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Original Research Article

Multiplex PCR assay findings of patients with clinically suspected acute viral encephalitis: an observational study in a tertiary care hospital

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ABSTRACT

Background: Acute viral encephalitis is a life-threatening neurological emergency characterized by fever, seizures, and altered consciousness. Rapid identification of viral pathogens is essential for timely management, yet conventional diagnostic tools often have limited sensitivity. This study aimed to determine the multiplex PCR assay findings and describe the clinical, MRI, and CSF characteristics of patients with clinically suspected acute viral encephalitis in a tertiary care hospital.
Methods: This observational study was carried out at the Department of Neurology during December 2024 to November 2025 in Chittagong Medical College Hospital, Chattogram, Bangladesh of 100 patients irrespective of age and sex with clinically suspected acute viral encephalitis. Detailed clinical evaluation, routine laboratory tests, CSF biochemical and cytological analysis, and MRI imaging were performed. CSF samples were tested using a multiplex PCR panel for detection of common viral pathogens.
Results: The mean age of patients was 45.28±19.95 years, with nearly equal male (51%) and female (49%) distribution. Fever (100%), altered consciousness (97%), and confusion (81%) were the predominant clinical features. MRI abnormalities were observed in 53% of patients, mainly FLAIR/T2 changes (51%), restricted diffusion (51%), and hypoxic changes (43%). CSF analysis revealed a predominantly lymphocytic profile (median lymphocytes 98%) with preserved CSF/blood glucose ratio (median 0.555). Multiplex PCR detected viral pathogens in 32% of cases. The majority (68%) of patients were mPCR-negative.
Conclusions: Despite characteristic clinical, MRI, and CSF features, most cases remained mPCR-negative, highlighting the limitations of current diagnostic panels and the likelihood of undetected viral etiologies. Integrating broader molecular testing may improve diagnostic yield in resource-limited settings.

Keywords: Acute viral encephalitis, CSF analysis, Epstein-Barr virus, MRI findings, Multiplex PCR

INTRODUCTION

Acute viral encephalitis is a potentially life-threatening neurological emergency characterized by inflammation of the brain parenchyma, typically presenting with fever, altered mental status, seizures, and focal neurological deficits. Despite advances in neurocritical care, the condition continues to cause substantial global morbidity and long-term disability, particularly among young adults and children. Early identification of the causative viral agent is essential for appropriate antiviral therapy, guiding

infection control and improving patient outcomes; however, this remains difficult in many healthcare settings due to overlapping clinical features, limited availability of diagnostic tools, and low viral loads in cerebrospinal fluid (CSF) samples.[1] In recent years, multiplex polymerase chain reaction (mPCR) assays, capable of detecting a broad panel of neurotropic viruses from a single CSF specimen within hours, have transformed the diagnostic landscape. These assays offer improved sensitivity compared with conventional PCR, shorten time to diagnosis, and reduce diagnostic uncertainty for clinicians who frequently manage encephalitis empirically.[2] Their use is expanding globally, yet the epidemiological context in which they are applied remains a key determinant of their clinical value. The burden of viral encephalitis is disproportionately higher in low- and middle-income countries (LMICs), where surveillance systems are limited and the true etiological spectrum remains incompletely understood. Global burden analyses show that South Asia in particular carries one of the highest rates of encephalitis-related disability and mortality worldwide, driven by the presence of endemic pathogens such as Japanese encephalitis virus, herpesviruses, enteroviruses, and emerging zoonotic threats like Nipah virus.[3] Recurrent outbreaks of acute encephalitis syndrome (AES) in the region highlight persistent gaps in timely diagnosis and pathogen identification.[4] Without confirmatory diagnostics, clinicians often rely on broad-spectrum antimicrobials and prolonged hospital stays, increasing cost and contributing to antimicrobial resistance.[5] Bangladesh remains one of the most affected countries in South Asia, reporting repeated outbreaks of viral encephalitis, including seasonal Nipah outbreaks with high case fatality, and sporadic clusters of encephalitis of unknown origin. Hospital-based and community surveillance consistently show that a significant proportion of encephalitis cases still lack laboratory confirmed etiologies, largely due to limitations in routine viral diagnostics, delays in sample processing, and lack of access to advanced molecular testing in many tertiary care hospitals.[6] Consequently, the true national burden of viral encephalitis is likely underestimated. A recent study also highlights challenges in clinical management, including delayed antiviral initiation and lack of pathogen-directed treatment strategies.[7] Strengthening diagnostic protocols through the integration of rapid molecular platforms such as multiplex PCR could therefore significantly improve clinical decision-making and surveillance capacity.[8] Although mPCR has been evaluated in several international cohorts demonstrating improved pathogen detection rates, reduced empirical antimicrobial use, and enhanced diagnostic confidence, there remains a clear evidence gap in its application within South Asian tertiary care settings.[9]

Therefore, this study aimed to describe the multiplex PCR assay findings among patients with clinically suspected acute viral encephalitis in a tertiary care hospital in Bangladesh.

