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Immunological dysregulation associated with herpes virus DNA in the serum of infertile women

J. E. Mahmood*, R. Bellagha*, O. Bahri**, W. Slama*, S. Q. Mohammad***

**University of Tunis El Manar, Tunis, Tunisia*
*** Aziza Othmana Hospital, Tunis, Tunisia*
****Middle Technical University, Diyala, Iraq*

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University of Tunis El Manar, Tunis, Tunisia. E-mail: jasim.e.mahmood@alamal.edu.iq, rahma_bellagha@live.fr, wided777slama@gmail.com

Laboratoire de Recherche LR16SP01, Hôpital Aziza Othmana, Tunis, 693210, Tunisia. E-mail: olfa.bahri@fmt.utm.tn

Middle Technical University, Technical Institute of Baqubah, Diyala, Iraq. E-mail: sarmadbio6@gmail.com

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Female infertility is a complex ailment, and the role of hidden viral infections could be more subtle but pivotal to achieving pregnancy. Herpes viruses: Herpes Simplex Virus (HSV), *Cytomegalovirus* (CMV), and Epstein-Barr Virus (EBV) are involved in the pathogenesis of reproductive failure, underlying the role of chronic inflammation and immunomodulation in this context. This study aimed to determine the presence of HSV, CMV, and EBV DNA in infertile women and evaluate associated serum cytokine levels (IL-6 and IL-10) to explore potential immunological signatures of viral involvement in infertility. The study was a case-control study that was conducted on 96 infertile and 96 fertile women. High-sensitivity ELISA was used to measure the levels of IL-6 and IL-10 in serum samples. qPCR was conducted in real time to detect viral DNA among the infertile participants. To determine relationships between viral load (Ct values), cytokines, infertility type, and age, statistical analysis was conducted with independent t-tests, chi-square tests, and Spearman correlation. qPCR showed the presence of CMV in 14.6% of the infertile women, HSV in 11.5%, and EBV in 8.3%. Women with the virus had small increases in cytokines (mean IL-6: 9.2–10.5 pg/mL; IL-10: 31.3–33.1 pg/mL) and a higher IL-6/IL-10 ratio (0.29–0.32), especially with EBV. There were significant inverse correlations between viral load and IL-6 in EBV-positive women ($r = –0.51$, $P = 0.029$). Secondary infertility had lower Ct values that are indicative of an increased viral activity. There were no considerable relationships with age. The results indicate a slight yet statistically significant relationship between latent infection with the herpes virus, imbalance of cytokines, and infertility. The increased IL-6/IL-10 rates and increased viral loads (EBV-positive and secondary infertility cases) suggest that chronic immune activation can be one of the factors in the disturbed reproductive outcomes. Molecular viral screening and immunological profiling should be integrated to improve the accuracy of the diagnosis of unexplained infertility.

Keywords: Infertility; HSV; CMV; EBV; qPCR; IL-6; IL-10; cytokine ratio; viral load.

Introduction

Infertility is a complicated clinical and community health issue with an approximated prevalence of 8–12% in reproductive couples in every age category around the world. The pathophysiology of infertility in women is complex and consists of anatomical defects, dysfunction of endocrine systems, environmental factors, and immunologic dysfunctions. The cause is, however, unknown in almost 15–30% of cases, and the issue of subclinical infection and possible consequences on reproductive health is gaining growing attention. One such group of pathogens, which is of interest in this respect, is the Herpesviridae viruses, including Herpes Simplex Virus (HSV), *Cytomegalovirus* (CMV), and Epstein-Barr Virus (EBV) (Vander Borght & Wyns, 2018; Akalewold et al., 2022).

These viruses have the major features of being capable of inducing a permanent latency phase after initial infection, and being reactivated periodically during a period of physiological stress or immunosuppression. Although the activation or latent infection by herpes virus remains clinically silent in immunocompromised individuals, inflammation of the reproductive tract, mechanism of endometria and other reproductive organs, and abnormal expression of cytokines, which can potentially affect fertility, have been linked to the activation or latent infection by herpesvirus (Pan et al., 2023).

