Research Article

DOI: 10.4274/vhd.galenos.2026.2025-10-4
Viral Hepat J

Antiviral Therapy Modulates Immune Responses in Chronic Hepatitis C: Implications Based on TH1/TH2 Cytokine Profiles

Kronik Hepatit C'de Antiviral Tedavi Bağışıklık Tepkilerini Modüle Ediyor: TH1/TH2 Sitokin Profillerine Dayalı Sonuçlar

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ABSTRACT

Objectives: Immune dysregulation and deranged cytokine networks are key features of chronic infection with hepatitis C virus (HCV) that causes the virus to persist and illness to worsen. Assessment of T-helper type 1 and 2 (Th1 and Th2) cytokine profiles may provide insight into immune modulation associated with disease status and antiviral therapy. To quantify the circulating levels of important Th1 and Th2 cytokines in treatment-naïve chronic HCV patients and compare them with post-treatment patients and healthy controls.

Materials and Methods: This cross-sectional study included three independent study groups: untreated chronic HCV patients, post-treatment HCV patients, and healthy controls. Serum levels of interferon-γ (IFN-γ), interleukin-2 (IL-2), IL-4, and IL-10 were measured using ELISA. Group comparisons were performed using Kruskal-Wallis and Mann-Whitney U tests.

Results: Each study group had a different cytokine profile. The lowest cytokine levels were consistently recorded in healthy controls. Treatment-naïve patients demonstrated a Th2-skewed profile with significantly elevated IL-2 (1.08±0.35 pg/mL), IL-4 (1.75±0.56 pg/mL), and IL-10 (1.63±0.65 pg/mL) compared with healthy controls. The post-treatment group showed higher IFN-γ levels (2.32±0.81 pg/mL) relative to both treatment-naïve patients and controls, along with reduced IL-4 and IL-10 levels compared with treatment-naïve patients. However, Th2 cytokines remained elevated in the post-treatment group compared with healthy controls.

Conclusion: Chronic HCV infection is linked with a Th2 cytokine predominance in untreated patients. Post-treatment patients exhibit a cytokine profile characterized by increased Th1-associated cytokines and partial attenuation of Th2 cytokines, suggesting treatment-associated immune modulation rather than complete immune normalization. Cytokine profiling may serve as a useful indicator of immune status in chronic HCV infection.

Keywords: Hepatitis C virus, cytokines, Th1 cells, Th2 cells

ÖZ

Amaç: Kronik hepatit C virüsü (HCV) enfeksiyonu, viral persistans ve hastalık progresyonuna katkıda bulunan bağışıklık düzensizliği ve bozulmuş sitokin ağları ile karakterizedir. T-yardımcı hücre tip 1 ve 2 (Th1 ve Th2) sitokin profillerinin değerlendirilmesi, hastalık durumu ve antiviral tedavi ile ilişkili immün modülasyonun anlaşılmasına katkı sağlayabilir. Tedavi almamış kronik HCV hastalarında dolaşımdaki Th1 ve Th2 sitokin düzeylerini nicel olarak belirlemek ve bu düzeyleri tedavi sonrası hastalar ile sağlıklı kontrollerle karşılaştırmak amaçlanmıştır.

Gereç ve Yöntemler: Bu kesitsel çalışmaya üç bağımsız grup dahil edilmiştir: tedavi almamış kronik HCV hastaları, tedavi sonrası HCV hastaları ve sağlıklı kontroller. Serum interferon-γ (IFN-γ), interlökin-2 (IL-2), IL-4 ve IL-10 düzeyleri ELISA yöntemi ile ölçülmüştür. Gruplar arası karşılaştırmalar Kruskal-Wallis ve Mann-Whitney U testleri kullanılarak yapılmıştır.

