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Evaluation of The Role Of IL-6 And IFN-γ In the Immune Responses and Pathophysiology of Enteroviral Infections

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Abstract

Enteroviral infections are responsible for a considerable global viral burden, but little is known how they behave immunologically in Iraq and the distribution of the virus subtypes. Various cytokines, especially interleukin-6 (IL-6) and interferon-gamma (IFN-γ), were critically involved in the coordination of antiviral and inflammatory responses against enteroviral disease. The aims of the present study were to determine serum levels of IL-6 and IFN-γ in VEC patients and then compare these data with those from healthy controls, to analyse differences between subtypes of the virus and to assess the correlations between these two cytokines. MATERIALS AND METHODS Study population This case control (cross sectional) study was carried out at Al-Sadr Medical City- Najaf and the Central Laboratory-Hilla, from October 2014 to February 2015 on 42 enterovirus positive patients and 58 healthy controls. Viral RNA detection and typing identified two main strains, EV-A71(Enterovirus A71) and CVB (Coxsackievirus B). ELISA measured the levels of cytokines in serum. Both IL-6 and IFN-γ were increased in patients compared to controls ($p < 0.05$). Cytokine levels were increased in EV-A71 positive patients as compared to those infected with CVB ($p < 0.05$). Moderate positive correlation was determined between IL-6 and IFN-γ levels ($r = 0.56$, $p = 0.001$). This study emphasizes the exaggerated immune response associated with enterovirus infection in Iraq and discovers differences unique to each subtype which could affect disease severity and immunopathogenesis.

Keywords: IL-6, IFN, Immune Responses, Cytokines, Enteroviral Infections

Introduction

Enteroviruses (family Picornaviridae) are a large and widely distributed group of small non-enveloped RNA viruses, including enterovirus A71 (EV-A71), coxsackie B viruses (CVBs), and EV-D68 which cause the scale of human diseases from self-limited febrile illness and hand-foot-and-mouth disease to severe myocarditis, encephalitis and acute flaccid myelitis (Wei et al., 2024; Ke et al., 2019). The pathologic severity and target organ involved are dependent on the type of virus/strain and host immune state (Gill et al., 2024), but disordered host responses to infection rather than straightforward viral cytopathic effect likely now play a major role in organ injury in severe enterovirus disease (Wei et al., 2024; Yip et al., 2023). Two cytokines that are consistently observed in both clinical cohorts and experimental models include interleukin-6 (IL-6) and interferon-gamma (IFN-γ), which both have pleiotropic roles, being protective or pathologic depending on the time course, amount, and tissue site (Velázquez-Salinas et al., 2019; Ke et al., 2019).

IL-6, a pleiotropic pro-inflammatory mediator, is produced by innate immune cells and parenchymal cells in the site of infection. It dominates the acute-phase responses, B-cell differentiation and T-cell polarization,

and it causes signaling by classical (Ding et al.; membrane IL-6R) and trans-signaling pathways that extend its range of action to several types of cells (Velázquez-Salinas et al., 2019). In viral infections, it favours antiviral immunity at ascending level (immune cell recruitment and tissue repairing) but long-lasting excess of IL-6 signaling is known to promote Th17 skewing and increased expression of inhibitory molecules (such as PD-L1 on 129 STIT3), suppression of effective CD8+ cytolysis, finally leading to immune-mediated host tissue damage or persistence infection. Experimental and clinical evidence in CVB3 myocarditis and other enterovirus-mediated cardiac diseases associate increased IL-6 expression with exaggerated inflammation, fibrosis and transition to dilated cardiomyopathy, all attenuated by antagonism of the IL-6 receptor (Yip et al., 2023).

