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ORIGINAL ARTICLE

Gene Expression of Viperin as A Key of Innate Response in HBV and HCV Infection

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ABSTRACT

Background: *Viperin is an essential antiviral protein its gene activated by interferons and plays a vital role in the innate immune response to viral infections.* ***Objective:*** *This study examined long-term cases of hepatitis B and C to evaluate viperin expression and function during viral infection.* ***Methodology:*** *The study included 80 patients ,40 with HBV and 40 with HCV. Peripheral blood samples were analyzed for viperin gene expression, serum interferon levels, and complete blood count (CBC). Viperin expression was measured using quantitative real-time PCR.* ***Results:*** *HBV patients showed mild lymphocytosis, whereas HCV patients exhibited mild leukocytosis, indicating differing immune responses between the two infections. Elevated serum interferon levels in all patients indicated an active immune response. Viperin gene expression was detected in 22 out of 80 samples: 10 in the HBV group (25%) and 12 in the HCV group (30%), with no statistically significant difference between the groups. Although viperin was expressed in both groups, the lack of significant difference suggests both viruses may activate viperin similarly despite their differing features.* ***Conclusion:*** *These results suggest that viperin induction is a general aspect of antiviral innate immunity and may not vary significantly across different virus species. However, the lack of sufficient viperin activation, despite elevated interferon levels, could contribute to inadequate viral clearance, potentially leading to chronic infection or even carcinogenesis. To better understand the regulatory mechanisms and consequences of viperin activity in viral infections, further investigations are necessary.*

INTRODUCTION

A variety of virus recognition sensing mechanisms prevent a number of viruses from reproducing. A variety of virus recognition sensing mechanisms combine to provide the common, highly effective pathways that support innate immunity. The system of interferon (IFN) is a common cellular defense against infections; it gets its name from its ability to prevent viral multiplication. This process exists in nearly every mammalian cell that has a nucleus and, when activated, results in hundreds Numerous interferon-stimulated genes (ISGs) possess direct antiviral characteristics. An example of an IFN-inducible protein that was identified roughly 20 years ago is viperin, which can prevent a number of viruses from reproducing. [1]. Viperin is an endoplasmic reticulum-associated viral inhibitory protein that is interferon-inducible. [2]. Numerous viruses, including HIV [3], the hepatitis B virus[4], and the human cytomegalovirus [5], have been linked to viperin and other viral types, including flaviviruses, which include the tick-borne encephalitis virus, dengue virus, hepatitis C virus, and Zika virus[6,7,8]. However, viperin's mechanism of inhibiting viral replication is yet unknown. [9]. The main antiviral mechanisms that have been proposed include localization to the endoplasmic reticulum (ER) and lipid droplets to stop the growth of viruses [10]. Guidance of viral proteins toward the ubiquitination pathway for degradation [11]. Activation of innate immune response pathways through interactions with signaling proteins within cells; [12]. Inhibition of viral genome replication through the synthesis of 3'-deoxy-3',4'-didehydro-cytidine triphosphate (ddhCTP) or interaction with viral replication complexes[13].

Aim of the Study

Viperin and interferons' function as an innate immune response against HBV and HCV infections was assessed in this study using the following goals: 1. Use a hematological analyzer to determine the proportion of neutrophils and lymphocytes in patients infected with HBV and HCV. 2. Use ELISA to determine those patients' levels of alpha interferon. 3. Use quantitative real-time PCR to examine viperin gene expression in HBV and HCV patients and assess the protein's function as an antiviral in the immune response.

METHODOLOGY

Ethical clearance:

This study is subjected to the qualifications of ethical considerations and according to the form prepared for this purpose by the Iraqi Ministry of Health. The research also got the agreement of the committee of ethical standards at the College of Science, Thi-Qar University, one of the colleges belonging to the Ministry of Higher Education and Scientific Research, Iraq. In addition, informed consent was obtained from all patients before we took samples.

