

Research article

**The impact of non-indicated CSF testing on the true detection rate of viral encephalitis
A single-centre retrospective study**

Abeywardana KDST, Priyanganie LUA, Susantha KMI

Sri Lankan Journal of Infectious Diseases 2026 Vol.16(2):E121:1-6

DOI: <https://doi.org/10.4038/sljid.v16i2.8908>

Abstract

Introduction: Timely and accurate diagnosis is required for viral encephalitis, which can result in devastating consequences. However, inappropriate ordering of cerebrospinal fluid (CSF) tests risks artificially lowering the documented positivity rates of viral encephalitis. Our study aimed to evaluate the ordering pattern of CSF viral testing and estimate the true incidence of herpes simplex virus (HSV) and varicella-zoster virus (VZV) infection in adult patients with clinical features of encephalitis.

Methods: This study retrospectively reviewed adult CSF specimens collected at National Hospital, Galle, from December 16, 2023, to December 15, 2024. Based on diagnostic thresholds outlined by the British Infection Association (BIA) and the Association of British Neurologists, medical request profiles were split into two cohorts: clinically justified encephalitis and non-encephalitic cases. We compared HSV and VZV incidence between the two groups to assess the appropriateness of viral testing and its diagnostic yield.

Results: Of 224 CSF specimens, 89 met the criteria for clinical encephalitis, while 135 were grouped as non-encephalitic. The incidence of HSV and VZV in the encephalitis group was 6.74%, whereas in the non-encephalitis group, it was 0%. Statistical analysis showed a higher diagnostic yield of viral infections in the clinical encephalitis group (p-value = 0.0035). Of the six positive samples, one was positive for HSV-1, and the remaining five were positive for VZV.

Conclusion: This study highlights that CSF viral testing is indicated and yields higher diagnostic value in patients with encephalitis. Non-indicated testing may underestimate the true incidence of HSV and VZV viral encephalitis. Optimising CSF testing by establishing diagnostic criteria is essential for improving diagnostic stewardship.

Keywords: *Diagnostic stewardship, Viral encephalitis, HSV, VZV, Incidence*

National Hospital, Galle, Sri Lanka.

Address for correspondence: Dr KDST Abeywardana; , Colombo North Teaching Hospital, Ragama, Sri Lanka

Tel: +94716367437; Email: sevwandiabeywardana@gmail.com  <https://orcid.org/0000-0002-1303-3114>

Received 1 April 2025 and revised version accepted 31 August 2026. Published on 27.10.26



This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Viral encephalitis is a critical medical emergency marked by acute inflammation of brain tissue secondary to viral invasion. Globally, herpes simplex virus (HSV) and varicella-zoster virus (VZV) represent the leading identifiable viral causes in adults, and accurate identification of these pathogens is critical for appropriate and prompt clinical management.¹ Modern molecular testing suites allow laboratories to quickly and accurately screen for a wide spectrum of central nervous system pathogens within cerebrospinal fluid (CSF). According to a meta-analysis comprising 71 studies, the overall prevalence of HSV encephalitis was 8%.² VZV encephalitis is less common than HSV encephalitis and accounts for 5% of viral encephalitis in adults.¹ A retrospective study conducted at General Hospital Kandy, Sri Lanka, revealed 2.3% positivity for VZV and 0.8% positivity for HSV among 352 CSF samples received from March 2017 to March 2019.³

Despite these baselines, clinicians frequently order broad diagnostic molecular panels defensively, seeking a positive target without strict symptomatic justification.⁴ Accurate quantification of the incidence of these viral infections requires the recruitment of well-defined cases of viral encephalitis since incomplete patient enrollment and misclassification skew prevalence estimates. A clear distinction between true clinical encephalitis and unrelated presentations is critical when tracking the true burden of VZV and HSV. Furthermore, over-testing consumes laboratory resources and delays turnaround times.

This retrospective study aimed to evaluate how unindicated CSF testing affects calculated HSV and VZV encephalitis statistics. Specifically, it sought to compare the diagnostic yield of HSV and VZV in CSF samples from clinically appropriate versus non-indicated patients and highlight the implications of estimating the true incidence of HSV and VZV encephalitis and optimising health resources.

Methods

This retrospective descriptive study analysed data from the Virology Laboratory, National Hospital, Galle. All CSF specimens from adults (aged >17 years) received for HSV and VZV PCR testing from December 2023 to December 2024 were included.

Data was retrieved from the electronic database, laboratory request forms, and laboratory result sheets. Every submission form was meticulously graded against diagnostic benchmarks set by the British Infection Association (BIA) and the Association of British Neurologists (ABN)⁵. Accordingly, patients were categorised into encephalitic and non-encephalitic groups.

Duplicate samples from the same patients, samples received for testing other than suspected CNS infections, and samples with incomplete clinical information not allowing proper group categorisation were excluded. Demographic information, clinical presentation, underlying comorbidities, and immunocompromised status were recorded from request forms and the electronic database. The results of CSF PCR testing for HSV and VZV were obtained from laboratory result sheets.