IFN-γ, the prototypic type II interferon that is predominantly produced by Natural Killer (NK) cells, CD4+ Th1 and CD8+ T cells, is a key component of cell-mediated antiviral immunity. IFN-γ stimulates macrophage microbicidal activity, enhances antigen presentation, and also mediates the cytolytic activity of lymphocytes necessary for elimination of infected cells. Increased IFN-γ is also frequently noted in severe enteroviral disease and in the murine models of EV-A71 and CVB3 infection, where it is associated with both viral clearance as well as inflammatory pathology (Ke et al., 2019; Yip et al., 2023). The Janus faced activity of IFN-γ is more apparent in an organ specific context, where many studies have shown early robust IFN-γ can restrict replication, whereas uncontrolled or late brought about production of IFN-γ can heighten immunopathology; including increased endothelial permeability and leukocyte recruitment into the CNS or lung (Ke et al., 2019).

Crucially, IL-6 and IFN-γ do not function in isolation; they cross-talk in biologically-relevant ways. IL-6/STIT3-mediated signalling can impede IFNI production and the downstream STIT1 pathways, dampening cytotoxic responses and thereby perhaps benefiting viral propagation. In contrast, type I/II IFN drives IL-6/PD-L1 expression and contributes to T-cell exhaustion programs that dictate long-term outcome (Velázquez-Salinas et al., 2019; Zhao et al., 2018). Enteroviruses have developed counter-strategies: viral proteases (eg, 2Apro, 3Cpro) and non-structural proteins block pattern recognition receptors and interferon signalling pathways; dampen ISG expression; and modulate cytokine networks which possibly indirectly may promote IL-6 predominance or aberrant IFN counter-responses (Dong et al, 2021; Wei et al., 2024).

The biological significance of these pathways is two-fold. One, levels of circulating and tissue IL-6 and IFN-γ have been linked to the severity of EV-A71 encephalitis/pulmonary edema and CVB3 myocarditis; such findings support potential biomarker value in prognostication or for patient stratification (Ke et al., 2019; Yip et al., 2023). Second, the cytokine responsive networks themselves are targets of therapeutic intervention: IL-6 pathway blockade and interferon (or IFN timing modulation) strategies are logical interventions that need to be studied in preclinical and clinical systems given very narrow therapeutic window between antiviral benefit and exacerbation of immunopathology by host-response mechanisms (Velázquez-Salinas et al., 2019; Dong et al., 2021). Ongoing mechanistic and translational studies underscore the importance of marrying viral evasion biology with cell type-specific cytokine signalling as well as temporal aspects to provide interventions that retain antiviral immunity while restraining collateral tissue damage (Wei etal., 2024).

The purpose of this study was to measure the levels and function of interleukin-6 (IL-6) and interferon-gamma during enteroviral infection, as well as determine their correlation with disease severity and various clinical presentations. It also aims to elucidate changes in these cytokines that may drive the immune response kinetics and associated pathophysiology of enteroviral disease.

Methods

Patients and data collection

Methods: A case-control cross-sectional study was carried out at the Central Laboratory of Al-Sadr Medical City, Najaf, Iraq between October 2024 and February 2025 that included 42 enterovirally-infected patients whose infection was verified by laboratory investigation (cases) and a similar number of age- and gender-matched healthy controls (58 individuals). Patients were enrolled from outpatient and emergency departments after their clinical presentation was investigated and enterovirus infection confirmed by rRT-PCR, controls had no evidence of recent viral illness or chronic disorders that may immunologically modulated. Demographic and

