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The Protective Role Of IL-12 And IL-23 In Host Immunity Against Dengue Virus Infection

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Abstract

Background: Dengue virus (DENV) infection still a series public health concern in endemic regions, with immune responses playing a decisive role in disease outcome. Proinflammatory cytokines such as IL-12, and IL-23 are thought to influence host protection and viral pathogenesis. **Objectives:** This study aimed to compare cytokine responses between DENV1 and DENV2 serotypes, evaluate the differences between patients and controls, and examine the correlation between IL-12 and IL-23 levels. **Materials and Methods:** A case–control study was conducted including 28 patients with confirmed dengue infection and 32 healthy controls. Serum cytokine levels (IL-12 and IL-23) were measured using ELISA. Statistical analyses included group comparisons and Pearson correlation tests. **Results:** Patients demonstrated significantly higher mean serum IL-12 and IL-23 levels compared with controls ($p<0.05$). IL-12 and IL-23 were slightly elevated in DENV1 cases compared with DENV2, although these variations were not statistically significant ($p>0.05$). A high positive correlation was found between IL-12 and IL-23 ($r=0.62$, $p<0.01$), suggesting coordinated immunological activity. **Conclusion:** Elevated IL-12 and IL-23 levels highlight their potential protective roles in host defense against DENV.

Keywords: DENV, Cytokine Protection, IL-12 and IL-23

Introduction

Viral diseases are regarded as major obstacles to international health security, particularly in tropical and subtropical climates where high morbidity and mortality rates are consistently reported. Among these pathogens, the dengue virus (DENV), classified as a mosquito-borne flavivirus, is responsible for infecting vast populations annually, producing clinical outcomes that range from clinically silent infections to severe manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Bhatt, Rahman, & Masyeni, 2024). Whether dengue infection progresses toward mild or life-threatening forms is strongly influenced by the host's immune profile. This response is orchestrated by multiple signaling molecules, with cytokines and chemokines serving as pivotal mediators. Within this network, interleukins (ILs) are considered fundamental modulators that coordinate microbial clearance, regulate immune activation, and restrain excessive inflammatory injury.

A prominent member of this group, interleukin-12 (IL-12), is widely documented as a promoter of Th1-type immunity. Through the induction of interferon-gamma (IFN-γ), enhancement of cytotoxic T lymphocyte (CTL) responses, and facilitation of macrophage activation, IL-12 has been associated with efficient viral control and elimination of intracellular pathogens (Rahman,

Masyeni, & Bhatt, 2023). In parallel, interleukin-23 (IL-23), which shares the p40 subunit with IL-12, has been highlighted for its role in sustaining Th17 differentiation and stimulating IL-17 release. These activities collectively contribute to protective immunity but may also intensify inflammatory cascades under dysregulated conditions (Masyeni, Bhatt, & Rahman, 2024).

In dengue pathogenesis, disruption of this immunological equilibrium is thought to underpin disease progression. Severe clinical presentations are frequently characterized by uncontrolled proinflammatory cytokine production, a phenomenon often described as a "cytokine storm," which precipitates vascular permeability alterations and subsequent organ impairment. Studies have shown that IL-12 levels are elevated in mild disease but tend to decline in more severe stages. For instance, in a Vietnamese cohort, IL-12 was significantly higher in dengue fever patients in comparison to healthy controls, with higher levels associated with less severe disease (Bhatt et al., 2024).

Likewise, IL-23 has been less studied in dengue but emerging research indicates that IL-23 correlates with IL-17 levels in dengue patients and might be part of the Th17 axis that can modulate the immune response during infection. While IL-17 has been associated with exacerbated pathology and inflammation, IL-23's role may be dual: on one hand, promoting immune responses that help control infection, and on the other, contributing to inflammatory damage if regulation fails (Sánchez-Vargas ET AL., 2020).

