

Research Article:

Immunomodulation and Molecular Diagnostic Aspects in Individuals with Hepatitis B Virus Infection

Soran Qader Abdel^{1,*}, Ahmed Farhan Shallal^{2,3}

¹Medical Laboratory Science, College of Science, University of Raparin, Rania, 46012, Sulaymaniyah, Kurdistan Region, Iraq

²College of Medicine, University of Sulaimani, Sulaymaniyah, 4600, Kurdistan Region, Iraq

³Department of Biology, College of Science, University of Raparin, Rania, 46012, Sulaymaniyah, Kurdistan Region, Iraq

<https://doi.org/10.31530/cjnst.2026.2.2.8>

Received: 06 May 2026

Revised: 03 July 2026

Accepted: 08 July 2026

*Corresponding author info: Soran

Qader Abdel,

Email: (soranqadir1994@gmail.com)

Copyright © 2026 Soran Qader Abdel, Ahmed Farhan Shallal, Charmo Journal of Natural Sciences and Technologies (CJNST).

This is an open access article published under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits use, distribution, and reproduction in any medium, provided the original work is properly cited.



Abstract

Background: Hepatitis B virus (HBV) is a major global health issue associated with severe liver complications. Outcomes depend on the immune response, which can sometimes fail, and are associated with chronic disease. Improved diagnostics and understanding of immune markers are crucial for more effective management and treatment of HBV infection.

Aims: The study aimed to investigate the association between the HBV viral load, viral antigens, including Hepatitis B surface antigen (HBsAg), Hepatitis B core antigen (HBcAg), Hepatitis B enveloped antigen (HBeAg), and pro-inflammatory cytokines, including Human Interferon γ (INF- γ), Human Tumour Necrosis Factor α (TNF- α), Soluble Human Leukocyte Antigen class I (sHLA-A), an antigen-presenting molecule, and Human soluble Cluster of Differentiation 8 (sCD8), a soluble T-cell activation marker. This study provides additional regional information on the associations between virological and immunological parameters in patients with HBV infection from the Kurdistan Region of Iraq.

Methodology: The study was a case-control study conducted on 120 individuals in the Kurdistan Region of Iraq, comprising 60 patients with HBV infection and 60 healthy controls. The investigation included both sexes aged 25 to 74 years, during the period from March to July 2025. RT-PCR was used to assess viral load, and ELISA was used to evaluate immunological markers.

Results: According to the study findings, Patients with HBV infection exhibited significantly higher immunological markers than healthy controls ($p < 0.0001$). Significant positive correlations were observed in the study between viral load and HBeAg ($r=0.9051$), as well as inflammatory cytokines INF- γ ($r=0.9189$), TNF- α ($r=0.832$), sCD8 ($r=0.888$), and HLA-A ($r=0.801$). The obtained results demonstrated significant positive correlations between viral load and immune activation markers.

Conclusion: Overall, the current study demonstrated significant positive correlations among viral load, viral antigens, and immunological markers in patients with chronic HBV infection and provides additional evidence of the immunological characteristics.

Keywords: Viral Load, RT-PCR, HBeAg, HBcAg, CD8, INF- γ , TNF- α , HLA-A

1. Introduction

A major regulatory mechanism involves the modification of immune responses by specific biobehavioural mediators, termed immunomodulation [1]. Both endogenous factors, such as cytokines and immune cells, and exogenous

factors, such as microbial products and therapeutic compounds, can act as immunomodulators of endogenous signalling pathways [2, 3]. In this way, via these modulatory processes, the immune system regulates the strength, quality, duration, and effectiveness of immune responses against pathogenic agents and some other pathological situations. In infectious diseases, it has been reported that HBV

represents a major global health problem [4, 5]. HBV infection has been reported more frequently among individuals with impaired or dysregulated immune responses. HBV is a small, enveloped DNA virus of the family Hepadnaviridae with a 3.2 kb partially double-stranded, circular genome [6]. HBV consists of an outer envelope matrix with HBsAg and a more interior nucleocapsid containing the HBcAg. A second major HBV-derived viral protein, HBeAg, is associated with viral replication and the disease phase in HBV infection; thus, these markers (HBsAg, HBcAg, HBeAg) are used to diagnose HBV infection [7, 8]. Structural and non-structural viral components are critical for virus entry, replication, and interaction with the host immune system. Background: HBV infection is a worldwide public health challenge. According to the World Health Organization (WHO), about 296 million people worldwide were living with chronic HBV in 2019, and approximately 1.5 million new infections occurred annually. Chronic HBV infection is one of the leading global causes of liver cirrhosis and hepatocellular carcinoma, accounting for more than 800,000 deaths each year [9]. HBV infection is markedly heterogeneous in its geographical distribution, with the majority of cases being detected in Asia and Sub-Saharan Africa, followed by the Middle East [10]. HBV transmission occurs through contact with infectious blood and body fluids, including sexual contact, non-sterile medical procedures, and transfusions. HBV infection is characterised by a complex interplay between viral replication and local and systemic innate and adaptive immune responses. Although cytopathic effects mediated by direct viral lysis underlie tissue injury in many viral infections, liver damage associated with HBV infection may be primarily immune-mediated [11]. Cellular immune responses, specifically those of CD8⁺ T cells, have been reported to be associated with the recognition and destruction of infected hepatocytes. These cells recognise viral peptides presented on the surface of infected cells via Major Histocompatibility Complex class I (MHC-I) molecules, including HLA molecules, and trigger antiviral immune responses [12]. Various immune mediators have been reported to be associated with different stages of the immune response in HBV infection. HLA class I molecules, particularly HLA-A, present viral antigenic peptides at the surface of infected cells and are recognised by CD8⁺ T lymphocytes to enable recognition of virus-infected cells. Cytotoxic T-cell responses rely on antigen presentation to couple viral clearance with T-cell activation. Ultimately, at least partly through cytotoxic activity and the secretion of antiviral cytokines, CD8⁺ T lymphocytes exert their antiviral effect [13]. For example, IFN- γ and TNF- α are considered key cytokines in mediating the antiviral immune response. IFN- γ and TNF- α have been widely associated with antiviral immune responses and have been reported to correlate with reduced viral replication in experimental and clinical studies, with the former enhancing antigen presentation and the latter exerting potent pro-inflammatory effects and inducing killing of infected hepatocytes. In addition to immune mechanisms, novel diagnostic techniques for HBV infection also involve molecular diagnostic methods. Internal quantification of viral DNA by Real-Time Polymerase Chain Reaction (RT-PCR) is a sensitive and

specific marker of viral load that reflects the extent of infection in individuals. Viral load measurements are a well-known surrogate marker, widely used in clinical practice for chronic viral hepatitis to assess disease progression, evaluate treatment response, and estimate the risk of complications [14]. Viral load has been associated with variations in immune responses across multiple studies, suggesting a potential relationship between viral replication and the expression of immune mediators. Many other prior studies have investigated immunological biomarkers in patients with HBV infection. Differences in CD8⁺ T-cell activity and cytokine production during HBV infection, acute, chronic and viral clearance have been reported [15]. Thus, these findings are consistent with previous studies reporting an association between elevated levels of IFN- γ and TNF- α and antiviral immune responses in patients with HBV infection. Meanwhile, the concurrent release of these proinflammatory cytokines favours viral persistence and chronic disease. Viral antigen reactivity has been associated with differences in disease severity and clinical outcomes [16]. Notably, data regarding the combined analysis of viral load and immune parameters remain limited in the Kurdistan Region of Iraq. This study provides additional region-specific information on the associations between virological and immunological parameters in patients with HBV infection from the Kurdistan Region of Iraq.