In a case study, the reactivation of CMV or HSV in the genital tract may cause local inflammation and alter the microenvironment of the endometria, which may affect the implantation. Similarly, EBV has also been involved in the regulation of immune tolerance dysregulation by way of viral IL-10 homologs and chronic antigenic stimulation (Cohen, 2020).

Interleukin-6 (IL-6) and interleukin-10 (IL-10) are now considered significant biomarkers of the association between infection, inflammation, and reproductive outcomes. The pleiotropic pro-inflammatory cytokine, IL-6, is important in the functioning of the ovaries, follicle development, and implantation; therefore, high levels of IL-6 have been linked to endometriosis, polycystic ovary syndrome (PCOS), and other infertility disorders. On the other hand, IL-10 is an anti-inflammatory cytokine needed to preserve maternal-fetal immune tolerance. Imbalances between IL-6 and IL-10 or a high ratio of IL-6/IL-10 are becoming recognized as markers of immune deregulation in the reproductive tract and would have fertility implications (Jafrin et al., 2022).

Although biologically latent herpesvirus infection, cytokine imbalance, and infertility can be related to each other, viral screening or immunological profiling is seldom part of routine fertility workups. Several studies performed in the past have examined seroprevalence, or shedding of the virus in samples of certain population groups, but only a few have examined the combination of viral DNA and inflammatory signs in infertile females. The gap will be bridged and could offer new information on poorly understood infertility and inform more specific diagnostic and treatment strategies (Akhigbe et al., 2022). Despite increasing recognition of the multidimensional relationship between infection, immunity, and fertility, the contribution of latent herpesvirus infections to female infertility remains underexplored, particularly with respect to their direct molecular detection and associated cytokine responses. Most previous studies have relied on serological markers or symptomatic viral reactivation, overlooking the potential role of subclinical DNAemia and immune dysregulation in reproductive failure. To address this gap, the present study aims to

investigate the presence of HSV, CMV, and EBV DNA in the serum of infertile women and to assess corresponding levels of pro- and anti-inflammatory cytokines (IL-6 and IL-10). By integrating molecular and immunological analyses, this research seeks to determine whether latent viral infections drive a measurable immune imbalance that may contribute to infertility, particularly in cases with no clear anatomical or hormonal cause.

Matherials and methods

The study protocol was reviewed and approved by the Institutional Review Board (IRB) of the Faculty of Science in Tunis, Tunisia, and all procedures were conducted following the Declaration of Helsinki. Written informed consent was obtained from all participants before their inclusion in the study.

This was a hospital-based case-control study conducted to evaluate the association between latent herpesvirus infections, serum cytokine levels (IL-6 and IL-10), and female infertility. A total of 192 women (96 infertile cases and 96 fertile controls) were recruited from gynecology and fertility clinics affiliated with tertiary hospitals between January to August, 2025. Sample size was calculated to provide 80% power to detect a minimum 15% difference in viral detection rates between groups at $\alpha = 0.05$ (Biau et al., 2008). Cases were defined as women aged 19–45 years with primary or secondary infertility (failure to conceive after ≥12 months of unprotected intercourse), confirmed by a gynecologist. Controls were age-matched fertile women with at least one live birth and no history of infertility. Exclusion criteria included autoimmune disease, chronic inflammatory disorders, malignancy, recent infection (<3 months), use of steroids / immunosuppressants, or refusal to consent.

The participants were subjected to a formal interview and physical check-up. Demographic and clinical data that were recorded were age, marital status, residence, reproductive history (gravidity, parity, miscarriage, infertility duration), and anthropometric measurements. The body mass index (BMI, kg/m²) was measured using standard equations and was taken as a possible confounding variable because of its established association with ovulatory functioning and hormone balance (Gesink et al., 2010; Rich-Edwards et al., 2002). The gynecological history was to be made to record the patterns of the menstrual cycle, dysmenorrhea, and clinical pregnancy diagnoses such as polycystic ovary syndrome (PCOS), endometriosis, or pelvic inflammatory disease (PID). These variables were only provided to do a descriptive analysis and not contrasted between groups.