Beyond human observational studies, in vitro and animal model work reveal that timing, magnitude, and cellular context of interleukin responses critically influence outcomes. For example, temporal cytokine profiling has shown that while IL-12 is elevated early, its levels may drop in severe secondary dengue, coinciding with elevated IL-6, IL-8, and IL-10. These data suggest that not only levels but timing and cellular context of IL-12/IL-23 expression are important in determining outcomes in DENV infection (Bhatt et al., 2024).

Despite these insights, there remain gaps in the literature. The dynamics of IL-23 in relation to disease progression and protection are not fully defined; also, how IL-12 and IL-23 interact (cross-regulation), the impact of different DENV serotypes, and the temporal kinetics of expression (early vs late infection) in humans need further elucidation. Current uncertainties in the literature have restricted progress toward establishing cytokine-driven biomarkers and therapeutic targets that could simultaneously reinforce host protection and reduce immune-mediated tissue injury (Masyeni, Rahman, & Bhatt, 2024).

The present investigation has been designed to explore the contribution of IL-12 and IL-23 to immune defense during dengue virus infection. Particular attention is directed toward examining the association between these cytokines and indicators of disease severity, viral burden, immune activation, and clinical outcome. To achieve this, data derived from patients will be integrated with complementary insights obtained through mechanistic studies in vitro and experimental models in vivo. Through this combined approach, the research seeks to determine whether IL-12 and IL-23 act merely as surrogate markers of immune function or if they actively participate as mediators conferring protection. Clarifying these roles is expected to provide critical knowledge that may facilitate the design of immunomodulatory interventions or vaccines capable of amplifying protective cytokine responses while simultaneously controlling pathological inflammation.

Methods

Patients and data collection

A cross-sectional case–control design was applied for this investigation, which took place at the Teaching Hospital in Thi-Qar City, Iraq, during a seven-month interval extending from December 2024 through June 2025. The study population comprised 28 patients with laboratory-confirmed dengue virus infection alongside 32 apparently healthy individuals serving as the control group. Recruitment of patients occurred within both inpatient and outpatient units, following initial clinical suspicion and subsequent confirmation by diagnostic testing. For comparison, controls were carefully selected to match cases by age and sex. Only individuals with no recent history of febrile or respiratory illness and without any underlying chronic medical conditions were included in the control group.

Inclusion criteria for patients were: clinical signs consistent with dengue virus infection, positive laboratory confirmation of infection, and willingness to provide informed consent. **Exclusion criteria** for both

groups included chronic illnesses or malignancies, as these may influence cytokine levels and immune responses.

Sample Collection and Laboratory Procedures

Demographic and clinical information were obtained through structured interviews and review of medical records, and clinical features were validated by attending physicians. Peripheral venous blood (5 mL) was collected aseptically from all participants, left to clot at 25 C, and subjected to centrifugation at 3,000 rpm for 10 minutes to get the serum.

For **viral confirmation and serotyping**, sterile flocked swabs were collected from patients and placed in viral transport medium. Viral RNA had been extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) based on the manufacturer's protocol. Serological testing and real-time RT-PCR were performed using dengue-specific primers and probes to identify dengue virus serotypes (DENV-1 and DENV-2) Samples with cycle threshold (Ct) values ≤38 were considered positive.

Measurement of Cytokine Levels

The serum concentrations of **IL-12** and **IL-23** were assessed using reliable sandwich ELISA kits (R&D Systems, USA). All reagents and serum samples were equilibrated to room temperature before testing. Briefly, 100 µL of serum was added to wells of 96-well microplates precoated with monoclonal antibodies specific for IL-12 or IL-23. Following incubation and washing, biotin-labeled detection antibodies and horseradish peroxidase-conjugated streptavidin were applied. Plates were developed with tetramethylbenzidine (TMB) substrate and absorbance was set at 450 nm using a microplate reader. Cytokine concentrations were calculated from standard curves generated with recombinant cytokine standards. All samples were tested in duplicate to ensure consistency, with intra-assay and inter-assay coefficients of variation kept below 10%.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Normality of data distribution was tested with the Kolmogorov–Smirnov test. Continuous variables were expressed as mean ± standard deviation (SD) for normally distributed data. Comparisons of cytokine levels between dengue patients and controls were conducted using independent sample t-tests. Associations between categorical variables (e.g., sex, serotype distribution) were assessed by Chi-square test. Pearson correlation coefficients were used to analyze the relationships between IL-12 and IL-23 concentrations and clinical parameters such as viral serotype and disease severity. A p-value <0.05 was indicated as statistically significant.