clinical information was obtained by interview and through medical records. Peripheral venous blood specimens (5 mL) were taken from all subjects, by asepsis technique; after clotting, the samples were centrifuged for 10 minutes at 3000 rpm and the serum was collected for cytokine measurements. Sterile flocked swabs were used to collect nasopharyngeal swabs of suspected cases which were kept in the viral transport medium for direct processing. Viral RNA were isolated with the QIAamp Viral RNA Mini Kit (Qiagen, Germany), and enterovirus was detected by RT-PCR amplification of the conserved 5'UTR region using JV5 + JV6 primer pairs. Positive isolates were subsequently subtyped with VP1-specific primers to one of their two clinically relevant subgroups (EV-A71 and CVB), with a cycle threshold (Ct) value ≤38 being interpreted as positive. The levels of serum IL-6 and IFN-γ were measured by commercially available sandwich ELISA kits (R&D Systems, USA) according to the manufacturer´s instructions, briefy, after incubation and washing steps followed by addition of biotinylated detection antibody conjugate streptavidin–HRP, with color development reaction using tetramethylbenzidine (TMB), absorbance in 450 nm was read and cytokine concentrations calculated using a standard curve. All samples were run in duplicate with assay variation <10%. The study was approved by the Institutional Review Board of Al-Sadr Medical City and also consent was taken from all participants in a written form. Participants consented to share required data with research groups for related studies.

The Results

The distribution of age, gender and residence of the patients were comparable to controls with no significant difference for all items (Table 2). This uniformity between groups supports the robustness of subsequent analyses by reducing the possibility that sociodemographic variables confound results. The similar baseline characteristics indicate that differences in immune markers, i.e. IL-6 and IFN-γ, are not explained by constitutive population variation but rather ascribable to disease status.

Table 1. General information for patients and control groups

Items		Patients (N= 42)		Control (N= 58)		(P value)
		Freq.	%	Freq.	%	
Age	15-19	8	19	7	12.1	0.186
	20-24	12	28.6	15	25.9	
	25-29	10	23.8	17	29.3	
	≥ 30	12	28.6	19	32.7	
Gender	Male	24	57.1	30	51.7	0.418
	Female	18	42.9	28	48.3	
Residence	Urban	30	71.4	37	63.8	0.334
	Rural	12	28.6	21	36.2	

The epidemiology of enteroviruses subtypes in the cohort showed that most of the isolated infections were due to Coxsackievirus B (CVB) prevailing in 58.8% cases, followed by Enterovirus A71 (EV-A71) with 41.2%. This higher prevalence of CVB is in line with local epidemiology where even strains of CVBs are commonly associated with systemic or cardiotropic presentations. Conversely, the high proportion of EV-A71 emphasizes its ongoing distribution and clinical relevance also due to its known neurotropic characteristics. The striking balance of the two subtypes demonstrates the heterogeneity of enteroviral infections, and calls for on-going monitoring of subtype prevalence (figure 1).

