

Assessment of Interleukin-31 and Calprotectin in Immunocompromised Patients with Epstein-Barr Virus Infection

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Abstract—Epstein-Barr virus (EBV) is among the most concerning herpesviruses, despite its typically asymptomatic or mildly self-limiting infections. The virus's carcinogenic features and its ability to modulate the immune system are critical. That is why more research is needed on the hidden aspects of the virus's life cycle, especially its oncogenic role and post-transplant complications. The study aims to harness EBV's unique characteristics to gain new insights into its pathogenesis. This study was designed to investigate immunological and inflammatory biomarkers among three immunocompromised groups compared with an immunocompetent group in Sulaimani City (n = 81), including identification of EBV seropositivity in association with interleukin (IL) 31, calprotectin, lactate dehydrogenase, total leukocytes, granulocytes, lymphocytes, and platelets. EBV viral capsid antigen (VCA) immunoglobulin (Ig)M seropositivity was 29%, 21%, 15%, and 0%, in the immunocompetent, renal transplant, bone marrow transplant (BMT), and hematological malignancy groups, respectively. In addition, VCA IgM was inversely correlated with age ($r = -0.353$, $p = 0.001$). Interestingly, a significant association between the EBV VCA IgM index and IL-31, and, consequently, calprotectin levels, was observed. Regarding hematological parameters, the study found no significant association with EBV seropositivity. In conclusion, EBV latent Ig was less commonly recorded in the hematology and BMT groups than in the kidney transplant and immunocompetent groups. The findings suggest a possible association between IL-31 and calprotectin levels with EBV seropositivity; however, additional mechanistic studies are required to determine causality or oncogenic relevance.

Index Terms—Calprotectin, Epstein-Barr virus, Immunocompromised patient, Interleukin 31, Sulaimani.

I. INTRODUCTION

Epstein-Barr virus (EBV), also known as human herpesvirus 4, is a double-stranded DNA virus that belongs

to the gamma herpesvirus family (Fournier and Latour, 2021). It is the first recognized carcinogenic virus, identified in connection with Burkitt's Lymphoma in the early 1960s, and is among the most prevalent lifelong viral infections (Jog et al., 2018; Frappier, 2021; Yu and Robertson, 2023). Recently, studies have indicated a significant prevalence of EBV in the Middle East, with 52.6% positive for viral DNA and 97.9%, 1.8%, and 91.5% seropositive for EBV viral capsid antigen (VCA) immunoglobulin (Ig)G, EBV VCA IgM, and EBV nuclear antigen (NA) IgG, respectively; the latter suggests a latent infection (Smatti et al., 2017). While EBV often resolves without many complications in immunocompetent individuals, it is closely linked to various cancers and immune dysfunctions, including Burkitt's Lymphoma, nasopharyngeal carcinoma, and lymphoproliferative disorders following organ transplantation (Kimura, 2006). Evidence supports that EBV manipulates various immune mechanisms to persist in the body, alternating between latency and the lytic cycle, which poses significant challenges, especially for immunocompromised patients who are consistently taking immunosuppressants (Ning, 2011; Jog et al., 2018; Todorović, Ambrosio and Amedei, 2024). EBV NA is a non-enzymatic protein; in addition to its carcinogenic and immunomodulatory characteristics, it is one of the unique antigens synthesized during all latency stages of EBV infection (Chakravorty, Afzali and Kazemian, 2022).

Interleukin 31 (IL-31) is a member of the IL-6 superfamily and has been associated with various disorders, including allergic conditions, oncology, and atopic dermatitis (Borgia et al., 2022). IL-31, primarily produced by activated CD4⁺ T helper type 2, macrophages, and dendritic cells also, by non-immune cells, including fibroblasts and keratinocytes (Dillon et al., 2004; Borgia et al., 2022; Jim et al., 2023). Its primary role is associated with tissue repair and remodeling (Borgia et al., 2022). There is growing interest in targeting IL-31 across immunological and oncological contexts (Borgia et al., 2022). Specifically, in the context of a viral infection, it has been reported that IL-31 is positively correlated with hepatitis B-mediated liver cirrhosis (Ming et al., 2015). Meanwhile, EBV modulates the immune response through cytokine interactions, including IL-1, IL-6, and IL-10 (Jog

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et al., 2018; Lehner et al., 2020; Liu et al., 2020; Ohashi et al., 2022).

Calprotectin is a calcium-binding protein that has been identified as a reliable, functional biomarker of inflammation, as it plays a role in the recruitment of inflammatory cells through interactions with endothelial cells (Toma et al., 2022). Recently, with the COVID-19 pandemic, research has been focused on its possible association with viral infection, typically serving as a prognostic biomarker in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (Toma et al., 2022).

