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RESEARCH ARTICLE

Immune dysregulation in herpes zoster: A correlative study of TLR7 gene expression, IFN-α, and CD4/CD8 T-cell

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ABSTRACT

Varicella-zoster virus (VZV) reactivation or herpes zoster (HZ) results from a decline in cell-mediated immunity. The precise immunological mechanisms driving this reactivation and determining its clinical severity remain unclear. To evaluate the expression of Toll-like receptor 7 (TLR7), serum levels of I interferon response (IFN-α), and the CD4/CD8 T-cell in patients with active HZ and to correlate these immunological markers with disease severity. A case control study was conducted with 50 patients with active HZ and 30 healthy controls. Whole blood and serum samples were collected. TLR7 gene expression was quantified using reverse-transcription quantitative real-time PCR (RT-qPCR), and the serum concentrations of IFN-α, soluble CD4 (sCD4) and soluble CD8 (sCD8) were quantitatively measured by sandwich enzyme-linked immunosorbent assay (ELISA). Patients with HZ exhibited significant upregulation of TLR7 gene expression ($p=0.0102$) and elevated serum IFN-α levels ($p<0.0001$) compared with controls. While IFN-α levels did not correlate with clinical severity, both CD4+ ($p=0.018$) and CD8+ ($p=0.016$) T cell levels increased significantly with greater disease severity. Sex-specific differences were observed, with males showing higher sCD4 levels and females showing higher sCD8 levels. In conclusion, the adaptive T-cell-derived response, rather than systemic IFN-α levels, is more closely associated with the clinical severity of herpes zoster. The paradoxical upregulation of TLR7 during active disease suggests a complex host–virus interaction involving potential viral evasion mechanisms. These preliminary findings, although limited by sample size, suggest that the sCD4/sCD8 balance and sex-specific immune profiles warrant further investigation as potential prognostic indicators in larger validation cohorts.

Keywords: Herpes Zoster; Varicella zoster virus infection; Toll-like receptor 7; interferon-alpha; CD4-CD8 ratio.

INTRODUCTION

Varicella-zoster virus (VZV), a ubiquitous human alphaherpesvirus, is the etiological agent of both primary varicella (chickenpox) and its reactivation, herpes zoster (shingles) (Laing *et al*., 2018). Following a primary infection, VZV establishes lifelong latency in the cranial nerve and dorsal root ganglia (Kennedy *et al*., 2021). A decline in VZV-specific cell-mediated immunity, often associated with aging, immunosuppressive therapy, or psychological stress, can lead to viral reactivation (Steain *et al*., 2014; Jassim *et al*., 2025; Son *et al*., 2025). Herpes zoster is a significant public health concern, characterized by a painful, unilateral vesicular rash and the potential for debilitating complications such as postherpetic neuralgia (PHN), which can severely affect the quality of life, particularly in the elderly and immunocompromised populations (Wang *et al*., 2025).

The host's ability to maintain VZV latency and prevent reactivation is critically dependent on a robust and coordinated immune response involving both the innate and adaptive systems (Gerada *et al*., 2020; Odhaib *et al*., 2020). The innate immune system provides the first line of defense, with pattern recognition receptors (PRRs) playing a pivotal role in detecting viral components and initiating antiviral responses (Paludan *et al*., 2011). Among these, Toll-like receptor 7 (TLR7), an endosomal receptor that recognizes single-stranded viral RNA, is crucial for triggering the production of type I interferons (IFNs) such as IFN-α (Chen *et al*., 2023; Xue *et al*., 2025). IFN-α, in turn, induces an antiviral state in neighboring cells and helps orchestrate the subsequent adaptive immune response (Dyavar *et al*., 2021). The adaptive immune response, primarily mediated by VZV-specific T lymphocytes, is essential for controlling and clearing virus (Abdullah *et al*., 2026). CD4+ T helper cells and CD8+ cytotoxic T lymphocytes work in concert to eliminate infected cells and are considered the principal mediators of long-term immunity to VZV (Almarjan *et al*., 2021; Jin *et al*., 2022).

Although VZV is a double-stranded DNA virus, it is sensed by multiple Toll-like receptors that act in concert during reactivation. TLR-2, located on the cell surface, recognizes VZV envelope glycoproteins and is the principal innate sensor that triggers pro-inflammatory cytokine production in monocytes and macrophages exposed to VZV (Wang *et al*., 2005). TLR-9, an endosomal sensor, recognizes unmethylated CpG motifs in

herpesvirus genomic DNA and contributes to IFN-α production by plasmacytoid dendritic cells, although VZV-induced IFN-α has been shown to occur via both TLR9-dependent and TLR9-independent pathways (Yu *et al*., 2011). TLR-7, in contrast, recognizes single-stranded viral RNA delivered into endolysosomes, and is engaged by the abundant viral mRNA transcripts and replication intermediates generated during lytic VZV replication. Recent direct evidence in herpes zoster patients showed significant upregulation of TLR-7 mRNA and protein in peripheral blood mononuclear cells (PBMCs), alongside altered TLR-2/TLR-9 protein levels, indicating a coordinated but dysregulated TLR network during active disease (Chen *et al*., 2023). The TLR-2/TLR-7/TLR-9 axis therefore represents complementary innate sensing arms whose relative activation may contribute to the variability of clinical outcomes in HZ.

