Section: Virology

Original Research Article

BEYOND RESPIRATORY VIRUSES: EPSTEIN-BARR VIRUS INFECTION IN FLU-LIKE ILLNESS

Ashok Viswanath Nalankilli[1], Poongodi Santhana Kumarasamy[2], Gowtham Burle[3], Anitha Shete Aich[4], Cinthujah Balachandra Perumal[5], Neeta Changdeo Thorat[6], Abrar Muhammath[7], Julie Jayachandra Baby Roselet[8], Jabesh Maria Vincent Jerome[9], Vinodhinie Indiran[10], Thendralarasi Muralidharan[11], Umeshkumar Chandrasekaran[12]

[1,10,11]D.M Virology - Superspeciality Postgraduate Senior Residents, Department of Virology, Government Tirunelveli Medical College & Hospital, Tirunelveli, Tamil Nadu, India.
[2]Professor & Head, Department of Virology, Government Tirunelveli Medical College & Hospital, Tirunelveli, Tamil Nadu, India.
[3]Consultant Microbiologist & Virologist, AIG Hospital, Gachibowli, Hyderabad, Telangana, India.
[4]Scientist E, National Institute of Virology, Pune, Maharashtra, India.
[5]Associate Professor, Department of Virology, Government Tirunelveli Medical College & Hospital, Tirunelveli, Tamil Nadu, India.
[6]Senior Technical Officer, National Institute of Virology, Pune, Maharashtra, India.
[7,8,9]Junior Resident, Department of Microbiology, Government Tirunelveli Medical College & Hospital, Tamil Nadu, India.
[12]Lab Director & Senior Consultant - Microbiology, Aarthi Scans & Labs, Chennai, Tamil Nadu, India.

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Corresponding Author:
Dr. Umeshkumar Chandrasekaran, Lab Director & Senior Consultant, Department of Microbiology, Aarthi Scans & Labs, Chennai, Tamil Nadu, India.
Email: ume361987@gmail.com

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ABSTRACT

Background: Epstein-Barr virus (EBV) is a herpesvirus with global seroprevalence exceeding 90% in adults. While primarily transmitted through saliva, emerging evidence suggests sexual transmission may contribute to EBV acquisition, particularly among individuals with multiple sexual partners. This study evaluated EBV VCA IgM prevalence in patients presenting with flu-like illness who tested negative for common respiratory viruses.

Materials and Methods: This cross-sectional study included 45 patients presenting with flu-like illness who tested negative for COVID-19, influenza A/H1N1, influenza A/H3N2, influenza B, respiratory syncytial virus A and B, and adenovirus. Participants were stratified into two groups: Group A (n=24) with history of exposure to multiple sexual partners and Group B (n=21) without such history. Serum samples were tested for EBV VCA IgM antibodies using enzyme-linked immunosorbent assay (ELISA). Statistical analysis included chi-square test, Fisher's exact test, and calculation of relative risk and risk difference with 95% confidence intervals.

Results: Overall EBV VCA IgM positivity was 22.2% (10/45). Positivity was higher in Group A than Group B (29.2%, 7/24 vs 14.3%, 3/21; relative risk 2.04, 95% CI 0.60–6.91; p=0.29, Fisher's exact test). The attributable risk was 14.9% (95% CI –8.7% to 38.4%), and the estimated number needed to harm was 7. EBV positivity was predominantly observed in the 19–37-year age group, with the highest frequency in individuals aged 21–30 years with a history of multiple sexual partners.

Conclusion: EBV infection contributes to flu-like illness presentations, particularly in young adults with history of multiple sexual partners, although the observed association did not reach statistical significance in this small sample. These findings underscore the importance of considering EBV in the differential diagnosis of unexplained flu-like symptoms when common respiratory viruses are excluded. Larger prospective studies are warranted to clarify the role of sexual behaviour in EBV transmission.

Keywords: Epstein-Barr virus; EBV VCA IgM; flu-like illness; sexual transmission; infectious mononucleosis; viral serology.