Five milliliters of venous blood were collected into BD Vacutainer® serum separator tubes. After clotting at room temperature (15–30 min), the samples were centrifuged at 3,000 rpm for 10 minutes. Serum was aliquoted and stored at –80 °C until analysis to prevent protein degradation (Rai et al., 2006; Arif et al., 2025).

Of the 96 serum samples collected from infertile women, 3 samples were excluded due to hemolysis (n = 2) or inadequate DNA purity (A260/A280 < 1.6; n = 1), resulting in 93 samples suitable for qPCR testing. Incomplete viral amplification results led to minor variations in sample size across viral targets (e.g., n = 94 for HSV, n = 90 for CMV).

Serum concentrations of IL-6 and IL-10 were quantified using Human Quantikine® High-Sensitivity ELISA Kits (R&D Systems, USA) according to manufacturer protocols. All samples were thawed once and run in duplicate. Optical density was measured at 450 nm using a BioTek ELx800® microplate reader. Standard curves were fitted using a four-parameter logistic regression model ($R^2 > 0.98$). The limit of detection was <1 pg/mL, and the inter-/intra-assay coefficient of variation was <10%. The same assays were performed in both infertile and fertile women to enable cross-group comparisons.

IL-6 and IL-10 were selected due to their established roles in reproductive immunology: IL-6 promotes inflammatory signaling and follicular maturation, while IL-10 maintains immune tolerance at the maternal-fetal interface (Caër et al., 2017).

Detection of IgG and IgM herpesvirus (HSV-1, HSV-2, CMV, EBV) serum antibodies was done by commercial ELISA kits (Bio-Rad, Germany). The samples were tested twice; the results were evaluated based on the recommendations of the manufacturer cut-off and reported as positive/negative. Serological assessments were done to offer a background on the previous exposure to the virus and to complement molecular results.

The 200-µL serum was subjected to the QIAamp Viral DNA Mini Kit to obtain the DNA (Qiagen, Germany). The concentration and purity of DNA were determined by NanoDrop 2000 (A260/A280 = 1.820). Viral DNA was quantified by TaqMan® probe-based real-time polymerase chain reaction (qPCR) using ABI 7500 and Rotor-Gene Q platforms.

Target genes included UL30 (HSV-1/2), UL54 (CMV), and EBNA-1 (EBV). UL30 (HSV-1/2), UL54 (CMV), and EBNA-1 (EBV) were considered target genes. Cycling conditions were 95°C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Samples were classified as qPCR-positive if the cycle threshold (Ct) was <36. Each run included positive and negative controls.

Data were analyzed using SPSS v29.0 (IBM Corp., USA) and GraphPad Prism v9.0. Continuous variables were expressed as mean ± SD or median (IQR) based on Shapiro–Wilk normality testing. Group differences were assessed using an independent samples t-test or Mann–Whitney U test, as appropriate. Categorical variables were compared using chi-square or Fisher's exact test. Correlations between viral load (Ct values) and cytokine levels were evaluated using Spearman's rank correlation. Multivariable binary logistic regression was used to adjust for confounders (age, BMI, and infertility type). Statistical significance was set at $P < 0.05$ (two-tailed) (Kim et al., 2017; Essa et al., 2024).

Laboratory personnel were blinded to participants' infertility status to minimize bias. No imputation was performed for missing laboratory data.

Results

Table 1 shows that the quantitative polymerase chain reaction (qPCR) was used to detect Herpes Simplex Virus (HSV-1/HSV-2), *Cytomegalovirus* (CMV), and Epstein–Barr Virus (EBV) in 96 infertile women. A cycle threshold (Ct) value of less than or equal to 36 was defined as positive with the presence of virus DNA.

The results indicated that HSV was positive in 11 cases (11.5%), CMV in 14 cases (14.6%), and EBV in eight patients (8.3%). CMV demonstrated the highest percent positivity and implied the fourth ranking in prevalence for the study population. The identification of these viruses suggests that latent or persistent viral infections may have potential roles in the etiology of infertility through chronic inflammatory and immune-mediated pathways.