The primary objective was to determine and compare HSV/VZV frequencies among the

encephalitic and non-encephalitic groups. Age variances were computed using the Mann-Whitney U test, while gender patterns were assessed via chi-square analysis. Fisher's exact tests determined differences in diagnostic yields, using a significance cutoff of $p < 0.05$.

Accurate case recruitment was essential to ensure the reliability and generalisability of the incidence estimates. All patients were therefore selected to ensure that both encephalitic and non-encephalitic cases were adequately represented, and categorisation was carried out by two investigators independently to ensure that patients were classified correctly based on clinical criteria. In addition, specimens with incomplete and unclear clinical data were excluded to avoid misclassification.

Because this study was retrospective, explicit patient consent was waived; however, full anonymity was strictly maintained by de-identifying all data inputs. Given the retrospective nature of the study, informed consent was not required for the use of existing clinical and laboratory data. However, patient confidentiality was strictly maintained, and all data were anonymised before analysis.

Results

A total of 237 CSF specimens were received for testing during the study period, and 13 samples were excluded due to insufficient clinical data. Among 224 samples analysed, 89 (39.7%) met the criteria for clinical encephalitis, while 135 (60.3%) were categorised as non-encephalitic.

The median age was 65 years (IQR 46–72 years) in the encephalitis group and 60 years (IQR 40–72 years) in the non-encephalitic group (Figure 1). Males comprised 50.6% ($n=45$) of the encephalitis group and 43% ($n=58$) of the non-encephalitic group (Figure 2), showing no statistically significant demographic differences.

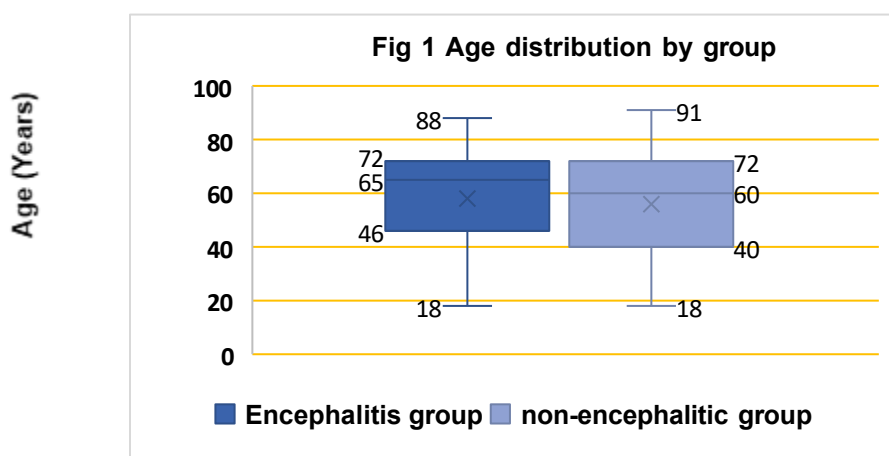


Figure 1: Age distribution by group

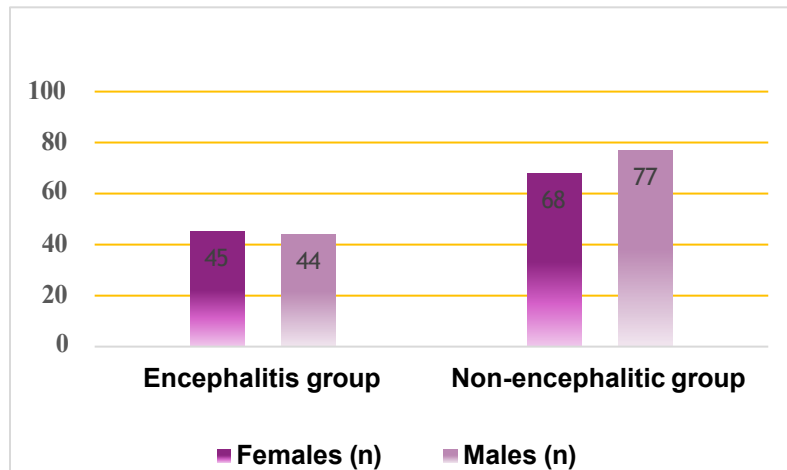


Figure 2: Gender distribution by group

Six of 89 specimens in the encephalitis group (6.74%) were positive for either HSV or VZV, whereas none of the 135 non-encephalitic samples was positive (0%). Statistical analysis showed a higher diagnostic yield of viral infections in the clinical encephalitis group (p -value = 0.0035). Of the six positive samples, one was positive for HSV- 1, and the remaining five were positive for VZV. There were no HSV-2 positives in this study cohort.