Numerous studies have evaluated peripheral blood cells associated with EBV infection, primarily establishing correlations with the infection itself. Nevertheless, there is a scarcity of research on blood cells from various immunosuppressed patients (Lin et al., 2021; Páez-Guillán et al., 2023).

Lactate dehydrogenase (LDH) is a key biomarker for evaluating inflammation and tissue injury, as well as for prognosis and outcomes in viral infections, such as COVID-19 (Zhang et al., 2023; Li and Wang, 2024).

This study hypothesizes that EBV seropositivity may dysregulate IL-31 and calprotectin, creating a feed-forward inflammatory loop that may potentially expand to broader immune signaling, including other cytokines and hormones, such as prolactin (Rasmi et al., 2023; Papageorgiou, Gogos and Akinosoglou, 2024), since it was reported that both EBV and IL-31 were associated with various cancer types, such as lymphoma and breast cancer (Ferretti et al., 2015; Muhealdeen et al., 2022). Thus, monitoring IL-31 and calprotectin in EBV-seropositive immunosuppressed hosts might yield early warning of subclinical viral reactivation or an impending cytokine storm, provide prognostic insight, and guide timely immunomodulatory therapy (Orfali and Aoki, 2023). The study aimed to investigate potential associations between the EBV-specific humoral immunity and immunological biomarkers, as well as the roles of these biomarkers in the virus's immunopathogenesis.

II. MATERIALS AND METHODS

A. Study Design and Setting

This case-control study was designed to prospectively assess the presence of IL-31, calprotectin, LDH, and differential leukocytes, as well as platelets, in EBV VCA IgM and NA IgG seropositivity. The study targeted three immunocompromised groups: The renal transplant (RT), $n = 19$; the bone marrow transplant (BMT), $n = 19$; and the hematological malignancy (HM), $n = 19$; and a control immunocompetent (IC) group, $n = 24$. The study participants were selected from Hiwa Hospital, the BMT center, and Dr. Shaxawan's clinic in Sulaimani city during the 2 months at the end of 2024. Overall, 81 participants have been included, given their dedication to studying immunocompromised and cancer patients and the limited availability of cases at regional facilities. The selected sample size balances the challenges of case availability and provides

meaningful biological analysis targeting medium-to-large effect sizes between subgroups.

B. Inclusion and Exclusion Criteria

This study included 81 cases in which participants provided consent to participate, regardless of age or gender. RT and BMT participants were recruited between post-transplant days 30 and 90, and almost all available cases at the unique centers in Sulaimani city were included. Regarding the HM group, the hospitalized patients in the Hiwa cancer-specific hospital with hematological malignancies who had received more than three doses of chemotherapy were selected. The immunocompetent (control) participants were randomly selected from the same study area to match the ages and genders of the already included study participants. Samples were excluded if hemolyzed, insufficient in volume, or failed quality control criteria during sample processing. In addition, the hematology group includes samples with lymphocytopenia (lymphocytes $< 1 \times 10^3$ cell/ μ L).

C. Demographic and Clinical Data Collection

The study was approved by the ethics committees of Sulaimani Polytechnic University (document number 26/245) and by the Sulaimani General Directorate of Health on 22 October 2024 (reference number 35). Physicians at the targeted hospitals and clinics then selected the individuals. Thereafter, the researchers obtained consent from the participants or their parents when the participant was a child, in addition to providing information about the study within a translated patient information sheet. Finally, the researchers collected demographic and primary clinical data using a validated questionnaire.

D. Sample Collection

The trained phlebotomists collected the sample using standard phlebotomy techniques simultaneously. The participant's laboratory monitoring was performed for immunocompromised cohorts to reduce the risk of infection and the invasiveness of the procedure. Sampling in the hematology and BMT groups was performed in the morning, whereas the IC and RT groups were sampled in the afternoon. Venous blood has been collected into an ethylenediaminetetraacetic acid tube for hematological measurements and into a clot-activator gel tube for serological parameters. The serum samples were aliquoted into multiple 0.4 mL portions after centrifugation at 3000 rpm for 5 min and stored at -20°C for further serological investigations.

E. Hematological Measurements

A complete blood count (CBC) was performed on all collected samples using a three-differential automated coulter counter (Swelab Alpha). The results were then recorded for further analysis.