Table 1
qPCR detection for HSV, CMV, and EBV in infertile women

Virus	n Positive (Ct < 36)	Positivity, %
HSV (HSV-1/HSV-2)	11	11.5
CMV	14	14.6
EBV	8	8.3

Among women who were qPCR-positive, Table 2 shows that the viral burden was modest (Ct medians ~30), with HSV at 30.6 (IQR 28.9–33.2), CMV at 29.8 (27.7–33.5), and EBV at 31.2 (28.5–34.1). Corresponding cytokine profiles showed IL-6 medians from 9.2 to 10.5 pg/mL and IL-10 medians from 31.3 to 33.1 pg/mL, indicating a consistent but mild inflammatory/immune-regulatory activation across viruses. EBV-positive cases had the highest central values for both IL-6 (10.5 pg/mL) and IL-10 (33.1 pg/mL), suggesting a comparatively stronger immunologic response in this subgroup. The IL-6/IL-10 ratio increased stepwise from HSV (0.29) to CMV (0.31) and EBV (0.32), implying a gradual shift toward a more pro-inflammatory balance as one moves from HSV to EBV.

Table 3 applied Spearman rank correlation to examine the correlation between the viral load in terms of cycle threshold (Ct) and the concentration of inflammatory markers (IL-6 and IL-6/IL-10 ratio) in infertile women with positive results regarding HSV, CMV, or EBV DNA. The analysis correlation coefficients reflected a negative value in all viral groups, which proves that low values of Ct (or high con-

centrations of viral DNA) are correlated with increased amounts of cytokines, a trend typical of active or stronger viral immune stimulation. Ct and IL-6 were moderately and negatively correlated in the case of HSV-positive patients (0.45, 0.042) which means that a high viral load has a positive correlation with the expression of the IL-6. The correlation between Ct and the IL-6/IL-10 ratio (r = –0.39, P = 0.078) was approaching the significant value meaning that more pro-inflammatory cytokines are being produced with a greater presence of the virus.

Table 2
Cytokine levels among qPCR-positive infertile women

Virus	N Positive	Ct Median (IQR)	IL-6 Median (IQR), pg/mL	IL-10 Median (IQR), pg/mL	IL-6/IL-10 Median (IQR)
HSV (min HSV-1/HSV-2)	11	30.6 (28.9–33.2)	9.2 (8.1–11.3)	31.3 (27.6–33.9)	0.29 (0.26–0.34)
CMV	14	29.8 (27.7–33.5)	10.1 (8.5–12.0)	32.4 (29.2–34.5)	0.31 (0.28–0.36)
EBV	8	31.2 (28.5–34.1)	10.5 (8.4–12.6)	33.1 (30.1–35.2)	0.32 (0.27–0.36)

On the same note, the correlations between Vs were negative, but not significant in CMV-positive women (IL-6, 0.061, 0.38; IL-6/IL-10, 0.102, 0.33). These findings confirm the assumption that CMV infection induces moderate cytokine activation, which is related to the amount of viral DNA, and an individual variation, and it might be linked to latency or reactivation stages. The EBV-positive ones exhibited the most most significant association between the Ct vs. IL-6 and Ct vs. IL-6/IL-10 ratios, with the values of -0.51 (p = 0.029) and -0.47 (P = 0.051), respectively. These results suggest that the viral DNA level has a negative relationship with the immune regulatory balance, which implies that the EBV infection is accompanied by a strong inflammatory response, and the levels of IL-6 are high in comparison to the level of IL-10.

The interleukin profile of women with positive viral DNA (HSV, CMV, or EBV) revealed in Table 4 showed different trends of cytokine stimulation that reflected different levels of the intensity of an immune response. The average levels of IL-6 were 9.2 to 10.5 pg/mL with the average levels of IL-10 being 31.3 to 33.1 pg/mL in the three viral groups. These values are a weak but consistent upregulation of both pro-inflammatory and anti-inflammatory cytokines in people who were infected by the virus. In patients with HSV-positive (HSV-1 / HSV-2) the mean level of IL-6 was 9.2 ± 1.3 pg/mL and IL-10 was 31.3 ± 2.8 pg/mL. About 36% of these women had IL-6 above 10 pg/mL and 45% had IL-10 above 33 pg/mL indicating a moderate activation of the immune system probably associated with subclinical viral persistence.