The Results

The comparative analysis presented in Table 1 demonstrates that gender distribution between patients and controls was not statistically significant (p = 0.232), suggesting that sex was unlikely to be a confounding factor in this study population. In contrast, residence showed a significant difference (p < 0.04), with a higher proportion of patients residing in rural areas compared to controls, indicating that environmental and ecological conditions in rural settings may play a role in dengue virus transmission.

Table 1. Comparison of gender and residence between patients and control

Items		Patients (N= 28)		Control (N= 32)		(P value)
		Freq.	%	Freq.	%	
Gender	Male	15	53.6	21	65.6	0.232
	Female	13	46.4	11	34.4	
Residence	Urban	18	64.3	28	87.5	< 0.04*
	Rural	10	35.7	4	12.5	

*** Significant at P value <0.05**

The pie chart illustrates the serotyping of dengue virus among patients revealed a predominance of DENV1 (57.1%), followed by DENV2 (42.9%), with the association between viral serotype and case status reaching statistical significance ($p < 0.03$). This finding highlights the circulation of multiple serotypes in the study area and underlines the importance of continuous surveillance to monitor shifts in serotype dominance, which may have implications for outbreak dynamics and disease severity (figure 1).

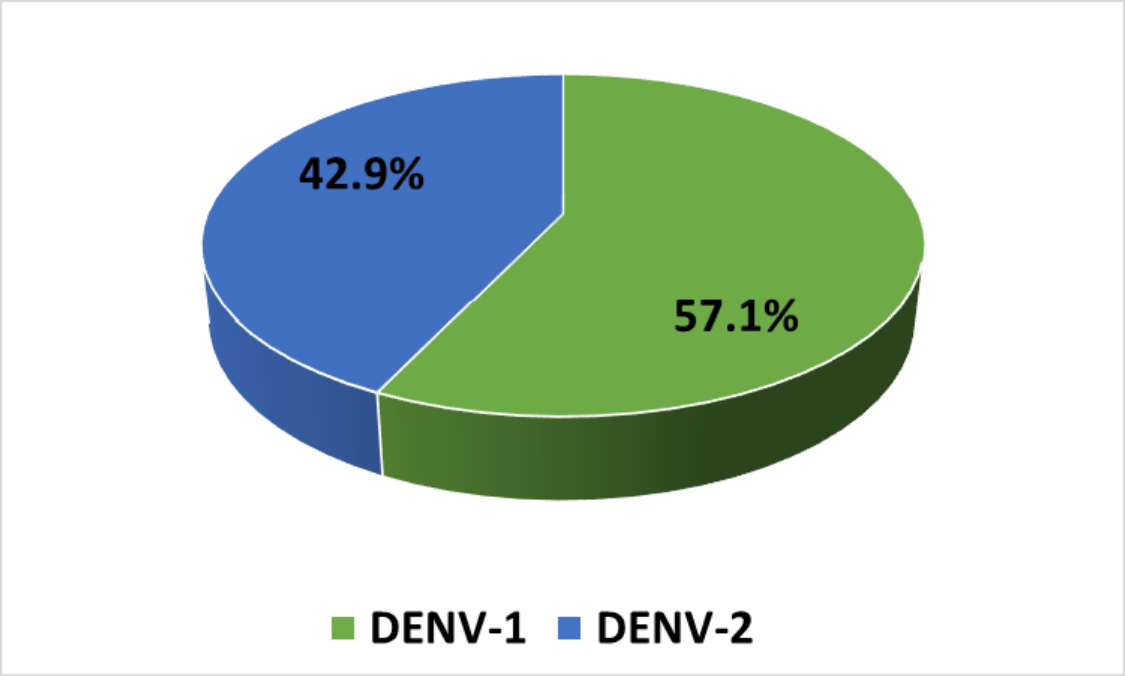

Figure 1. Percentage of patients according to viral serotypes

The results presented in Table 2 demonstrate that both IL-12 and IL-23 levels were significantly elevated in patients with dengue virus infection compared to healthy controls. The increase in IL-12 suggests its essential role in activating Th1 immune responses and enhancing interferon-γ production, which are critical for viral clearance. Similarly, the higher IL-23 concentrations observed in patients highlight its contribution to sustaining proinflammatory pathways, particularly through the IL-23/IL-17 axis. These findings are consistent with previous studies that have emphasized the protective and regulatory functions of IL-12 and IL-23 during viral infections, while also suggesting that dysregulated cytokine responses may contribute to disease severity. Collectively, the observed cytokine patterns provide further evidence supporting the protective immunological role of IL-12 and IL-23 against dengue virus infectio.