F. EBV Detections (Seropositivity)

Serological EBV identification has been performed using the EBV NA IgG and EBV VCA IgM qualitative research

ELISA kit (BT-LAB, China). First, the test kit's performance was evaluated by running the manufacturer-provided negative and positive controls, and the results confirmed that the kit's performance and calibration setup were effective. The procedure was then applied to all samples as instructed by the manufacturer. The optical densities were measured at 450 nm using an ELISA microplate reader (BioTek 800 TS) that had been calibrated, and the cutoff value was calculated from the negative control.

G. Immunological and Inflammatory Markers

IL-31 and calprotectin were quantified using the Quantification Research ELISA kit (BT LAB, China). The assay was calibrated by diluting the manufacturer-provided standard solution to generate six serial dilutions, and the optical density at 450 nm was measured for each dilution, yielding standard curves with $R^2 > 0.98$ for IL-31 and $R^2 > 0.99$ for calprotectin. Accordingly, GraphPad Prism version 9.0 was used to compute the actual values of IL-31 (ng/L) and calprotectin (ng/mL) for each sample. Finally, LDH was measured using a dry-chemistry colorimetric analyzer (FUJIFILM DRI-CHEM NX500i) after electrical calibration with the provided quality control card, and the kits were validated in accordance with the internal quality control system and an interlaboratory comparison.

H. Data Analysis

The study data were recorded and organized confidentially, then uploaded to SPSS 27 for further analysis. Next, normality tests were performed on the continuous variables. The data deviated slightly from normality, with minor non-linearity, and exhibited approximate monotonicity. In addition, standardizing the variables did not affect the correlation coefficients; therefore, Spearman's Rho with 1000 bootstrap samples was selected to test the correlations among the continuous variables. For categorical comparisons, Fisher's exact test and bootstrapping were used to estimate risk. Furthermore, Kruskal–Wallis and Mann–Whitney U tests were used to identify statistically significant differences; moreover, variables were ranked, and one-way ANOVA was performed to calculate effect sizes with confidence intervals for more reliable subgroup analysis. In EBV seropositivity, the exact p-value was computed within the subgroup, and the Monte Carlo p-value was used for other analysis levels. Finally, multivariate quantile regression analyses were performed to identify consistent predictors of correlated outcomes. A $p \leq 0.05$ is interpreted as indicating a statistically significant result.

III. RESULTS

Demographic data includes age, gender, and body mass index (BMI). The mean and standard deviation for age and BMI are 36 ± 20 and 23.7 ± 6.18 , respectively. The gender characteristics of participants were 45 (55.6%) males and 36 (44.4%) females. The frequency of seropositivity in stratified age groups at 10-year intervals (Yamako et al.,

2017) is presented in Fig. 1. Interestingly, the mean age of seropositive participants is significantly lower than that of seronegative cases, as shown in Fig. 2c. On the other hand, EBV seropositivity was not associated with gender ($p = 0.398$; Fisher's exact 2-sided).

Regarding the clinical data, fever, pain, skin rash, cough, and lymphadenopathies were recorded with frequencies of 14, 11, 4, 7, and 2, respectively. The statistical analysis found no association between clinical records and Ig levels, except for a significant difference in IL-31 levels between cough-positive and cough-negative patients (data not shown).

EBV seropositivity differed among the study groups. The overall seropositivity is 8.64% for NA IgG and 17.28% for VCA IgM. Fig. 3 shows group-specific positivity. Risk estimation testing for HM group participants showed a 12.5% lower risk of being positive for NA IgG (Risk Ratio (RR) = 0.875, Bootstrap 95% CI = 0.720–0.962; Bias = 0.012; Standard Error (SE) = 0.088). The HM group participants have a 29.2% lower risk of VCA IgM positivity (RR = 0.708; Bootstrap 95% CI: 0.520–0.877; Bias = 0.025; SE = 0.208), with a bias of 0.019 and a standard error of 0.095. Regarding NA IgG, the BMT group has a 12.5% lower risk of NA IgG positivity (RR = 0.875; Bootstrap 95% CI: 0.737–0.962; Bias = 0.011; SE = 0.087). However, Fisher's exact test showed a significant difference in VCA IgM positivity between the HM and immunocompetent groups ($p = 0.012$; Monte Carlo).

The non-parametric comparison of NA IgG and VCA IgM indexes between the immunocompetent group and the other study groups is shown in Table I.