In the CMV-positive patients, the mean IL-6 was moderately elevated (10.1 ± 1.8 pg/mL) together with a mean IL-10 of 32.4 ± 2.5 pg/mL. Almost 43% and 57% of CMV-positive women had high amounts of IL-6 and IL-10 respectively. This indicates the immunostimulatory effect of CMV which is associated with the stimulation of chronic, low-grade inflammation and persistent cytokine release especially in viral latency or reactivation. The EBV-positive group had the highest cytokine values, being 10.5 ± 1.9 pg/mL in IL-6 and 33.1 ± 2.1 pg/mL in IL-10 with 50% (IL-6) and 62% (IL-10) subjects having high cytokine values. The fact that EBV is well documented to mediate B-cell and cytokine pathways is consistent with these results suggesting a strong inflammatory and immunoregulatory response.

Table 3
Correlation between cycle threshold (Ct) and cytokine markers among qPCR-positive infertile women

Virus	ρ (Ct vs IL-6)	p-value	N	ρ (Ct vs IL-6/IL-10)	P-value	N
HSV (min HSV-1/HSV-2)	−0.45	0.042	11	−0.39	0.078	11
CMV	−0.38	0.061	14	−0.33	0.102	14
EBV	−0.51	0.029	8	−0.47	0.051	8

Table 4
Interleukin profiles among virus-positive cases

Virus	N Positive	Mean IL-6 (pg/ml) ± SD	Mean IL-10 (pg/mL) ± SD	% with High IL-6 (>10)	% with High IL-10 (>33)
HSV (HSV-1/HSV-2)	11	9.2 ± 1.3	31.3 ± 2.8	36	45
CMV	14	10.1 ± 1.8	32.4 ± 2.5	43	57
EBV	8	10.5 ± 1.9	33.1 ± 2.1	50	62

In the study, Spearman correlation analysis was conducted to assess the relationship between cytokine levels (IL-6, IL-10) and selected clinical indicators, specifically age and duration of infertility, in virus-positive infertile women (Table 5). The findings demonstrated weak to moderate positive correlations, implying that cytokine increase is likely to correlate with extending periods of infertility and, to a lesser degree, with growing age. In HSV-positive patients, IL-6 and age (ρ = +0.28, P = 0.18) as well as IL-10 and infertility duration (ρ = +0.29, P = 0.15) had a weak positive relationship, but neither was statistically significant. These data are indicative of a small role of cytokines in HSV-induced infertility, which may indicate less systemic inflammatory reactivity.

The CMV-positive women exhibited a strong correlation between IL-6 levels and the duration of infertility (ρ = +0.45, P = 0.04), indicating that a longer infertility duration is associated with increased IL-6 levels. A significant IL-10 relationship was identified, approaching significance (ρ = +0.38, P = 0.06), indicating that anti-inflammatory mechanisms are concurrently activated with the persistence of infertility. This is an indicator of a chronic inflammatory, smoldering state, which could be sustained by latent CMV infection. In women who are EBV-positive, the same trends were noted, as IL-6 showed a strong positive correlation with the amount of infertility (ρ = +0.49, P = 0.03) and IL-10 showed a marginal correlation (ρ = +0.41, P = 0.07). These findings support the hypothesis that the long-term presence of the EBV infection can be the cause of chronic activation of the immune systems, which may worsen infertility due to a long-term cytokine imbalance.

Table 5
Correlation between interleukin levels and clinical features

Variable	ρ (IL-6 vs. Age)	p-value	ρ (IL-6 vs. Infertility Duration)	p-value	ρ (IL-10 vs. Infertility Duration)	p-value
HSV-positive	+0.28	0.18	+0.34	0.10	+0.29	0.15
CMV-positive	+0.31	0.12	+0.45	0.04*	+0.38	0.06
EBV-positive	+0.24	0.22	+0.49	0.03*	+0.41	0.07

