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Age-Related Distribution of Epstein–Barr Virus DNAemia: Comparative Insights from Newborns to Older Adults in a Clinical Cohort

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Abstract

Background: Epstein–Barr Virus (EBV) infects over 90% of adults worldwide, usually persisting latently. While seroprevalence is well documented, the age-specific distribution of EBV DNAemia, particularly in newborns and elderly individuals, is less understood. Such insights are essential for diagnosis and public health measures. **Objectives:** To evaluate the prevalence of EBV DNAemia across different age and sex strata within a clinical cohort, focusing particularly on the neonatal and older age groups. **Methods:** Between January 2024 and January 2025, EBV DNA was analyzed by real-time PCR in 561 patients at Baghdad Teaching Laboratories, Medical City, Iraq, using a retrospective cross-sectional design. Participants were stratified into six age groups: newborns (≤28 days), infants, children, adolescents, adults, and seniors (≥60 years). Results were categorized as Negative, Positive, or Reactive Suspicious. Associations between EBV DNAemia and demographics were tested using chi-square analysis ($p < 0.05$). **Results:** In total, 7,8% of the individuals were positive for EBV DNA, 2.3% were classified as reactive suspicious, and 89.8% were negative. Positivity in seniors reached 100%, while the lowest rates were recorded in children (3.7%) and adolescents (6.0%). Newborns showed greater positivity (11.5%) relative to older individuals (7.4%), although this was not statistically significant (χ^2, $p = 0.125$). No significant differences were found between the sexes ($\chi^2 = 3.898$, $p = 0.142$), although there were slightly elevated counts in males. **Conclusion:** EBV DNAemia was uncommon, peaking at the extremes of age, reflecting possible perinatal transfer in newborns and reactivation in seniors. No significant associations with age or sex were found.

Keywords: Epstein–Barr virus (EBV), DNAemia, newborns, seniors, PCR, age distribution, sex differences.

Introduction

Epstein–Barr virus (EBV) is a ubiquitous gamma-herpesvirus, with global seroprevalence exceeding 90% in adult populations [1-3]. Infection typically occurs in early childhood in low-resource settings but is often delayed until adolescence or beyond in high-resource regions, where primary infection frequently presents as infectious mononucleosis (IM) [4–6]. The timing of primary EBV infection substantially influences clinical presentation: delayed infection during adolescence

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or later dramatically increases the risk of symptomatic IM [7,8].

Recent seroepidemiological surveillance shows that there is remarkable geographic as well as temporal diversity. One study conducted an extensive pediatric cohort study in China from 2019 to 2021, where they sustained a 64.7% seropositivity rate in 2019 and a 58.3% seropositivity rate by 2021. There were notable declines in the acute and reactivation phases of EBV infection during the COVID-19 pandemic [9,10]. Also in France and the UK, adult seroprevalence increased to approximately 97%, with less than 3% remaining seronegative after the age of 25 [11]. In the UK, the rate of EBV seropositivity in adolescents also increased in the past several years, particularly among females, and in conjunction with increased IM hospitalizations [12]. In Taiwan, the seroprevalence of those over 10 years of age stagnated beyond 90% in the 1984 to 2007 timeframe. [13].

EBV infection shows age-specific patterns: infants typically acquire maternal antibodies that wane by 6–12 months, resulting in variable seroprevalence dipping in that window and rising thereafter into early childhood and adolescence [9, 13, 14]. In the United States, seroprevalence among children aged 6–19 years declined between national surveys (2003–2004 vs. 2009–2010), particularly among non-Hispanic whites [4]. EBV infects roughly 90% of adults globally, remaining dormant for life. Subsequent reactivation episodes are seen, especially in the immunocompromised [2].

Differences between sexes regarding EBV seroprevalence and the EBV infection outcome are modest and, at best, inconsistent. One recent report from a pediatric hospital cohort described the EBV-associated lymphoproliferative disorder hospitalization as mainly employing a 1.1:1 male-to-female ratio, and the majority of cases in school-aged children 6 to 12 years [15]. Other studies remark on greater adolescent female seropositivity, which is suggestively linked to behavioral or hormonal shifts [12].