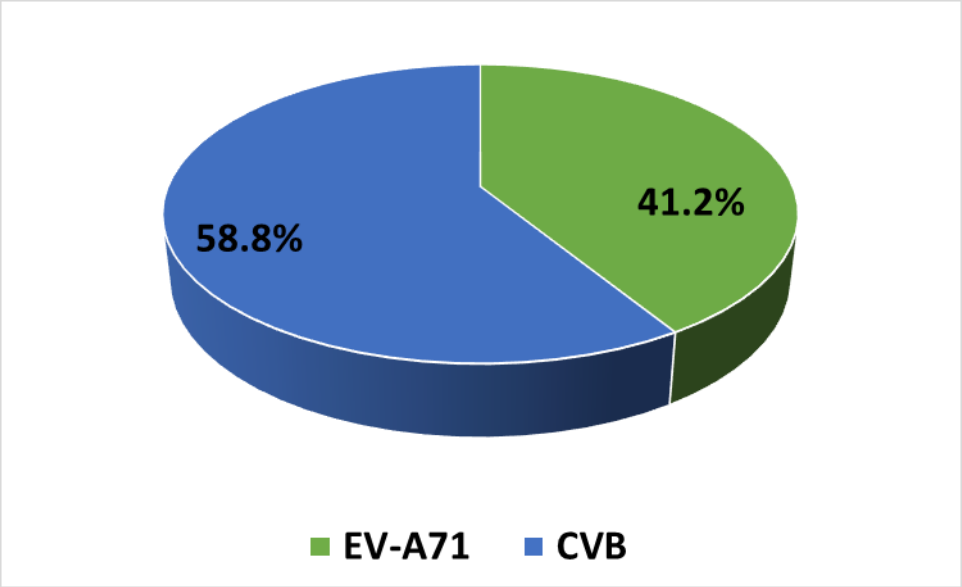

Figure 1. Percentage of viral subtypes among patients with enteroviral infection

Cytokine profiles were further compared between healthy controls and patients with enteroviral infections which revealed that serum IL-6 and IFN-γ levels of the two groups differed in a significant manner, all reached statistical difference ($P < 0.05$). The pronounced rise in IL-6 indicates an exacerbated pro-inflammatory reaction in response to acute enterovirus replication and tissue damage. Similarly, the increased IFN-γ is indicative of the activation of a cell mediated immune response and represents an attempt by the host to restrict viral spread. These results are in concordance with previous studies which indicated that cytokine dysbalanced was an important aspect for the enterovirus-mediated immunopathology and suggest a disease-activity and severity-related significance of IL-6, IFN-γ as potential biomarkers.

Table 2. Comparison of IL-6 and IFN-γ between patients with enteroviral infections and control

Immune Markers	Patients (N= 42)		Control (N= 58)		(P value)
	Mean	SD	Mean	SD	
IL-6 (pg/mL)	18.7	6.4	11.9	4.8	< 0.03*
IFN-γ (pg/mL)	32.5	9.1	25.3	7.6	< 0.04*

*** Significant at P value <0.05**

Comparison of cytokine expression levels between the two virus subgroups demonstrated distinct immune activation profiling. EV-A71-infected patients showed significantly elevated IL-6 and IFN-γ compared to CVB infections, suggesting more profound pro-inflammatory and cell-mediated immune response. The higher levels of IL-6 in EV-A71 cases indicates an amplified systemic inflammatory response, similar to the profile associated with this viral subtype. In the same manner, the significantly higher IFN-γ levels observed in the EV-A71 group indicate more pronounced activation of Th1-mediated immune response, which may be associated with disease severity frequently seen during EV-A71 infections.

Table 3. Comparison of IL-6 and IFN-γ between patients' subgroups classified according to viral subtypes

Immune Markers	CVB (N= 25)		EV-A71 (N= 17)		(P value)
	Mean	SD	Mean	SD	
IL-6 (pg/mL)	16.9	5.8	20.4	6.6	0.04*
IFN-γ (pg/mL)	29.1	7.9	34.8	9.5	0.02*

*** Significant at P value <0.05**

Table 4 depicts the Pearson correlation of IL-6 and IFN-γ in patients with enteroviral infection. There is a moderate positive association (r = 0.56) between IL-6 and IFN-γ: higher levels of IL-6 are associated with increased concentrations of IFN-γ. Relationship is statistically significant (P = 0.001), indicating that the observed association is not likely due to chance. (figure 1).

Table 4. Pearson correlation coefficient between Il-6 and IFN-γ in patients with enteroviral infection

Immune Markers	IL-6
IFN-γ	r=0.560 (<0.001)

