

IMMUNOLOGICAL REFLEX OF TLR-4 AND TNF-AN OF HEPATITIS C VIRUS  
INFECTED PATIENTS IN BABYLON PROVINCE<sup>\*1</sup>Raheem Tuama Obayes Al –Mammori, <sup>2</sup>Marwan Hamid Al- Alwany, <sup>3</sup>Ammar Malik Dokhin<sup>1</sup>Clinical Immunology / PCR and Immunology Unit, Babylon GIT Center. Babylon, Iraq.<sup>2</sup>Endoscopy Unit / Babylon GIT Center. Babylon, Iraq.<sup>3</sup>Endoscopy Unit / Babylon GIT Center. Babylon, Iraq.

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**ABSTRACT**

**Background:** Hepatitis C virus (HCV) infection is a major global health problem and a leading cause of chronic liver disease. Viral load assessment by real-time polymerase chain reaction (RT-PCR) is the standard method for diagnosing active infection and predicting therapeutic outcomes. Immunological pathways, particularly Toll-like receptors (TLRs) and pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ), may play a role in disease progression and chronicity. This study aimed to evaluate the prognostic significance of Toll-like receptor-4 (TLR-4) and TNF- $\alpha$  levels in patients with HCV infection and to assess their association with viral load.

**Methods:** A case–control study was conducted at the Babylon Gastrointestinal and Liver Center from January to September 2023. Fifty patients with confirmed HCV infection were compared with 50 apparently healthy individuals. Diagnosis was established by positive screening tests and confirmed using HCV RNA quantification via RT-PCR. Serum levels of TLR-4 and TNF- $\alpha$  were measured, and statistical analysis was performed using SPSS software.

**Results:** Most patients exhibited a low viral load, with a higher proportion of females affected than males. A statistically significant decrease in TLR-4 levels and a significant increase in TNF- $\alpha$  concentrations were observed in HCV patients compared with controls. These findings suggest an altered immune response associated with HCV infection.

**Conclusion:** Viral load assessment, together with immunological markers such as TLR-4 and TNF- $\alpha$ , may provide valuable insights into disease prognosis and management in HCV patients. Reduced TLR-4 and elevated TNF- $\alpha$  levels may be associated with disease progression and liver damage. Further large-scale studies are recommended to explore their role in predicting cirrhosis and long-term outcomes.

**KEYWORDS:** Immunological, reflex, TLR-4, TNF, Hepatitis C virus.**INTRODUCTION**

Hepatitis C virus (HCV) infection remains a major global public health problem, causing both acute and chronic liver disease that can progress to liver cirrhosis and hepatocellular carcinoma (HCC). The virus is primarily transmitted through blood exposure, including unsafe medical practices, blood transfusion, and intravenous drug use. It is estimated that more than 70 million individuals worldwide are chronically infected with HCV, with significant morbidity and mortality associated with its complications.<sup>[1]</sup> In response to this burden, global strategies have aimed to eliminate viral hepatitis as a public health threat by reducing incidence

and mortality rates.<sup>[1]</sup> HCV is considered one of the most important causes of liver disease worldwide. The detection and quantification of HCV RNA remain the gold standard for diagnosis, while viral load and genotype are critical determinants of disease progression and response to antiviral therapy.<sup>[2,3]</sup> Viral load, in particular, serves as an important predictor of treatment outcomes and disease severity, as higher viral loads are often associated with increased hepatic damage and chronicity of infection.<sup>[3]</sup> The innate immune response plays a central role in the early defense against HCV infection. Toll-like receptors (TLRs), especially Toll-like receptor 4 (TLR4), are key components of this response,