2. Materials and Methods

2.1. Materials and Instruments

The immunological and virological parameters investigated in this study were measured using commercially available kits and standard laboratory equipment. HBsAg, HBeAg, HBcAg, sCD8, TNF- α , HLA-A and IFN- γ were quantified using Enzyme-Linked Immunosorbent Assay (ELISA) kits supplied by SUNLONG (China). All ELISA assays were performed according to the manufacturer's instructions using a Chromate 4300 microplate reader. Viral nucleic acid extraction was carried out using the PureLink™ Viral RNA/DNA Mini Kit (Thermo Fisher Scientific, USA). HBV viral load was quantified by RT-PCR using the Bosphore HBV Quantification Kit (Anatolia Genetworks, Turkey) according to the manufacturer's protocol.

2.2. Study Design and Methods

A case-control study was conducted in 2025 at the Rania Central Laboratory in Rania, Kurdistan Region, Iraq. This investigation was systematically designed to evaluate the associations between molecular diagnostic parameters and immunological markers in HBV infection. The immunological markers evaluated in this study included the viral antigens HBsAg, HBeAg, and HBcAg; the pro-inflammatory cytokines IFN- γ and TNF- α ; HLA-A; and sCD8. The molecular evaluation involved DNA extraction and viral load assessment. This study includes two different groups. The first group comprised male and female patients who were diagnosed with HBV infection, and their ages were between 25 and 74 years. The second group, identified as

the control group, comprised 60 individuals without HBV infection, both male and female, aged 25–55 years. Although the groups were selected from similar geographical and socioeconomic backgrounds, a significant difference in sex distribution was observed. All participants in the current study were recruited from neighbouring areas within Sulaymaniyah governorate. Under controlled and standardised conditions, samples were collected from each person and then sectioned into two parts. The first was established to study immunological markers, and the second to conduct molecular studies. Sera were obtained by centrifugation and divided into two tubes. The first tube was used to quantify viral load by RT-PCR (Anatolia Geneworks). The second tube was used to detect viral antigens (HBsAg, HBeAg, and HBcAg). Serum-circulating HBcAg was measured to investigate its associations with inflammatory cytokines, including TNF- α and IFN- γ , and with HBeAg and viral DNA replication. Immunological markers (IFN- γ , TNF- α) and soluble HLA-A and sCD8 were measured by sandwich ELISA, and read on the Chromate 4300 microplate reader. The absorbance of the viral markers was converted to concentration using a cut-off, while the absorbance of the immunological markers was converted to concentration using the standard curve, as were HLA-A and sCD8 as well. Patients with HBV were recruited based on a confirmed laboratory diagnosis of HBV infection obtained at Rania Central Laboratory through serological and molecular testing.

2.3. Inclusion Criteria

Individuals aged 25–74 years in the HBV group and 25–55 years in the control group, with confirmed HBV infection based on serological markers, readiness to participate in the study, and provision of informed consent.

2.4. Exclusion Criteria

Patients with co-infection with other hepatitis viruses (e.g., HCV or HDV), those receiving immunosuppressive therapy, and individuals with chronic infectious diseases or severe systemic disorders were excluded from the study to minimize potential confounding factors that could influence immune responses and biomarker levels.

2.5. Ethical Considerations

Ethics approval for the study proposal was granted by the Institutional Review Board (IRB) Ethical Committee of the University of Raparin, with approval number (1228) and date (6/11/2024). Reference: 2866/28-5-2023. The IRB confirms that this study complies with all relevant ethical guidelines and has identified no legal concerns.

2.6. Statistical Analysis

All statistical analyses were performed in GraphPad Prism version 8.0.2. Shapiro examined the distribution of the data using the Shapiro–Wilk normality test. Continuous variables were assessed for normality using the Shapiro–Wilk test. Normally distributed variables were expressed as mean $\pm$ standard deviation (SD) and compared using the unpaired Student's t-test. In contrast, non-normally distributed variables were reported as medians (interquartile ranges) and compared using the Mann–Whitney U test. The Spearman rank correlation coefficient was calculated to assess associations between HBV viral load levels and immunological biomarkers. P-value < 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Demographic Characteristics

A total of 120 participants were included in this study, comprising 60 patients with HBV infection and 60 healthy controls. The mean age of HBV patients was 46.37 ± 12.34 years, compared with 34.05 ± 7.368 years in the control group; the difference was statistically significant ($p < 0.0001$). Regarding biological sex distribution, males (75%) and females (25%) were present in the patient group, and males (50%) and females (50%) were present in the control group, with a statistically significant difference between the groups ($p = 0.0080$), as shown in Table 1.

Table 1: Age and sex distribution of HBV patients and healthy controls.

Parameter	HBV Patients (n = 60)	Control (n = 60)	P-value
Age (years)	46.37 ± 12.34	34.05 ± 7.368	<0.0001 ****
Sex (M/F)	45/15	30/30	0.0080 **

3.2. Viral Load Assessment and Cytokine Analysis

Molecular assessment of viral load was performed for both patient and control groups. HBV DNA was detected only in infected patients; the median HBV DNA level was 3.535 (IQR 2.374–6.681) log₁₀ IU/mL, and a significant difference was observed. The immunological markers were evaluated: TNF- α (pg/ml) was 83.68 ± 6.671 in patients and 40.10 ± 2.373 in controls. IFN- γ (pg/ml) was 32.59 ± 0.8845 in patients and 15.88 ± 0.5377 in controls. The results were highly significant in patients compared to the controls, as shown in Table 2. As shown in Figures 1 and 2 (panel A), this study examines serum levels of viral load, TNF- α , and IFN- γ in participants with HBV infection compared with those in the control group.

Table 2: Immunological and antigen-presenting molecule markers in both groups

Marker	HBV Patients (n = 60)	Controls (n = 60)	P-value
TNF- α (pg/ml)	83.68 $\pm$ 6.671	40.10 $\pm$ 2.373	<0.0001****
IFN- γ (pg/ml)	32.59 $\pm$ 0.884	15.88 $\pm$ 0.537	<0.0001****
sCD8 (ng/ml)	11.09 $\pm$ 0.533	6.221 $\pm$ 0.279	<0.0001****
sHLA-A (ng/ml)	3.217 $\pm$ 0.199	1.181 $\pm$ 0.086	<0.0001****

The data are presented as (Mean $\pm$ standard error of the mean (SEM)).