The three leukocyte types are shown in Fig. 4. Statistical analysis showed that the parameters varied significantly across groups ($p \leq 0.001$). Platelet count, which is considered a non-immune cell, also showed the highest mean level in the immunocompetent group, at (269 ± 79), followed by the RT group with (222 ± 71), and the BMT group had a mean of (151 ± 55), the lowest level recorded in HM participants, at (124 ± 92.8). In addition, the variations were statistically significant, $H(3) = 29.88$, $p \leq 0.001$. All hematological parameters were significantly lower in the

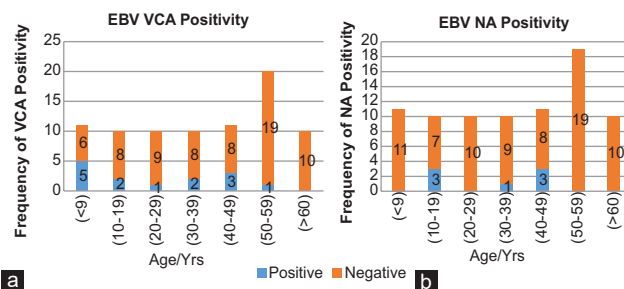


Fig. 1. Presents the frequencies of Epstein-Barr virus (EBV) positivity and participation by age group. (a) Represents the frequency of EBV viral capsid antigen immunoglobulin (IgM) positivity. (b) Represents the frequency of EBV nuclear antigen IgG positivity. The X-axis represents age groups, and the Y-axis represents the frequency of EBV positivity. The blue area indicates the EBV-negative participants, whereas the red area indicates the EBV-positive participants.

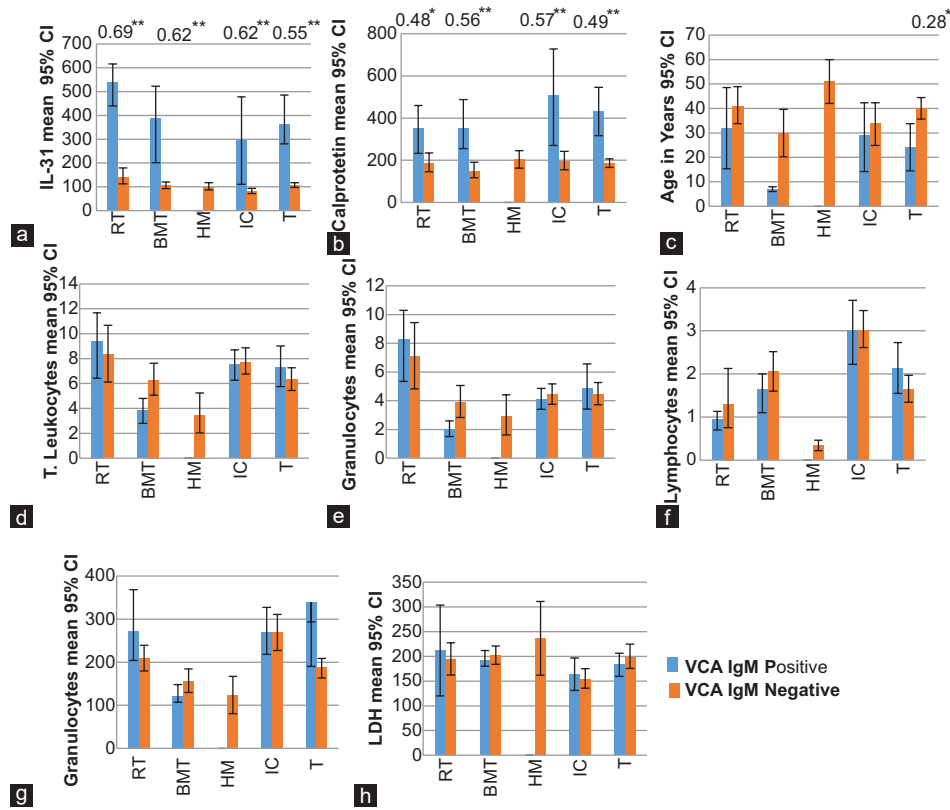


Fig. 2. Comparison cluster graph of viral capsid antigen (VCA) immunoglobulin Mu (IgM) positivity regarding numerical variables. Mean serum (a) IL-31, (b) Calprotectin, (c) Age, (d) Total leukocyte, (e) Granulocyte, (f) Lymphocytes, (g) Platelets, and (h) Lactate dehydrogenase (LDH) concentration across study groups stratified by Epstein-Barr virus VCA-IgM serostatus. The X-axis represents the study groups: Renal transplant (n = 19), Bone marrow transplant (n = 19), hematological malignancy (n = 19), immunocompetent (n = 24), and total (All Groups, n = 81). The Y-axis represents the numerical variable means, and the error bars represent the 95% bootstrapping (nearly 1000 samples) confidence interval of the mean. The values above the bars in the figures a-c for the compared group represent rank-biserial correlations (rb). Effect sizes were estimated with robustness assessed via bootstrap resampling (10,000 samples), with (0.1–0.29 = small), (0.3–0.49 = moderate), and (≥ 0.50 = large) effect sizes. (*) represent significance at the 0.05 level, and (**) significance at the 0.01 level, two-tailed; the non-significant values are not mentioned.