METHODS

This observational study was carried out at the Department of Neurology during December 2024 to November 2025 in, Chittagong Medical College Hospital (CMCH), Chattogram, Bangladesh. A total of 100 patients irrespective of age and sex with clinically suspected acute viral encephalitis (AVE) were consecutively enrolled in this study during the study period. Patients with confirmed bacterial meningitis, metabolic encephalopathy, pre-existing chronic neurological disorders, or traumatic brain injury were excluded from this study. All patients underwent routine laboratory and clinical investigations following existing standard protocols. MRI was performed on all patients and findings were categorized as normal or abnormal and were further assessed the clinical features associated with MRI findings. Then all patients underwent cerebrospinal fluid (CSF) analysis following lumbar puncture under aseptic conditions. CSF parameters measured included glucose level, CSF-to-blood glucose ratio, total protein, red blood cell (RBC) count, leukocyte count, and differential cell percentage (neutrophils and lymphocytes). Values were summarized using median and interquartile range (IQR An aliquot of each CSF sample was tested using a multiplex polymerase chain reaction (mPCR) assay to detect common neurotropic viral pathogens according to manufacturer protocols. Data were collected using a prestructured case record form (CRF). All collected data were analyzed using SPSS version 23.0, Continuous data were summarized as means with standard deviation or medians with interquartile range, and categorical data presented as frequencies and percentages in tables and charts. Ethical approval was obtained from the Institutional Review Board (IRB) of Chittagong Medical College, Chattogram, Bangladesh and confidentiality of patients' information was strictly maintained throughout the study in accordance with Helsinki declaration of 1964.

RESULTS

A total of 100 patients presenting with clinically suspected acute viral encephalitis, irrespective of age and sex, were consecutively enrolled in this study. The most frequent 24 ((24%) patients were aged <26 years and followed by, 37-46 years 17 (17%), 47-56 years 15(15%), 27-36 years 13 (13%), and 57-66 years 11 (11%) ≥ 67 years 20 (20%). The mean age was 45.28±19.95 years, median 42 years, and mode 40 years. Gender distribution of the patients was nearly equal, with males comprising 51 (51%) and females 49 (49%). The mean pulse rate was counted 101.69±17.78 beats/min, respiratory rate 22.44±5.64 beats/min, temperature 38.69±1.06°C, systolic blood pressure 121.20±30.32 mmHg, and diastolic blood pressure 74.14±15.82 mmHg. Laboratory parameters showed mean hemoglobin level of the patients was 12.01±1.71 g/dl, WBC 11.46±4.32×10³/µl, random blood sugar 155.69±63.41 mg/dL, serum creatinine 0.96±0.43 mg/dl, sodium 138.26±7.90 mmol/l, and potassium 3.83±0.52 mmol/l. Mean Glasgow Coma Scale scores were: eye

2.76±1.05, motor 4.03±1.20, and verbal 3.15±1.30 (Table 1).

Table 1: Demographic and baseline characteristics of the study patents (n=100).

Variables	Frequency	Percent
Age groups (years)		
<26	24	24
27-36	13	13
37-46	17	17
47-56	15	15
57-66	11	11
≥ 67	20	20
Total	100	100.0
Age: (Mean±SD) (years)	45.28±19.95	
Median	42	
Mode	40	
Range	17-85	
Gender		
Male	51	51
Female	49	49
Vital signs		
Pulse rate (beats/min) (Mean±SD)	101.69±17.78	
Respiratory rate (beats/min) (Mean±SD)	22.44±5.64	
Temperature (°C): (Mean±SD)	38.69±1.06	
Systolic BP (mmHg): (Mean±SD)	121.20±30.32	
Diastolic BP (mmHg) (Mean±SD)	74.14±15.82	
Laboratory parameters		
Hemoglobin (Hb, g/dl) (Mean ±SD)	12.01±1.71	
(WBC,×10³/µl) (Mean±SD)	11.46±4.32	
(RBS, mg/dl) (Mean±SD)	155.69±63.41	
S. creatinine (mg/dl) (Mean±SD)	0.96±0.43	
Serum electrolyte		
Sodium (Na^+, mmol/l) (Mean±SD)	138.26±7.90	
Potassium (K^+, mmol/L) (Mean±SD)	3.83±0.52	
Glasgow Coma Scale (GCS)		
Eye (Mean±SD)	2.76±1.05	
Motor (Mean±SD)	4.03±1.20	
Verbal (Mean±SD)	3.15±1.30	

Most patients had no comorbidity with them 58 (58%). Among those with comorbidities (n=42, 42%), hypertension was the most common 24 (24%), followed by diabetes mellitus 15 (15%) and ischemic heart disease 3 (3%) (Table 2).