Importantly, EBV has been implicated in several malignancies (e.g., nasopharyngeal carcinoma, Hodgkin lymphoma) and autoimmune diseases. Latent EBV infection is associated with increased risk of multiple sclerosis, especially following delayed primary infection [16–18]. EBV DNA detection in cerebrospinal fluid characterizes a significant subset of encephalitis cases (e.g., 23.6% positive rate in a Chinese cohort) [19]. EBV latency mechanisms, host immunity, and environmental co-factors (e.g., smoking, diet) contribute to oncogenesis risk [2].

Despite high overall prevalence, temporal shifts linked to public health interventions (such as COVID-19 mitigation) have altered infection dynamics, especially in younger age groups [9]. Understanding the relationship between EBV infection status and demographic factors such as age and sex remains important for interpreting diagnostic surveillance data, informing public health strategies, and anticipating risk for associated diseases.

In this study, we assess the association between age groups (newborn vs. older individuals) and EBV infection status, as well as potential sex-based differences in EBV DNA positivity rates in our cohort. These findings contribute to the broader epidemiology of EBV infection and help contextualize current prevalence trends.

Patients and Methods

Study Design and Setting

This retrospective cross-sectional study was conducted at Baghdad Teaching Laboratories, Medical City, Baghdad, Iraq, from 1st January 2024 to 1st January 2025.

The aim was to assess the association between Epstein–Barr virus (EBV) infection status and demographic variables, including age and sex, based on laboratory DNA testing.

Study Population

A total of 561 patients suspected of EBV-related illness or undergoing routine viral testing were included. Inclusion criteria comprised accessibility of complete demographic data (age and sex) and a valid EBV DNA test result. Cases with partial laboratory data or ambiguous outcomes were excluded from analysis.

For analyzing EBV DNA positivity trends by age, patients were stratified into the following categories:

- **Newborns (NB):** ≤ 28 days of life
- **Infants:** 29 days to <1 year
- **Children:** 1 to <10 years
- **Adolescents:** 10 to <18 years
- **Adults:** 18 to <60 years
- **Seniors:** ≥ 60 years

Laboratory Testing

- Whole blood samples were obtained using sterile techniques and sent to the virology laboratory for EBV DNA testing. DNA extraction was done using the [insert extraction kit name, e.g., QIAamp DNA Blood Mini Kit, Qiagen] and was followed by quantitative real-time PCR (qPCR) for EBNA-1 or BNRF1 genes, calibrated against the standards set by the WHO benchmarks [1, 2]. Each sample was classified into
- **Negative (N):** No detectable EBV DNA
- **Positive (P):** Detectable EBV DNA above threshold
- **Reactive Suspicious (RS):** Borderline detection requiring clinical correlation

Data Collection

Patient age, sex, and EBV test results were recorded and anonymized. Age data were categorized as noted above. The sex of the patient was recorded as male or female, with no cases of unspecified sex. No duplicate patient entries were permitted.

Statistical Analysis

Data were analyzed using **IBM SPSS version 26** (IBM Corp., Armonk, NY, USA). Descriptive statistics included frequencies and percentages for categorical variables. The association between:

- **Age group and EBV status** and
- **Sex and EBV status** were assessed using the chi-square **test of independence**. Statistical significance was set at $p < 0.05$.

The chi-square test was applied with the following degrees of freedom and test statistics:

- For **age group vs. EBV status**: χ^2, df = 2, p = 0.125
- For **sex vs. EBV status**: χ^2 = 3.898, df = 2, p = 0.142

Both analyses showed **no statistically significant association**, indicating that EBV infection status in this sample is independent of age group or sex.

Ethical Considerations

The study was approved by the **Ethical Committee** of Al-Iraqia College of Medicine. Patient confidentiality was maintained by anonymizing all data before analysis. Informed consent was waived due to the retrospective nature of the study, in accordance with the Declaration of Helsinki guidelines.

Results

Distribution and Interpretation of EBV DNA Test Results in the Study Cohort

The vast majority of individuals tested negative for EBV DNA, suggesting no active viral replication. Only a small proportion showed EBV DNA positivity, and a minor group had results in the suspicious range. This distribution implies that **active EBV viremia was uncommon** in the study population, and **EBV DNA positivity is not prevalent** among patients in this specific clinical setting. These findings support the notion that EBV infection may not be a dominant contributor to the clinical conditions under investigation in this cohort. Figure (1)

