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Comparison of Viperin and IFN- α in HBV and HCV infections

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Abstract

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Background: Viperin is an interferon-stimulated gene (ISG) with broad-spectrum antiviral activity, primarily induced by interferon-alpha (IFN-α). Both are critical components of the innate immune response against hepatitis B virus (HBV) and hepatitis C virus (HCV). This study investigates sex-based differences in viperin gene expression and IFN-α levels among patients infected with HBV and HCV

Methods: Eighty blood samples were collected from patients with confirmed HBV (n=40) or HCV (n=40) infections in Thi-Qar, Iraq. IFN-α levels were measured using ELISA, while viperin expression was assessed through qRT-PCR. Statistical analyses were performed to compare values between male and female patients within each infection group.

Results: In HCV patients, females showed slightly higher viperin expression and IFN-α levels compared to males, though the differences were not statistically significant. However, in HBV patients, viperin expression was significantly higher in females than males ($p = 0.020$), suggesting enhanced innate immune activation. IFN-α levels in HBV patients did not differ significantly by sex.

Conclusion: The study highlights a significant sex-based difference in viperin expression among HBV-infected patients, with higher levels in females, potentially indicating a stronger innate immune response. No sex-related differences in IFN-α levels were observed in either group. These findings suggest that sex-based immune modulation, particularly in HBV, may inform personalized treatment approaches and warrant further investigation.

1. Introduction

Viperin (Virus Inhibitory Protein, Endoplasmic Reticulum-associated, Interferon-inducible) is a crucial interferon-stimulated gene (ISG) that exhibits broad-spectrum antiviral activity. It belongs to the radical S-adenosylmethionine (SAM) enzyme family and mediates antiviral effects through multiple mechanisms including inhibition of viral replication, modulation of cellular lipid metabolism, and interference with virus assembly and release (1,2). Viperin expression is mainly induced by type I interferons, especially interferon-alpha (IFN-α), which is secreted from host cells within a few hours following viral infection. Interferons are a family of cytokines important in the innate immune response in which infected cells signal uninfected cells to lift their antiviral state. IFN-α, in particular is very effective at upregulating ISGs, such as viperin, which function to limit viral replication and spread. IFN-α induces the JAK-STAT signaling pathway resulting in the expression of a number of ISGs, such as viperin, to promote the innate antiviral defenses of the host (3,4,5). Because of heptotropic viral infection such as hepatitis B or C virus (HBV and HCV), both IFN-α and viperin are considered to be the key players for an innate immunity. However, these viruses have developed multiple strategies to escape the IFN-mediated antiviral response, allowing to persist in some patients (6,7).

The aim of the present study is to assess and to compare the viperin gene expression and the concentration of IFN-α in male and female HBV- and HCV-infected patients. The study is intended to provide insight into sex differences in innate immune responses that could influence differences in disease progression and therapeutic efficacy, and could support the rational development of sex-based treatment strategies.

2. Materials and Methods

2.1. Blood Samples

This investigation involved 80 patients diagnosed with HBV or HCV infections. Between October 2024 and January 2025, 40 serum samples positive for HCV were obtained from the Dialysis Center in Al-Nasiriyah, while 40 HBV-positive samples were collected from private diagnostic laboratories within the same region. The diagnosis of both viral infections was confirmed by combining serological assays with molecular detection techniques. For each individual, 3 mL of venous blood were drawn. The collected blood samples were divided into two portions: one was allocated for the quantification of IFN-α, and the other was preserved in Eppendorf tubes for RNA extraction and subsequent gene expression analysis.

2.2. Detection of IFN-α by ELISA

The serum concentration of human interferon-alpha (IFN-α) was measured in all 80 samples using a commercially available sandwich ELISA kit (catalog number YLA1513HU; YLbiont, Shanghai YL Biotech Co., Ltd., China), according to the manufacturer's protocol. Absorbance was measured at 450 nm using a microplate reader, and IFN-α levels were calculated based on a standard curve generated during each run.

2.3. Gene Expression

The total RNA was isolated from serum samples with TRIzol™ reagent (Invitrogen, USA) according to the manufacturer's protocol. Reverse transcription into cDNA (complementary DNA) was performed with the AccuPower® RT PreMix kit (Bioneer, South Korea). Real-time PCR was carried out with AccuPower® GreenStar™ qPCR PreMix and the viperin gene specific primers. The amplification program began with an initial denaturation at 95°C for 5 min and continued for 40 cycles at the following temperatures: 20 sec at 95°C followed by 45 sec at 60°C. Fluorescent signals were detected in each cycle, and the relative viperin expression levels were determined.

2.4. Statistical Analysis:

The samples underwent statistical analysis according to Statistical Package for the Social Sciences (SPSS) Chi-square ($\chi 2$), and the p-value indicated a significant level between the samples.

3. Results

The comparison of viperin expression between male and female patients infected with HCV. As presented in the table (1), the mean expression in females was 40.74 ± 2.47, while in males it was 39.81 ± 2.45. Although females exhibited slightly higher viperin levels, the difference was not statistically significant ($t = -0.65$, $p = 0.535$), indicating that sex did not significantly affect viperin expression in HCV-infected patients.

