sekali sahaja pada hari tersebut. Sekiranya anda didapati mengalami jangkitan mata, rawatan yang sewajarnya, dan jika perlu, temujanji susulan akan diberi.

Tujuan Kajian

Kajian ini adalah bertujuan untuk menentukan prevalens atau kadar kejadian jangkitan virus Herpes simplex dalam kes–kes jangkitan mata yang disebabkan oleh virus (konjunktivitis virus)

Kelayakan Penyertaan

Doktor yang bertanggungjawab dalam kajian ini telah membincangkan kelayakan untuk menyertai kajian ini dengan anda. Adalah penting anda berterus terang dengan doktor tersebut tentang sejarah kesihatan anda. Anda tidak seharusnya menyertai kajian ini sekiranya anda tidak memenuhi semua syarat kelayakan.