INTRODUCTION

Epstein-Barr virus (EBV), a double-stranded DNA virus belonging to the Herpesviridae family, is one of the most common human viruses worldwide. It is estimated that more than 90-95% of the adult population is infected globally, with primary infection typically occurring during childhood or adolescence. While early childhood infection is often asymptomatic, delayed primary infection in adolescents and adults frequently manifests as infectious mononucleosis.[1,2]

EBV is primarily transmitted through close personal contact, particularly via saliva; however, increasing evidence suggests that sexual contact may play a role in viral transmission, especially among adolescents and young adults. Studies have demonstrated higher EBV seropositivity rates among sexually active individuals and those with multiple sexual partners, indicating that sexual behavior may influence the timing and risk of primary infection.[3-5]

Serological testing remains the cornerstone for diagnosing EBV infection. EBV VCA IgM antibodies appear within the first week of symptoms and generally disappear within four to six weeks, although they may persist for up to three months. Detection of viral capsid antigen (VCA) IgM antibodies indicates acute or recent infection, while VCA IgG and Epstein-Barr nuclear antigen (EBNA) antibodies are markers of past infection. The presence of VCA IgM is particularly useful in epidemiological studies to estimate the burden of recent EBV infection in specific populations.[6,7]

Although EBV infection is usually self-limiting, it has significant clinical and public health implications. EBV is associated with several malignancies, including nasopharyngeal carcinoma, Burkitt lymphoma, Hodgkin lymphoma, and certain gastric carcinomas, as well as autoimmune conditions such as multiple sclerosis. Understanding the epidemiology and risk factors associated with EBV infection is therefore essential for early detection, prevention strategies, and risk stratification.[8]

Despite the high global prevalence of EBV, data on the association between sexual behavior, particularly exposure to multiple sexual partners, and EBV seropositivity remain limited in many regions. This study aims to evaluate the prevalence of EBV VCA IgM antibodies and to assess the relationship between recent EBV infection and a history of multiple sexual partners, thereby contributing to a better understanding of EBV transmission dynamics in high-risk populations.[9]

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional study was conducted at the Department of Virology, Government Tirunelveli Medical College & Hospital, Tirunelveli, Tamil Nadu, India, during January 2025 to December 2025.

Study Population

A total of 45 serum samples by consecutive sampling from patients attending the General medicine outpatient department presenting with flu-like illness were included in this study. All patients had tested negative for COVID-19, influenza A/H1N1, influenza A/H3N2, influenza B, respiratory syncytial virus (RSV) A, RSV B, and adenovirus. Participants were stratified into two groups based on sexual exposure history: Group A consisted of 24 patients with documented history of recent exposure to multiple sexual partners, and Group B consisted of 21 patients without such history.

Inclusion Criteria

Patients aged ≥18 years presenting with influenza-like illness (fever, malaise, sore throat, myalgia, and fatigue), who tested negative by nasopharyngeal swabs PCR for SARS-CoV-2 (COVID-19), influenza A (H1N1 and H3N2), influenza B, respiratory syncytial virus (RSV) A and B, and adenovirus, and who provided written informed consent to participate in the study were included.

Exclusion Criteria

Patients were excluded if they declined to participate, had positive test results for any of the aforementioned respiratory viruses, had known immunodeficiency disorders or were receiving immunosuppressive therapy, or had incomplete clinical or demographic data.

Sample Collection and Processing

Venous blood samples (5 mL) were collected from each participant using standard phlebotomy techniques in red vacutainer tube. Samples were allowed to clot at room temperature for 30 minutes, then centrifuged at 3000 rpm for 10 minutes. Serum was separated, aliquoted, and stored at -20°C until testing. All samples were processed within 24 hours of collection.

Laboratory Methods

The serological assay utilized Anti-EBV VCA IgM ELISA which is an in-vitro diagnostic device for the detection of IgM antibodies against the VCA antigens p23 and p18 of EBV.The Anti-EBV VCA IgM ELISA is a highly sensitive IgM (μ-chain) specific capture enzyme immunosorbent assay (ELISA) for the detection of EBV specific antibodies in serum or plasma. During the first incubation step, IgM antibodies of the sample will bind to the microplate. Other immunoglobulin types will be removed by washing. During a second incubation, captured VCA p23-18-specific IgM antibodies will be detected. This is performed by the addition of an antigen-enzyme conjugate. The recombinant VCA p23-18 is directly and covalently labelled with horseradish peroxidase (HRP). Non-specifically bound conjugate is removed by another washing step. For the last incubation, the substrate solution (TMB, 3,3′,5,5′-Tetramethylbenzidine) is filled into the wells. The enzyme reaction is stopped by adding sulphuric acid (colour change from blue to yellow) and the optical density is measured with a spectrophotometer at 450 nm and a reference wavelength of 615-690 nm.