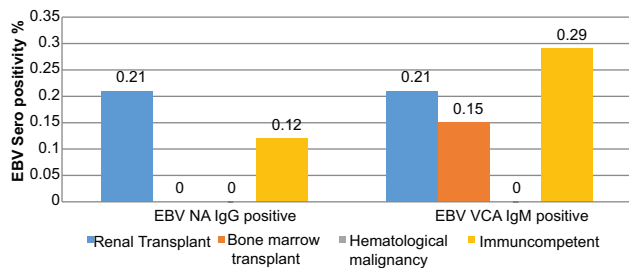


Fig. 3. Rate of Epstein-Barr virus (EBV) seropositivity in study groups. The X-axis shows EBV nuclear antigen immunoglobulin (Ig)G and viral capsid antigen (VCA) IgM positivity across the study groups, and the Y-axis represents the corresponding positivity rates. Regarding nuclear antigen IgG positivity, none was observed in the hematology and bone marrow transplant groups; however, only the Hematology group lacked VCA IgM positivity.

immunocompromised groups than in the immunocompetent group ($p \leq 0.05$), except for total leukocyte count in the RT group.

Hematological parameters and LDH levels were analyzed in conjunction with EBV seropositivity. The results showed

no significant difference between EBV seropositivity and either parameter, as shown in Fig. 2.

The IL-31 mean concentration was (154 ± 136) at the study level. Across the study groups, the mean IL-31 level differed significantly ($p = 0.003$). The IL-31 mean rank was significantly higher in EBV-seropositive cases than in seronegative participants (Fig. 2a).

In addition, the calprotectin mean concentration was (231 ± 169) at the study level. Grossly, the observed means varied among the study groups; however, the differences were not significant at the study level or between the immunocompetent and case groups. On the other hand, as shown in Fig. 2b, a significant moderate-to-large effect size difference in calprotectin levels was observed between VCA IgM seropositive and seronegative cases.

Statistical correlation analysis (Spearman's correlation coefficient, 2-tailed) was used to screen for possible associations between the VCA IgM index and IL-31, Calprotectin, and various hematological and demographic numerical variables. Spearman's rho quantifies the degree and direction of association between variables. The key results are summarized in Fig. 5, which presents the heat

TABLE I
COMPARISONS OF EBV NA IGG AND VCA IGM MEANS AMONG STUDY GROUPS

Nonparametric difference analysis					
Parameters	Compared groups	Mean±SD	F-value	ω ² (CI)	OP
EBV NA IgG	All groups	0.89±2.37	4.609**	0.118 (-0.021-0.244)	0.875
	RT versus IC	2.24±4.54 versus 0.77±1.15	0.844	-0.003 (-0.024-0.141)	0.151
	BMT versus IC	0.32±0.19 versus 0.77±1.15	5.55*	0.096 (-0.023-0.286)	0.63
	HM versus IC	0.25±0.11 versus 0.77±1.15	17.02**	0.271 (0.058-0.458)	0.981
EBV VCA IgM	All groups	1.34±2.94	11.689**	0.284 (0.097-0.412)	0.999
	RT versus IC	2.74±4.95 versus 1.52±2.80	0.326	-0.016 (-0.024-0.107)	0.086
	BMT versus IC	0.64±0.63 versus 1.52±2.80	8.60**	0.15 (-0.007-0.345)	0.817
	HM versus IC	0.43±0.13 versus 1.52±2.80	17.02**	0.325 (0.097-0.503)	0.981

EBV NA IgG: EBV Nuclear Antigen Immunoglobulin Gamma, EBV VCA IgM: EBV viral capsid antigen immunoglobulin Mu, RT: Renal transplant, BMT: Bone marrow transplant, HM: Hematological malignancy, IC: Immunocompetent, SD: Standard deviation, F-value: Statistics (*) significant at 0.05, (**) Significant at 0.01 levels, ω²(CI): Omega-squared (95% confidence intervals) indicating unbiased effect size (0.01: Small), (0.06: Medium), (≥0.14: Large) effects, OP: Observed power, EBV: Epstein-Barr virus. The bolded values highlight the statistically significant results.

map correlation. Regarding the BMI, it was not correlated with the VCA IgM index.