Bulgular: Çalışma grupları arasında belirgin sitokin profili farklılıkları saptanmıştır. Sağlıklı kontroller tüm sitokinler için en düşük düzeyleri göstermiştir. Tedavi almamış hastalarda IL-2 (1,08±0,35 pg/mL), IL-4 (1,75±0,56 pg/mL) ve IL-10 (1,63±0,65 pg/mL) düzeyleri sağlıklı kontrollere kıyasla anlamlı derecede yüksek bulunmuş ve Th2 ağırlıklı bir sitokin profili izlenmiştir. Tedavi sonrası grupta IFN-γ düzeyleri (2,32±0,81 pg/mL) hem tedavi almamış hastalara hem de kontrollere göre daha yüksek bulunmuş, IL-4 ve IL-10 düzeylerinde ise tedavi almamış gruba kıyasla azalma gözlenmiştir. Bununla birlikte, Th2 sitokin düzeyleri tedavi sonrası grupta sağlıklı kontrollere göre yüksek kalmaya devam etmiştir.

Sonuç: Kronik HCV enfeksiyonu, tedavi almamış hastalarda Th2 sitokin baskınlığı ile ilişkilidir. Tedavi sonrası hastalarda Th1 ile ilişkili sitokinlerde artış ve Th2 sitokinlerinde kısmi azalma gözlenmesi, tam bir immün normalizasyon yerine antiviral tedavi ile ilişkili immün modülasyonu düşündürmektedir. Sitokin profillemesi, kronik HCV enfeksiyonunda bağışıklık durumunun değerlendirilmesinde yararlı bir gösterge olabilir.

Anahtar Kelimeler: Hepatit C virüsü, sitokinler, Th1 hücreleri, Th2 hücreleri

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Received: 24.10.2025 **Accepted:** 17.07.2026 **Epub:** 18.09.2026

Cite this article as: Ravichandran J, Kuberan D, Balasubramanian R. Antiviral therapy modulates immune responses in chronic hepatitis C: implications based on TH1/TH2 cytokine profiles. Viral Hepat J. [Epub Ahead of Print]

Introduction

Hepatitis C virus (HCV), a positive-sense single-stranded ribonucleic acid (RNA) virus of the *Flaviviridae* family, is a major cause of chronic liver disease leading to cirrhosis and hepatocellular carcinoma. Although acute infection is often asymptomatic, approximately 50-80% of infected individuals progress to chronic disease, reflecting the virus's ability to evade host immune responses. The contribution of inflammatory and regulatory cytokines to disease progression and hepatocarcinogenesis remains incompletely understood; however, available evidence suggests that these mediators influence viral clearance or persistence through protective or immunopathological immune responses. Disease outcome in HCV infection is therefore thought to depend on the balance between pro-inflammatory and regulatory cytokines.

Cytokines play a central role in immune regulation by coordinating innate and adaptive immune responses. During HCV infection, type I interferon (IFN) produced by infected hepatocytes activates natural killer (NK) cells, contributing to antiviral defense (1). NK cells and dendritic cells further promote antiviral immunity through IFN-γ production and antigen presentation to T lymphocytes via major histocompatibility complex molecules (1). Adaptive immune responses are mediated by T lymphocytes, with $CD8^+$ cytotoxic T-cells limiting viral replication and $CD4^+$ helper T-cells modulating immune responses through the secretion of T-helper type 1 (Th1) and T-helper type 2 (Th2) cytokines (2).

Th1 and Th2 immune responses exist in a dynamic balance, with Th1 cytokines such as IFN-γ and interleukin-2 (IL-2) supporting effective antiviral immunity, while Th2 cytokines including IL-4 and IL-10 promote humoral responses and immune regulation. A shift toward Th2 dominance has been associated with viral persistence and disease progression, whereas Th1-biased responses are linked to viral control (3,4). Despite growing interest, studies evaluating Th1/Th2 cytokine profiles in chronic HCV infection have yielded inconsistent results, reporting Th2 predominance, mixed cytokine expression, or Th1 dominance (4,5).