Interpretation: Results were interpreted according to the manufacturer's instructions. Samples with optical density values above the cut-off were considered positive for EBV VCA IgM, indicating acute or recent EBV infection.

Data Collection

Demographic data (age, sex) and clinical history, including sexual exposure history, were collected through structured questionnaires. Confidentiality and privacy were strictly maintained throughout the study.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics software (version 25; IBM Corp, Armonk, NY, USA). Descriptive statistics were calculated for demographic and clinical variables, categorical variables were expressed as frequencies and percentages. Associations between sexual exposure history and EBV VCA IgM positivity were assessed using Fisher's exact test, with effect measures including OR, RR, absolute risk difference, NNH, and 95% CIs. Effect size was estimated using Cohen's h. Statistical significance was defined as $p < 0.05$. Age distribution was examined using frequency histograms.

Ethical Considerations

The study was carried out in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment.

RESULTS

Among 45 samples tested, overall EBV VCA IgM positivity was detected in 10 cases (22.2%), while 35 cases (77.8%) were negative (Table 1). The study population had a minimum age of 19 years and maximum age of 61 years (range 19-61 years; mean age 35 years).

VCA IgM positivity among those with history of recent exposure to multiple sexual partners (Group A) was 29.16% (7/24), compared to 14.28% (3/21) among those without such history (Group B) (Table 2). Although Group A demonstrated numerically higher seropositivity, the difference was not statistically significant (odds ratio [OR] = 2.47; 95% confidence interval [CI] 0.55-11.14; p = 0.2956, Fisher's exact test).

The relative risk of EBV VCA IgM positivity in Group A compared to Group B was 2.04 (95% CI 0.60-6.91), indicating that individuals with exposure to multiple sexual partners had approximately twice the risk of recent EBV infection, though this did not achieve statistical significance. The attributable risk was 14.9% (95% CI -8.7% to 38.4%), suggesting that approximately 51% of EBV VCA IgM positivity in the exposed group could be attributed to the exposure history. The number needed to harm (NNH) was calculated as 6.7, indicating that for every ~7 individuals exposed to multiple sexual partners, approximately 1 additional case of EBV VCA IgM positivity would occur. Chi-square analysis with Yates correction yielded $\chi^2 = 0.70$, $p = 0.40$, further confirming the lack of statistical significance. Effect size analysis revealed Cohen's h = 0.364, indicating a small effect size.

EBV positivity clustered predominantly in the 19-37 year age group, with fewer positive cases observed at older ages. The highest frequency among individuals with exposure to multiple sexual partners (Group A) was observed in the 21-30 age group (40%), followed by the 31-40 age group (20%). In contrast, individuals without such exposure (Group B) showed lower distribution with 10% frequency in the 11-20 age group, relatively uniform frequency of 10% in both groups among the 21-30 and 51-60 age groups. No cases were observed in the 41-50 age group in either category. This pattern suggests that exposure to multiple sexual partners is more prevalent among younger adults, particularly those aged 21-30 years. [Figure 1]

Table 1: EBV VCA IgM test results among study participants (N=45)

EBV VCA IgM Status	Number (n)	Percentage (%)
Positive	10	22.2
Negative	35	77.8
Total	45	100

Table 2: EBV VCA IgM positivity stratified by sexual exposure history (OR = 2.47, 95% CI 0.55-11.14; p = 0.2956, Fisher's exact test; RR = 2.04, 95% CI 0.60-6.91)

History of Exposure to Multiple Sexual Partners	EBV VCA IgM Positive	EBV VCA IgM Negative	EBV Positivity Percentage (%)
Yes (Group A)	7/24	17/24	29.16
No (Group B)	3/21	18/21	14.28
Total	10/45	35/45	22.22