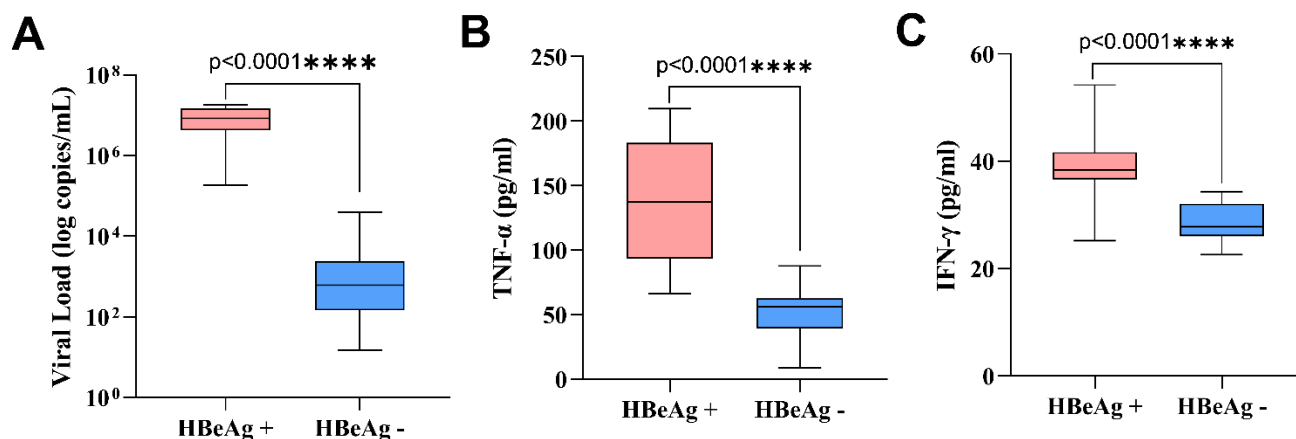


Figure 1: Comparison of HBV viral load and immune biomarkers according to HBeAg status. Panel A: Comparison of HBV viral load between HBeAg-positive and HBeAg-negative individuals. Panel B: Serum TNF- α concentrations according to HBeAg status. Panel C: Serum IFN- γ concentrations according to HBeAg status. HBeAg-positive individuals had significantly higher viral loads and elevated immune biomarkers than HBeAg-negative individuals, indicating enhanced viral replication and increased immune activation during active HBV infection. Data are presented as box-and-whisker plots showing median, interquartile range, and minimum–maximum values. Statistical significance was determined using the Mann–Whitney U test. ****P < 0.0001.

This study offers a comprehensive assessment of the association between HBV replication and host immune responses measured at both molecular and immunological levels. However, HBV DNA was detected only in infected patients and was positively correlated with HBeAg, possibly implying ongoing replication via an increased burden of infectious virus [17, 18]. Recent clinical data suggest that viral load should be viewed as a relatively dynamic biomarker, mediated primarily by host and disease determinants, rather than a linear function of age or duration of exposure. Associations with patient characteristics, such as age, have been reported in some studies. However, the data remain largely inconclusive and are closely linked to aspects of the immunology of infection and viral activity [19, 20]. Patients exhibited significantly higher serum IFN- γ concentrations than healthy controls, indicating an association between HBV infection and increased inflammatory immune activity. The present findings are consistent with these reported associations but do not permit conclusions regarding causality or immune regulation. The high IFN- γ levels found in our population are consistent with recent literature showing that IFN- γ production can be upregulated in individuals with active HBV infection and that IFN- γ is positively correlated with immune-mediated control of

HBV [21]. These findings suggest that elevated IFN- γ levels are associated with immune activation in HBV infection; however, the cross-sectional design does not permit conclusions regarding their role in viral control [22]. On the other hand, this clear correlation has indicated that the role of IFN- γ in HBV infection is complex and has not been consistently reported across studies. Several studies have indicated that the associations of some cytokines with HBV DNA were variable or not significant across populations or disease stages [23, 24]. Another marker was elevated TNF- α levels compared with controls. There was also a highly statistically significant difference, which was associated with higher inflammatory and immune activation markers in patients with HBV than in healthy controls. [25, 26]. Taken together, these data corroborate our findings and suggest that higher TNF- α concentrations were associated with HBV infection and may indicate increased inflammatory activity. However, their biological significance should be interpreted within the context of the study design.

3.3. HLA-A and Soluble CD8

Another finding of our research was the determination of serum levels of sCD8 (ng/ml) in patients (11.09 ± 0.533);

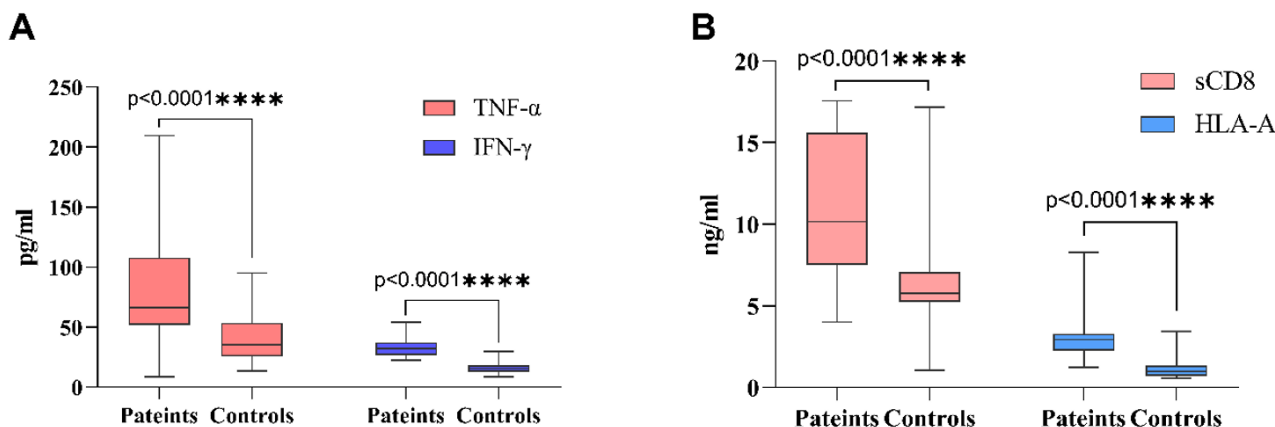


Figure 2: Comparison of inflammatory and cellular immune markers between HBV patients and healthy controls. Panel A: Serum concentrations of TNF- α and IFN- γ in HBV-infected patients and healthy controls. Panel B: Serum concentrations of sCD8 and HLA-A in HBV-infected patients and healthy controls. HBV patients exhibited significantly elevated levels of all investigated immunological markers compared with controls, indicating enhanced inflammatory responses, T-cell activation, and antigen-presentation activity associated with chronic HBV infection. Data are presented as box-and-whisker plots. Statistical comparisons were performed using the Mann–Whitney U test. ****P < 0.0001.

sCD8 was higher than in the control group, and the difference between groups was highly significant. Our results indicate significantly elevated levels, consistent with recent immunology studies showing that CD8+ T cells are central to antiviral immunity, particularly in restricting HBV infection through cytotoxic activity and cytokine release [27, 28]. These findings indicate that higher sCD8 levels were associated with immune activation markers but not with indicators of viral control. The upregulated expression of HLA-A observed in the current study was significantly higher than in the control group, suggesting increased antigen presentation and stimulation of cellular immune responses in HBV patients [29]. These findings are strongly consistent with our results, indicating that higher HLA-A concentrations were associated with HBV infection and may represent enhanced antigen-presentation activity; however, the present study cannot determine whether these changes contribute to effective antiviral immunity [30]. Genetic studies have also demonstrated differential susceptibility to infection, viral clearance, and disease progression associated with variability in HLA alleles and diversity, with certain alleles protective and others non-significant [31]. There was limited information about the immune profiles of patients with chronic HBV infection in the

the control was 6.221 ± 0.279 , and HLA-A (ng/ml) was 3.217 ± 0.199 in patients and 1.181 ± 0.086 in controls. The results were significant in patients compared to the controls, as shown in Figure 2 (panel B).