Given these discrepancies, the present study aimed to characterize circulating Th1 (IFN-γ, IL-2) and Th2 (IL-4, IL-10) cytokine profiles in chronic HCV infection by comparing treatment-naïve patients, post-treatment patients, and healthy controls, in order to better understand immune modulation associated with disease status and antiviral therapy.

Materials and Methods

Study Population

Group 1: Healthy controls (n=24),

Group 2: Treatment-naïve chronic HCV patients (n=103) (adult and pediatric participants),

Group 3: Post-treatment chronic HCV patients (n=50) (adult and pediatric participants).

This study was designed as a cross-sectional analysis. The treatment-naïve and post-treatment groups consisted of independent patient cohorts, and individual patients were not followed longitudinally from pre-treatment to post-treatment phases. Consequently, comparisons between groups reflect differences associated with treatment status at the time of sampling and do not represent within-subject longitudinal immune changes. The healthy control group comprised exclusively adult participants. Pediatric patients were included only in the chronic HCV cohorts, with 19 pediatric patients in the treatment-naïve group and 11 pediatric patients in the post-treatment group, reflecting the clinical population presenting to the participating centers during the study period.

Collection of Sample

The study comprised 177 participants which include 24 healthy controls and 153 patients with chronic HCV infection who were admitted to government general hospitals in and around Chennai. Table 1 of the results section details the demographic and gender distribution of the research participants in Groups 2 and 3 and Figure 1 shows the general study population profile. The Institutional Human Ethics Committee of Dr. ALM PG IBMS, University of Madras (approval no: UM/IHEC/F.RM/2021-II, date: 12.07.2021) approved the study. A questionnaire with the participants' medical history was obtained and all adult patients and the guardians of pediatric patients provided written informed consent.

From each participant, peripheral blood samples were collected and transported at 2-8 °C to the lab (Department of Microbiology, Dr. ALM Post Graduate Institute of Basic Medical Science, University of Madras, Chennai, Tamil Nadu, India). The serum was separated and kept for analysis at -80 °C after the blood samples were centrifuged for 10 minutes at 2000 rpm at room temperature. Patients with HCV infection who participated in this study tested positive for HCV-RNA using the reverse transcriptase polymerase chain reaction and

Table 1. Cytokine mean and standard deviation values for each group

Cytokine	Healthy control	Treatment-naïve	Post-treatment
IFN-γ	0.75±0.18	0.66±0.36	2.32±0.81
IL-2	0.51±0.19	1.08±0.35	1.40±0.57
IL-4	0.48±0.21	1.75±0.56	1.28±0.48
IL-10	0.77±0.39	1.63±0.65	1.26±0.42

IFN: Interferon, IL: Interleukin

seropositive for anti-HCV antibodies. To ensure diagnostic precision, samples were re-confirmed using a commercial HCV detection kit (GB-CHEK HCV25, Genuine Biosystems Private Limited).

Antiviral Treatment and Sample Collection

Patients in the post-treatment group had completed antiviral therapy as per standard clinical practice during the study period. Treatment consisted of direct-acting antiviral (DAA)-based oral regimens, which included combinations such as sofosbuvir with velpatasvir, glecaprevir with pibrentasvir, or ledipasvir with sofosbuvir, administered as once-daily tablet or capsule formulations. The duration of therapy ranged from 12 to 48 weeks, depending on clinical factors such as viral genotype, disease severity, and treatment response. IFN-based therapy was not used, as it is no longer part of standard HCV management.

Serum samples for cytokine analysis were collected after completion of antiviral therapy during routine follow-up visits. Sustained virological response (SVR) data and the exact timing of post-treatment sample collection were not uniformly available for all participants due to the retrospective nature of clinical record retrieval. However, all post-treatment patients had completed standard-of-care DAA therapy and were clinically categorized as post-treatment at the time of sampling. As a result, subgroup analysis based on SVR status or precise post-treatment intervals could not be performed.