Despite our understanding of these individual components, the precise mechanisms of immune dysregulation that permit VZV reactivation and dictate the clinical severity of herpes zoster remain incompletely understood (Jalil & Al Atbee, 2022; Peng *et al*., 2022). While the importance of IFN-α and T-cell responses in controlling VZV is well established, the upstream signaling events that initiate these responses during reactivation, and how their dysregulation might contribute to disease, are less clear (Haberthur *et al*., 2011). Specifically, the role of TLR7 in VZV reactivation has not yet been extensively investigated. It is plausible that alterations in TLR7 expression or function could lead to a suboptimal IFN-α response, thereby impairing the downstream activation and recruitment of effector T cells and allowing for uncontrolled viral replication and more severe clinical manifestations (Ghimire *et al*., 2025).

This represents a notable knowledge gap in our comprehension of the immunopathogenesis of herpes zoster. Co-study of the mediators of this immune axis, ranging from initial viral sensing by TLR7 to downstream IFN-α production and resulting T-cell-derived response profile (sCD4/sCD8 ratio), is required. It would be informative to determine the relationship between these components in patients with active herpes zoster and to correlate these immunological parameters with clinical severity, which may yield new insights into disease pathogenesis (Dendouga *et al*., 2012). Such research could not only enhance our fundamental understanding of host-virus interactions, but also pave the way for the identification of new prognostic biomarkers and the development of targeted therapeutic strategies aimed at modulating these specific immune pathways to improve patient outcomes (Walsh *et al*., 2012; Duncan & Hambleton, 2015). Therefore, this study aimed to investigate the interplay between the innate and adaptive immune responses during VZV reactivation. Specifically, we evaluated the gene expression of TLR7, serum levels of IFN-α, and the CD4/CD8 T-cell in patients with active herpes zoster. Furthermore, we aim to correlate these immunological parameters with clinical severity to provide novel insights into the immunopathogenesis of the disease and identify potential prognostic biomarkers.

METHODOLOGY

Study Design and Participants

A case control study was carried out to examine the immune profiles of patients who have herpes zoster (HZ) in Maysan Province, Iraq. Enrolment and sample collection took place between October 2024 and May 2025. The source population enrolled in this study were patients who attended the dermatology and infectious disease outpatient clinics in two central hospitals; Al-Sader City Hospital and Al-Hakeem Hospital within Maysan.

Clinical Diagnosis of Herpes Zoster

Diagnosis of herpes zoster was made clinically by board-certified dermatologists based on the characteristic unilateral dermatomal distribution of vesicular eruption, accompanied by prodromal or concurrent neuropathic pain. This is the standard diagnostic approach recommended by international guidelines (Gross *et al*., 2020). PCR-based laboratory confirmation of VZV DNA was not performed because of resource limitations at the participating centres; this is acknowledged in the Limitations section.

Inclusion and Exclusion Criteria

A sample of 80 subjects whom 50 clinically diagnosed herpes zoster patients and 30 controls as healthy volunteers were included who frequency-matched with the similar source population (age-sexes), study population from Maysan Province, Iraq. Patients of any age and either sex were eligible if they had fresh, vesicular skin lesions suggestive of herpes zoster, but were ineligible if the lesions became crusted, scabbed or exhibited evidence of secondary bacterial infection; with inappropriate collection. Healthy control subjects who met the following criteria were selected: absence of active herpes zoster and a history of past herpes zoster, no signs or symptoms of acute disease, no evidence of current infection, no known immunodeficiency and none taking immunosuppressant drugs, as well sex matched and age comparable with patients. Healthy controls were excluded if they had a chronic disease that might alter immune function (e.g., diabetes mellitus, autoimmune diseases, cancers), if they had received vaccination recently (within 4 weeks prior to sampling), current use of medications known to modulate immune responses, or improperly collected samples. Samples were also excluded if they were not collected properly. The collected samples included whole blood and serum samples.

Clinical Severity Classification

Lacking a standardized composite score for acute herpes zoster, disease severity was operationalized using a study-specific construct requiring concordance across at least two of three validated components assessed at peak severity: pain intensity (VAS: 1–3 [mild], 4–6 [moderate], 7–10 [severe]), dermatomal extent (single vs. ≥2 dermatomes), and rash severity (<25%, 25–75%, or >75% vesicular coverage of the affected dermatome) (Higa *et al*., 1997; Boonstra *et al*., 2014).

Sample Size Justification

The study enrolled 80 subjects (50 cases, 30 controls) over an eight-month enrolment window. The sample size was constrained by the number of incident cases meeting the inclusion criteria during the study period and the logistical capacity of the molecular laboratory. A post-hoc power analysis confirmed adequacy for the primary comparisons: with the observed effect size for serum IFN-α (Cohen's $d \approx 1.26$ between cases and controls), the achieved statistical power exceeded 0.99 at $\alpha = 0.05$ (two-tailed), well above the conventional 0.80 threshold. For the smaller subgroup analyses (sex, age strata, and severity strata), the study should be regarded as exploratory; this is acknowledged in the Limitations section. The exclusion criteria above and the demographic distribution of the cohort are presented in Table 3.