Kurdistan Region of Iraq. Examining cytokines, viral antigens, HLA-A, and soluble CD8 in this population will provide extra regional data. Factors such as genetics, the environment, healthcare access, and Viral transmission patterns were associated with variations in immune responses and clinical characteristics of HBV infection.

3.4. HBV Antigen Profiles

The data on HBV viral antigens among the study participants are informative. All 60 patients with HBV infection tested positive for HBsAg, as shown in Figure 3, panel A. Among HBeAg results, 21 of the 60 patients were positive, representing about 35%. The remaining 39 patients were negative for HBeAg, as shown in Figure 3, panel B. Of the 60 HBV patients, 13 were positive for HBcAg (about 21.67%). At the same time, 47 were negative, as shown in Figure 3, panel C. Overall, these findings indicate that HBsAg is universally present among HBV patients. In contrast, HBeAg and HBcAg are found only in certain individuals, indicating varying stages of infection and levels of viral activity.

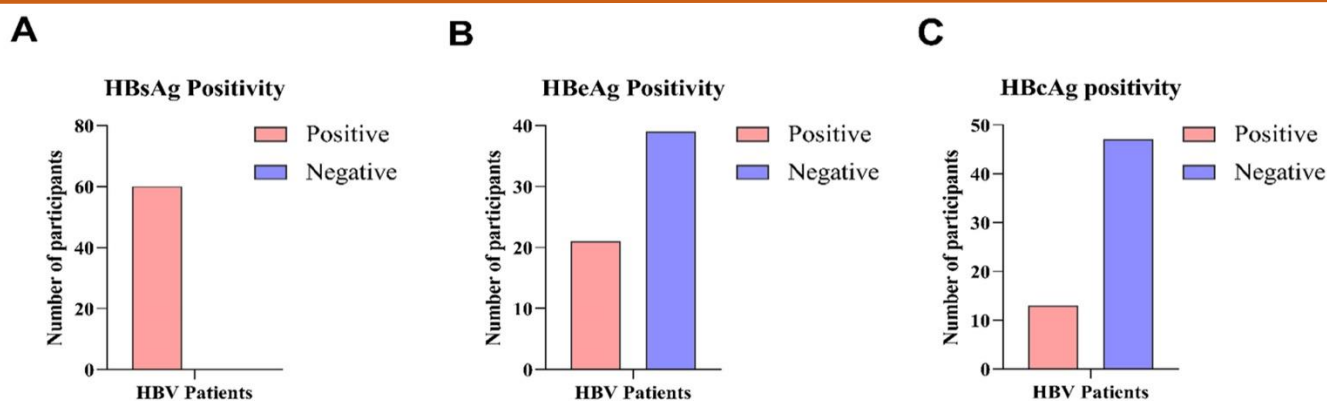


Figure 3: Serological distribution of HBV viral antigens in patients. Panel A: HBsAg positivity and negativity among HBV patients. Panel B: HBeAg positivity and negativity among HBV patients. Panel C: HBcAg positivity and negativity among HBV patients. All HBV patients were HBsAg-positive, whereas HBeAg and HBcAg positivity were observed in a subset of infected individuals. The results demonstrate the serological characteristics and replication-associated antigen profile of HBV infection.

All patients with HBV infection had positive HBsAg, which positively correlated with HBV viral load, and a moderately positive correlation was observed between viral infection and HBsAg expression [32]. These results are also consistent with data from the past few years, which show a moderate correlation between HBsAg and HBV DNA [32, 33]. This finding suggests that HBeAg positivity was associated with higher HBV replication markers in the study population [34]. HBeAg-positive patients in the present study were more frequently observed to have higher HBV viral load than HBeAg-negative patients. HBcAg, a core structural component of the HBV that captures nucleocapsids produced in infected hepatocytes, is more strongly associated with active viral replication than circulating antigens. [35]. This finding is consistent with recent evaluations showing that HBcAg-related markers, most notably HBcAg, are strongly associated with serum HBV DNA levels and serve as reliable surrogate markers of infection-wide replication status and host immune control. Furthermore, recent studies have confirmed the link between HBcAg expression and immune-mediated hepatitis, cytokine activation, and T-cell responses in chronic HBV infection in humans, which is consistent with the observed association between sCD8 and conventional immunological markers [36]. Still, while there are clearly strong correlations between the two markers, that correlation is not consistently linear with

respect to viral activity. It has more recently been shown that HBcAg levels can be detectable during HBV treatment, while on antiviral therapy or under immune control conditions, and can decline with continued suppression in a setting of low-level replication [37]. This heterogeneity may be associated with viral genetic variability reported in patients with chronic HBV infection, host immune pressure and differences in disease stage. Consequently, although HBcAg is a predictor of clinical events and a suitable measure of activity according to the pathophysiological and biological rationale, its interpretation does not rely solely on this. Therefore, when combined with HBV DNA quantification and immunological markers, it may have greater clinical significance, as they together form a comprehensive portrait of viral replication dynamics and disease evolution [38, 39].

3.5. Correlation Analysis

This study assesses the relationship among viral load, HBsAg, HBcAg, HBeAg, TNF- α , IFN- γ , sCD8, and HLA-A using Spearman's rank correlation analysis. The strong correlations identified between the markers suggest interrelated pathways of immunomodulation and viral infection, as shown in Table 3 and Figures 4 and 5.

Table 3: Correlations among viral load and immunological markers in HBV patients

Variable pair	r_s -value	95 % CI Confidence interval	P-value
Viral load vs IFN- γ	0.92	0.82 to 0.97	<0.0001****
Viral load vs TNF- α	0.80	0.68 to 0.88	<0.0001****
Viral load vs sCD8	0.89	0.82 to 0.93	<0.0001****
Viral load vs sHLA-A	0.83	0.73 to 0.90	<0.0001****
Viral load vs HBeAg	0.91	0.86 to 0.95	<0.0001****
TNF- α vs IFN- γ	0.80	0.68 to 0.87	<0.0001****
sHLA-A vs sCD8	0.76	0.62 to 0.85	<0.0001****

Data distribution was assessed using the Shapiro–Wilk test prior to statistical analysis. Continuous variables that were not normally distributed were summarised as median (interquartile range) and compared using the Mann–

Whitney U test. Correlations were evaluated using Spearman's rank correlation coefficient. A two-sided p-value <0.05 was considered statistically significant.