Blood Samples

This study included 80 patients diagnosed with either hepatitis B or C infections. Between October 2024 and January 2025, 40 HCV-positive samples were obtained from the Dialysis Center in Al-Nasiriyah, Iraq, while 40 HBV-positive samples were collected from private laboratories within the same city. The diagnosis of HBV and HCV infections was confirmed using both serological and molecular methods. For each patient, 3 mL of venous blood were collected. The samples were processed as follows: one portion was used for complete blood count (CBC) analysis, another portion was allocated for interferon-alpha (IFN-α) testing using ELISA, and the remaining volume was transferred to Eppendorf tubes for RNA extraction and gene expression.

Detection of IFN-α by ELISA

The human interferon-alpha (IFN-α) levels in 80 serum samples were determined in accordance with the manufacturer's instructions using a commercial sandwich ELISA kit (YLA1513HU, YLbiont, Shanghai YL Biotech Co, Ltd, China).

Gene expression

Total RNA was manually extracted from all 80 serum samples using the TRIzol™ reagent, following the instructions provided by the manufacturer. After completion of the extraction process, the RNA was used to synthesize complementary DNA (cDNA). This step was performed using the AccuPower® RT PreMix kit supplied by Bioneer, South Korea. The viperin gene expression was conducted using real-time polymerase chain reaction (qRT-PCR). For this purpose, the AccuPower® GreenStar™ qPCR PreMix kit (Bioneer, South Korea) was used, along with primers specific to the viperin gene *(viperin1-F* and *viperin1-R).* Sample preparation involved preparing the positive control, negative control, and cDNA samples. All components were mixed and vortexed to ensure proper homogenization.

RT-PCR Setup:

After the preparation, the tubes were placed in the PCR machine. The following cycling conditions were applied:

Step	Temp.	Time	Number of Cycles
Pre-denaturation	95°C	5 min	1
Denaturation	95°C	20 sec	40
Annealing & Extension	60°C	45 sec	40

- Fluorescence readings were recorded at each cycle to quantify the expression level.

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.

RESULTS

Distribution of patients according to age group

According to the patient age distribution, (35%) of HBV patients were between the ages of 35 and 49, whereas (35%) of HCV patients were between the ages of 50 and 64. However, the two groups' age distributions did not differ in a way that was statistically significant, as shown in Table 1.

Table 1: Distribution of patients according to age group

Age group	HCV n (%)	HBV n (%)	P. value
20-34	10 (25%)	12 (30%)	0.431
35-49	9 (22.5%)	14 (35%)	
50-64	14 (35%)	10 (25%)	
65-80	7 (17.5%)	4 (10%)	
Total	40 (100%)	40 (100%)	

df=3, p. value ≤0.05 mean significant

Distribution of patients according to sex

HCV infection was more prevalent in females, with 23 females (57.5%) and 17 males (42.5%) affected. In contrast, HBV infection showed a higher prevalence in males, with 28 males (70%) and 12 females (30%) infected, as indicated in Table 2. These findings highlight a notable difference in the sex distribution of both infections.

Table 2: Distribution of patients according to sex

Sex	HCV n (%)	HBV n (%)	P. value
Man	17 (42.5%)	28 (70%)	0.01*
Women	23 (57.5)	12 (30%)	
Total	40 (100%)	40(100%)	

df= 1, p. value ≤0.05 mean significant

CBC values in HCV & HBV patients

Table (3) showed that the HBV and HCV groups had white blood cell (WBC) counts of 6.43 ± 2.26 and 5.51 ± 2.19, respectively. A t-test analysis showed no statistically significant difference between the two groups, with a t-value of -1.70 and a p-value of 0.09. The mean neutrophil count (NEU) for the HCV group was 58.03 ± 15.54, while the HBV group's was 46.11 ± 18.96. The HCV group exhibited higher NEU levels, and a statistically significant difference was demonstrated by a t-test with a t-value of 3.07 and a p-value of 0.003. The mean lymphocyte percentage (LYM%) was significantly higher in HBV patients (37.56 ± 14.96) compared to HCV patients (30.22 ± 12.52), with a statistically significant difference (P = 0.02).

Table 3: CBC values in HCV & HBV patients

Parameters	HCV n (%)	HBV n (%)	t. test	P. value
Total WBC	5.51± 2.19	6.43± 2.62	-1.70	0.09
NEU%	58.03± 15.54	46.11± 18.96	3.07	0.003*
LYM%	30.22± 12.52	37.56± 14.96	-2.37	0.02*

df= 78, p. value ≤0.05 mean significant

IFN -α value in HCV & HBV patients

Interferon (IFN) levels in HCV and HBV patients differ statistically significantly, as presented in the table (4). IFN levels were greater in HCV patients (150.33 ± 95.8) than in HBV patients (107.79 ± 69.6), suggesting that IFN expression was much greater in the HCV group.