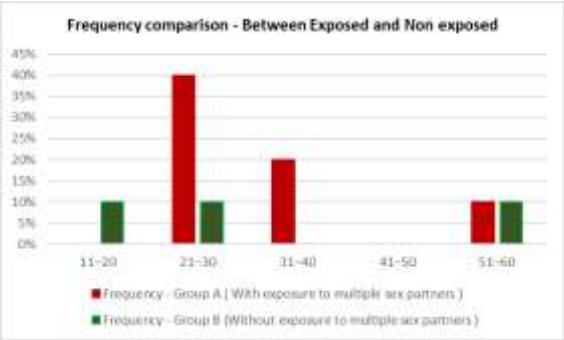

Figure 1: Age distribution histogram comparing frequencies between Group A (with exposure to multiple sexual partners) and Group B (without exposure to multiple sexual partners) among EBV VCA IgM positive cases

DISCUSSION

In this study, EBV VCA IgM testing was conducted among 45 patients presenting with flu-like illness who tested negative for other common respiratory viral infections. The overall EBV VCA IgM positivity of 22.2% observed in our study is consistent with previous reports.

A higher proportion of EBV VCA IgM positivity was observed among individuals with a history of exposure to multiple sexual partners (29.16%) compared to those without such history (14.28%), though this difference did not reach statistical significance (p=0.29). This trend is consistent with epidemiological data linking sexual behavior to EBV transmission risk.[10] Crawford et al. demonstrated that having multiple sexual partners was a highly significant risk factor for EBV seropositivity, with odds ratios increasing proportionally with the number of partners.[3] Our findings support this association, though the limited sample size may have precluded demonstration of statistical significance.

Previous studies have demonstrated that delayed primary EBV infection, which is more frequent in individuals with increased close or sexual contact, is more likely to be symptomatic and may present as infectious mononucleosis or nonspecific flu-like illness.[11] The clinical manifestations of EBV infection often overlap with those of common respiratory viral infections, including fever, malaise, sore throat, and lymphadenopathy, making clinical differentiation challenging.[12]

The detection of EBV VCA IgM among patients initially investigated for respiratory viral infections highlights the potential for underdiagnosis of EBV in routine clinical practice.[13] This finding underscores the importance of considering EBV infection in the differential diagnosis of flu-like illness, especially when routine respiratory viral testing is negative and when relevant behavioral risk factors, such as multiple sexual partners, are present.

The age distribution observed in our study, with predominance in the 21-30 year age group, aligns with known epidemiological patterns of delayed primary EBV infection in young adults. This age group represents a period of increased social and sexual activity, which may facilitate viral transmission through close personal contact and exchange of saliva or other bodily fluids.[4,5]

Clinical and Public Health Implications

These findings have important clinical implications. Healthcare providers evaluating patients with flu-like illness should maintain a high index of suspicion for EBV infection, particularly in young adults presenting with persistent symptoms despite negative testing for common respiratory pathogens. Sexual history should be considered as part of comprehensive risk assessment. From a public health perspective, understanding EBV transmission dynamics can inform targeted prevention strategies and health education interventions, particularly for high-risk populations

CONCLUSION

EBV VCA IgM positivity was observed in 22.2% of patients presenting with flu-like illness who tested negative for common respiratory viruses. A higher proportion of seropositivity was noted in individuals with a history of exposure to multiple sexual partners (29.16%) compared to those without such history (14.28%), though this difference did not reach statistical significance in our limited sample. These findings suggest that EBV infection should be considered in the differential diagnosis of flu-like illness, particularly in young adults with relevant sexual exposure history, even when other respiratory viral infections are suspected or excluded.

Future research should focus on larger multicenter prospective studies with adequate statistical power to definitively establish the association between sexual behavior and EBV transmission. Additionally, comprehensive serological panels including EBV VCA IgG, EBNA-1 IgG, and molecular detection methods such as EBV PCR would provide more complete characterization of infection status and viral load dynamics.

Limitations of the study

1. EBV PCR was not performed, limiting the ability to quantify viral load and confirm active viral replication
2. EBV VCA IgG and EBNA-1 IgG were not tested, not including definitive differentiation between primary and reactivated infection
3. Indirect fluorescent antibody test (IFA), considered the gold standard for detecting IgM antibodies against EBV VCA, was not performed; ELISA methodology was used instead
4. Cross-reactivity of EBV VCA IgM with other herpesviruses (CMV, HSV, VZV) was not ruled out through additional testing.

Author Contributions

All authors participated in the writing of this manuscript. The final manuscript has been read by all authors and is their work.

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Conflict of Interest

The authors declare no conflicts of interest related to this study.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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