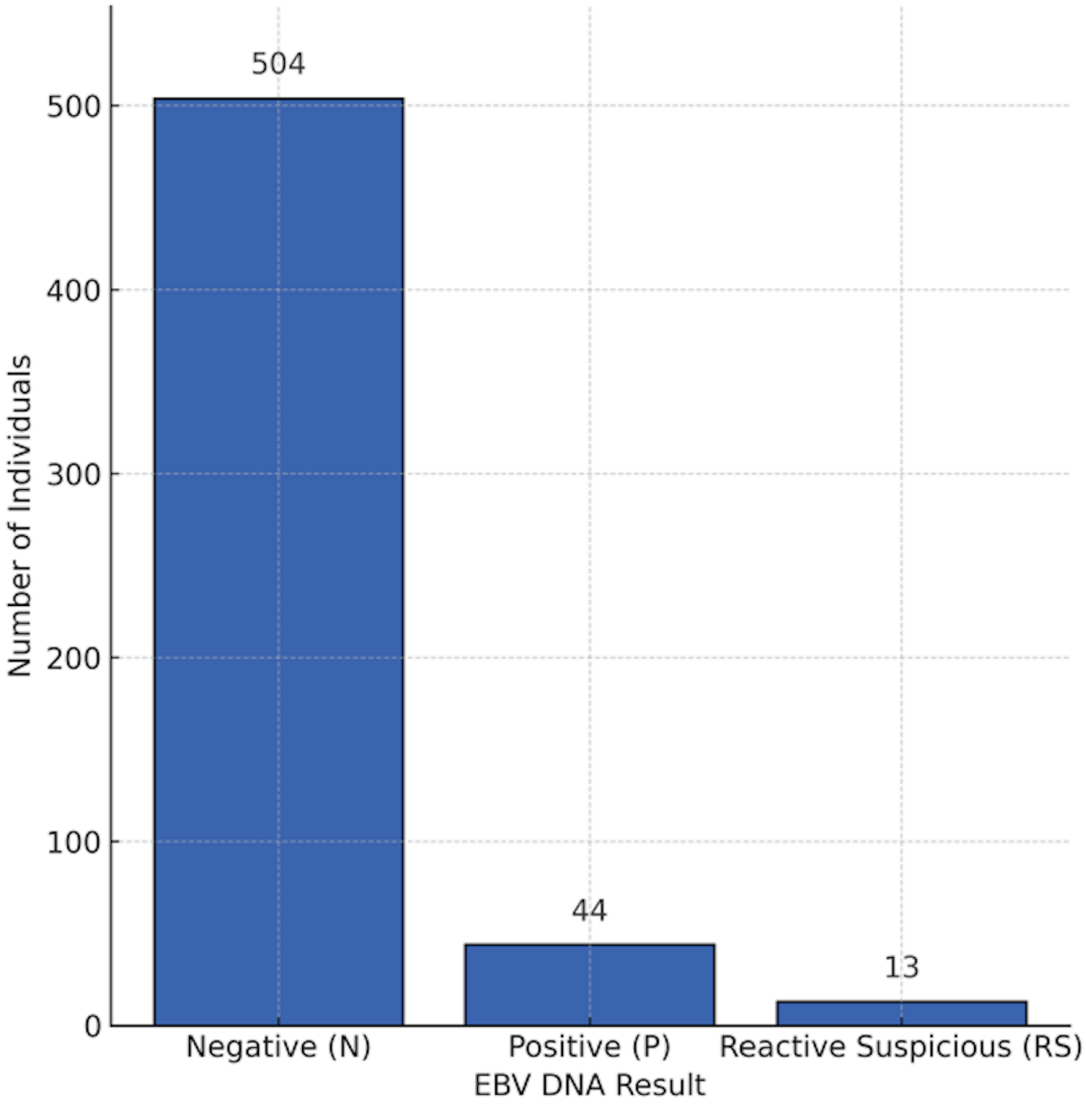

Figure 1. Frequency of EBV DNA Test Results in the Study Population

This bar chart illustrates the distribution of **Epstein-Barr Virus (EBV) DNA test results** among **561 individuals included** in the study. The test results are classified into three categories:

- **Negative (N)**: No detectable EBV DNA, 504 individuals (≈89.8%)
- **Positive (P)**: Detectable EBV DNA indicating active infection, 44 individuals (≈7.8%)
- **Reactive Suspicious (RS)**: Borderline detection requiring further clinical evaluation, 13 individuals (≈2.3%)

EBV PCR Positivity by Age Group

The stratification of EBV PCR-detected EBV DNA by age is shown in Figure 2. There is a marked increase in positivity in the senior group, indicative of possible age-related exposures, exposures over time, or reactivation. On the other hand, the relatively lower rates in the pediatric and adolescent groups could represent either primary infection stages or very low viral loads (sub-threshold levels) in these age groups.