Table 4: IFN -α value in HCV & HBV patients

Parameters	HCV n (%)	HBV n (%)	t. test	P. value
IFN -α	150.33 ± 95.8	107.79± 69.6	2.41	0.02*

df=78, p.value ≤0.05 mean significant

Cycle Threshold (CT) of viperin in HBV & HCV

The mean viperin levels were slightly higher in HCV patients (40.34 ± 2.39) than in those with HBV (38.90 ± 2.37); however, as seen in Table (5), this difference did not reach statistical significance.

Table 5: Cycle Threshold (CT) of viperin in HBV & HCV

Parameters	HCV n (%)	HBV n (%)	t. test	P. value
Viperin	40.34±2.39	38.90±2.37	1.41	0.51

df= 20, p.value ≤0.05 mean significant

Viperin levels in patients

Viperin gene expression was identified in 12 out of 40 HCV patients, representing 30%, while the remaining 28 patients (70%) showed no expression. In the HBV group, 10 patients (25%) expressed the viperin gene, whereas 30 patients (75%) did not. As shown in Table (6), these results demonstrate that viperin gene expression was limited to a small portion of patients in both groups.

Table 6: Viperin levels in patients

Virus type	No. Sample	Viperin (+)	Viperin (-)
HCV	40	12 (30%)	28 (70%)
HBV	40	10 (25%)	30 (75%)

IFN -α–Viperin Correlation in Viral Hepatitis Patients

The correlation between viperin gene expression and interferon (IFN) levels was analyzed. In the overall sample, the Pearson correlation coefficient was very low (r = 0.093) and did not reach statistical significance, suggesting a lack of meaningful association regardless of viral infection type. When the analysis was stratified by viral group, no significant correlation was observed in either cohort. Among HCV patients, a negligible positive correlation was found (r = 0.01), while HBV patients showed a slightly higher, yet still weak, correlation (r = 0.08). These results indicate that viperin gene expression does not appear to be significantly linked to interferon levels in either group or in the study population as a whole illustrated in Tables (7) and (8).

Table 7: Correlation between IFN-α in both HBV and HCV infection with viperin

Correlation	Viperin	P. value
IFN -α	r= 0.093	0.76

Table 8: Correlation of IFN-α in each patient group with viperin

	Correlation	Viperin	P. value
HCV	IFN	r= 0. 01	0.97
HBV		r= 0.08	0.83

cDNA concentration

Table (9) the study samples had an average cDNA concentration of 239.20 ng/µL, with a standard deviation of 74.25.

Table 9: The cDNA concentration

	Mean	SD
cDNA concentration	239.20	74.25

DISCUSSION

According to are study, the majority of HBV infections occurred in people aged 35 to 49, whereas the majority of HCV cases were found in those aged 50 to 64 years. This age pattern wasn't statistically significant, but it matches global trends showing that older people are more likely to have HCV due to past exposure to unscreened blood and unsterilized medical tools before widespread screening began. Due to the fact that early-life or intrafamilial transmission is widespread in endemic areas, the greater frequency of HBV in the 35–49 age range may be explained. Other research has confirmed similar results, highlighting the importance of early exposure in HBV persistence [14, 15, 16]. In several studies, it was found that HBV is more common in men, while HCV is more prevalent in women. Estrogen is thought to enhance innate immunity and antiviral responses in females, improving the detection of viruses like HCV [17, 18]. Conversely, behavioral variables such as unprotected sexual activity and intravenous drug use may be the cause of male predominance in HBV [17]. Moreover, greater exposure to blood products during pregnancy may be the cause of women's higher HCV prevalence[19]. In Thi-Qar Province, a study of individuals with multi-transfused thalassemia revealed that HBV was more prevalent in men and HCV in women. This could be related to the immunological responses of the sexes and the increased probability of blood product exposure in women [20]. Similarly,a research showed that medical treatments and greater exposure during pregnancy increase the risk of HCV infection in females. These results emphasize the necessity for region-specific preventive and treatment measures, as well as the need to take hormonal and behavioral variations into account when examining the incidence of viral hepatitis between sexes [21].