Table 2. Comparison of Il-12 and Il-23 between patients with SI and control

Interleukins	Patients (N= 28)		Control (N= 32)		(P value)
	Mean	SD	Mean	SD	
IL-12 (pg/mL)	92.4	18.6	76.3	15.2	< 0.04*
IL-23 (pg/mL)	68.7	14.5	54.1	12.8	< 0.02*

*** Significant at P value <0.05**

The comparison of cytokine responses between DENV1 and DENV2 serotypes demonstrated slightly higher mean IL-12 and IL-23 levels in the DENV1 group; however, these variations were not statistically significant ($p > 0.05$). This suggests that although proinflammatory activity may vary across serotypes, the immune response patterns were largely comparable in this cohort. The unequal group sizes, with a smaller DENV1 sample, may have reduced statistical power, highlighting the need for larger, well-balanced studies to

clarify potential serotype-specific immunological differences.

Table 3. Comparison of Il-6 and Il-17 between vaccinated and non-vaccinated patients with SI

Interleukins	DENV1 (N= 12)		DENV2 (N= 16)		(P value)
	Mean	SD	Mean	SD	
IL-6 (pg/mL)	78.5	16.2	72.1	18.4	0.14 (NS)
IL-17 (pg/mL)	43.7	9.8	39.9	10.5	0.08 (NS)

NS: Non-Significant at P value >0.05

The analysis presented in Table 4 demonstrates a **significant positive correlation** between IL-12 and IL-23 levels in patients with dengue virus infection ($r = 0.62$, $p = 0.001$). This indicates that as IL-12 concentrations increase, IL-23 levels tend to rise concurrently, suggesting a coordinated regulation of these proinflammatory cytokines during the host immune response. The strong correlation supports the concept that IL-12 and IL-23 may act synergistically to enhance Th1 and Th17-mediated pathways, contributing to viral clearance and modulation of inflammation. These findings are in line with previous studies highlighting the interplay between IL-12 and IL-23 in viral infections, reinforcing their potential role as biomarkers for immune activation and targets for immunomodulatory interventions in dengue. (figure 1).

Table 4. Pearson correlation coefficient between Il-12 and IL-23 in patients with DENV

Interleukins	IL-12
IL-23	r=0.620 (P value= 0.001)