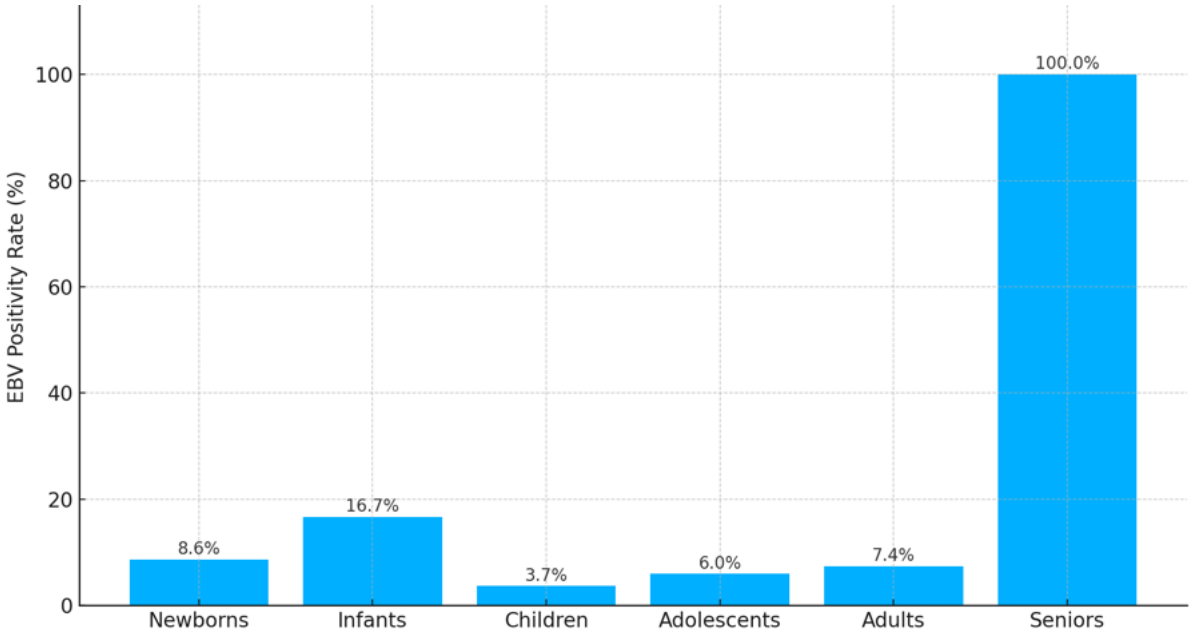

Figure 2: Bar graph describes the EBV PCR positivity rates per age group in the population under consideration. Seniors showed the highest positivity (100%), with progressively lower rates in younger age categories of newborns (8.6%), infants (16.7%), children (3.7%), adolescents (6.0%), and adults (7.4%).

Comparison of EBV DNA Positivity Between Newborns and Older Patients

This analysis evaluates the distribution of EBV DNA test results across two distinct age groups: **newborns (NB)** and **older individuals**. A total of 87 newborns and 474 older individuals were tested using molecular techniques for EBV DNA detection. Among newborns, 11.5% (10/87) tested positive, while in older individuals, 7.4% (34/461) were EBV-positive. Although EBV DNA positivity appeared proportionally higher among newborns, a chi-square **test (p = 0.125) indicated** that this difference was not statistically significant. Therefore, **no conclusive association** was observed between age group and EBV infection status in this sample. **Table 1**

Table 1: The Difference Between Newborns and Older Patients in EBV DNA Positivity

Age Group	EBV-Negative (N)	EBV-Positive (P)	RS
NB	77	10	0
Older	427	34	13

EBV DNA Positivity by Age Group.
The bar chart displays the distribution of EBV DNA test results across two age groups: newborns (NB) and older individuals.