The quantile regression results for parameters with significant correlations are presented in Table II. The VCA IgM index was consistently positive and a significant predictor of IL-31 across all quantiles. However, participant ages had diverse non-significant effects. The intercept increased with the quantile level, indicating a right-skewed distribution. Regarding the calprotectin as a dependent variable of VCA IgM, age, and IL-31. The intercept was statistically significant across all quantiles, indicating elevated baseline calprotectin levels in the higher quantiles. VCA IgM has a significant positive effect only at the upper quantile. Age did not have a significant impact across all quantiles. Interestingly, IL-31 has a consistent prediction effect on calprotectin concentrations in all quantiles.

IV. DISCUSSION

This study found that EBV seropositivity is consistent across participant genders, and a similar result was reported in other studies (Elansary et al., 2016; Kareem, Abd and Sahib, 2025). Participant age was weakly negatively associated with VCA IgM positivity, with a small effect at the study level. The present finding is relative to Kareem's study, which stated that they are not correlated (Kareem, Abd, and Sahib, 2025). Nevertheless, as in Elansary's study, participants' ages were significantly associated with seropositivity. A reason that can be suggested is diversity in participant age among the studies; the present one includes participants aged 3–80 years, whereas the included cases in Kareem's study range from 9 months to 14 years old, mimicking the high positivity observed in the first two groups included in the present study, and Elansary's study includes participants between 18 and 59 years old. Compared with the present observation in the same age groups, seropositivity gradually increased with age. The findings are consistent with Freuer's study on EBV seroprevalence, which found no significant association with obesity in either sex (Freuer et al., 2020).

The EBV VCA IgM seropositivity rate in this study was 29%, which is higher than the 4.4% reported in a study among blood donors in Egypt (Elansary et al., 2016).

TABLE II
REGRESSION ANALYSIS RESULT

Parameter estimates by different quantiles ^{a,b}			
IL-31 ^a	β ^p (95% CI)		
	q=0.25	q=0.5	q=0.75
VCA IgM ^b	39.20** (36-42)	38.28** (34-41)	44.02** (36-51)
Age ^b	-0.17 (-0.58-0.24)	-0.24 (-0.75-0.28)	-1.03 (-2.05-0.004)
Intercept	72.60** (54-90)	93.87** (71-116)	160** (115-205)
Pseudo-R ²	0.36	0.44	0.54
Calprotectin ^a	B ^p (95% CI)		
	q=0.25	q=0.5	q=0.75
VCA IgM ^b	-1.77 (-19-15)	-15.70 (-34-3)	28.96* (0.73-0.57)
Age ^b	-0.15 (-1.4-1.7)	-0.80 (-2.2-0.6)	-0.001(-2.1-2.1)
IL-31 ^b	0.48* (0.09-0.85)	0.84** (0.42-1.26)	0.79* (0.16-1.40)
Intercept	86.14* (10-161)	127.17** (43-210)	133.38* (10-255)
Pseudo-R ²	0.10	0.16	0.28

^aDependent variable, ^bIndependent variables, β^p: Coefficient value, (*) Significant at 0.05, and (**) Significant at 0.01 level, CI: Confidence intervals. The Bolded values highlight the statistically significant results.

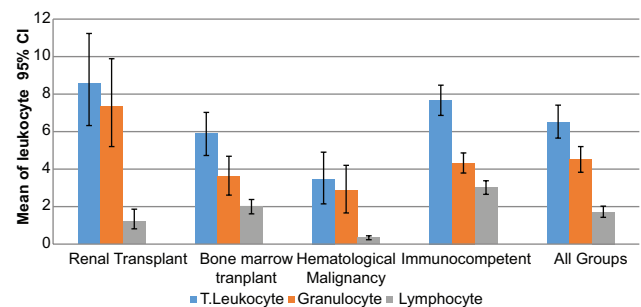


Fig. 4. Mean of leukocytes in the study groups. Demonstrates the mean and 95% confidence intervals for total leukocytes, granulocytes, and lymphocytes in the study group. The X axis represents the study groups, while the Y axis represents the mean of each leukocyte count (10³ cells/μL). The error bars represent the confidence intervals of means for each parameter.