The IL-6/IL-10 ratio is a vital measure of the activation or suppression of the pro-inflammatory and anti-inflammatory mechanisms, respectively. Whereas IL-6 enhances inflammation, IL-10 is an immunoregulatory cytokine that neutralizes the overactivity of the immune system. Consequently, an increase in the ratio of IL-6 / IL-10 is an indicator of a shift towards inflammatory control, which may carry

negative consequences in terms of reproductive health. As was revealed in the findings of Table 6, the virus-negative group had an average level of IL-6/IL-10 of 0.27 ± 0.05, which indicated a balanced immunological environment characteristic of the normal state of physiological processes. Conversely, the ratio of HSV-positive women, who were considered as having a mild inflammatory tilt, increased progressively within the virus-positive groups, as HSV-positive women had a mean of 0.29 ± 0.06. The CMV-positive group exhibited a mean ratio of 0.31 ± 0.07, which indicates a moderate level of systemic inflammation potentially caused by repeated reactivation of the virus and ongoing immune signaling. The highest ratio was observed in the EBV-positive women (0.32 ± 0.08), which indicated a high degree of pro-inflammatory bias. This trend is consistent with the established ability of EBV to cause chronic immune response by stimulating B-cells and cytokines.

Table 6
Cytokine ratio (IL-6/IL-10) and immune balance

Group	Mean ± SD	Immunological trend
Virus-negative	0.27 ± 0.05	balanced
HSV-positive	0.29 ± 0.06	slight inflammatory tilt
CMV-positive	0.31 ± 0.07	moderate inflammation
EBV-positive	0.32 ± 0.08	strong inflammatory tilt

Table 7 shows that infertile women with measurable viral DNA (Ct < 36) had the highest means of Ct values obtained with HSV, then EBV, and CMV (note: lower Ct = higher load of DNA). The HSV (minimum of HSV-1/HSV-2) was 19.48 ± 8.90, EBV was 26.97 ± 7.46, and CMV was 28.74 ± 5.65. The relatively reduced mean Ct of HSV indicates that it becomes activated more frequently when it is present. The larger SDs, especially of HSV and EBV, indicate that there is viral burden heterogeneity among individuals, which is consistent with variability in the dynamics of latency/reactivation.

Table 7
Cycle threshold (Ct) measurements from qPCR tests
for each virus in cases of infertile women

Virus	N positive	Ct
HSV	94	19.43 ± 8.90
CMV	90	28.73 ± 5.64
EBV	93	26.97 ± 7.45

Table 8 displayed both the inflammatory (IL-6) and regulatory (IL-10) cytokines in the virus-positive subgroups. In all three viruses, mean IL-6 was about 10–11 pg/mL, and mean IL-10 was about 28.73 ± 7.51 and 28.70 ± 7.49, respectively. The mean IL-6/IL-10 ratio was of the order of 0.41-0.43, with an EBV mean ratio of 0.425 ± 0.272 being slightly higher than the HSV and CMV ones (0.41), indicating a slightly more pro-inflammatory bias in EBV-positive women. All in all, these data show the presence of a steady, but weak, immune response that is accompanied by a viral detection.

Table 8
Cytokine measurements from qPCR analyses
for each virus in cases of infertile women

Virus	N	IL-6	IL-10	IL-6/IL-10 Ratio
HSV	44	10.46 ± 3.72	28.72 ± 7.51	0.411 ± 0.263
CMV	43	10.60 ± 4.14	28.70 ± 7.56	0.425 ± 0.26
EBV	44	10.87 ± 4.51	28.69 ± 7.49	0.424 ± 0.71

The stratification by infertility type provided a consistent result, as shown in Table 9. The women who had secondary infertility were more likely to have a lower mean Ct (i.e., a higher viral burden) than women having primary infertility, with the most significant difference in the case of HSV and EBV. In the case of HSV, primary vs. secondary Ct was 20.45 ± 8.62 vs. 17.93 ± 9.25; in the case of EBV, 27.65 ± 7.16 vs. 25.89 ± 7.89. There was less difference between CMV (primary 29.09 ± 4.50 vs. secondary 28.24 ± 7.01). These data are consistent with the hypothesis that previous reproductive events or pathologies of the pelvis can be related to viral persistence/reactivation and thus have a minor but significant impact on viral DNA load in women with secondary infertility.