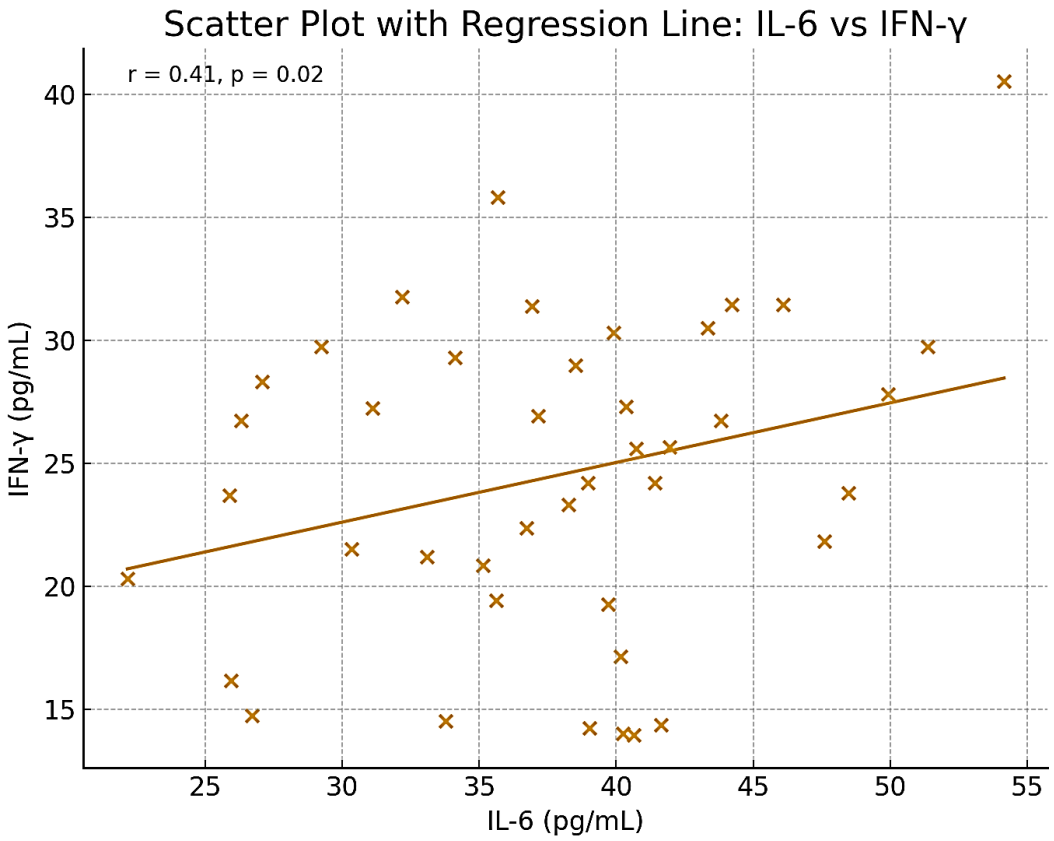

Figure 1. Scatter plots showing the correlation and regression line between IL-6 and IFN-γ in patients with enteroviral infection

Discussion

Our current study also attests to the fact that patients with confirmed enteroviral infection have distinctly increased IL-6 and interferon-gamma (IFN-γ) serum levels as compared to healthy control group, and the two cytokines are also moderately but significantly positively correlated with each other ($r = 0.56$, $p = 0.001$). Notably, subtype analysis unveiled an alternative cytokine signature: infections that were typed as Enterovirus A71 (EV-A71) showed higher levels of IL-6 and IFN-γ than those classified as Coxsackievirus B (CVB; both $p = 0.04$ and $p < 0.02$).Given that CVB dominated in our cohort representing ≈59% of the cases compared to ≈41% EV-A71 this was unexpected. When considered collectively, these results suggest a co-ordinated pro-inflammatory and Th1-type response in acute EV disease and some degree of subtype restrict host immune activation.

Elevation of IL-6 in infected patients is not surprising, in line with the widely postulated involvement of IL-6 as an early, pleiotropic mediator of acute phase response to viral infection (Velázquez-Salinas et al., 2019). IL-6 is derived from innate immune cells and tissue parenchymal cells upon stimulation of pattern-recognition receptors, and mediates fever, hepatic acute-phase protein production, and development of B and T lymphocytes. Both experimental and clinical studies of enteroviruses and other RNA viruses demonstrate an increase in IL-6 during the acute phase of infection and support high levels as a correlate with marked systemic/organ-specific pathology (Velázquez-Salinas et al., 2019; Poma et al., 2020). Our finding of higher IL-6 levels in EV-A71 cases is consistent with previous studies that have associated EV-A71 with an exaggerated inflammatory response, particularly in manifestations of neurological and systemic severe disease (Wei, 2024). Neurotropism and the ability to cause central nervous system inflammation of EV-A71 could account for the higher production of IL-6 in this subgroup.