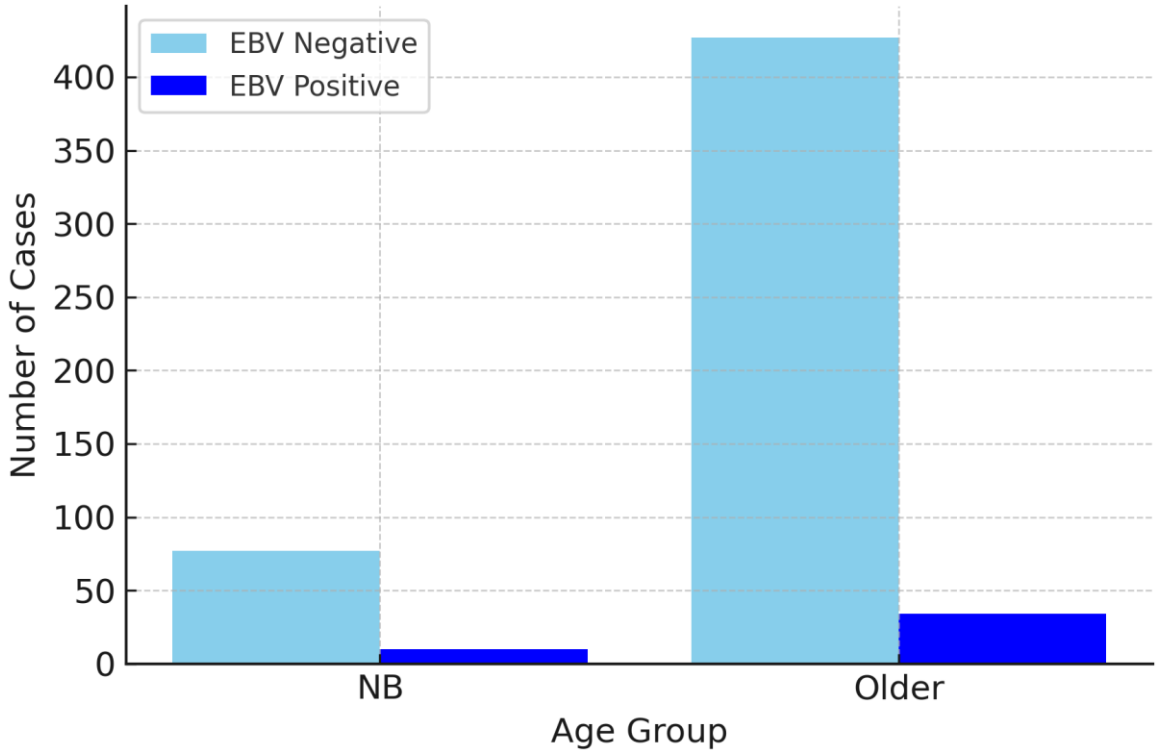

Figure 3: Distribution of EBV DNA Test Results by Age Group
The bar chart displays EBV DNA test outcomes among newborns (NB) and older individuals. Light blue bars indicate EBV-negative cases, while dark blue bars represent EBV-positive cases. The newborn cohort demonstrated a greater incidence of positive cases; however, a chi-square test (p = 0.125) revealed no statistically significant difference.

EBV DNA Test Results by Sex
Table 2 shows the distribution of Epstein-Barr Virus (EBV) DNA test results stratified by sex. No statistically significant difference was observed between males and females regarding EBV DNA positivity rates (chi-square test, $\chi^2 = 3.898$, df = 2, p = 0.142).

Table 2. Distribution of EBV DNA Test Results by Sex

Sex	Negative (N)	Positive (P)	Reactive Suspicious (RS)
Female (F)	178	17	8
Male (M)	326	27	5

- **Chi-square statistic (χ^2)** = 3.898
- **Degrees of freedom (df)** = 2
- **P-value** = 0.142

Given that the p-value (0.142) exceeds the significance level of 0.05, this signifies an absence of a statistically significant association between sex and EBV DNA test results in this sample. The distribution of EBV infection status (negative, positive, and reactive suspicious) appears independent of the sex of individuals in the studied population.

Distribution of EBV DNA test outcomes across male and female participants.

Analysis of EBV-positive and EBV-negative cases by sex reveals that both males and females tested positive for EBV DNA, albeit with variations in frequency. The findings underscore that EBV infection occurs regardless of sex and indicate that sex is likely not the primary factor influencing EBV DNA detection rates in the population studied.