Cytokine Evaluation

The IFN-γ, IL-2, IL-4 and IL-10 levels were quantified from serum samples using a Sandwich ELISA technique, by applying Biolegend Human ELISA MAX™ Deluxe set (Cat no: 430104, 431804, 430304, 430604) following the manufacturer's instructions. Briefly, each cytokine's mouse monoclonal human capture antibody was prepared, coated in 96-well plates, and stored at 2-8 °C overnight. After coating, assay diluent A was added and incubated at room temperature for 1 hour to avoid non-specific binding. After washing the plates with wash buffer, 100 µL of samples and standards were added to each well, and kept for a two-hour incubation period. In order to detect IFN-γ, IL-2, IL-4 and IL-10, 100 µL of biotinylated mouse monoclonal anti-human antibodies were then added, and the mixture was left for an hour. Following a wash, 100 µL of the Avidin-HRP conjugate was added to the plates and left for half an hour. After further washing, 100 µL of freshly prepared tetramethylbenzidine substrate were added and kept in dark for 20 minutes to develop color. To halt the reaction, 100 µL of stop solution was added which caused the color to change from blue to yellow. The absorbance was measured at 450 nm (Biotek Gen5 Microplate reader). Cytokine concentration (pg/mL) was determined by means of standard curve plots.

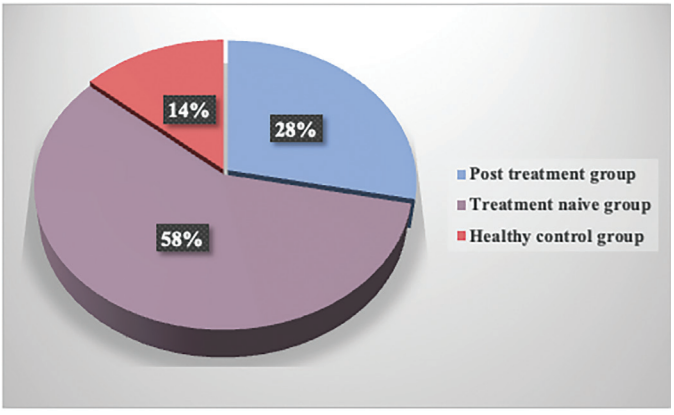

Figure 1. Distribution of study population

Statistical Analysis

The cytokine levels in each group were compared using the Kruskal-Wallis test because of the data's non-normal distribution. To compare groups pairwise, the Mann-Whitney U test was employed. To account for multiple pairwise comparisons, p-values obtained from the Mann-Whitney U test were adjusted using the Benjamini-Hochberg false discovery rate (FDR) correction. Adjusted p-values (q-values) <0.05 were considered statistically significant.

Age-Stratified Analysis

To address potential age-related confounding due to inclusion of pediatric participants in the HCV groups and exclusively adult healthy controls, a subgroup analysis restricted to adult participants (≥18 years) was performed. Cytokine levels among adult participants in the three study groups were compared using the same non-parametric statistical methods (Kruskal-Wallis and Mann-Whitney U tests). This analysis was undertaken to evaluate whether the observed differences in cytokine profiles remained consistent after controlling for age.

Results

The mean age of the study population (n=177) was 37.1±17.8 years. Group wise comparison revealed healthy controls to have mean age 44.1±16.8 years, Treatment-naïve to have mean age 36.7±17.1 years, and post-treatment to have mean age 38.4±19.4 years. Total male-to-female ratio was roughly 1.6:1 (110 men, 67 women). Group-wise distribution revealed an equal distribution in healthy controls (1:1), but treatment-naïve and post-treatment groups revealed male predominance, with 1.6:1 and 2.1:1 ratios, respectively.