Biological Sample Collection and Processing

Venous blood (5 ml) was collected from each participant by a trained phlebotomist, using a sterile disposable syringe. The blood sample was divided into two tubes; the first 750 µL was placed in a labelled sterile EDTA tube for RNA extraction, and the remaining was placed in a clotting activator gel tube for serum isolation to measure immunological markers. Samples were immediately transported on ice to the laboratory.

Immunological Assays

Serum concentrations of IFN-α, soluble CD4 (sCD4) and soluble CD8 (sCD8) were measured using sandwich ELISA kits (ELK Biotechnology, USA). The 96-well plates were pre-coated with specific primary antibodies, and a biotin-conjugated antibody (specific to each kit)

was used as the detection antibody. It is important to clarify that this assay does NOT measure absolute T-cell counts (which would require flow cytometry of fresh whole blood or PBMCs); rather, it quantifies the soluble (shed) ectodomain forms of CD4 and CD8 that are released into circulation by activated T lymphocytes. Soluble CD4 and CD8 have been validated as biomarkers of in vivo T-cell activation and have been used to monitor immune activation in viral infections including measles, infectious mononucleosis, and chronic viral hepatitis. Lack of parallel flow cytometric phenotyping is acknowledged as a limitation.

Gene Expression

Total RNA was extracted from whole blood samples using SRC® Green-Zol Total RNA Extraction Reagent (TrizolScientific Researcher), strictly following the manufacturer's instructions. RNA quality was verified spectrophotometrically; samples were eligible for downstream RT-qPCR analysis only if the A260/A280 ratio was within 1.8–2.1, the A260/A230 ratio was ≥ 1.8, and the housekeeping gene (β-actin) successfully amplified at Ct ≤ 35. Samples that failed any of these quality criteria were excluded from molecular analysis. Of the 80 enrolled participants, 33 (13 controls and 20 patients) were excluded from RT-qPCR analysis on the basis of these pre-specified MIQE-aligned quality criteria (Bustin *et al*., 2009); exclusion was performed BLINDED to clinical group assignment to avoid selection bias. The final RT-qPCR dataset therefore comprised 47 participants (17 controls and 30 patients). Although a 33/80 (~41%) exclusion rate is higher than ideal, it reflects the practical realities of whole-blood RNA extraction in a resource-limited setting and is consistent with the MIQE-recommended exclusion of analytically unreliable samples; this is further discussed in the Limitations section.

The cDNA synthesis was performed using the GoScript™ Reverse Transcription System (Promega). To determine the expression of the TLR7 gene, the synthesised cDNA was amplified by RT-qPCR using specific forward and reverse primers for the TLR7 gene along with the housekeeping gene β-actin. All primers were supplied by Macrogen Company (South Korea). Primer details are listed in Table 1. The gene expression process used in this study was performed as described by (Alberts *et al*., 2002), and the RT-qPCR thermal cycling conditions Table 2.

Statistical analysis

The statistical analysis employed a rigorous methodological approach. Data were systematically summarized, entered into an Excel spreadsheet, and then transferred to GraphPad Prism version 9 for analysis. Descriptive statistics were reported as frequencies and percentages for categorical variables (e.g., sex) or mean ± standard deviation (SD) with range for numerical variables (e.g., age, cytokine levels). For inferential analysis, independent samples t-test was used for categorical variables (i.e. interferon-α, CD4 and CD8) across control vs HZV infected patients), whereas chi-square test was applied to study the nominal association between the groups (e.g., sex distribution). To compare the levels of any groups (mild, moderate and severe) one-way ANOVA with Tukey's post-hoc test was used. Correlations between immune parameters were computed using Pearson correlation coefficients. The diagnostic performance of the cytokines was analyzed by determining sensitivity, specificity, and area under (AUC) the ROC curve with 95% confidence intervals. The level of significance was set at $p < 0.05$.

RESULTS

The demographic and clinical characteristics of the study cohorts are summarised in Table 3. Of the 50 enrolled HZ patients, 33 (66%) were males and 17 (34%) were females, with ages ranging from 10 to 85 years. Of the 30 healthy controls, 19 (63%) were males and 11 (37%) were females, with ages ranging from 11 to 85 years. There was no statistically significant difference between the groups with respect to sex ($p = 0.8$), and the mean age of healthy controls (44.6 ± 20 years) did not differ significantly from that of HZ patients (42.1 ± 21.6 years; $p = 0.6$). The mean duration from rash onset to sample collection was 4.8 ± 2.1 days (range, 2–9 days). Among the 50 patients, the most frequently reported precipitating or contributing factors were psychological/physical stress (n = 18; 36%), aging (≥ 60 years; n = 14; 28%), comorbid diabetes mellitus (n = 9; 18%), recent intercurrent infection (n = 6; 12%), and no identifiable trigger (n = 13; 26%); some patients had more than one factor.

Regarding the immunological markers, mean IFN-α was significantly elevated in patients (15.2 ± 7.0 pg/mL) compared with controls (7.53 ± 5.0 pg/mL; $p < 0.0001$). Similarly, sCD4 (0.74 ± 0.47 ng/mL) and sCD8 (33.8 ± 13.1 ng/mL) were significantly higher in patients than in controls (sCD4 0.51 ± 0.32 ng/mL, $p = 0.032$; sCD8 27.7 ± 6.6 ng/mL, $p = 0.034$).