Table 2: Distribution of comorbidities among the study patents (n=100).

Distribution of comorbidities	Frequency	Percent
Absent	58	58
Present	42	42
Hypertension (HTN)	24	24
Diabetes Mellitus (DM)	15	14
Ischemic Heart Disease (IHD)	3	3

Fever was the most common clinical presentation, observed in all patients (n=100, 100%), followed by altered consciousness 97 (97%), confusion 81 (81%), headache 55 (55%), vomiting 52 (52%), photophobia 45 (45%), seizures 39 (39%), and status epilepticus 12 (12%) (Table 3).

Table 3: Distribution of clinical presentation among the study patents (n=100).

Clinical presentations	Frequency	Percent
Fever	100	100
Headache	55	55
Vomiting	52	52
Seizure	39	39
Status epilepticus	12	12
Altered consciousness	97	97
Confusion	81	81
Photophobia	45	45

Abnormal MRI findings were present in 53 (53%) patients, while normal scans were observed in 47 (47%) patients. Among the clinical associated features with MRI, focal deficits were seen in 23 (23%) patients and followed by planter response was predominantly extensor 66 (66%), compared to flexor 34 (34%). Oedema was noted in 27 (27%), restricted diffusion in 5 (51%), FLAIR T2 abnormalities in 51 (51%), hypodense areas of hypoxia in 43 (43%), and contrast enhancement in 21 (21%) (Table 4).

Regarding CSF biochemical and cytological parameters distribution, the median (IQR) values were CSF lymphocytes 98% (90-100), CSF glucose 76 mg/dl (67.5-91.5), CSF protein 51.25 mg/dl (39.15-68.98), CSF RBCs 600 cells/µl (300-1000), CSF leukocytes 9 cells/µl (5-17), CSF neutrophils 10% (8-25), and CSF/blood glucose ratio 0.555 (0.43-0.685) (Table 5).

According to multiplex PCR assay results, most patients tested negative (n=68, 68%), while 32% (n=32) were positive (Table 6). Among positive cases, Epstein Barr virus was the most frequently detected 14 (14%), followed by adenoviruses 8 (8%), herpes simplex 1 and 2 (7, 7%), cytomegalovirus 2, (2%), and human herpesvirus 6 and 7 (n=1, 1%) (Table 7).

Table 4: MRI findings and associated clinical features among the study patents (n=100).

	Frequency	Percent
MRI findings		
Normal	47	47
Abnormal	53	53
Associated clinical features		
Focal deficit		
Absent	77	77
Present	23	23
Planter response		
Flexor	34	34
Extensor	66	66
Oedema		
Absent	73	73
Present	27	27
Restricted diffusion		
Absent	49	49
Present	51	41
Hypodense areas of hypoxia		
Absent	57	57
Present	43	42
FLAIR T2 abnormalities		
Absent	49	49
Present	51	51
Contrast enhancement		
Absent	79	79
Present	21	21

Table 5: Distribution of CSF biochemical and cytological parameters of the study patents (n=100).

CSF parameter	Number	Median (IQR)
CSF glucose (mg/dl)	100	76 (67.5-91.5)
CSF/blood glucose ratio	100	0.555 (0.43-0.685)
CSF protein (mg/dl)	100	51.25 (39.15-68.98)
CSF RBCs (cells/µl)	100	600 (300-1000)
CSF leukocytes (cells/µL)	100	9 (5-17)
CSF neutrophils (%)	100	10 (8-25)
CSF lymphocytes (%)	100	98 (90-100)

Median (IQR) represents median with interquartile range (25th-75th percentile)

Table 6: Distribution of multiplex PCR assay findings among the study patents (n=100).

Multiplex PCR-assay results	Frequency	Percent
Negative	68	68.0
Positive	32	32.0
Total	100	100.0

Table 7: Distribution of pathogens detected by multiplex PCR assay test among the study patents (n=100).

Detected pathogens	Frequency	Percent
Adenoviruses	8	8
Cytomegalovirus	2	2
Epstein-barrvirus	14	14
Herpes simplex 1 and 2	7	7
Human herpes 6 and 7	1	1