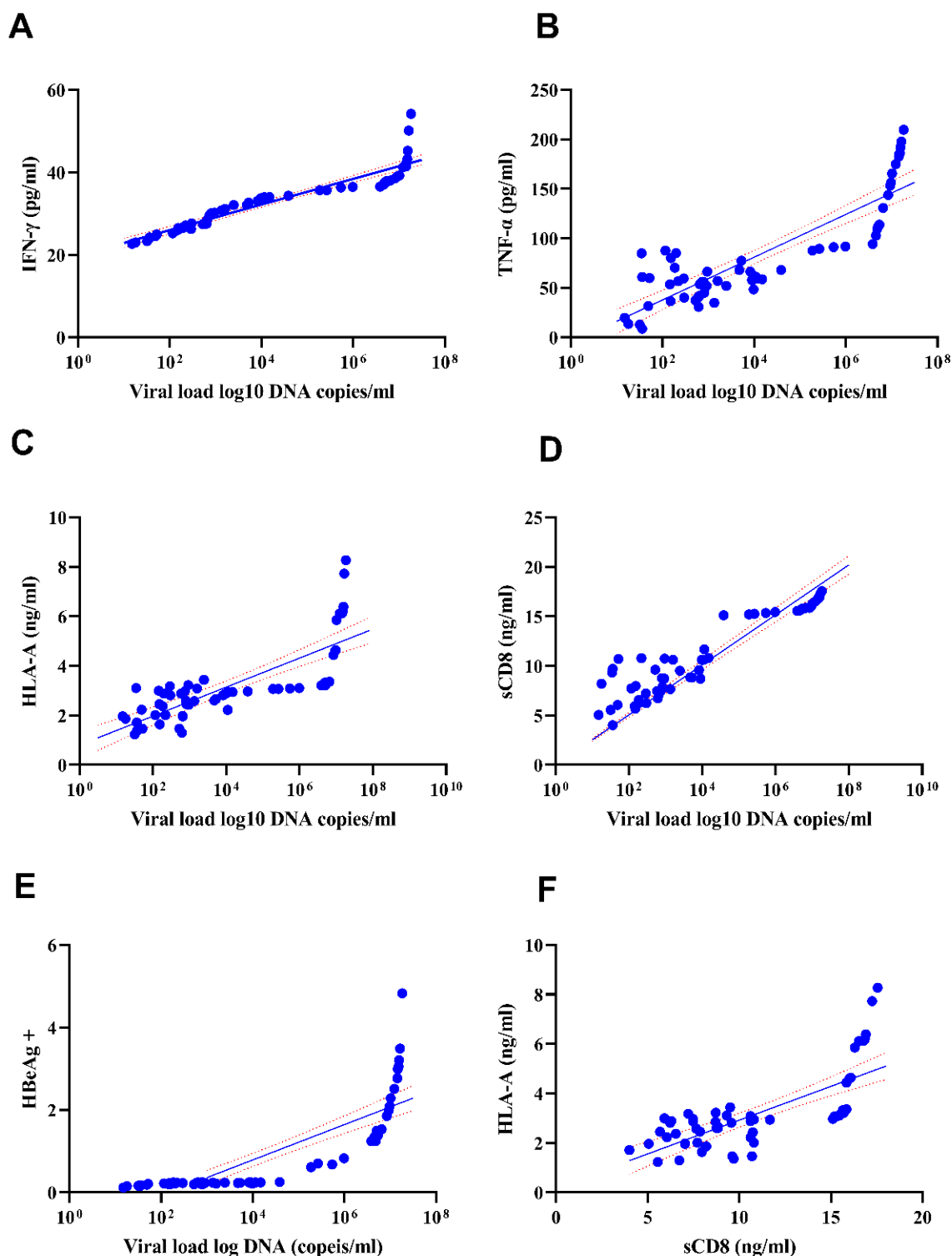


Figure 4: Correlation analysis between HBV viral load and immunological markers. Scatter plots illustrating Spearman correlation analyses among virological and immunological parameters in HBV patients. Panel A: Correlation between viral load and IFN- γ ($r = 0.92$, $P < 0.0001$). Panel B: Correlation between viral load and TNF- α ($r = 0.80$, $P < 0.0001$). Panel C: Correlation between viral load and sCD8 ($r = 0.89$, $P < 0.0001$). Panel D: Correlation between viral load and HLA-A ($r = 0.83$, $P < 0.0001$). Panel E: Correlation between viral load and HBeAg ($r = 0.91$, $P < 0.0001$). Panel F: Correlation between sCD8 and HLA-A ($r = 0.76$, $P < 0.0001$). Trend lines represent the direction of association. The strong positive correlations observed between viral load and immunological markers suggest that higher HBV viral loads were associated with elevated immune-response markers in the study population.

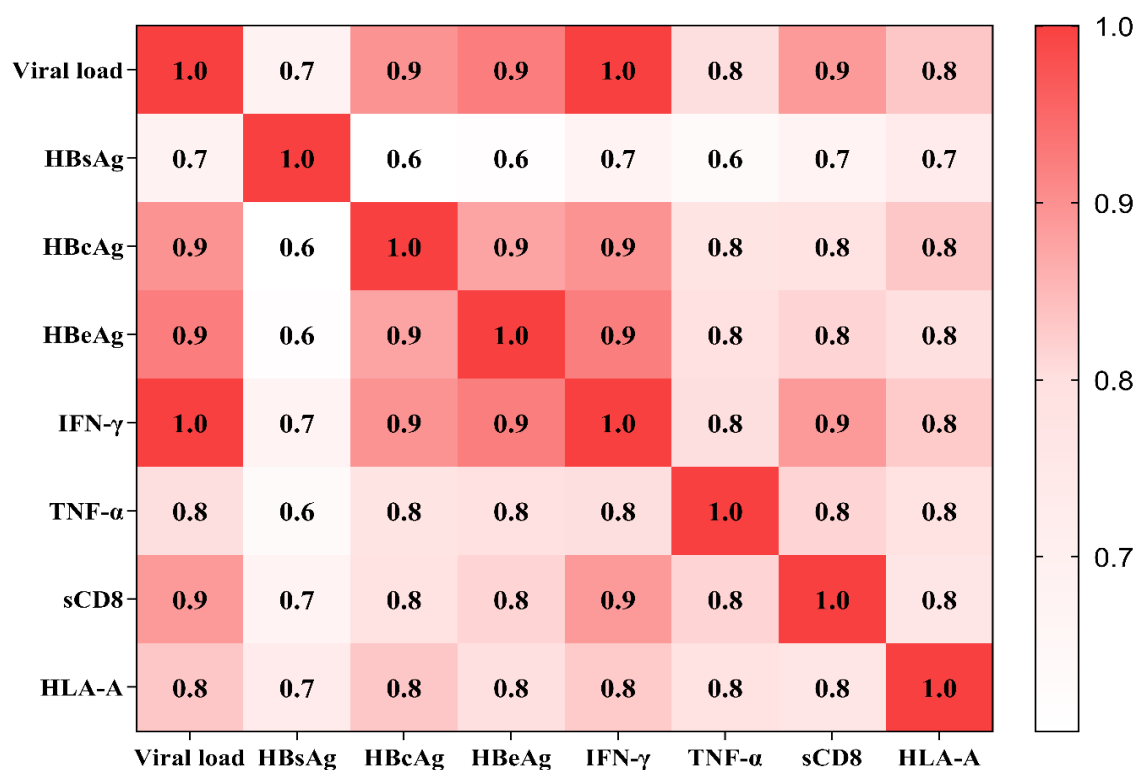


Figure 5: The Heatmap that presents Spearman correlation coefficients. Heatmap representing the correlation matrix between HBV viral load, viral antigens (HBsAg, HBcAg, HBeAg), and immune markers (TNF- α , IFN- γ , CD8⁺ T cells, and sHLA-A). Colour intensity reflects the strength and direction of correlations based on Spearman's correlation coefficients.