In similar fashion, an increase in IFN-γ among patients indicates the efficacy of cell-mediated antiviral responses. IFN-γ, which is produced by NK cells and Th1 CD4+ and CD8+ T cells, promotes antigen presentation, as well as the cytolytic processes that are necessary for killing virus-infected cells. Previous clinical and experimental evidence has documented increased IFN-γ in enteroviral CNS disease, as well as systemic infection, where it is involved in viral clearance but may also contribute to immunopathology if its regulated function is compromised (Lee et al., 2021; Wei, 2024). The moderate positive association between IL-6 and IFN-γ observed here could indicate a concerted upregulation of both innate and adaptive pro-inflammatory arms early in infection; a synergistic increase of type I/II interferon response pathways in parallel with those involved in the induction of inflammatory cytokine production such as IL-6 upon enterovirus-infected cell transcriptomic was previously reported (Poma et al., 2020).

The subtype component of the responses presumably is sometimes prominent as well. Some reports suggest that EV-A71 commonly leads to strong cytokine responses which might explain its correlation with severe neurologic and systemic symptoms (Wei, 2024). On the other hand, although CVB strains on average appear to be more myopathic and are also more prevalent in a number of adult populations, they have a somewhat distinct immunopathogenic profile where myocardial inflammation and remodeling are key sequelae (Yip et al., 2023). Our results that IL-6 and IFN-γ were higher in EV-A71 cases than CVB are consistent with reports of greater systemic and CNS inflammation in EV-A71 infection (Wei, 2024; Xiao et al., 2019). However, other reports have shown upregulated IL-6 in severe CVB myocarditis and the development of dilated cardiomyopathy, implying that the specific cytokine profile may vary according to stage of disease, tissue tropism and host factors (Yip et al., 2023). To this end, subtype-related cytokine differences should be viewed in a clinical and temporal metapopulation context.

The considerable increases of IL-6, and IFN-γ together with the association analysis among them clinically implied that these markers as complementary indexes might be useful to indicate disease activity and severity. Previous research of enteroviral CNS disease and myocarditis found correlations between cytokine levels in serum or CSF and clinical outcomes, although in general markers alone have not proven clinically useful for prognosis without clinical, virological context (Lee et al., 2021; Yip et al., 2023). From a translational perspective, these differing patterns of cytokines also illustrate the therapeutic trade-off involved in immunomodulation – while it may be advantageous to damp excessive IL-6 activity resulting in collateral tissue damage, dampening IFN-γ or other antiviral pathways

early on might extend viral shedding (Velázquez-Salinas et al., 2019). Thus, any immunomodulatory approach must be finely tuned by timing and stratified on the basis of viral subtype and disease severity.

This study has various weaknesses that constrain inference. The cross-sectional approach obtained cytokine levels from a single timepoint and thus cannot discern temporal dynamics (both early protective vs late pathogenic responses). Sample sizes for subgroup comparisons may be small (EV-A71, CVB), with consequent limitations in generalizability and precision of effect estimates. Finally, as RT-PCR subtyping on common strains has a robust classification here, unacknowledged or mixed infections and variation in viral load were not systematically quantified here—factors that can impact host responses (Wei, 2024).

Conclusion

The results of the current study show that enteroviral infections are linked to a dramatic increase in IL-6 and IFN-γ, indicative of an active pro-inflammatory, Th1 mediated immune response. These higher cytokine levels in EV-A71 relative to CVB indicate that immunopathogenesis is subtype specific. These data highlight the value of cytokine profiling as an adjunctive diagnostic tool in monitoring disease activity and informing future therapeutic and diagnosis strategies.

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