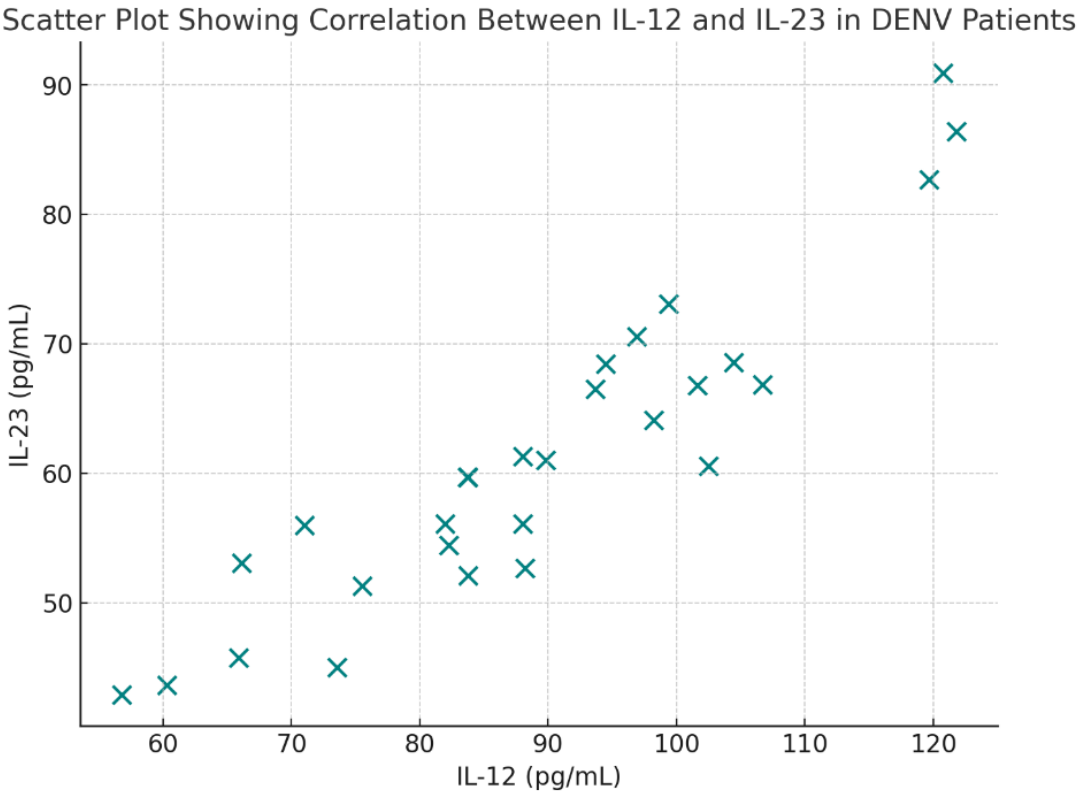

Figure 1. Scatter plots showing the correlation and regression line between IL-12 and IL-23 in patients with DENV

Discussion

he presents study found that serum levels of IL-12 and IL-23 were significantly elevated in patients with dengue virus (DENV) infection compared with healthy controls, and that these two cytokines were moderately and positively correlated ($r = 0.62$, $p = 0.001$). Conversely, IL-6 and IL-17 showed only modest, non-significant differences between serotypes (slightly higher in DENV1), suggesting broadly comparable proinflammatory profiles across the two dominant serotypes in this cohort. Taken together, these results support a model in which coordinated activation of IL-12/IL-23 pathways accompanies acute DENV infection and may contribute to host defense while also linking to inflammatory processes previously associated with disease severity.

IL-12 is classically associated with Th1 polarization, NK cell activation, and IFN-γ induction—responses that promote antiviral activity and viral clearance (Rahman et al., 2023). The significantly higher IL-12 levels in patients versus controls in our study are therefore consistent with an activated antiviral immune milieu during acute dengue. Several recent reviews and experimental studies emphasize IL-12 family cytokines as pivotal modulators of antiviral immunity and as potential therapeutic adjuvants, underscoring the plausibility that IL-12 production represents a protective host response in early infection (Rahman et al., 2023; Tjan et al., 2021). However, temporal dynamics must be considered: some longitudinal analyses indicate that IL-12 signatures may be higher in early or mild disease and decline in severe phases, implying that low or waning IL-12 could be associated with progression to severe dengue (Bhatt et al., 2024). Our cross-sectional finding of elevated IL-12 in the patient group is therefore most consistent with a protective/acute activation role, but without serial sampling we cannot determine whether IL-12 trajectories predict clinical progression.