These results demonstrate positive associations among HBV viral load, antigen expression, and immune activation markers in the study population. It has been previously shown that HBcAg is strongly associated with HBV DNA and intrahepatic viral activity [40]. On the other hand, the relatively weak correlation between viral load and HBsAg corroborates earlier findings that HBsAg is a viral antigen and that its circulating levels have shown variable associations with markers of active HBV replication. [41]. A positive association was observed between HBV viral load and immune activation markers in the present study, as evidenced by significant positive correlations between viral load and several immunological markers (IFN- γ , TNF- α , sCD8, and HLA-A). Both IFN- γ and TNF- α are master regulators of antiviral immunity and inflammation. Recent studies have found that these cytokines are markedly upregulated in patients with HBV infection and correlate with disease activity and liver inflammation [42, 43]. In addition, cytokine profiling studies have shown that immune markers and virological parameters tend to cluster together, reflecting distinct HBV-infected immune states [44]. High correlations with sCD8 and HLA-A also emphasise the contribution of cytotoxic T-cell activation and MHC class I-mediated antigen presentation to effective antiviral defence [45]. Additionally, recent data revealed the plasticity of these responses: cytokine levels may not directly correlate with viral load, and CD8⁺ T cells can become functionally exhausted in the presence of chronic antigen exposure [44, 46]. The observed associations among viral antigens are consistent with coordinated patterns of viral replication, although temporal relationships cannot be established from the

present study and antigen expression. The positive correlation between HBcAg and HBeAg suggests that both antigens are products of active viral transcription. In contrast, the lower correlation with HBsAg confirms its role as a product of HBV biology. Recent studies have shown an association between HBcAg and viral replication rather than HBsAg, which is consistent with the pattern observed here [47]. In contrast, the multinomial distribution of antigen expression has been observed across clinical stages, especially in individuals with HBeAg-negative infection and during therapeutic intervention; antigen production may not equal actual viral replication [48, 49]. Intracellular levels of IFN- γ and TNF- α . Higher IFN- γ and TNF- α concentrations were observed concurrently with viral antigen positivity and inflammatory markers, suggesting an association between virological and immunological parameters [42, 50]. Finally, the strong correlations between immune markers and viral load suggest a close association between virological and immunological parameters, involving enhanced coordinated cytokine signalling, T-cell activation, and probably antigen presentation. This is consistent with recent mechanistic studies pointing to the role of MHC class I interactions between cytokines and CD8⁺ T cells in mediating antiviral immunity [51]. However, more recent work also indicates that this immune activation signals pathology, chronic inflammatory responses to the persistent viral infection, rather than effective viral control, especially for a persistent infection [29, 52, 53].

4. Limitations

This study has some limitations that are important to consider when looking at the findings. First, there is a clear difference in age and sex between the HBV patients and the healthy controls, and the study design is cross-sectional. The HBV patients were older and had a higher proportion of males than the control group. Given the significant differences in age and sex between the study groups, and because these demographic factors have been reported to be associated with immune and inflammatory profiles, the observed biomarker differences should be interpreted cautiously. The present cross-sectional study cannot determine the independent contribution of these demographic variables to the observed associations. As a result, we should interpret the findings of this study carefully. Future research should use control groups that match the age and sex of the HBV patients, or adjust for these factors using statistical methods, to reduce confounding and improve the reliability of the results. Second, we did not check liver function tests like alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin levels. These tests are key indicators of liver inflammation and disease in chronic HBV infection. Without this information, we could not connect the immune markers we measured to liver health and function. Third, we did not categorise patients based on the phase of their chronic HBV infection, such as immune-tolerant, immune-active, inactive carrier, or HBeAg-positive/-negative chronic hepatitis. Immune responses can vary significantly in these phases. Not having this classification made it harder to understand the observed biomarker profiles and may have contributed to differences among patients. Finally, our study design examined immune marker levels at a single time point. This means we could not see how these levels change over time or establish any cause-and-effect relationships between immune markers and disease progression. Future studies should include repeated measurements, thorough liver function tests, and clear clinical classifications to confirm and expand on these findings.

5. Conclusion

Our findings demonstrate an association between HBV viral load and immune markers, including IFN- γ , TNF- α , CD8⁺ T cells, and HLA-A, which have been understudied in the Kurdistan region of Iraq. The results demonstrated positive associations between HBV infection and elevated immune-response markers and the virological parameters evaluated in the present study. This study expands the available regional data on the associations between HBV virological and immunological markers in patients from the Kurdistan Region of Iraq by integrating medical tests with immune system analyses, potentially improving patient care, risk assessment, and outcome prediction. However, these findings should be interpreted with caution given the cross-sectional design and demographic differences between study groups.

Contribution statement

Ahmed Farhan Shallal contributed to the study design, study supervision, and manuscript review and editing. Soran Qader Abdel was responsible for data collection, statistical analysis of the obtained data, and writing the original draft of the manuscript. All authors reviewed and approved the final version of the manuscript.

Declarations

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interests

The authors declare no competing interests.

Data availability

All data generated or analysed during this study are included in this published article. Additional datasets are available from the corresponding author upon reasonable request.

Acknowledgments:

The authors thank the Department of Biology/College of Science at the University of Raparin for the facilities and technical assistance.

Declaration of AI use (ChatGPT)

During the preparation of this manuscript, the authors used (ChatGPT) for language editing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the accuracy and integrity of the final manuscript.