Table 1. Specific primer sequences used in the present study for RT-qPCR amplification of the TLR7 gene of interest and the β-actin housekeeping reference gene. Primers were designed against the reference human transcripts; β-actin was selected as the internal calibrator because of its stable expression across human peripheral blood mononuclear cell preparations

Genes	Primer sequences (5'→3')	Length (bp)	Reference
β-actin (forward)	GGCTGCTTCCAGCTCCTCCC	99	(Chen *et al*., 2023)
β-actin (reverse)	AAGAGTGCCTCAGGGCAGCG		
TLR7 (forward)	CCCCATTTCCTTGTGCGCCG	132	(Chen *et al*., 2023)
TLR7 (reverse)	ACCATCTTGGGGGCACATGCT		

Table 2. RT-qPCR thermal cycling conditions employed in the present study. The protocol comprised an initial reverse-transcription/hold stage, followed by 40 amplification cycles, and a melt-curve stage to confirm amplicon specificity

Stage	Step 1	Step 2	Step 3	Cycles
Hold stage	50°C, 2 min	95°C, 30 Sec	—	1×
PCR stage	95°C, 10 Sec	60°C, 15 Sec	—	40×
Melt curve	95°C, 15 Sec	60°C, 1 min	95°C, 15 Sec	1×

Table 3. Demographic and clinical characteristics of control participants and HZV-infected patients

Characteristic	Control *n* = 30	Patients infected with HZV *n* = 50	*p*
Age (years)			
Mean ±SD	44.6 ± 20	42.1 ± 21.6	0.6
Range	11 – 85	10 – 85	
Gender			
Male, *n* (%)	19 (63 %)	33 (66 %)	0.8
Female, *n* (%)	11 (37 %)	17 (33 %)	
Interferon-α (pg/mL)			
Mean ±SD	7.53 ± 5.0	15.2 ± 7.0	< 0.0001***
Range	1.83 – 18.3	2.23 – 40.6	
Cluster of Differentiation 4 (ng/mL)			
Mean ±SD	0.51 ± 0.32	0.74 ± 0.47	0.032*
Range	0.013 – 1.38	0.13 – 2.4	
Cluster of Differentiation 8 (ng/mL)			
Mean ±SD	27.7 ± 6.6	33.8 ± 13.1	0.034*
Range	10.8 – 39.8	15.5 – 65.6	

Data presented as mean ± standard deviation (SD) or frequency (%). p-values from independent samples t-test (age) or χ^2 test (gender); NS (not significant, $p \geq 0.05$).

Table 4. Comparison of Serum Immune Marker Levels by Sex in Patients Infected with HZV

Characteristic	Male *n* = 33	Female *n* = 17	*p*
Interferon-α (pg/mL) Mean ±SD	15.9 ± 7.8	13.8 ± 5.36	0.23
Cluster of Differentiation 4 (ng/mL) Mean ±SD	0.86 ± 0.53	0.65 ± 0.25	0.048*
Cluster of Differentiation 8 (ng/mL) Mean ±SD	30.7 ± 11.29	38.7 ± 13.2	0.042*

Data as mean ± SD. Independent samples t-test; *p < 0.05 considered significant; NS (p ≥ 0.05).

According to sex distribution (Table 4), there was no statistically significant difference in IFN-α levels (p = 0.23) between male and female patients. In male patients, sCD4 levels (0.86 ± 0.53 ng/mL) were significantly higher compared to females (0.65 ± 0.25 ng/mL; p = 0.048). Conversely, sCD8 levels were significantly higher in females (38.7 ± 13.2 ng/mL) than in males (30.7 ± 11.3 ng/mL; p = 0.042).

Patients were stratified into three age groups (≤ 20 years, 21–40 years, and ≥ 41 years) and three severity categories (mild, moderate, severe). To provide a comprehensive overview as requested, all four immunological parameters (IFN-α, sCD4, sCD8, and TLR7 fold change) are presented in a single comparative matrix in Table 5. There was no statistically significant difference in IFN-α levels across age groups (p = 0.6) or across severity categories (p = 0.7). sCD4 levels increased significantly with age (p = 0.013), with values of 0.42 ± 0.2, 0.68 ± 0.41, and 0.91 ± 0.5 ng/mL for the ≤ 20, 21–40, and ≥ 41 years groups respectively, and also increased significantly with severity (p = 0.018), with values of 0.46 ± 0.22, 0.84 ± 0.57, and 0.82 ± 0.24 ng/mL for mild, moderate, and severe categories respectively as showed Table 6. sCD8 followed a similar pattern, increasing significantly with both age (p = 0.026) and severity (p = 0.016). TLR7 fold change was strongly associated with severity (p = 0.039), with median values of 0.18, 35.6 and 60.9 in the mild, moderate, and severe categories respectively.