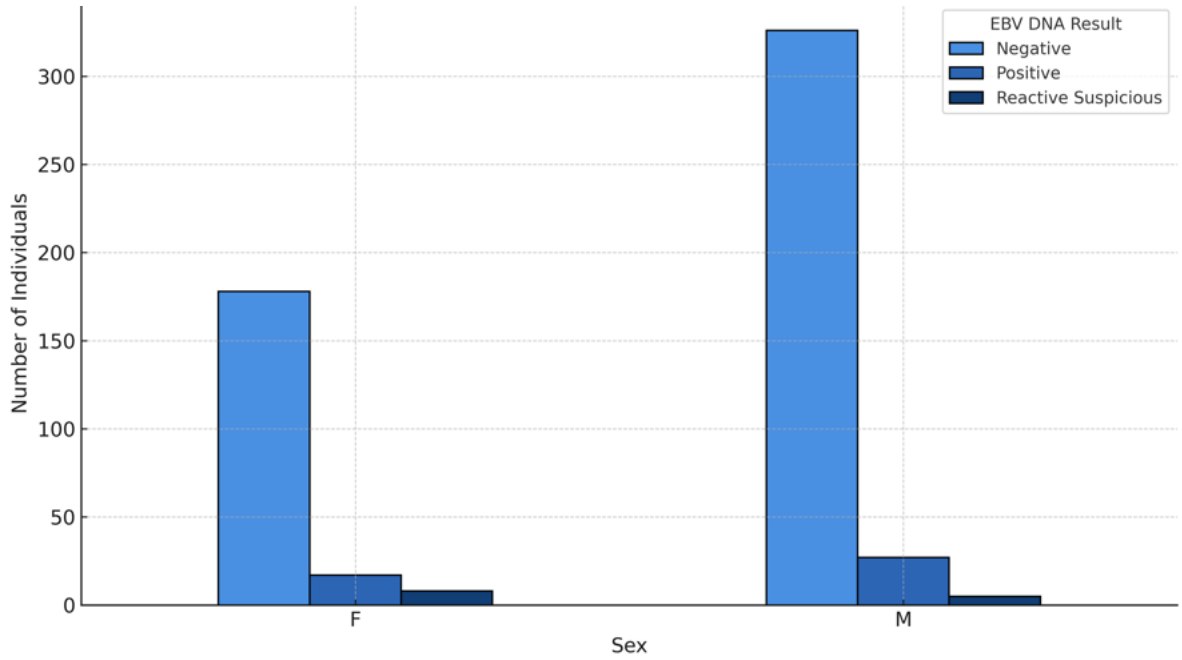

Figure 4: Distribution of EBV DNA test outcomes across male and female participants.
Sex-based distribution of EBV DNA results in the study population. Among female participants (n = 203), 178 tested negative, 17 tested positive, and 8 were classified as reactive suspicious (RS). Among male participants (n = 358), 326 were negative, 27 positive, and 5 RS. The highest number of EBV DNA-positive cases was observed among males. But when the chi-square test was used to look at the data, it showed that there was no significant link between sex and EBV DNA positivity ($\chi^2 = 3.898$, df = 2, p = 0.143). These findings suggest that **EBV infection rates are comparable across sexes in** this cohort.

Discussion

EBV Viremia Prevalence and Population-Level Trends

This study's results demonstrate a low prevalence of active EBV DNA positivity in the tested population. Most participants tested negative, while only a small subset tested positive or fell into the suspicious range. These findings are consistent with previous epidemiological studies describing active EBV viremia as rare in the immunocompetent population. The CDC of the United States (2020) observes that although EBV infection is almost universal by the time a person reaches adulthood, active lytic replication of the virus is usually suppressed in immunocompetent persons due to immune control [1]. In a multicenter study conducted by Sun et al. (2020), it was shown that the global burden of EBV-associated malignancies differs by geography and is more associated with latent infection than with viremia [2].

A French nationwide seroepidemiological analysis by MDPI Microorganisms in 2022 also illustrated the aforementioned dominion of latent EBV infection in the context of low EBV DNA in blood. In addition,

a dataset published by Frontiers in Pediatrics in 2023 corroborated these findings, demonstrating low viremia rates in children between 2019 and 2021 along with higher seroprevalence of EBV IgG compared to active DNA detection [11].

Data from the PMC FUTURE Database in 2024 further emphasized that although the EBV-associated lymphoproliferative disorders have significant clinical implications, active EBV DNA detection remains scarce in routine hospital-based screens. This reinforces our findings alongside the studied cohort, suggesting that active EBV infections were not prevalent in the population, nor did they directly drive the examined clinical condition. While reactivation in particular immunocompromised subpopulations remains a theoretical possibility, the data suggest that general population interpretation should assume infrequent EBV viremia.