IL-23, a member of the IL-12 cytokine family that shares the p40 subunit with IL-12, is a principal driver of Th17 responses and acts in concert with IL-1β and IL-6 to sustain IL-17 production (Dash et al., 2024). The elevated IL-23 in patients and its strong positive association with IL-12 observed here suggest coordinated activation of these cytokine families during dengue. This coupling may reflect a complex host strategy: IL-12-driven Th1 responses limit viral replication, while IL-23/IL-17-associated pathways recruit neutrophils and reinforce barrier defenses. Yet, excessive IL-23/IL-17 activity has been implicated in immunopathology and vascular leakage—features central to severe dengue (Dash et al., 2024; Yong et al., 2022). The coexistence of higher IL-12

and IL-23 might therefore indicate a balanced but heightened immune activation that could be protective when regulated, but potentially pathogenic if dysregulated. This duality aligns with contemporary characterizations of cytokine roles in dengue, where timing, magnitude, and cellular context determine beneficial versus harmful outcomes (Bhatt et al., 2024; Jiravejchakul et al., 2025).

The positive correlation between IL-12 and IL-23 (r = 0.62) in our cohort corroborates emerging evidence for cross-talk between IL-12 family members during viral infections (Rahman et al., 2023). Mechanistically, antigen-presenting cells exposed to viral ligands can co-express IL-12p35/p40 and IL-23p19/p40 subunits under distinct pattern-recognition receptor signaling contexts; thus, simultaneous elevation of IL-12 and IL-23 may reflect shared upstream triggers such as Toll-like receptor activation by DENV components (Rahman et al., 2023; Kombe Kombe et al., 2024). Clinically, the observed correlation supports the notion of using combined cytokine signatures (rather than single analytes) to better capture host immune states and potential prognostic signals.

Comparing serotypes, IL-6 and IL-17 were slightly greater in DENV1 than in DENV2 but did not reach statistical significance—an outcome that may stem from limited sample size (particularly the smaller DENV1 group) and natural interindividual variability. Large cohort studies show that while certain serotypes or genotypes can influence disease phenotype and cytokine patterns, host factors and timing frequently exert stronger effects than serotype alone (de Arruda et al., 2023; Yong et al., 2022). Therefore, although our serotype comparison suggests a trend, it should be interpreted cautiously and warrants confirmation in larger, balanced samples with longitudinal sampling to capture peak cytokine windows.

Our findings are broadly consistent with recent studies that document complex cytokine storms in dengue, featuring elevated IL-6, IL-10, and other inflammatory mediators, while also pointing to nuanced roles for IL-12 family members (Bhatt et al., 2024; Dash et al., 2024; de Arruda et al., 2023). Notably, some reports link reduced IL-12 in later or severe disease stages, supporting the interpretation that sustained or appropriately timed IL-12 may be protective (Bhatt et al., 2024). Similarly, elevated IL-23 and IL-17 have been associated with severity in several cohorts, confirming our concern about the potential pathogenicity of an overactive IL-23/IL-17 axis (Dash et al., 2024).

Although major chronic comorbidities were excluded from the analysis, it cannot be ruled out that unmeasured confounders—such as previous dengue infection with the potential for antibody-dependent enhancement, nutritional factors, or concurrent infections—may have shaped the observed cytokine responses (de Arruda et al., 2023). To address these uncertainties, forthcoming investigations would benefit from longitudinal sampling across different stages of illness, recruitment of larger and geographically diverse cohorts, and the integration of virological assessments (including viremia levels and viral genotype) with advanced immune-profiling techniques. Such approaches may provide more precise insights into the temporal regulation and cellular origins of IL-12 and IL-23 during dengue pathogenesis.

Conclusion

In summary, the findings of this study contribute to the expanding evidence base highlighting the involvement of IL-12 and IL-23 in host responses to dengue virus infection. The observed co-elevation and correlation between these cytokines are suggestive of synchronized antiviral and inflammatory pathways. Consequently, IL-12 and IL-23 may hold promise both as components of multiplex biomarker panels and as therapeutic targets within immunomodulatory approaches designed to strengthen protective immunity while reducing pathological inflammation.

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