ROC analysis (Figure 1) was used to evaluate the diagnostic performance of the immunological markers in differentiating HZV-infected patients from healthy controls. IFN-α (cut-off > 6.63 pg/mL) demonstrated high sensitivity (93%) and moderate specificity (69%), with an area under the curve (AUC) of 0.81 (95% CI: 0.708–0.895;

Table 5. Comprehensive comparative matrix of immunological parameters (IFN-α, sCD4, sCD8 and TLR7 fold change) stratified by age group and clinical severity in HZV-infected patients

Stratum	n	IFN-α (pg/mL)	sCD4 (ng/mL)	sCD8 (ng/mL)	TLR7 fold-change (median)	p-value (overall)
Age ≤ 20 yrs	12	13.5 ± 7.3	0.42 ± 0.20	27.1 ± 7.0	0.42	Age: 0.6 (IFN-α); 0.013 (sCD4)*; 0.026 (sCD8)*
Age 21 – 40 yrs	14	15.5 ± 8.5	0.68 ± 0.41	34.7 ± 14.5	29.8	
Age ≥ 41 yrs	24	15.8 ± 6.1	0.91 ± 0.50	39.4 ± 12.8	47.5	
Severity – Mild	26	14.9 ± 4.1	0.46 ± 0.22	27.7 ± 9.6	0.18	Severity: 0.7 (IFN-α); 0.018 (sCD4)*; 0.016 (sCD8)*; 0.039 (TLR7)*
Severity – Moderate	19	15.9 ± 7.4	0.84 ± 0.57	30.5 ± 10.9	35.63	
Severity – Severe	15	13.9 ± 7.5	0.82 ± 0.24	41.2 ± 14.5	60.94	

Table 6. Association of serum immune biomarkers with clinical severity in HZV-infected patients (re-presented from Table 5 for direct comparison)

Marker	Mild (n = 26)	Moderate (n = 19)	Severe (n = 15)	p-value
IFN-α (pg/mL), mean ± SD	14.9 ± 4.1	15.9 ± 7.4	13.9 ± 7.5	0.7
sCD4 (ng/mL), mean ± SD	0.46 ± 0.22^{A}	0.84 ± 0.57^{B}	0.82 ± 0.24^{B}	0.018*
sCD8 (ng/mL), mean ± SD	27.7 ± 9.6^{A}	30.5 ± 10.9^{B}	41.2 ± 14.5^{B}	0.016*

Data as mean ± SD. One-way ANOVA with Tukey's post hoc test. Different superscript letters (A, B) indicate significant differences between groups (*p < 0.05). **<0.01. similar letters indicate no significant difference.

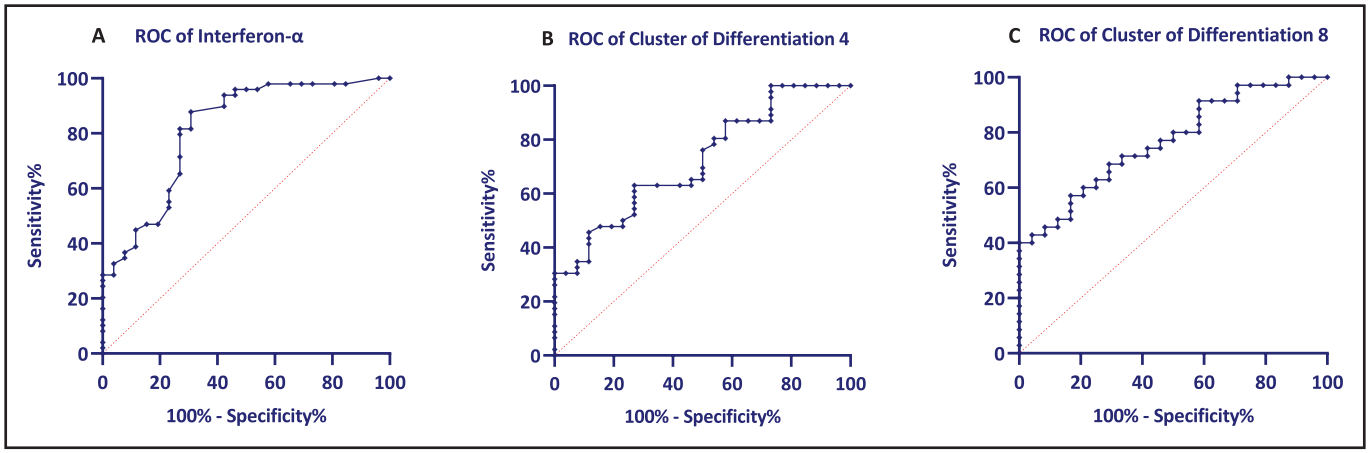

Figure 1. ROC Curve for Diagnostic Performance of immunological markers (A. IFN-α B. CD4. C. CD8).

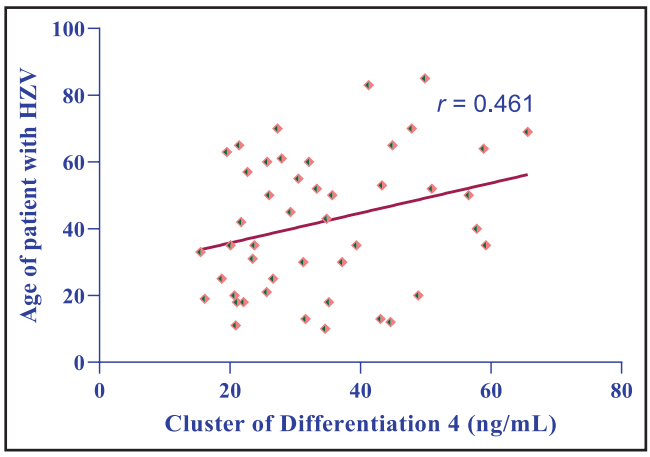

Figure 2. Correlation between Cluster of Differentiation 4 and age of patients with HZV.