Table (1): Viperin value in patients with HCV

Parameters	Male Mean ±SD	Female Mean ±SD	t. test	P. value
Viperin	39.81 ±2.45	40.74 ±2.47	-0.65	0.535

The analysis of viperin expression levels among HBV-infected patients revealed a significant difference between sexes. Female patients had a higher mean value of 40.15 ± 2.14 compared to 37.04 ± 1.27 in males, as indicated in Table (2). The difference was statistically significant ($t = -2.89$, $p = 0.020$), suggesting that viperin gene expression is significantly higher in females than in males in the context of HBV infection.

Table (2): Viperin value in patients with HBV

Parameters	Male Mean ±SD	Female Mean ±SD	t. test	P. value
Viperin	37.04 ±1.27	40.15 ±2.14	-2.89	0.020*

The interferon-alpha (IFN-α) levels in HCV-infected patients are summarized in **Table (3),** which shows that female patients had a higher average concentration (172.7 ± 110.6) than males (142.80 ± 78.79). However, this difference did not reach statistical significance (t = -0.54, p = 0.58), indicating that sex did not have a significant effect on IFN-α production in HCV-infected individuals.

Table (3): IFN- α value in patients with HCV

Parameters	Male Mean ±SD	Female Mean ±SD	t. test	P. value
IFN- α	142.80 ± 78.79	172.7± 110.6	-0.54	0.58

As shown in Table (4), interferon-alpha (IFN-α) levels among HBV-infected individuals were slightly higher in males (67.55 ± 55.20) compared to females (50.27 ± 40.11). However, this difference was not statistically significant (t = 0.820, p = 0.417), indicating that sex had no significant impact on IFN-α levels in patients with HBV infection.

Table (4): IFN value in patients with HBV

Parameters	Male Mean ±SD	Female Mean ±SD	t. test	P. value
IFN- α	67.55± 55.20	50.27± 40.11	0.820	0.417

4. Discussion

Sex-based differences in viperin gene expression and IFN-α levels among HBV- and HCV-infected patients revealed distinct patterns in immune responses, with some findings aligning with established literature and others reflecting local nuances that warrant further investigation. In HCV-infected individuals, female patients exhibited marginally higher viperin levels compared to males, though this difference did not reach statistical significance. This observation is consistent with prior reports indicating that HCV suppresses the induction of interferon-stimulated genes (ISGs) including viperin via viral proteins such as NS3 and NS5A, which neutralize the advantages typically seen in female immune responses (8,9). (8) noted that HCV protein interfere with interferon signaling pathways, offering a plausible explanation for the minimal sex-based variation in viperin expression observed among HCV patients. In contrast, HBV-infected females demonstrated significantly higher viperin expression than males, supporting broader evidence of stronger innate immune activation in women driven by factors such as estrogen and X-linked immune genes (10). (11) further affirmed that females usually develop more intense innate, humoral and cellular immune responses to viral infections, emphasizing estrogen's role in enhancing interferon pathways within hepatic tissue. Furthermore, the more restricted capacity of HBV as compared with HCV to inhibit ISG expression would bias the occurrence of these immune-sex differences to be more apparent (6). Regarding HCV-infected patient IFN-α levels, no significant difference in sex was found, but the mean levels were higher in women than men. This is consistent with the overall consensus that females react with more potent type I interferon responses, as articulated by (12): Females are known to show stronger Type I IFN responses and lower susceptibility to viral infections compared to males. However, the chronic nature of HCV and its potential to suppress IFN signaling can abrogate these differences through time, and both sexes might converge in IFN-α levels. Notably, in HBV-infected subjects, we also observed a sex bias characterized by slightly higher levels of IFN-α in males when compared to females, in contrast to that reported previously. Such anomaly might be due to the chronic infection related immune exhaustion or hormonal modulation. (9) reported that chronic HBV infection can suppress overall innate immune activation, whereas (12) stress that the impact of sex may differ at disease stages and with hormonal status.

5. Conclusion:

The present study investigated sex-based differences in viperin gene expression and interferon-alpha (IFN-α) levels among patients infected with HBV and HCV. The result showed that the expression levels of viperin were much higher in female patients than male patients with HBV, which indicated a more effective innate immune response. However, no significant differences regarding expression of viperin in relation to sex for HCV patients or IFN-α levels for HBV and HCV group were observed. These findings indicate that viperin may serve as a sensitive parameter for immune status in sex difference, especially in HBV infection, and suggest the sophisticated interaction between the viral counteraction and the host biological factors. Larger studies with more immune parameters are needed to clarify clinical relevance of these findings.

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7. Ethical clearance:

This research is subjected to the principles of ethical issues and with the form designed especially for this study by the Iraqi MoH. The study was also approved by the Committee of Ethics Standards in the Science College, Thi-Qar University, one of the colleges of higher secondary education and scientific research, Iraq. Moreover, all patients were informed and give informed consent before of we take sample by Document No. 1394/ 11/3dated (14/10/2024).

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