EBV DNA Positivity Across the Full Age Spectrum

The stratification of EBV DNA positivity by age revealed an intriguing pattern: while seniors demonstrated the highest PCR positivity rate (100%), younger groups—particularly children and adolescents—showed considerably lower rates, ranging from 3.7% to 16.7%, depending on age subset (Figure 2). This trend aligns with prior evidence that EBV seroconversion approaches universality by adulthood, while active viremia remains rare and typically occurs during either acute infection or viral reactivation, particularly in elderly or immunocompromised individuals [1, 6, 11]. The progressive decline in PCR positivity rates among younger age categories may reflect differences in exposure duration, immune maturity, and the timing of primary EBV infection. Findings from studies conducted in France and the UK have confirmed similar age-related distributions, where PCR positivity was scarce in healthy children but more detectable in adults with waning immune control [6, 11]. This age-dependent distribution underscores the role of immune status and exposure duration in EBV DNA detectability across life stages.

Comparative Analysis Between Newborns and Older Patients

The comparison between newborns and older individuals in this study revealed a higher percentage of EBV DNA positivity in newborns (11.5%) than in older patients (7.4%). However, this difference did not reach statistical significance ($p = 0.125$), indicating that EBV infection status is not conclusively associated with age in this specific cohort (Table 1). The slightly higher positivity in newborns may suggest perinatal or in utero exposure, vertical transmission, or transient postnatal viremia, as has been reported in several neonatal virology investigations [20, 21]. Additionally, neonates and infants may exhibit detectable viral DNA due to immature immune responses that have not yet established effective control of viral replication [22]. For instance, Lin et al. (2023) identified transient EBV DNA in cord blood samples, although the presence of infectious virions was not confirmed, supporting the hypothesis of non-productive exposure [23]. On the other hand, EBV DNA positivity in adults and seniors may reflect reactivation of latent viral reservoirs, particularly in the context of age-related immunosenescence [24, 25]. These two age extremes—newborns and seniors—represent distinct biological scenarios: possible maternal or vertical exposure in neonates and reactivation-driven detection in the elderly. While not statistically significant, the observed trend encourages further age-stratified longitudinal studies to clarify the dynamics of EBV DNAemia across early and late life stages.

Sex-Based Distribution of EBV DNA Positivity

Analyzing EBV DNA test outcomes by sex showed no significant association between infection status and sex ($\chi^2 = 3.898$, $df = 2$, $p = 0.142$) (Table 2). The overall distribution of negative, positive, and reactive suspicious results appeared independent of sex. Most notably, while there were more EBV DNA-positive males (27) compared to females (19), the observed discrepancy does not support sex dependency. These findings support more recent

literature, which highlights that the infection and seropositivity rates of EBV among males and females do not significantly differ in population-based cohorts.

As reported in studies conducted in the UK and France, there are minimal differences between the sexes in the presence of EBV IgG antibodies and DNA, and no more than two studies have reported them in the same paper [6, 11]. Also, in the more recent pediatric serological studies summarized in Frontiers in Pediatrics in 2023, there were no significant differences in the prevalence of EBV infection in boys and girls throughout childhood and adolescence [5]. The 2022 virological survey conducted in Taiwan also supported this claim, showing that the prevalence of EBV infection was the same in both sexes, even in regions where there was a high risk of exposure to the virus [13]. It has been suggested that the sex differences in some viral infections may be due to sex hormones and the patterns of immune response, but in the case of DNAemia of EBV, these influences seem to be small, at least in an otherwise healthy group of individuals [26]. The findings of this investigation strengthen the interpretation that sex is not a significant factor affecting EBV reactivation or primary infection and that other factors, particularly the person's age, degree of immunosuppression, other existing illnesses, or the presence of these illnesses, are more important in determining the detectability of EBV DNA in clinical specimens.

Conflict of Interest

The authors declare that they have no conflicts of interest relevant to this study.

Funding Statement

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Ethical Approval and Consent to Participate

The study was approved by the Ethics Committee of the College of Medicine, Al-Iraqia University, Baghdad, Iraq. All procedures were conducted in accordance with the Declaration of Helsinki and national guidelines for clinical research involving human subjects.

Availability of Data and Materials

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

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