p < 0.001). sCD4 (cut-off > 0.26 ng/mL) exhibited perfect specificity (100%) but modest sensitivity (62%), yielding an AUC of 0.708 (95% CI: 0.588–0.811; p < 0.001). sCD8 (cut-off > 39.78 ng/mL) showed balanced sensitivity (91%) and specificity (63%), achieving an AUC of 0.76 (95% CI: 0.637–0.868; p < 0.001). All biomarkers demonstrated statistically significant discriminatory power (AUC > 0.5; p < 0.001).

Pearson correlation analysis (Figure 2) examined associations between age and serum immune biomarkers (sCD8, sCD4, and IFN-α). A statistically significant positive correlation was observed between age and sCD8 (r = 0.461, p = 0.0011), indicating that older participants exhibited higher sCD8 concentrations. No significant correlations were observed between age and sCD4 (r = 0.115, p = 0.4357) or IFN-α (r = -0.194, p = 0.1825). Pairwise comparisons among immune markers also showed no significant interrelationships (sCD8 vs sCD4: r = 0.101, p = 0.5024; sCD8 vs IFN-α: r = -0.207, p = 0.1673; sCD4 vs IFN-α: r = 0.151, p = 0.3108).

Among the 47 participants with successful RT-qPCR (17 controls, 30 patients), TLR7 gene expression analysis revealed significant differences (p = 0.0102) between patients (median fold change = 36.74) and healthy controls (median fold change = 0.79), indicating pronounced TLR7 upregulation (Table 7).

In the patient group, 73.3% (22/30) showed upregulated TLR7 expression compared with 47.1% (8/17) in the healthy control group (Table 8). The ratio of upregulated to downregulated subjects was 2.75:1 in patients versus 0.89:1 in controls. Fisher's exact test indicated that this difference in expression direction was significantly associated with group classification (p = 0.048), providing additional evidence for TLR7 dysregulation in HZ.

The ROC curve (Figure 3) was used to assess the diagnostic performance of TLR7 fold change. The analysis showed statistically significant moderate diagnostic power, with an AUC of 0.72 (p = 0.003). The optimal cut-off for TLR7 fold change was > 3.604, providing a sensitivity of 70.0% and specificity of 70.6%.

Patients were further analysed by clinical severity (mild, moderate, severe), as shown in Table 9. The moderate and severe categories showed significantly elevated TLR7 fold change compared with the mild category (p = 0.039).

Table 7. Descriptive Statistics of ΔCt and Fold Change for TLR7 Expression

Group	N	ΔCt Median	ΔCt IQR	Fold Change Median	Fold Change IQR	P-value
Healthy	17	2.34	4.42	0.79	7.8	0.0102
Patient	30	-3.20	6.09	36.74	66.3	

Mann Whitney test.

Table 8. Frequency and Proportion of TLR7 Expression Direction in Study Cohorts

Group	N	Up-regulated n(%)	Down-regulated n(%)	Ratio (Up:Down)	p-value
Healthy	17	8 (47.1%)	9 (52.9%)	0.89:1	0.048
Patient	30	22 (73.3%)	8 (26.7%)	2.75:1	

Fisher's Exact Test.

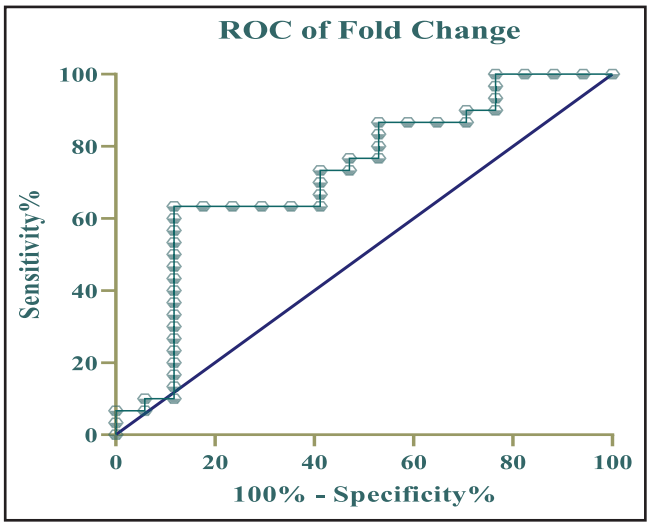

Figure 3. ROC Curve for Fold Change in Discriminating Patient and Healthy Cohorts.

Table 9. Association Between TLR7 Expression and Clinical Severity in HZV-Infected Patients

Clinical Severity	Mean Fold Change ± SD	Median Fold Change	Range (Min – Max)	p-value
Mild	7.92 ± 8.85^{A}	0.18	0.18 – 15.65	0.039
Moderate	35.39 ± 49.6^{B}	35.63	0.19 – 191.89	
Severe	49.31 ± 39.7^{B}	60.94	0.48 – 110.29	

Data as mean ± SD. One-way ANOVA with Tukey's post hoc test. Different superscript letters (A, B) indicate significant differences between groups (*p < 0.05). **<0.01. similar letters indicate no significant difference.