Table 9
Ct measurements for each virus based on the type
of infertility in cases of infertile women

Virus	Infertility type	N	Ct
HSV	primary	58	20.44 ± 8.61
	secondary	36	17.93 ± 9.25
CMV	primary	53	29.08 ± 4.50
	secondary	37	28.23 ± 7.00
EBV	primary	57	27.65 ± 7.15
	secondary	36	25.89 ± 7.88

Age-stratified analyses (<30, 30–34, ≥35 years) showed only modest differences in Table 10. For HSV, mean Ct varied narrowly (18.34 ± 8.66, 20.73 ± 9.42, 19.79 ± 8.93 across ascending age bands), indicating no marked age gradient. CMV Ct remained highly similar across groups (28.85 ± 6.07, 28.21 ± 5.78, 28.97 ± 5.34). EBV displayed a weak trend toward lower Ct (higher load) in women ≥30 years (<30: 28.30 ± 3.52; 30–34: 26.07 ± 8.45; ≥35: 26.34 ± 9.21), but variability was large and group means overlapped. Collectively, the age effects appear limited in magnitude relative to the differences seen by infertility types.

Table 10
Ct measurements for each virus
categorized by age group among cases of infertile women

Virus	Age group	N	Ct
HSV	<30	34	18.33 ± 8.65
	30–34	22	20.73 ± 9.41
	≥35	38	19.78 ± 8.92
CMV	<30	30	28.85 ± 6.07
	30–34	23	28.21 ± 5.77
	≥35	37	28.96 ± 5.33
EBV	<30	33	28.29 ± 3.52
	30–34	23	26.07 ± 8.44
	≥35	37	26.34 ± 9.21

Discussion

This study has explored the possibility of latent viral infections and latent cytokine reactions in the cause of female infertility, specifically HSV, CMV, and EBV. Viral DNA was identified in a proportion of infertile women with CMV (14.6%), HSV (11.5%), and EBV (8.3%), with the latter being the most common. Those results represent the immunotropic character of herpesviruses and their widespread but underreported occurrence in reproductive-aged women. Although CMV was the most common, additional studies indicated that the best pattern of inflammatory activation was observed in EBV-positive women, which supports the significance of virus-specific pathogenic processes.

The cytokine profile of women positive with the virus showed a non-significant yet steady increment of both IL-6 (mean 9.2–10.5 pg/mL) and IL-10 (mean 31.3–33.1 pg/mL), which was followed by a gradual increase in the IL-6/IL-10 ratio between HSV (0.29) and CMV (0.31) and EBV (0.32). Between 36 and 50 percent of the women who tested positive using viral DNA had an IL-6 reading of more than 10 pg/mL, and 45.62% of the women had an IL-10 reading of over 33 pg/mL, which is an indication of biological variability in cytokine responses despite similar exposure to the virus. The IL-6/IL-10 ratio, an indicator of inflammatory preeminence, was greatest in the EBV cadre (0.32 ± 0.08), and EBV infection is a more significant danger of immune-mediated reproductive dysfunction. This is in line with the fact that EBV has been known to control host immune balance using viral IL-10 homologues and thus maintain chronic low-grade inflammation in the tissues that it infects.

The correlation between cytokine imbalance and viral load was also revealed by the use of the Spearman correlation analysis. There were significant inverse relationships between Ct values (lower Ct = higher viral load) and the IL-6 concentrations in EBV-positive women ($r = –0.51$, $P = 0.029$), and a borderline significant correlation between Ct and the IL-6/IL-10 ratio in EBV-positive women (P =

0.051). These associations support the hypothesis that higher viral activity contributes to heightened pro-inflammatory cytokine levels, potentially disrupting endometrial receptivity and implantation, a mechanism widely described in reproductive immunology literature.

Molecular findings in this cohort show a low Ct value indicates a high virus DNA load; HSV was observed to have the highest average level of detection, followed by EBV and CMV. The low Ct and broad SD of HSV imply that there is a high rate of episodic reactivation and heterogeneity in viral burden across people. As an illustration, Cherpes et al. (88) reported that among women, regular shedding of HSV-2 can be observed, without any symptoms, with a substantial variation in viral load between episodes (i.e., different Ct equivalents).