6. References

- [1] A. F. Shallal, "Immunomodulatory Pathways of Some Biomarkers in Individuals with Renal Failure," *Aro-the Scientific Journal of Koya University*, 2025, vol. 13, no.(2), p. 326–333, DOI: <https://doi.org/10.14500/aro.12474>
- [2] A. F. Shallal, "Modulation of immune pathway and biomarkers: impacts of lifestyle, nutrition, and therapeutic interventions," *Journal of Pharmaceutical Research*, 2025, vol. 14, no.(24), p. 419–430, DOI: <https://doi.org/10.5281/ZENODO.17949376>
- [3] A. F. Shallal, "Association of Immunomodulation with Biological Agents: A Review," *World Journal of Pharmaceutical Research*, 2026, vol. 15, no.(6), p. 849–860, DOI: <https://doi.org/10.5281/ZENODO.19045651>
- [4] A. F. SHALLAL, C. H. RAZAQ, and M. N. OWAID, "Immunomodulatory Effects of *Ganoderma lucidum* on NK Cells: From Traditional Use to Modern

- Immunotherapy," *Journal of Macrofungi*, 2025, vol. 1, no.(1), p. 14–29, DOI: <https://doi.org/10.65999/macrofungi.2025.27>
- [5] A. F. Shallal, "Immune System Modulation: The Roles of Leukocytes, Vitamin D, Plant Extracts, Nanotechnology, and Biomarkers in Disease and Health," *World Journal of Pharmaceutical Research*, 2026, vol. 15, no.(3), p. 250–263, DOI: <https://doi.org/10.5281/ZENODO.18427459>
- [6] C. Seeger and W. S. Mason, "Molecular Biology of Hepatitis B Virus Infection," *Virology*, 2015, vol. 479, p. 672–686, DOI: <https://doi.org/10.1016/j.virol.2015.02.031>
- [7] J. E. Song, "Diagnosis of hepatitis B," *Annals of Translational Medicine*, 2016, vol. 4, no.(18), p. 338, DOI: <https://doi.org/10.21037/atm.2016.09.11>
- [8] G. C. Fanning, F. Zoulim, J. Hou, and A. Bertoletti, "Therapeutic strategies for hepatitis B virus infection: towards a cure," *Nature Reviews Drug Discovery*, 2019, vol. 18, no.(11), p. 827–844, DOI: <https://doi.org/10.1038/s41573-019-0037-0>
- [9] P. J. Easterbrook, N. Luhmann, S. Bajis, M. S. Min, M. Newman, O. Lesi, and M. C. Doherty, "WHO 2024 hepatitis B guidelines: an opportunity to transform care," *The lancet Gastroenterology & hepatology*, 2024, vol. 9, no.(6), p. 493–495, DOI: [https://doi.org/10.1016/S2468-1253\(24\)00089-X](https://doi.org/10.1016/S2468-1253(24)00089-X)
- [10] W. H. Organization, *Global hepatitis report 2024: action for access in low-and middle-income countries*. 2024: World Health Organization.
- [11] S. Sabeena and N. Ravishankar, "Horizontal modes of transmission of hepatitis B virus (HBV): a systematic review and meta-analysis," *Iranian Journal of Public Health*, 2022, vol. 51, no.(10), p. 2181, DOI: <https://doi.org/10.18502/ijph.v51i10.10977>
- [12] Y. Shi and M. Zheng, "Hepatitis B virus persistence and reactivation," *BMJ*, 2020, vol. 370, DOI: <https://doi.org/10.1136/bmj.m2200>
- [13] P. Zheng, Y. Dou, and Q. Wang, "Immune response and treatment targets of chronic hepatitis B virus infection: innate and adaptive immunity," *Frontiers in Cellular and Infection Microbiology*, 2023, vol. 13, p. 1206720, DOI: <https://doi.org/10.3389/fcimb.2023.1206720>
- [14] Y.-P. Liu and C.-Y. Yao, "Rapid and quantitative detection of hepatitis B virus," *World Journal of Gastroenterology*, 2015, vol. 21, no.(42), p. 11954–11963, DOI: <https://doi.org/10.3748/wjg.v21.i42.11954>
- [15] A. Bertoletti and P. T. Kennedy, "The immune tolerant phase of chronic HBV infection: new perspectives on an old concept," *Cellular & Molecular Immunology*, 2015, vol. 12, no.(3), p. 258–263, DOI: <https://doi.org/10.1038/cmi.2014.79>
- [16] M. K. Maini and A. R. Burton, "Restoring, releasing or replacing adaptive immunity in chronic hepatitis B," *Nature Reviews Gastroenterology & Hepatology*, 2019, vol. 16, no.(11), p. 662–675, DOI: <https://doi.org/10.1038/s41575-019-0196-9>
- [17] S. Donica, C. N. Ciurea, M. A. Budianu, A. Cighir, R. L. Coşeriu, A. M. Bunea, and A. Man, "Correlation between HBV viral load and other paraclinical parameters in patients with chronic hepatitis B," *Acta Marisiensis-Seria Medica*, 2025, vol. 71, no.(3), p. 223–226, DOI: <https://doi.org/10.2478/amma-2025-0036>
- [18] T.-C. Tseng, C.-J. Liu, C.-Y. Hsu, C.-M. Hong, T.-H. Su, W.-T. Yang, C.-L. Chen, H.-C. Yang, Y.-T. Huang, and S. F.-T. Kuo, "High level of hepatitis B core-related antigen associated with increased risk of hepatocellular carcinoma in patients with chronic HBV infection of intermediate viral load," *Gastroenterology*, 2019, vol. 157, no.(6), p. 1518–1529. e3, DOI: <https://doi.org/10.1053/j.gastro.2019.08.028>
- [19] G. L. H. Wong, "Updated guidelines for the prevention and management of chronic hepatitis B—World Health Organization 2024 compared with China 2022 HBV guidelines," *Journal of Viral Hepatitis*, 2024, vol. 31, p. 13–22, DOI: <https://doi.org/10.1111/jvh.14032>
- [20] F. S. Ali, M. H. Nguyen, R. Hernaez, D. Q. Huang, J. Wilder, A. Piscocoy, T. G. Simon, and Y. Falck-Ytter, "AGA clinical practice guideline on the prevention and treatment of hepatitis B virus reactivation in at-risk individuals," *Gastroenterology*, 2025, vol. 168, no.(2), p. 267–284, DOI: <https://doi.org/10.1053/j.gastro.2024.11.008>
- [21] N. Le Bert, A. Koffas, R. Kumar, F. Facchetti, A. T. Tan, U. S. Gill, L.-Y. Mak, S. W. Stretch, T. Miao, and S. Hariharaputran, "Rapid whole-blood immune profiling reveals heterogeneous HBV-specific T cell response patterns independent of clinical disease phases," *Journal of Hepatology*, 2025, vol. 83 no.(6), p. 1292–1304, DOI: <https://doi.org/10.1016/j.jhep.2025.06.012>
- [22] X. Yuan, Y. Shao, R. Huang, S. Seery, H. Wang, N. Hu, L. Wen, X. Lin, and L. Zhang, "Understanding the influence of cytokines in intrauterine hepatitis B transmission: A cross-sectional study in China," *Cytokine*, 2024, vol. 181, p. 156670, DOI: <https://doi.org/10.1016/j.cyto.2024.156670>
- [23] Y. Shu, S. Li, Y. Du, and X. Zheng, "Anti-HBV treatment partially restores the dysfunction of innate immune cells and unconventional T cells during chronic HBV infection," *Frontiers in Immunology*, 2025, vol. 16, p. 1611976, DOI: <https://doi.org/10.3389/fimmu.2025.1611976>
- [24] S. H. Hosseini Khorami, F. Nejatollahi, and M. A. Davarpanah, "Serum Levels of Interleukin-4, Interleukin-10 and Interferon- γ in Patients with Chronic Hepatitis B Infection," *Hepatitis Monthly*, 2018, vol. 18, no.(4), DOI: <https://doi.org/10.5812/hepatmon.60377>
- [25] T. Thu, "Serum Level of IL-6, TNF-A, IL-4, IL-10 in Patients with Cirrhosis Due to Chronic Hepatitis B," *Vietnam Journal of Infectious Diseases*, 2024, vol. Vol. 4 No. 36 (2021), DOI: <https://doi.org/10.59873/vjid.v4i36.206>
- [26] M. Holmberg, H. C. D. Aass, O. Dalgard, E. Samuelsen, D. Sun, N. K. Björkström, A. Johannessen, and D. H. Reikvam, "Treatment cessation in HBeAg-negative chronic hepatitis B: clinical response is associated with increase in specific proinflammatory cytokines," *Scientific Reports*, 2023, vol. 13, no.(1), p. 22590, DOI: <https://doi.org/10.1038/s41598-023-50216-y>
- [27] H. Li, S. Xiao, C. Huo, S. Yang, J. Wang, X. Lan, M. Li, L. Shi, L. Zhuo, and J. Zhang, "Targeting ferroptosis restores the antiviral activity of CD8⁺ T cells during chronic HBV infection," *Cellular and Molecular Gastroenterology and Hepatology*, 2025, p. 101612, DOI: <https://doi.org/10.1016/j.jcmgh.2025.101612>
- [28] F. Andreatta, K. D. Moynihan, V. Fumagalli, P. Di Lucia, D. C. Pappas, K. Kawashima, I. Ni, P. H. Bessette, C. Percuchini, and E. Bono, "CD8 cis-targeted IL-2 drives potent antiviral activity against hepatitis B virus," *Science Translational Medicine*, 2024, vol. 16, no.(729), p. eadi1572, DOI: <https://doi.org/10.1126/scitranslmed.adi1572>

