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ORIGINAL ARTICLE

Prevalence and Genotyping of Herpes Simplex Virus-1 Among the Patients with Keratoconjunctivitis in Andaman Islands, India

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Abstract

Background: Various microbes and allergens are responsible to cause keratoconjunctivitis. But the viral agents are mostly associated with this ocular illness. Adenovirus, Herpes simplex virus (HSV), and enterovirus are the main causative agents. Among those, HSV is of great concern as it can lead to encephalitis.**Aim:** To determine the prevalence and know the circulating genotype of Herpes Virus among the population of Andaman Island.**Methods:** Conjunctival swabs from 470 Keratoconjunctivitis suspects were collected (August 2017-January 2022). Diagnosis of HSV-1 and HSV-2 carried out by PCR. **Result:** Positives were typed with Sanger's Sequencing and sequences analyzed with MEGA software. 20 patients found positive for HSV-1 and typing revealed HSV Strain circulating in Andaman Islands was of AES origin.**Conclusion** This is first report on Ocular HSV prevalence and strain connected to AES origin in Andaman Islands. To prevent encephalitis epidemics in Andaman population, HSV screening for cases is needed.

Keywords

Keratoconjunctivitis, Herpes Simplex Virus, Ocular Infections, Andaman Islands

Introduction

Keratoconjunctivitis is an inflammation of the conjunctiva and superficial cornea mainly caused by Allergens and microorganisms. It was noted that 20%–70% of conjunctivitis cases are caused by viruses,among those 8–75% patients have acute infection.[1,2] The most common viral agents that cause ocular infections are herpes simplex, enteroviruses, adenoviruses, and herpes zoster. HSV-1 (Herpes Simplex Virus 1) causes encephalitis, keratitis, and mucocutaneous ulcers. It is the most prevalent virus causing spontaneous encephalitis and blindness in the USand spreading worldwide and affects people of all ethnicities.[3-5]Alpha-, Beta-, and Gamma-herpesviruses have large 152kb double-stranded DNA encoding 84 polypeptides. Even in immune-competent people, herpes simplex virus (HSV) is most often spread through eye, oral, or vaginal contact.[6]

HSV-1 is assumed to reside in the trigeminal ganglia and trigger oral (cold sores, fever blisters) and ocular

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(keratitis) outbreaks.[6,7] In Africa, HSV-1 prevalence is estimated to be 87%, while in the America, it is estimated to be 40%–50%.[8,9] While 88% of ocular HSV-1 cases are unilateral, 12% are bilateral.[5,9] The symptoms of ocular HSV1 infection includes redness, photophobia, excessive tears, pain, impaired vision, irritation,foreign body sensation.[8,10] Despite this, there exists some evidence that suggests viral shedding during the asymptomatic phase is a significant route of transmission.[11]

Studies of viral gene sequence and glycoprotein variation are of interest due to their clinical implications and infection severity.[12,13] However, understanding HSV-1 sequences and clades in various clinical conditions is crucial. Thus, Andaman Islands have no data on the prevalence of herpes simplex virus (HSV) and the HSV type-specific strain in circulation. The Keratoconjunctivitis is mainly associated with HSV1. So, the study aims to characterize the clinical presentation, molecular profile, and genotype of ocular herpes simplex virus (HSV) strains.

Material and Methods

Ethical clearance was obtained prior to initiate the study.

We collected conjunctival swabs in VTM vials from 470 individuals with keratoconjunctivitis symptoms who visited eye hospitals between August 2017 and January 2022. The swab specimens were pelleted and re-suspended in 400µl PBS for genomic DNA extraction. Viral DNA was extracted from the 200µl sample suspension using the Pure Link Viral RNA/DNA Mini Kit (Invitrogen, USA -cat no.12280-050) according to the manufacturer's instructions (including negative controls), and the DNA was eluted with 45µl of Elution buffer. The identification of HSV-1 targeting TK gene (F-5'-AGCGTCTTGTCATTGGCGAA-3'; R-5'-TTTTCTGCTCCAGGCGGACT-3') and HSV-2 pol gene (F-5'-GTCCCACCTCAGCGATCTGCCT-3'; R-5'-CAGCAGCGAGTCCTGCACAA-3') was performed using conventional PCR.[14] The amplification range for the HSV 1 primer set was 342bp, while the range for the HSV 2 primer set was 490bp. To achieve maximum amplification, a final volume of 25µl PCR Reaction mix was used, which contained 7µl of DNA template, 12.5µl of 2x PCR Master mix (Cat. No. K0171), 0.7µl of forward and reverse primers, and 4.1µl of Nuclease free water. The amplification was carried out using the following cycling conditions on an Applied Biosystems (Model no.9902, Singapore)veriti 96 well PCR thermal cycler: Initial denaturation for 12 minutes at 94^0C followed by Denaturation for 30 seconds at 94^oC, annealing for 30 seconds at 55^oC, extension for 30 seconds at 72^oC (up to 40 cycles) and final extension at 72^oC for 3 minutes followed by a holding stage at 4^0C. To ensure accurate results, each PCR experiment also included negative and positive controls. The â-globin gene was utilized as an internal check to ensure the integrity and purity of the extracted DNA.[15]Following amplification, 10µl of PCR products were run on a 2% agarose gel electrophoresis to detect the presence of specific bandsagainst a 100 bp DNA ladder. And the gel run was observed under the UV transilluminator to look for HSV 1 and HSV 2 bands.