The mean and standard deviation (SD) of the serum cytokine levels for each of the three groups are displayed in Table 1. Healthy controls had the lowest levels of all cytokines, whereas treatment-naïve patients had higher levels of IL-2, IL-4, and IL-10. Post-treatment patients demonstrated higher levels of IFN-γ and IL-2 and lower levels of IL-4 and IL-10 compared with treatment-naïve patients, while remaining distinct from healthy controls. Among the three groups, IFN-γ levels were highest in the post-treatment group (mean=2.32, SD=0.81). Although IL-2 levels were elevated in treatment-naïve patients compared with healthy controls, the highest IL-2 levels were observed in the post-treatment group (mean=1.40, SD=0.57). IL-4 levels were maximum in treatment-naïve group with a mean and SD of 1.75 and 0.56 respectively. In post-treatment group, IL-4 levels were found to be intermediate (mean=1.28, SD=0.48). Similarly, IL-10 levels were also maximum in treatment-naïve group (mean=1.63,

SD=0.65), while in post-treatment group the levels were in between the other two groups (mean=1.26, SD=0.42).

Group Comparisons

The cytokine levels in each group were compared using the Kruskal-Wallis test because of the data's non-normal distribution. The cytokine values varied significantly with a p-value <0.001 among the three groups. To compare groups pairwise, the Mann-Whitney U test was employed.

Mann-Whitney U test revealed statistically significant differences for all pairwise comparisons between groups. IFN-γ levels were significantly higher in the post-treatment group compared with both healthy controls (U=44.0, $p=1.40\times10^{-10}$) and treatment-naïve patients (U=168.5, $p=7.86\times10^{-21}$). A smaller, significant, difference between healthy controls and treatment-naïve individuals was also observed (U=1749.0, p=0.0016). The IL-2 levels were significantly altered between all groups, with increased levels in the treatment-naïve in comparison to the healthy controls (U=256.5, $p=1.64\times10^{-9}$), again increased in post-treatment group in comparison to both the treatment-naïve (U=1715.5, p=0.00083) and healthy controls (U=40.0, $p=1.04\times10^{-10}$). The IL-4 levels were significantly increased in the treatment-naïve in comparison to the healthy controls (U=0.0, $p=2.75\times10^{-14}$) and still increased in comparison to the post-treatment individuals (U=3703.0, $p=1.15\times10^{-5}$), whilst levels in the post-treatment individuals were also increased in comparison to the healthy controls (U=105.0, $p=1.12\times10^{-8}$). IL-10 levels were significantly higher in treatment-naïve patients compared with healthy controls (U=292.0, $p=6.20\times10^{-9}$) and remained elevated, though at lower levels, in the post-treatment group (U=232.0, $p=2.20\times10^{-5}$). Comparing the IL-10 levels of treatment-naïve individuals to the post-treatment individuals indicated a statistically significant difference (U=3374.5, p=0.0019). All statistically significant differences remained significant after FDR correction (Table 2), confirming the robustness of the findings.

As this study employed a cross-sectional design with independent cohorts, the observed differences reflect cytokine profiles associated with treatment status rather than within-subject longitudinal changes.

Age-Restricted (Adult-Only) Subgroup Analysis

To address potential age-related confounding, an analysis restricted to adult participants (≥18 years) was performed. The cytokine distribution patterns in the adult subgroup were consistent with those observed in the overall cohort. Kruskal-Wallis analysis demonstrated statistically significant differences among the three groups for all cytokines (IFN-γ, IL-2, IL-4, and IL-10) (p<0.001) (Supplementary Table S1). Pairwise comparisons using the Mann-Whitney U test confirmed that post-treatment patients had significantly higher IFN-γ levels compared with both treatment-naïve patients and healthy controls, while IL-4 and IL-10 levels were significantly elevated in treatment-naïve patients and showed partial reduction in post-treatment patients, though remaining higher than healthy controls (Supplementary Table S2). IL-2 levels were significantly increased in both treatment-naïve and post-treatment groups compared with controls, with higher levels observed in the post-treatment group. Overall, these findings indicate that the observed cytokine differences persist after restricting the analysis to adults and are not solely attributable to age-related variation.