However, a regional study also reported the highest EBV prevalence associated with high lymphoma incidence (Muhealdeem et al., 2022). In addition, there may be two reasons for the differences in frequency. First, the Egyptian study participants were adults only, and all cases that were

EBV VCA IgM Index

IL-31 ng/L	0.525** (0.307-0.708)	0.833** (0.464-0.961)	0.368	0.299	0.617** (0.182-0.874)	1
Calprotectin ng/mL	0.508** (0.314-0.669)	0.66** (0.306-0.871)	0.653** (0.277-0.914)	-0.041	0.683** (0.342-0.895)	0.9
LDH U/L	0.084	0.276	-0.300	0.359	0.207	0.8
T. WBC 10³ cell/μL	0.298** (0.074-0.500)	0.206	-0.263	0.386	-0.041	0.7
Lymphocyte 10³ cell/μL	0.182	-0.219	-0.327	0.231	0.092	0.6
Granulocyte 10³ cell/μL	0.293** (0.073-0.486)	0.336	-0.08	0.362	-0.109	0.5
Platelets 10³ cell/μL	0.258* (0.057-0.434)	0.19	0.254	0.009	-0.114	0.4
Age/years	-0.353** (-0.542 -(-0.139))	-0.427	-0.484* (-0.719 - 0.145)	-0.413	-0.259	0.3
	Total	RT	BMT	HM	IC	0.2
						0.1
						0
						-0.1
						-0.2
						-0.3
						-0.4
						-0.5
						-0.6
						-0.7
						-0.8
						-0.9
						-1

Fig. 5. Correlation heat map of Epstein-Barr virus (EBV) viral capsid antigen (VCA) immunoglobulin Mu (IgM) with numerical variables. EBV VCA IgM (EBV VCA Mu Globulin), Total (all participants n = 81), Renal transplant n = 19, bone marrow transplant (n = 19), hematological malignancy (n = 19), immunocompetent (n = 24), scales indicate Spearman's Rho value, and (**) indicates significance at the 0.01 level (2-tailed). While (*) indicates that the correlation is significant at the 0.05 level (2-tailed). Each cell denotes the strength and direction of the correlation: the cooler the color, the stronger the positive correlation; the warmer the color, the stronger the negative correlation. A $p \leq 0.05$ is considered statistically significant; bootstrap confidence intervals are presented for significant correlations based on 1000 samples. Rho's value (<0.19) is very weak; (0.20–0.39) is weak; (0.40–0.59) is moderate; (0.60–0.79) is strong; and (≥ 0.80) indicates very strong correlations.

positive for other infectious agents, such as HBV, HCV, HIV, and *Treponema pallidum*, were excluded. Second, a cohort study reported that high EBV salivary shedding declined gradually over 6 months following the onset of primary infection. It also discussed how younger immunocompetent EBV-positive individuals experience greater viral shedding because less time has elapsed since their primary infection (Johnson et al., 2016). In this study's context, targeting the immunocompetent group, 2 of 7 seropositive cases tested positive for NA IgG, suggesting that 71% of seropositive participants had no evidence of EBV latency. This positivity rate may reflect residual viral shedding or subclinical re-exposure to the virus.

A statistically significant difference with a medium-to-large effect size, supported by a reliable power analysis, was observed at the study level for NA IgG and VCA IgM. Thus, the study groups show diversity in the two specific Ig levels. Although the difference between immunocompetent and RT groups is not statistically significant, the result suggests that renal transplantation does not affect EBV seropositivity. However, the significantly lower Ig levels in the HM and BMT groups, with moderate to large effect sizes, and their low protective rate against seropositivity suggest that the chemotherapy regimen and BMT process may be associated with EBV humoral immunity, as indicated by the measured Igs. Another point of view is that, in RTs, seropositive

cases were reactive to both Igs, as in immunocompetent; this may suggest latent activities, in addition to the present infection, since NA is the only protein expressed in the three latency stages (Chakravorty, Afzali and Kazemian, 2022). Interestingly, in the BMT group, seropositive cases were negative for NA IgG, suggesting that most infections were current, with no latent activity. Regarding the hematology group, zero positivity may have two possible etiologies. First, this study observed that leukocytes, including lymphocytes, are periodically eliminated by chemotherapy; therefore, EBV-latent cells, particularly B lymphocytes, may also be eliminated, leading to the body's clearance of EBV latency. Second, it is well documented that chemotherapies decrease Ig levels both during and after treatment (Zugmaier et al., 2014). In these contexts, latency detection using advanced molecular techniques and a large cohort is recommended to mitigate the risk of cross-reaction in ELISA and to address humoral immunity inhibition in immunocompromised participants; the findings may be clinically insightful.

This pioneering study observed a significant, moderate, and monotonic positive correlation between IL-31 concentration and the EBV VCA IgM index. Moreover, the present study highlighted that IL-31 is a consistent predictor for both EBV IgM and calprotectin levels. However, the correlation is not significant in the BMT and HM groups; this finding suggests that the association may be potentiated by seropositivity in other groups. To the best of the researchers' knowledge, based on currently indexed and publicly available scientific publications, no dedicated research study exists, particularly in the Iraqi scientific literature, that directly assesses the correlation between EBV infection status with IL-31 and calprotectin levels. Except for a Russian study that concludes an association between several cytokines, including IL-31, with herpes viruses, without specifying EBV (Natalia et al., 2023). Relatedly, a positive association of IL-31 with HIV infection is documented (Yan et al., 2022).