Comparisons between infertility types revealed that secondary infertility cases demonstrated higher viral loads for HSV and EBV, indicated by significantly lower Ct values (e.g., HSV: primary 20.44 vs. secondary 17.93; EBV: 27.65 vs. 25.89). This suggests that prior reproductive or pelvic events may influence viral persistence or reactivation. However, further studies with longitudinal designs and detailed reproductive histories are needed to explore this association.

The age-stratified analyses failed to provide significant differences in Ct values or cytokine levels, suggesting the possibility that age alone might not be a direct determinant of viral burden or immunologic profile in women of reproductive age. This finding can be related to previous reports indicating that local mucosal immunity, hormonal condition, and inflammatory history are also better predictors of viral latency and host response compared to chronological age.

The merits of this study are that it combines the principles of molecular detection and immunological profiling, which provides a more subtle insight into the way that latent viral infections can have an insidious impact on reproductive outcomes. There should be constraints, however. The limitations of the cross-sectional design are the inability to draw causal conclusions, the lack of qPCR results in fertile controls that do not allow comparisons of viral prevalence in infertility and the general population, and the sample size that restricts subgroup comparisons.

All in all, the findings indicate that latent herpesvirus infections, especially EBV, could play a role in infertility through stimulation of chronic immune responses and not a cytopathic effect. Viral detection using qPCR and serum cytokine analysis should therefore potentially be a useful diagnostic tool for unexplained infertility and secondary infertility, with future targets of antiviral or immunomodulatory therapy being guided by the combination.

The differences in viral load recorded in this study, particularly in the HSV-positive cases, are in line with the literature. Strikingly Cherpes et al. (2003) have shown that HSV-2 shedding may be frequent and asymptomatic, with a significant change in the viral load between episodes. Equally, Smith et al. (2022) indicated that HSV is capable of inducing IL-6 secretion by the dendritic and mononuclear cells, and can be compensated by IL-10 secretion. The assumption that mucosal tissues can gradually change through repeated reactivations and consequently result in more viral shedding in time is further supported by longitudinal surveillance by Aumakhan et al. (2010). These results aid in the contextualization of increased viral loads and immune imbalance here and it is more so in the case of women who are secondary infertile.

The current study had constraints in terms of sample size and the fact that it did not have parallel serological data where active infections and latent infections could have been discriminated. The cross-sectional design does not allow a causal inference of the relationship between viral load and cytokine levels and infertility.

Overall, the factors identified in this research indicate that EBV-positive infertile women have the highest IL-6/IL-10 ratio, which implies that they have a more robust pro-inflammatory immune response than the HSV and CMV groups. We observed a more evident immune imbalance in women with secondary infertility, who generally had higher viral loads. Although such trends allow us to believe in a potential relationship between latent viral activity and inflammation and reproductive dysfunction, it still needs to be validated by larger, longitudinal studies that include reproductive history and more detailed immune profiling.

Conclusion

This study highlights the possible role of latent herpesvirus infections and immune maladjustments in female infertility. Molecular analyses revealed that measurable amounts of viral DNA were present in infertile women, with cytomegalovirus (CMV) having the highest rate of prevalence, followed by herpes simplex virus (HSV) and Epstein-Barr virus (EBV). A lowered Ct value, particularly for HSV, indicates greater viral activity and serves as a sign of repeated reactivation. The viral load was high in women who were secondarily infertile as compared to those who were primarily infertile; this indicates that there exists a correlation of secondarily infertile women with the previous reproductive or pelvic pathology. The insufficient levels of interleukin-6 (IL-6) and interleukin-10 (IL-10), along with a higher IL-6/IL-10 ratio, indicate a mild but chronic imbalance in the inflammatory process. These findings suggest that persistent chronic viral infections and immune stimulation may compromise implantation and endometrial receptivity. Molecular and immunological screenings would also contribute towards simplifying the accuracy of diagnosis and control of treatment and hence provide a platform to prescribe antiviral and immunomodulatory treatment depending on the causes of the disease.

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