recognizing pathogen-associated molecular patterns and initiating signaling cascades that lead to the production of pro-inflammatory cytokines.<sup>[4,5]</sup> Activation of TLR4 triggers intracellular signaling pathways that promote the release of cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), and interferons, which contribute to antiviral defense mechanisms.<sup>[5,6]</sup> TLR4 has been implicated in the pathogenesis of various viral infections, including HCV, where it influences both immune activation and disease progression. Studies have shown that HCV infection can modulate TLR4 expression, leading to altered immune responses and contributing to viral persistence.<sup>[7,8]</sup> Furthermore, TLR4 signaling has been associated with hepatic inflammation, fibrosis progression, and carcinogenesis, suggesting its potential role as a biomarker for disease severity.<sup>[9,10]</sup> Tumor necrosis factor-alpha (TNF- $\alpha$ ) is a key pro-inflammatory cytokine involved in the regulation of immune responses during HCV infection. It is produced by multiple cell types, including macrophages, lymphocytes, and hepatic cells, and plays a dual role in both antiviral defense and liver injury.<sup>[11]</sup> Elevated TNF- $\alpha$  levels have been linked to chronic inflammation, hepatocellular damage, and progression to fibrosis and cirrhosis.<sup>[12]</sup> Moreover, interactions between TLR4 signaling and TNF- $\alpha$  production highlight the importance of innate immune pathways in shaping disease outcomes.<sup>[13]</sup> Chronic HCV infection is characterized by persistent immune activation and dysregulation, which may lead to immune exhaustion and impaired viral clearance. Cytokine imbalance and altered TLR expression contribute to the complexity of host-virus interactions and influence disease progression and

therapeutic response.<sup>[14]</sup> Several host-related factors, including age, gender, immune status, and metabolic conditions, further modulate the risk of fibrosis and disease severity.<sup>[15]</sup> Despite advances in understanding HCV immunopathogenesis, limited data are available regarding the relationship between TLR4 expression, TNF- $\alpha$  levels, and viral load in Iraqi patients. Therefore, the present study aimed to evaluate the role of TLR4 and TNF- $\alpha$  in HCV-infected patients and to explore their association with viral load as indicators of disease prognosis.

**METHOD**

In order to assess the efficacy of TLR-4 and TNF-a in the prognosis of hepatitis C infection, 50 patients were selected as HCV infected and 50 individuals who appeared to be healthy were selected as control samples. The viral load in RT-PCR was confirmed for all patients who had a positive screening test. The Saccacie procedure was employed to conclude the viral burden in the tower thermocycler at the GIT and liver center in Babylon from January to September 2023. The TLR-4 and TNF-a, as well as HCV antibody, were analyzed using the manual ELISA principle of Elabscience Company. SPSS was employed to conduct the statistical analysis of the data in the form of Chi-Square, ANOVA, T-Test, and Correlation.

**RESULTS AND DISCUSSION**

The viral load distribution indicated that the majority of patients had a low viral load (< 20.000 iu/ml) at (36: 72%), while only 14: 28% of patients had a viral load > 20.000 iu/ml at P. value < 0.05. as illustrated in table (1).

**Table 1: The viral load distribution of studied patients.**

HCV infected patients		Frequency	Percent	P-Value
Viral Load	< 20.000	36	72%	0.006
	>20.000	14	28%	
Total		50	100%	

The female patients have higher percentage than male (29:58%, 21: 42%) respectively at p value < 0.05 as in table (2).

HCV infected patients		Frequency	Percent	P.Value
Gender	Male	21	42%	0.042
	Female	29	58%	
Total		50	100%	

This table show that statistically significant decreased in TLR-4 concentration in hepatitis patients. While increased in TNF-a concentration in hepatitis patients after comparison with control.

**Table 3: The result of TLR-4 and TNF-a concentration in HCV patients.**

Group	Variable	N	Mean	SD	P. Value
TLR-4	Test	50	8.25	3.61	0.000
	Control	50	12.25	2.93	
TNF-a	Test	50	50.40	7.33	0.000
	Control	50	31.56	86	

The level of TLR-4 in association with viral load show significant decreased (P < 0.05) in < 20.000 viral load, and > 20.000, after comparison with control at (M $\pm$  SD,

8.24 $\pm$  3.92, 7.66  $\pm$  2.86 and 12.25  $\pm$  2.93) respectively, as mentioned in figure (1). This result might be referring to that increased of viral concentration lead to lower the

signaling activity of TLR-4, in which show that the strong activity of virus against hepatic tissue or cells.