Information regarding SVR status and exact timing of post-treatment sampling was not consistently available across all participants; therefore, stratified analysis based on virological response or time since therapy completion could not be performed.

Discussion

The present study included three groups-healthy controls, treatment-naïve chronic HCV patients and post-treatment chronic HCV patients. The overall male-to-female ratio was 1.6:1, and the

Table 2. Inter-group comparisons for cytokine levels across pairs of groups using Mann-Whitney U test

Cytokine	Groups compared	Test statistic U	p-value	FDR-adjusted p-value (q)	Interpretation
IFN-γ	Healthy vs. treatment-naïve	1749.0	0.0016	0.00213	Significant difference
	Treatment-naïve vs. post-treatment	168.5	7.86×10^{-21}	9.43×10^{-20}	Highly significant difference
	Healthy vs. post-treatment	44.0	1.40×10^{-10}	4.20×10^{-10}	Highly significant difference
IL-2	Healthy vs. treatment-naïve	256.5	1.64×10^{-9}	3.28×10^{-9}	Highly significant difference
	Treatment-naïve vs. post-treatment	1715.5	0.00083	0.00142	Significant difference
	Healthy vs. post-treatment	40.0	1.04×10^{-10}	3.12×10^{-10}	Highly significant difference
IL-4	Healthy vs. treatment-naïve	0.0	2.75×10^{-14}	5.50×10^{-14}	Highly significant difference
	Treatment-naïve vs. post-treatment	3703.0	1.15×10^{-5}	1.73×10^{-5}	Significant difference
	Healthy vs. post-treatment	105.0	1.12×10^{-8}	1.68×10^{-8}	Highly significant difference
IL-10	Healthy vs. treatment-naïve	292.0	6.20×10^{-9}	1.24×10^{-8}	Highly significant difference
	Treatment-naïve vs. post-treatment	3374.5	0.0019	0.00228	Significant difference
	Healthy vs. post-treatment	232.0	2.20×10^{-5}	2.93×10^{-5}	Significant difference

IFN: Interferon, IL: Interleukin, FDR: False discovery rate

study participants' mean age were 37.1±17.8 years. This study examined differences in immune profiles associated with treatment status in chronic HCV by comparing treatment-naïve patients, post-treatment patients, and healthy controls. There was notable alteration in key Th1 (IFN-γ, IL-2) and Th2 (IL-4, IL-10) cytokine profiles amongst the different study groups. Such differences in immune profiles provide insight into the immunological milieu associated with chronic HCV infection and treatment status.

The peak levels of IFN-γ were noticed in post-treatment group (2.32±0.81). There was a significant elevation when compared to the other two groups-healthy controls ($U=44.0$, $p=1.40\times10^{-10}$) and treatment-naïve patients ($U=168.5$, $p=7.86\times10^{-21}$). The higher IFN-γ levels observed in the post-treatment group may reflect a Th1-skewed immune profile associated with antiviral therapy status. Zhang et al. (1), observed that higher IFN-γ levels in naïve patients at baseline and week 4 was linked with HCV eradication and suggested IFN-γ to be a possible predictor of viral response. A recent study by Kishida (2), also focused the anti-viral role of IFN-γ secreted by NK cells and proved that it inhibits viral replication. Contrastingly, reduced IFN-γ levels were reported in patients chronically infected with HCV when compared to healthy controls by (3). According to Ribeiro et al. (4), patients with co-occurring occult hepatitis B virus (OBI)/HCV and healthy controls had nearly identical levels of IFN-γ (4). The IFN-γ levels are found to be influenced by viral etiology as claimed by Estevez et al. (5), who stated that HBV-infected patients had higher plasma values of IFN-γ than both HCV-infected and healthy controls.