DISCUSSION

The reactivation of VZV, leading to HZ, is a stark manifestation of the decline in host-specific cell-mediated immunity. This study provides a comprehensive immunological snapshot of patients with active HZ, revealing a complex interplay between innate sensing pathways, cytokine responses, and adaptive T cell dynamics. Our principal findings of significant upregulation of TLR7 gene expression, elevated serum IFN-α, and a strong correlation between T-cell subsets and disease severity offer novel insights into the immunopathogenesis of VZV reactivation, and align with an evolving understanding of the nuanced and sometimes paradoxical nature of the host immune response to this ubiquitous herpesvirus.

A central and intriguing finding of our study is the significant upregulation of TLR7 gene expression in HZ patients compared with healthy controls. Although VZV is a double-stranded DNA virus, this is not inconsistent with TLR7 engagement: during lytic

VZV replication, abundant viral mRNA transcripts and ssRNA replication intermediates are produced and are delivered into the endolysosomal compartment via autophagy (Lee *et al*., 2007), where they engage TLR7 (Heil *et al*., 2004). This pathway has been demonstrated for other DNA herpesviruses, notably EBV (Quan *et al*., 2010), and is consistent with the observation that VZV-induced IFN-α production occurs through both TLR9-dependent and TLR9-independent endosomal sensing pathways (Yu *et al*., 2011). Our finding directly aligns with the recent observation by Chen *et al*., who demonstrated upregulated TLR7 mRNA and protein in PBMCs of HZ patients (Chen *et al*., 2023). However, the occurrence of TLR7 upregulation in the context of active viral reactivation remains paradoxical; an effective TLR7 response would be expected to control viral replication. This may reflect post-transcriptional or post-translational regulatory mechanisms that subvert downstream effector function, as suggested by the discrepancy between TLR mRNA and protein levels reported by Chen *et al*. It is plausible that VZV deploys immune-evasion strategies that counteract downstream effects of TLR7 sensing, a tactic common among herpesviruses (Gerada *et al*., 2020; Zheng *et al*., 2020). Future studies should focus on the functional capacity of upregulated TLR7 and the integrity of its downstream signalling cascade, particularly NF-κB activation, which has been shown to be crucial for suppressing herpesvirus reactivation (Haas *et al*., 2014).

Beyond TLR7, the broader TLR network must be considered to interpret the present findings. Wang *et al*. demonstrated that VZV activates inflammatory cytokine production in human monocytes and macrophages through TLR-2 recognition of viral envelope glycoproteins (Wang *et al*., 2005), while Sato *et al*. established that TLR-9 senses unmethylated CpG motifs in herpesvirus DNA in plasmacytoid dendritic cells (Sato *et al*., 2006). In direct VZV studies, Yu *et al*. showed that VZV-induced IFN-α production in human mononuclear cells is only partially TLR9-dependent, supporting the existence of additional sensing routes such as TLR7 (Yu *et al*., 2011). Notably, Chen *et al*. found that TLR-2, TLR-7 and TLR-9 mRNA were all upregulated in PBMCs of HZ patients, but only TLR-7 (and TLR-4) protein levels followed suit, while TLR-2 and TLR-9 protein levels were paradoxically reduced pointing to differential post-transcriptional control across the TLR family during reactivation (Chen *et al*., 2023). Our findings, which document significant TLR-7 gene upregulation, are therefore best interpreted as one element of a broader, dysregulated innate-sensing network rather than as an isolated event.

The present results demonstrate a significant elevation of serum IFN-α in patients with HZ, confirming the activation of a type I interferon response during VZV reactivation. This is consistent with the established role of IFN-α in controlling VZV replication by inducing an antiviral state and orchestrating an adaptive immune response (Yu *et al*., 2011). However, a key observation in our study was the lack of a significant correlation between IFN-α levels and the clinical severity of HZ. This suggests that while the IFN-α response is initiated, its magnitude alone may not be the primary determinant of the clinical outcome. This could be attributed to several factors. First, VZV has evolved mechanisms to evade the effects of interferons, such as the action of viral proteins like IE62, which can suppress VZV gene transcription by impeding the formation of essential transcriptional complexes (Ku *et al*., 2016). Second, the timing and location of the IFN response are critical. A systemic elevation in serum IFN-α levels may not accurately reflect the local cytokine milieu within the affected ganglia and skin, where the battle between the virus and the host is the most intense. Furthermore, recent studies have highlighted that pre-existing autoantibodies neutralizing IFN-α can be associated with a higher risk of HZ, suggesting that in some individuals, IFN-α production may be functionally impaired (Mathian *et al*., 2022).