Chronic HBV patients in this study had higher lymphocyte (LYM) levels, which is in line with other studies that found an increase in lymphocytes as a result of immunological responses against the virus [22]. Lower lymphocyte counts were noted in chronic HBV patients, according to another research [23]. This suggests that the prolonged viral contact has exhausted the immune system[24].

Higher lymphocyte counts were also seen in chronic HCV patients, underscoring the role of lymphocytes in the immunological response to HCV[17]. Nonetheless, other research pointed out that the degree of the sickness may affect the lymphocyte growth[18]. Higher amounts of neutrophils (NEU) and white blood cells (WBC) were seen in HCV patients, which is in line with a research that links these markers to the chronic inflammation brought on by HCV[17].[25]. On the other hand, another research revealed that chronic HBV patients had reduced WBC and NEU levels, which may indicate a weakened immune response in persistent infections[23]. The severity of the illness, genetic differences, viral exposure, and whether the infection is acute or persistent can all affect the results of different research. Immunological responses and immunological marker levels, such as LYM, WBC, and NEU, are influenced by these factors. IFN-α levels were greater in HCV patients than in HBV patients in this research. This is understandable given that HCV increases the production of interferon by potently activating innate immune sensors such as RIG-I and TLR3[26]. However, by inhibiting interferon responses, HBV tends to evade early immune detection, which accounts for the decreased levels of IFN-α[25]. But not every study is in agreement. Even if IFN-α is high, it may not effectively manage the virus since certain chronic HCV patients exhibit interferon resistance or immunological fatigue. IFN-α levels for HBV can occasionally increase during flare-ups or therapy, albeit this is less frequent [27].

The expression of the viperin gene was found to be comparatively low in this investigation. Only 25% of HBV patients and 30% of HCV patients had detectable levels. These results are significantly less than those of earlier research. We find significant discrepancies in viperin expression levels between our results and those of another research. In HCV-infected cells, viperin expression ranged from 40% to 70%, suggesting a robust immunological response [28]. However, our analysis revealed a lower level of gene expression in HCV patients, reaching just 30% expression. Similarly, it was shown that HCV-infected liver cells expressed viperin at 50% to 60% [26]. The local immunological context in HBV-infected tissues, especially placental tissue, was shown to have increased viperin expression in the study. The placental tissue showed a stronger immune response, which was clear from comparing viperin levels in infected and non-infected tissues. An exact proportion of viperin expression in these tissues was not mentioned in the study[4].

Conversely, our research revealed that 25% of HBV patients had viperin expression, which is a lower level of expression than the patterns seen in other tissues with a robust immune response. Viperin mRNA detectability may also be impacted by other variables, such as RNA quality, illness stage, and inter-individual variability, including previous interferon therapy[28]. Even though HCV patients had greater levels of interferon-alpha, there was only a weak and statistically insignificant connection between IFN-α and viperin expression. This finding implies that there are probably more regulatory layers at play and that viperin is not only controlled by interferon levels[29]. Post-transcriptional regulation is a mechanism by which hepatitis viruses evade the immune system. Even while host cells may have viperin mRNA, RNA-binding proteins and microRNAs (miRNAs) can actively inhibit its translation into a functional protein, reducing its antiviral action even in the presence of interferon[24,28]. Furthermore, interferon-

stimulated genes like viperin can have their expression decreased by viral interference with the JAK-STAT signaling pathway, which is essential for interferon signaling.

This promotes viral persistence and immune evasion[24].

CONCLUSION:

Viperin, a crucial interferon-stimulated gene with a wide range of antiviral properties, is essential to the innate immune system's defense against viral infections. Its significance is not diminished by the fact that, despite high interferon levels, its expression was modest in the instances under study. Rather, it draws attention to how intricately it is regulated and raises the possibility that other viral or host variables may affect viperin's activation. Since an inadequate viperin response may hinder viral clearance and lead to persistent infection or illness development, it is imperative to comprehend these regulatory mechanisms.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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