In case of IL-2, though treatment-naïve group had higher levels compared to healthy controls ($U=256.5$, $p=1.64\times10^{-9}$), post-treatment group had further elevated levels with significant difference compared to treatment naïve ($U=1715.5$, $p=0.00083$) and healthy controls ($U=40.0$, $p=1.04\times10^{-10}$). As a Th1 cytokine, IL-2 is essential for triggering strong immune responses by promoting activation of T-cell and proliferation. This may explain the higher IL-2 levels observed in the post-treatment group compared with treatment-naïve patients. OBI/HCV-infected patients showed a similar increase in IL-2 levels, compared to healthy controls (4). Whereas, decreased IL-2 levels correlated with anti-viral induced reduction in viral titer, especially in high-responsive groups in the studies done by Wright et al. (6) and Zhang et al. (1). However, another study found that controls had higher IL-2 levels than HCV patients (7). The complex nature of IL-2 patterns in chronic HCV infection is demonstrated by these conflicting results. Variability in IL-2 levels across studies highlights the complex and context-dependent regulation of this cytokine in chronic HCV infection.

Treatment-naïve patients had the highest IL-4 levels (1.75±0.56) when compared to other two groups. In spite of a decrease in post-treatment IL-4 levels (1.28±0.48), they still remained elevated compared to healthy controls ($U=105.0$, $p=1.12\times10^{-8}$). Elevated IL-4 levels in treatment-naïve chronic infection reflects an immune response skewed to Th2 arm. This immunological pattern is linked with HCV immune evasion and persistence as IL-4 suppresses Th1 responses and promote immune tolerance. Similar conclusions have been reached by Ribeiro et al. (4) and Fan et al. (8). IL-4 and other Th2 cytokines were found to be elevated in the serum of patients with chronic HCV and OBI/HCV. Contrastingly, Hengst et al. (9), reported low levels of IL-4 in patients with chronic HCV when compared to healthy controls. Study done by Zhang et al. (1), was in line with our results as it reported a link between lower baseline IL-4 levels and HCV clearance and established that transition from Th2 predominance will aid in treatment response.

Peak values of IL-10 were observed in treatment-naïve patients (1.63±0.65). Despite having lower values than treatment-naïve, post-treatment IL-10 levels (1.26±0.42) were more than healthy controls ($U=232.0$, $p=2.20\times10^{-5}$). A Th2 cytokine with immunosuppressive and anti-inflammatory properties, IL-10 promotes immune evasion and viral infection persistence. As a result, the current study's findings align with existing literature. According to a study by Han et al. (3), when compared to healthy controls, HCV patients had substantially high levels of IL-10. The viral load was found to be directly proportional to the IL-10 values. Additionally the study also demonstrated a fall in IL-10 levels following successful antiviral therapy, especially in rapid and early virological responders. Based on Kishida (2), in HCV patients IL-10 induction results in the formation of Treg cells and suppression of $CD4^+$ T cell function. Additionally, NK cells are also adversely regulated by IL-10. A similar finding was reported by Ribeiro et al. (4), showing that OBI/HCV patients had significantly higher levels of IL-10 than controls. The reduced IL-10 levels observed in the post-treatment group in the present study may indicate attenuation of the immunosuppressive cytokine milieu associated with chronic HCV infection.

According to the current study's findings, Th1 and Th2 cytokines have complex interactions in chronic HCV, and these interactions differ across patient groups with distinct treatment status. The observed higher levels of Th1 cytokines and lower levels of Th2 cytokines (though still elevated in comparison to healthy controls) in the post-treatment patient group in this study suggest partial immune rebalancing associated with antiviral therapy status. This highlights the importance of effective treatment in the management of chronic HCV. Nevertheless, to further elucidate the mechanisms underlying persistent Th2 cytokine elevation following treatment, more research is required to examine the causes of the ongoing elevation of Th2 cytokines after treatment, particularly in relation to confirmed virological clearance.