In stark contrast to the findings for IFN-α, the present study revealed a significant association between T cell subsets and disease severity. The observed increase in both CD4+ and CD8+ T cell levels with increasing clinical severity points towards the adaptive immune response as the central battleground in determining the course of HZ. This finding strongly resonates with the growing body of literature emphasizing the critical role of VZV-specific T-cells in controlling infection. A recent study by Wang *et al*. identified the CD4+/CD8+ as an independent risk factor for the development of postherpetic neuralgia (PHN), which is the most debilitating complication of HZ (Wang *et al*., 2025). Our results, showing a progressive increase in T-cell markers with severity, likely reflect a more extensive and prolonged inflammatory response required to combat a higher viral load or a more widespread infection in severe cases. Peng *et al*. provided a temporal context, showing that VZV-specific CD4+ T-cells peak approximately two weeks after HZ onset, highlighting the dynamic nature of this response (Peng *et al*., 2022). The correlation we observed between age and CD8+ levels also aligns with the concept of immunosenescence, where age-related changes in T cell populations can lead to a less effective, albeit numerically present, immune response (Wei *et al*., 2017).

Stratification by severity provides additional mechanistic insight. In the MILD category (n = 26), patients exhibited the lowest sCD4 (0.46 ± 0.22 ng/mL), the lowest sCD8 (27.7 ± 9.6 ng/mL), and a markedly suppressed TLR7 fold change (median 0.18). This pattern suggests a contained reactivation event in which the limited viral load triggers only minimal T-cell activation and minimal innate sensing. In the MODERATE category (n = 19), TLR7 fold change rose sharply (median 35.6) and sCD4 increased to 0.84 ± 0.57 ng/mL, indicating that as viral spread expands, both arms of immunity engage. In the SEVERE category (n = 15), TLR7 fold change peaked (median 60.9) and sCD8 rose further to 41.2 ± 14.5 ng/mL, while sCD4 remained near the moderate level. The disproportionate rise in sCD8 with severity, in the setting of robust TLR7 induction, is consistent with a cytotoxic-dominant adaptive response to a higher viral burden, but also with the host's failure to clear the virus before tissue damage accumulates. Notably, IFN-α levels did not stratify with severity ($p = 0.7$), supporting the interpretation that adaptive T-cell-derived responses, rather than systemic innate type I IFN, dictate clinical course in established HZ. The lack of a comparative pre-reactivation immune profile is a limitation; longitudinal studies of latency-to-reactivation are required to confirm this pattern (Steain *et al*., 2014; Peng *et al*., 2022).

One of the most novel findings of our study was the identification of sex-specific differences in the T-cell-derived response to VZV reactivation, with males exhibiting significantly higher sCD4 levels and females showing significantly higher sCD8 levels. This observation contributes to an expanding area of research on sex-based disparities in immunity to viral infections and vaccination (Klein *et al*., 2006). Although the incidence of HZ is often reported to be higher in females, the underlying immunological mechanisms are not well understood (Opstelten *et al*., 2006). Our data suggest that males and females employ different T-cell-mediated strategies to control VZV: higher sCD4 levels in males could reflect a more robust T-helper response, whereas elevated sCD8 levels in females may reflect a more vigorous cytotoxic T-lymphocyte response. These findings are consistent with recent studies reporting that males exhibit a lower percentage of VZV-specific IFN-γ-secreting CD4+ and CD8+ T cells than females, suggesting functional sex-based differences in T-cell responsiveness (Mangmee *et al*., 2025). These sex-specific signatures warrant further investigation, as they could have important implications for personalised risk assessment and vaccination strategies.

Limitations and Future Directions

This study has several limitations. First, the cross-sectional design precludes tracking immune dynamics over time, highlighting the need for longitudinal studies and in vitro functional assays to evaluate VZV-specific T-cell activity and TLR7 regulation. Second, limited subgroup sample sizes warrant larger, multi-center prospective cohorts to improve generalizability. Third, a high exclusion rate (41%) in RT-qPCR analysis due to stringent quality criteria may reduce the precision of TLR7 estimates, necessitating optimized RNA extraction protocols in future work. Fourth, while soluble CD4 and CD8 levels indicate T-cell activation, flow cytometric confirmation is required for comprehensive immunophenotyping. Fifth, diagnoses relied on expert clinical evaluation; future studies should incorporate laboratory confirmation via PCR or serology. Finally, the study-specific composite severity score requires validation against standardized instruments.

CONCLUSION

This study provides a detailed analysis of the immune dysregulation that characterises VZV reactivation in a single-centre Iraqi cohort. We confirmed activation of the TLR7–IFN-α axis but demonstrated that the magnitude of the T-cell-derived soluble response, particularly the balance between sCD4 and sCD8, is more closely correlated with clinical severity than systemic IFN-α levels. Our findings are consistent with and add to the growing recognition of cell-mediated immunity as central to controlling HZ, and provide preliminary evidence for sex-specific differences in the adaptive immune response. The paradox of TLR7 upregulation in the face of active disease highlights a sophisticated interplay between host defence and viral evasion that deserves further mechanistic investigation. While these observations should be interpreted in the context of the limited sample size and the methodological caveats acknowledged above, they nevertheless suggest that the sCD4/sCD8 balance and sex-specific immune profiles merit further evaluation as candidate prognostic markers and as targets for future therapeutic strategies.

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Ethical approval

This study has followed the ethical guidelines in the Helsinki Declaration. The research protocol, informed consent documents and study subject recruitment materials were reviewed by the publishing ethics committee of Basrah University (Ethical approval Ref.number: 5823 in date 21st October 2024).

Use of Artificial Intelligence

Used artificial intelligence to improve sentence structure and clarity.

Competing interests

The author declare that they have no competing interests

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