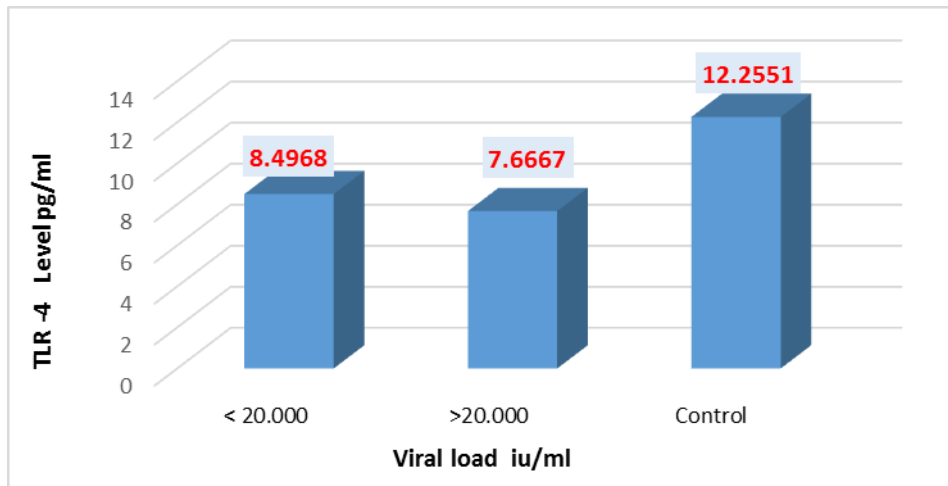


Figure 1: The relationship between TLR-4 and Viral load of Hepatitis C patients.

In comparison to the control group, the level of TNF-a is significantly increased ( $P < 0.05$ ) in viral loads less than 20.000 and greater than 20.000 ( $M \pm SD$ ,  $49.25 \pm 17.2$ ,  $53.07 \pm 18.29$ , and  $31.56 \pm 4.86$ , respectively), as illustrated in Figure 2. This finding may indicate that

pro-inflammatory cytokines (TNF-a) exhibit greater activity against viral particles and may suggest that the infection is more severe on liver cells and tissues due to the nature of TNF-a's role in the clearance of apoptotic cells.

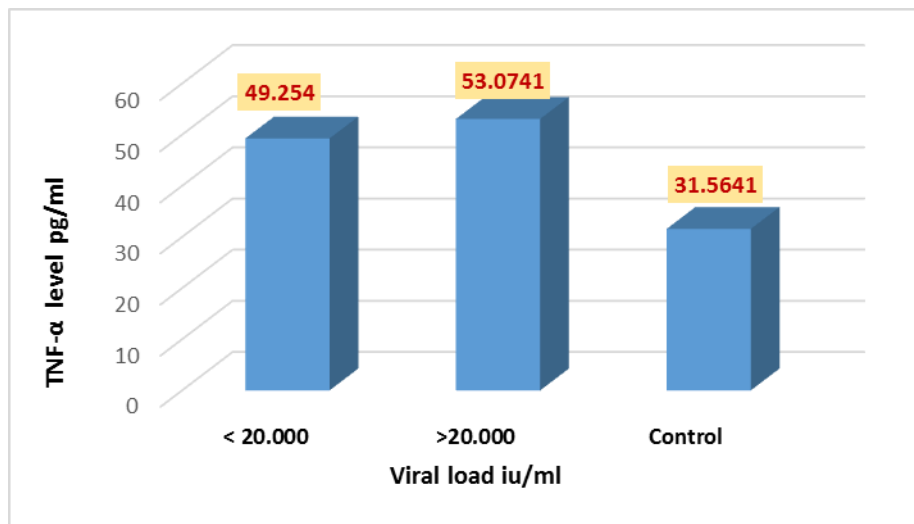


Figure (2): The relationship between TNF-an and Viral load of Hepatitis C patients.

The correlation between TLR-4 and TNF-a indicates an indirect relationship between the two. This result may indicate that an increase in TLR-4 results in a decrease in

TNF-a, as illustrated in story (4). This result provides insight into the severity of the disease or its prognosis.

Table 4: Correlation between TLR-4 and TNF-a among Hepatitis B- patients.