An important limitation of this study is the lack of complete data on SVR status and the exact timing of post-treatment sample collection. As a result, it was not possible to determine whether the observed cytokine changes, including increased IFN-γ and reduced IL-4 and IL-10 levels, are directly associated with virological cure or influenced by variability in post-treatment intervals. The post-treatment group in this study represents patients who completed antiviral therapy, but heterogeneity in virological outcomes and timing of immune assessment may have influenced cytokine profiles. Nevertheless, further studies with confirmed SVR status and standardized post-treatment sampling are required to determine the mechanisms underlying persistent Th2 cytokine elevation following antiviral therapy.

Study Limitations

This study has certain limitations. The cross-sectional design involving independent cohorts limits inference on within-subject longitudinal immune changes, and the observed cytokine differences should therefore be interpreted as associations with treatment status rather than causal or treatment-induced immune restoration. Detailed virological outcome data, including SVR status and exact timing of post-treatment sample collection, were not uniformly available, precluding stratified analyses based on virological cure and limiting causal interpretation of treatment-associated immune changes. Although all post-treatment patients received DAA-based therapy, variability in treatment regimens and duration may have influenced cytokine profiles. Additionally, pediatric patients were included only in the chronic HCV cohorts, while healthy controls comprised adults exclusively, introducing potential age-related confounding. Finally, cytokine levels were measured at a single time point, which may not fully capture the dynamic nature of immune responses in chronic HCV infection.

Despite these limitations, the study provides valuable insight into cytokine profiles associated with treatment status in chronic HCV infection and highlights areas for future longitudinal investigation. These findings should be interpreted in the context of unavailable SVR and post-treatment timing data.

Conclusion

Cytokine profiling revealed distinct immune signatures among healthy controls, treatment-naïve chronic HCV patients, and post-treatment patients. Treatment-naïve patients exhibited a predominantly Th2-skewed cytokine profile characterized by significantly elevated IL-4 and IL-10 levels when compared with healthy controls. Concurrently, the principal Th1 antiviral cytokine, IFN-γ, remained markedly lower than that observed in post-treatment patients, whereas IL-2 levels were elevated despite the absence of a corresponding increase in IFN-γ. This pattern suggests an altered immune milieu associated with active chronic HCV infection. In contrast, post-treatment patients demonstrated higher IFN-γ and IL-2 levels together with reduced IL-4 and IL-10 levels when compared with treatment-naïve patients, indicating a shift towards a Th1-associated cytokine profile. However, Th2 cytokine levels remained higher than those of healthy controls, suggesting only partial attenuation of the immunoregulatory environment rather than complete immune normalization. These findings indicate that cytokine profiling may serve as a useful adjunct for assessing immune status in chronic HCV infection and provide further insight into immune modulation associated with antiviral treatment. Prospective longitudinal studies with standardized post-treatment follow-up and confirmed SVR are warranted to further elucidate the relationship between viral clearance and cytokine dynamics.

Ethics

Ethics Committee Approval: The Institutional Human Ethics Committee of Dr. ALM PG IBMS, University of Madras (approval no: UM/IHEC/F.RM/2021-II, date: 12.07.2021) approved the study.

Informed Consent: A questionnaire with the participants' medical history was obtained and all adult patients and the guardians of pediatric patients provided written informed consent.

Acknowledgments

The authors gratefully acknowledge Dr. M. Elanchezhiyan, former Professor and Head, Department of Microbiology, University of Madras, for facilitating the initial administrative and ethical approval processes during the commencement of this research when the first author was registered under his supervision. The authors also thank the University of Madras for approving the subsequent transfer of PhD guidance.

Footnotes

Authorship Contributions

Surgical and Medical Practices: J.R., Concept: J.R., R.B., Design: J.R., R.B., Data Collection or Processing: J.R., Analysis or Interpretation: J.R., D.K., Literature Search: J.R., Writing: J.R., D.K., R.B.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare no financial support.

Supplementary Tables Links: https://d2v96fxpocvxx.cloudfront.net/cf9d60d6-523c-458a-a2e6-78728d3ffbb0/content-images/1382a128-1c9a-4d15-b063-dd8785e67670.pdf

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