The positive HSV-1 samples were typed using Sanger's Sequencing (Big Dye Chain Termination Method), and the raw Sequence obtained was analyzed using Mega software (MEGA version 11.0). The sequence was aligned and analyzed against worldwide reference sequences from the Genbank database. A total of 39 Genbank reference sequences were obtained and used in the phylogenetic analysis to determine the circulating clade in the study area. The ClustalW method, which was included in mega software version 11.0, was used to align Genbank sequences.

Results

Conjunctival swab specimens from 470 patients were analysed for the presence of HSV-1 and HSV-2 over the course of the study, which spanned from August 2017 to January 2022. Research was done in order to assess the prevalence of HSV infection that is related with keratoconjunctivitis. Twenty samples (4.26%) of the total, tested positive for HSV-1, while none tested positive for HSV-2. All those who tested positive for HSV-1 showed symptoms within a week of testing. In comparison to the diagnosed female patients (N=7, 35%), the number of male patients (N=13, 65%) were found to be more with HSV-1 infection. The majority of patients with HSV-1 linked keratoconjunctivitis had unilateral eye involvement. Redness, ocular pain, increased tear production, and blurred vision were the most often experienced symptoms among the study population (*Table 1*).

Genome retrieval and phylogenetic analysis

The Neighbor-joining bootstrap consensus phylogenetic

Table 1:-Percentage Distribution of Symptoms Among HSV-1 Mediated Keratoconjunctivitis Patients.

Total positives	Percentage (%)
Male Patients positive for HSV-1	65
Female Patients Positive for HSV-1	35
Unilateral	95
Bilateral	5
Symptoms	%
Blurry Vision	50
Swelling of eyelid	35
Swelling of Conjunctiva	30
Redness	90
Follicles	15
SPK	30
Eye pain	75
Cloudy Cornea	5
Photophobia	10
Excessive tearing	65
FB sensation	5
Fever	15
Itching	20

tree analysis showed that the HSV-1 that was found in the Andaman Islands was related to other strains of the virus and had 0.00% genetic distance with F-strain (GU734771) isolated in the United States which belongs to HSV-1 clade I (*Fig.1*).

Additional research was conducted in order to gain a better understanding of the genetic connection that exists between the HSV1 strains found on these distant islands and those found in other regions of mainland India.HSV-1 sequences from the Andaman Islands had a very small genetic distance (K2P=0.003%) with two Indian strains (KJ847330 and MH319852) isolated from the Cerebrospinal Fluid (CSF)of patients suspected of having encephalitis, then an isolate (MG646679) from patient with throat infection in mainland India (K2P=0.003%).

Discussion

Based on geographic location and genetic variation, HSV-1 is classified into five subgroups / clades (I-V). Viruses of clade I are more common in the North and South America, whereas those of clade II are more common in East Asian nations. The remaining clades (III-V) trace their ancestry to nations in East Africa.[12]

Viral encephalitis can cause symptoms that are extraordinarily severe, such as encephalopathy, epileptic