DISCUSSION

Acute viral encephalitis (AVE) remains a significant neurological emergency in low- and middle-income countries such as Bangladesh, where rapid diagnosis and early treatment continue to be a challenge. In this study of 100 clinically suspected AVE patients, the age pattern demonstrated a bimodal distribution, with younger adults and elderly individuals being the most affected. This distribution is consistent with earlier observations from Bangladesh, where encephalitis disproportionately affects physiologically vulnerable groups such as young adults and older populations.[10] Similar demographic patterns have also been reported in Nepal and Northern India, reflecting regional susceptibility linked to socioeconomic, environmental, and vector-related determinants.[11,13] The nearly equal male-female distribution in the present cohort aligns with surveillance data from Bangladesh, suggesting that AVE does not strongly discriminate by sex.[12] Vital signs in this study indicated notable systemic derangements, especially fever and tachycardia, which are hallmarks of viral neuroinflammation. These findings parallel those from Indian and Southeast Asian cohorts, where severe AVE presentations often include hemodynamic instability and persistent high-grade fever.[13,14] Laboratory parameters, particularly leukocytosis and elevated random blood glucose, reflect physiologic stress and inflammatory activation. Similar laboratory trends have been reported in both Vietnamese and Indian studies, supporting the concept that metabolic dysregulation commonly accompanies viral CNS infections.[14,15] Comorbidities such as hypertension and diabetes were present in 42% of patients. This is clinically important because previous South Asian studies have identified cardiometabolic conditions as predictors of poor neurological outcomes, prolonged hospitalization, and increased mortality in viral encephalitis.[15,16] Clinical features in this cohort were dominated by fever, altered consciousness, and confusion, symptoms strongly associated with diffuse cerebral involvement. This pattern aligns with previous findings from India, Bhutan, and Sri Lanka, where disturbances in mental status are consistently reported as early and prominent manifestations of AVE.[17-19] Seizures, observed in nearly 40% of cases, further highlight the severity of cortical involvement. Recent Sri Lankan research similarly documented a high incidence of seizure activity, emphasizing the need for early seizure control to prevent secondary brain injury.[19] MRI abnormalities were detected

in 53% of cases, reinforcing its diagnostic value in AVE. Features such as FLAIR/T2 hyperintensities, restricted diffusion, and oedema reflect the underlying inflammatory and cytotoxic processes. Similar imaging findings have been reported from Indian, Japanese, and Korean cohorts, where MRI often reveals focal or diffuse lesions corresponding to viral tropism and host inflammatory response.[20-22] The presence of contrast enhancement in a subset of patients may indicate a breach in the blood-brain barrier, a phenomenon highlighted in global radiological study of viral encephalitis.[23] CSF analysis in this study revealed marked lymphocytic predominance, modest protein elevation, and preserved glucose ratios, hallmarks of viral pathology. These findings closely resemble earlier CSF studies conducted in Bangladesh and India, which describe similar inflammatory profiles.[24,25] The median CSF/blood glucose ratio (0.555) strongly supports a viral rather than pyogenic etiology. Interestingly, the median CSF RBC count was relatively high, which may reflect traumatic taps or viral-induced meningoencephalitic irritation, a pattern occasionally observed in South Asian studies.[25] Multiplex PCR identified viral pathogens in 32% of cases, a detection rate consistent with other South Asian and global PCR-based studies utilizing similar platforms.[26] The prominence of Epstein Barr virus (14%) in this study is noteworthy. EBV-associated encephalitis has emerged as a growing concern in Bangladesh, particularly among immunocompetent adults, as highlighted in recent neurovirology reports.[27] Adenoviruses and HSV-1/2 were also identified, mirroring the diverse viral landscape seen in Nepal, India, and Japan.[28,29] However, strengthening molecular diagnostic capacity, incorporating serological panels, and improving early referral pathways could substantially enhance diagnostic accuracy of suspected acute viral encephalitis in resource limited settings.

This study has several limitations. It was conducted in a single tertiary care hospital, which may limit the generalizability of the findings. Consecutive sampling may have introduced selection bias, as the included patients might not fully represent all cases of acute viral encephalitis in the broader population. Diagnostic confirmation relied on clinical evaluation, MRI, and a multiplex PCR panel with limited pathogen coverage, which may have led to under- diagnosis of certain viruses.

CONCLUSION

In this study, Multiplex PCR (mPCR) detected viral pathogens in one-third of patients with suspected acute viral encephalitis, with Epstein Barr virus being the most common, followed by adenoviruses and herpesviruses. Despite typical clinical, MRI, and CSF findings, most cases were mPCR-negative, highlighting limitations of the panel and the potential role of undetected viruses or low viral load. These results emphasize the value of mPCR in pathogen identification while underlining the need for broader diagnostic panels and complementary strategies to improve viral encephalitis detection in resource-limited settings.

Recommendations

Based on the findings, it is recommended to expand the use of Multiplex PCR panels with broader viral coverage for suspected acute viral encephalitis, alongside timely collection of CSF and blood samples to improve pathogen detection. Strengthening laboratory capacity, integrating molecular diagnostics with clinical and MRI evaluation, and establishing standardized protocols for early recognition and management can enhance diagnostic accuracy and patient outcomes. Additionally, ongoing epidemiological surveillance is essential to identify emerging viral pathogens and guide public health interventions in resource-limited settings.

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