Correlations		TLR-4	TNF-a
TLR-4	Pearson Correlation	1	-.363**
	Sig. (2-tailed)		.006
	N	56	56
TNF-a	Pearson Correlation	-.363**	1
	Sig. (2-tailed)	.006	
	N	56	56

## DISCUSSION

The present study evaluated the relationship between hepatitis C virus (HCV) viral load and the immunological markers Toll-like receptor 4 (TLR-4) and tumor necrosis factor-alpha (TNF- $\alpha$ ) in infected patients. The findings revealed that the majority of patients had a low viral load (<20,000 IU/mL), which may indicate an early or relatively controlled stage of infection. Viral load is considered a key determinant of treatment response and disease progression, and it plays an essential role in guiding therapeutic decisions.<sup>[4,15]</sup> The predominance of low viral load observed in this study is consistent with previous reports indicating that viral load and genotype are important predictors of treatment outcomes among HCV-infected patients.<sup>[4,9]</sup> However, regional variations in genotype distribution, particularly genotype 4 in Middle Eastern populations, may influence disease progression and response to therapy.<sup>[1,16]</sup> These findings emphasize the importance of considering both viral characteristics and host factors in disease management. In the present study, a significant decrease in TLR-4 levels was observed in HCV patients compared with healthy controls. This finding differs from some previous studies reporting increased TLR expression in chronic HCV infection.<sup>[17]</sup> Reduced TLR-4 expression may reflect viral immune evasion strategies, as HCV has the ability to modulate innate immune signaling pathways to enhance its persistence.<sup>[7,12]</sup> TLR-4 is essential for recognizing viral components and initiating immune responses; thus, its downregulation may impair effective antiviral defense mechanisms.<sup>[2,3]</sup> Conversely, TNF- $\alpha$  levels were significantly elevated in HCV-infected patients compared to controls. TNF- $\alpha$  is a major pro-inflammatory cytokine that contributes to hepatic inflammation and immune activation. Increased TNF- $\alpha$  levels may reflect ongoing inflammatory processes within the liver and the host response to viral infection.<sup>[5,18]</sup> Chronic elevation of TNF- $\alpha$  has been associated with hepatocellular injury, fibrosis progression, and the development of hepatocellular carcinoma.<sup>[6,19]</sup> The inverse correlation observed between TLR-4 and TNF- $\alpha$  in this study suggests a complex interaction between innate immune signaling and inflammatory responses. This negative association may indicate that decreased TLR-4 activity is accompanied by increased inflammatory cytokine production, contributing to disease severity and tissue damage. Similar interactions between TLR signaling and cytokine production have been reported in previous studies.<sup>[5,20]</sup> Regarding demographic characteristics, the current study showed a higher prevalence of HCV infection among females, which contrasts with some epidemiological data indicating higher infection rates in males due to increased exposure to risk factors such as unsafe practices and high-risk behaviors.<sup>[21]</sup> Such discrepancies may be attributed to local population characteristics and healthcare accessibility. Chronic HCV infection is characterized by persistent immune activation and dysregulation, leading to immune exhaustion and impaired viral clearance. Cytokine imbalance, including

increased TNF- $\alpha$  and altered TLR signaling, plays a critical role in disease progression and the development of complications such as fibrosis and cirrhosis.<sup>[19]</sup> Additionally, genetic polymorphisms in immune-related genes, including TLRs and cytokines, have been shown to influence viral clearance, disease susceptibility, and response to therapy.<sup>[22]</sup> Furthermore, HCV infection induces inflammatory signaling pathways that contribute to liver injury and carcinogenesis. The interaction between TLR-4 signaling in Kupffer cells and hepatocytes may represent a key mechanism linking chronic inflammation to hepatocellular carcinoma.<sup>[13,18]</sup> These findings highlight the importance of understanding the immunological mechanisms underlying HCV infection. Despite these findings, the present study has limitations, including a relatively small sample size and single-center design, which may limit the generalizability of the results. Future studies with larger sample sizes and broader immunological profiling are recommended to better elucidate the role of TLR-4 and TNF- $\alpha$  in HCV pathogenesis and their potential as prognostic biomarkers.

## CONCLUSION

The investigation is the first to examine the distribution of HCV viral load and TLR and TNF- $\alpha$  in Babylon HCV-infected patients. The viral load may provide insight into the treatment protocol, which is crucial for the patient's management and prognosis. The chronicity or tissue injury that has been associated with decreased TLR-4 and increased TNF- $\alpha$  levels is suggested by the negative correlation between both markers (TNF- $\alpha$  and TLR-4). A large-scale study was suggested for the assessment of liver cirrhosis or hepatocellular carcinoma in conjunction with TLR-4 and TNF- $\alpha$ . In addition to the patient's response to the treatment.

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