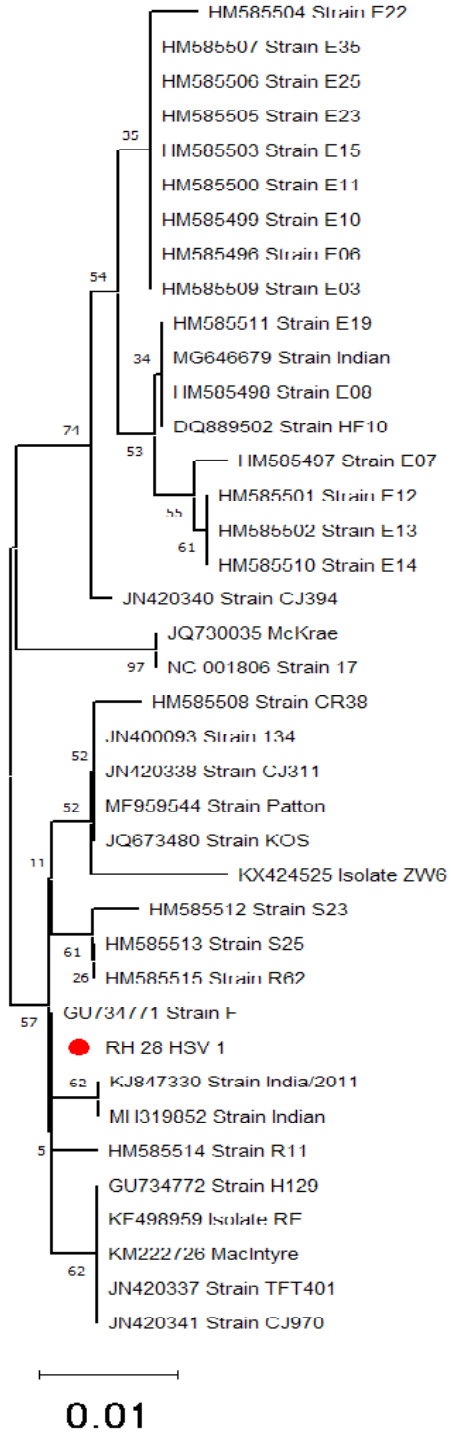

Fig 1: The Neighbor-joining bootstrap consensus phylogenetic tree shows the genetic relationship between HSV-1 gene sequence from Andaman and Nicobar Islands, India, and 39 reference sequences of worldwide. (Red round denotes circulating strain in Andaman)

convulsions, focal deficits, neurological sequelae, and even death in the most serious cases. Despite the fact that neurotropic herpes viruses (HSV and VZV) are among the most frequently reported agents of cause and effect, it is possible for other forms of infections to manifest in a certain set of conditions. However, neuroimaging and molecular biology (PCR, metagenomics) have recently made significant strides, enabling faster and more precise etiological diagnosis.[16]In 2016, Pfaff and colleagues performed whole-genome sequencing of HSV-1 and discovered three main phylogroups correlating to geographic locations and reflecting human migration history.[17]There is a growing understanding in India in terms of the association between the HSV-1 and sporadic acute encephalitis.[18-20]In addition, it is primarily accountable for around 10% of the fatalities resulting from reiterated episodes of acute encephalitis syndrome (AES).[21]

During the course of this research, a total of twenty people who tested positive for HSV-1 were identified using molecular diagnostics. Clinical indicators and symptoms experienced by the patient are the main criteria used to diagnose herpes simplex virus keratitis. Although HSV-1 infectious keratoconjuncitvitis are typically unilateral. The symptoms can manifest in a variety of ways, including painful blisters or lesions at the site, redness, irritation, pain, blurred vision, excessive weeping, photophobia, blepharitis, foreign body sensation, and conjunctivitis.[25]The majority of patients showed up with unilateral symptoms, with the eye redness (90%), ocular pain(75%), increased tearing(65%), and blurred vision(50%) were the most often experienced symptoms. Besides this, the majority of patients reported within a week after the commencement of symptoms. In addition, typing and sequence analysis revealed that the circulating HSV-1 strain of ocular origin seen in Andaman Islanders is of a genetically related AES ancestry. This information was gleaned through the study of suspected cases of Keratoconjunctivitis among the population of Andaman Island. Aside from that, this is the first report on ocular HSV to come from this remote geographic location.From March 2016 to February 2017,researchers from Rajnandgaon, India, identified 25% of patients with HSV infection during a prospective study.[24]

HSV-1 is one of the most common cause of fatal viral encephalitis in adults, and it occurs without any identifiable seasonality everywhere in the world, after the neonatal period.[22] In addition, HSV-1 is the most common cause of fatal viral meningitis. In the same vein, the findings of this analysis revealed that there was no seasonal variation noticed during the diagnosis of HSV positive cases, and that patients with HSV infection came from all age groups.According to the findings of our research, out of a total number of cases only twenty were positive, and upon sequencing it was analysis that the circulating strain of HSV- 1 among this tribal population were recognized as of AES origin. No matter how well the disease is treated, herpes simplex encephalitis is almost always deadly in very young children. This includes the infants and the toddlers. Despite the fact that it strikes younger age groups more frequently, the elderly and very young children are at an especially high risk for contracting the disease.[22]Hence our study strongly suggests that all keratoconjuctivitis cases must be effectively screened for viral etiologies to prevent suddenAES outbreaks among all the age groups. Topical antivirals are used to treat HSV-1 associated keratoconjunctivitis. In cases of systemic infections, oral and intra-venous acyclovir remains the therapy of choice.[18] With the increasing reports on antiviral drug resistance of HSV strains, acyclovir is a growing concern. Therefore, for the effective management of HSV infection among the population of any geography can be achieved by combination of acyclovir, valacyclovir, and famciclovir, presently which is considered as the backbone of HSV-1 treatment.[23]Recurrent infections often lead to visual loss, which usually manifests as stromal corneal involvement. Dendritic keratitis, an infection of the corneal epithelium, is the most typical site of HSV manifestation in the eye, though it can appear anywhere in the body. There is a higher chance of recurrence and worse visual impairment if it has invaded the corneal stroma.[25]

Conclusion

In addition to exhibiting a rising medication resistance quality, the HSV-1 strains that are circulating on these far-flung islands share close genetic characteristics with the isolates of acute encephalitis syndrome strains. These strains can also be found elsewhere in the world. As a result, testing for HSV is necessary to be in place for each and all instances in which viral encephalitis is suspected. In addition, it is crucial to rule out the HSV-1 associated with keratoconjunctivitis on a regular basis as a part of preventative measures against the future outbreaks that could lead to acute encephalitis, particularly

among the children. This is particularly an important aspect when it comes to paediatric patients.

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