The overall diagnostic yield was 2.68% (6/224). But the yield rose to 6.74% when limited to clinically indicated encephalitic cases, highlighting the significant effect of appropriate test selection on diagnostic utility.

Discussion

In our study, if all 224 samples were included in the calculation of incidence, the positivity rate of HSV/VZV would be only 2.68%, underestimating the true rate of 6.74% among the correctly indicated patient cohort. Therefore, inclusion of non- encephalitic cases leads to underestimation of the true disease burden of viral encephalitis. Interestingly, VZV accounted for most of the detected cases of viral encephalitis, and no HSV-2-positive cases were identified. This may reflect the local epidemiological pattern and is consistent with the findings of the study conducted at Kandy, Sri Lanka.³

Diagnostic stewardship acts as a systematic tool to refine ordering practices, reduce unnecessary testing, enhance diagnostic accuracy, ensure clinical utility, and optimise resource utilisation.^{6,7} Furthermore, unnecessary tests increase the burden on microbiology/virology laboratories and contribute to increased healthcare costs.

Limitations: This study had several limitations due to its retrospective design. These include a lack of broad screening for alternative pathogens like enteroviruses, cytomegalovirus (CMV), or arboviruses. Other limitations included missing details of illness duration, antiviral treatment and the specific timing of cerebrospinal fluid (CSF) sample collection relative to symptom onset in the accompanying request forms - critical factors that significantly influence diagnostic positivity rates. Nevertheless, these data offer crucial perspectives into contemporary clinical testing

behaviour and its operational impact. The study was also limited by a lack of clinical data regarding the clinical presentation of immunocompromised individuals and atypical viral infections, such as enteroviruses. Because these populations can present atypically and bypass standard diagnostic criteria, our application of the term 'non-indicated testing' may not fully capture these clinical instances and should be interpreted with caution.

We recommend establishing institutional CSF viral PCR request guidelines aligned with BIA criteria and conducting regular audits to monitor test utilisation to improve diagnostic stewardship. In addition, we recommend future multicentre studies in this area with added testing for other viral pathogens.

Conclusion

This study highlights that inappropriate testing significantly affects the detection rate of HSV/VZV viral encephalitis and also leads to inefficient use of diagnostic resources. It is therefore essential to implement a diagnostic stewardship programme, establish guideline-based testing criteria, and provide clinical education to ensure better patient outcomes, cost-effective laboratory utilisation, and accurate surveillance.

Declaration

Acknowledgement: Not applicable

Conflicts of Interest: No conflicts of interest.

Funding: The authors did not receive any funding for conducting the study.

Ethics statement: The administrative approval taken from the NH Galle and ethics approval and consent to participate were not applicable as retrospective laboratory data were used for the study.

Authors' contributions:

KDST Abeywardana: The main author responsible for the research conceptualisation, supervision of the project, writing, editing, and reviewing.

LUA priyanganie-: Responsible for data collection, investigation, and writing

KMI Susantha: data collection, writing

All authors read and approved the manuscript.

References

1. Granerod J, Ambrose, HE Davies, NW S *et al.* Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. *The Lancet Infectious Diseases* 2010; 10(12):835–844. doi: 10.1016/S1473-3099(10)70222-X.
2. Rohani H, Arjmand R, Mozhgani SH, *et al.* The Worldwide Prevalence of Herpes Simplex Virus Encephalitis and Meningitis: A Systematic Review and Meta-Analysis. *Turk Arch Pediatr* 2023;58(6):580-587. doi: 10.5152/TurkArchPediatr.2023.23007.
3. Dheeraseskara W, Attanayake W, Raziya M, *et al.* Alpha herpes virus infections in a group of patients clinically suspected of central nervous system infections in Sri Lanka. A brief laboratory report. *Sri Lankan J Infect Dis* 2020;10(1):72-75. doi:10.4038/sljid.v10i1.8277.
4. Leber AL, Everhart K, Balada-Llasat JM, *et al.* Multicenter Evaluation of BioFire FilmArray Meningitis/Encephalitis Panel for the Detection of Bacteria, Viruses, and Yeast in CSF Specimens. *J Clin Microbiol* 2016; 54(9):2251–61. doi: 10.1128/JCM.00730-16.
5. Solomon T, Michael BD, Smith PE, *et al.* Management of suspected viral encephalitis in adults – Association of British Neurologists and British Infection Association National Guidelines. *J Infect.* 2012; 64(4):347–73. doi: 10.1016/j.jinf.2011.11.014.

6. Patel R, Fang FC. Diagnostic Stewardship: Opportunity for Laboratory Medicine. *Clin Infect Dis* 2018; 67(5):799–801. doi: 10.1093/cid/ciy077.
7. Messacar K, Parker SK, Todd JK, Dominguez SR. Implementation of Rapid Molecular Infectious Disease Diagnostics: The Role of Diagnostic Stewardship. *J Clin Microbiol* 2017; 55(3):715–23. doi:10.1128/jcm.02264-16.