This study also observed an association between calprotectin and EBV seropositivity, similar to that observed in IL-31. However, regression analysis indicates that IL-31 levels are a consistent predictor of calprotectin levels across the three quantiles, whereas VCA positivity shows heterogeneous, non-significant associations in the two lower quantiles. Literally, there is limited research, at least in human blood, on measuring calprotectin in viral infections, aside from studies reporting it as an effective prognostic biomarker in SARS-CoV-2 infection (Toma et al., 2022; Keller et al., 2024). Furthermore, Keller et al. concluded that calprotectin levels are not associated with herpes simplex virus in patients coinfecting with COVID-19.

Therefore, the present study's findings may offer new insights into the pathogenic patterns of EBV infection. Mechanistically, in addition to its primary role in inducing epithelial cell proliferation and tissue repair, IL-31 can also induce paracrine and/or autocrine signaling in localized follicular lymphoma tissues via IL-31/IL-31 receptor alpha, thereby prolonging cell proliferation (Ferretti et al., 2015; Borgia et al., 2022). Concurrently, prolactin, which acts as a cytokine during viral infections, would potentiate this

response: Prolactin receptor engagement activates the Janus-activated kinase two (JAK2)/Signal transducer and activator of transcription (STAT) and mitogen-activated protein kinase (MAPK) cascades in macrophages and lymphocytes, thereby promoting T/B-cell activation (Rasmi et al., 2023). Regarding calprotectin, EBV reactivation can precipitate macrophage activation syndrome with massive cytokine release. In this context, innate alarmins, such as neutrophil-derived calprotectin, would likely increase alongside IL-1, IL-6, tumor necrosis factor-alpha, and other cytokines, reflecting uncontrolled myeloid activation (Papageorgiou, Gogos and Akinosoglou, 2024). These pathways converge on IL-31 and prolactin, both of which feed into the (STAT)/(MAPK) axes, while calprotectin triggers TLR4/NF- κ B, suggesting a synergistic amplification of inflammation during EBV flares. Further exploration of the mechanism beyond the observed association in the present study, using advanced molecular and immunological techniques on a large cohort, is needed. Aiming to manipulate IL-31 therapeutically to prevent and better control both EBV infection and its related pathological complications, as such efforts are currently under trial to control atopic dermatitis (Orfali and Aoki, 2023).

Regarding hematological parameters, the weak correlation observed at the study level only (Fig. 5) may reflect group heterogeneity rather than an association with EBV seropositivity, as no significant association was observed within the study groups. However, leukocytosis in infectious mononucleosis and pancytopenia in EBV infection have been reported (Lin et al., 2021; Páez-Guillán et al., 2023). Interpretation suggests that hematological parameters, including leukocytes and platelets, may be associated with EBV activity, and that their role is finished once the infection is controlled. EBV genome quantification and early antigen detection may require distinguishing between lytic (active) infection and past recovered seropositivity, whereas NA positivity supports EBV latency states without addressing lytic activities.

The study did not observe a significant association between seropositivity and LDH levels. However, such a correlation was reported in studies involving primary (active) EBV infection among children (Zhang et al., 2023; Li and Wang, 2024). Thus, the elevation in LDH during active EBV infection is possible because inflammation and tissue damage may occur. However, some seropositive cases in the present study may not have experienced significant inflammation or tissue injury, as all seropositive cases in the immunocompetent group were asymptomatic.

V. CONCLUSION

Despite variation in the literature on the prevalence of EBV seropositivity across age and gender, this study found that EBV seropositivity was more frequent among younger individuals, regardless of gender. In contrast, EBV latent activity was more common among adults and older individuals. Hematological malignancies and chemotherapeutic approaches may eliminate specific humoral immunity against EBV or against lymphocytes harboring the

latent virus, so inhibition of EBV latent activity may be more likely, at least in cases involving lymphocyte elimination. Whereas, after kidney transplantation, latent activities are also present, which is why isolating kidney recipient persons alone may not keep them away from EBV. Total leukocytes, granulocytes, lymphocytes, platelets, and LDH may not be associated with EBV VCA IgM seropositivity. The findings suggest a possible association between IL-31 and potentially calprotectin levels with EBV seropositivity; however, additional mechanistic studies are required to determine causality or oncogenic relevance.

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