- [29] K. Heim, Sagar, Ö. Sogukpinar, S. Llewellyn-Lacey, D. A. Price, F. Emmerich, A. R. Kraft, M. Cornberg, S. Kiellbassa, and P. Knolle, "Attenuated effector T cells are linked to control of chronic HBV infection," *Nature Immunology*, 2024, vol. 25, no.(9), p. 1650–1662, DOI: <https://doi.org/10.1038/s41590-024-01928-4>
- [30] A. Letafati, O. S. Ardekani, M. Naderisemiromi, M. Norouzi, M. Shafiei, S. Nik, and S.-H. Mozhgani, "Unraveling the dynamic mechanisms of natural killer cells in viral infections: insights and implications," *Virology Journal*, 2024, vol. 21, no.(1), p. 18, DOI: <https://doi.org/10.1186/s12985-024-02287-0>
- [31] A. Tălăngescu, M. Tizu, B. Calenic, D. F. Mihăilescu, A. E. Constantinescu, and I. Constantinescu, "HLA genetic diversity and chronic hepatitis B virus infection: effect of heterozygosity advantage," *Medical Sciences*, 2024, vol. 12, no.(3), p. 44, DOI: <https://www.mdpi.com/2076-3271/12/3/44#>
- [32] U. Maimunah, P. Wardhani, C. D. K. Wungu, A. F. Rahmanda, A. A. Kurniawan, and A. Palayukan, "Correlation between quantitative HBsAg and quantitative HBV DNA in chronic hepatitis B patients: a systematic review and meta-analysis," *Egyptian Liver Journal*, 2024, vol. 14, no.(1), p. 35, DOI: <https://doi.org/10.1186/s43066-024-00336-5>
- [33] B. Garba, A. A. Mohamud, F. H. Mohamed, A. A. Moussa, Y. Yakubu, N. I. Dirie, A. M. Abdulatif, J. H. Mohamoud, A. S. Ali, and A. H. Jibril, "Prevalence, risk factors, and viral load quantification of HBV infection among hospital patients: A cross-sectional investigation in Mogadishu, Somalia," *PLoS One*, 2026, vol. 21, no.(1), p. e0340800, DOI: <https://doi.org/10.1371/journal.pone.0340800>
- [34] K.-N. Tsai and J.-H. J. Ou, "Hepatitis B virus e antigen and viral persistence," *Current Opinion in Virology*, 2021, vol. 51, p. 158–163, DOI: <https://doi.org/10.1016/j.coviro.2021.10.003>
- [35] Y. Qiu, Q. Tang, X.-Q. Liu, Y.-L. Xue, Y. Zeng, and P. Hu, "Hepatitis B core-related antigen as a promising serological marker for monitoring hepatitis B virus cure," *World Journal of Hepatology*, 2025, vol. 17, no.(1), p. 98658, DOI: <https://doi.org/10.4254/wjh.v17.i1.98658>
- [36] L. O. Downs, M. Delphin, M. van Schalkwyk, S. Hugo, S. F. Lumley, E. Waddilove, T. Wang, J. Martin, C. de Lara, and A. Babbs, "Evaluation of Hepatitis B core related antigen (HBcrAg) as a biomarker in cohorts from the United Kingdom and South Africa," *Journal of Infection*, 2025, vol. 91, no.(3), p. 106601, DOI: <https://doi.org/10.1016/j.jinf.2025.106601>
- [37] Z. Zhu, J. Wu, J. Li, and T. Wu, "Challenges and Solutions in pgRNA Measurement: Toward Improved Monitoring of Hepatitis B Therapy," *Pathogens*, 2026, vol. 15, no.(2), p. 153, DOI: <https://www.mdpi.com/2076-0817/15/2/153#>
- [38] L. I. Bozomitu, A. Lupu, V. V. Lupu, N. Gimiga, D. T. Anton Paduraru, D. E. Mindru, M. Mihai, C. Anton, E. Anton, and M. Mitrea, "Accuracy of Diagnostic Investigations in Monitoring Hepatitis B Virus Infection: Strengths, Limitations, and Emerging Biomarkers," *International Journal of Molecular Sciences*, 2026, vol. 27, no.(5), p. 2464, DOI: <https://www.mdpi.com/1422-0067/27/5/2464#>
- [39] J. Lin, S. Jiang, X. Chen, M. Zhu, and H. Zhang, "The significance of detecting HBV pgRNA and HBcrAg in HBV patients treated with NAs," *Medicine*, 2024, vol. 103, no.(14), p. e37752, DOI: <https://doi.org/10.1097/MD.00000000000037752>
- [40] T. Inoue, T. Watanabe, and Y. Tanaka, "Hepatitis B core-related antigen: A novel and promising surrogate biomarker to guide anti-hepatitis B virus therapy," *Clin Mol Hepatol*, 2023, vol. 29, no.(4), p. 851–868, DOI: <https://doi.org/10.3350/cmh.2022.0434>
- [41] Y. Liu, D. Wu, K. Zhang, R. Ren, Y. Liu, S. Zhang, X. Zhang, J. Cheng, L. Chen, and J. Huang, "Detection technology and clinical applications of serum viral products of hepatitis B virus infection," *Frontiers in Cellular and Infection Microbiology*, 2024, vol. 14, p. 1402001, DOI: <https://doi.org/10.3389/fcimb.2024.1402001>
- [42] B. Jiang, L. Wang, H. Liu, L. Wang, R. Su, L. Xu, G. Wei, J. Li, F. Lu, and X. Chen, "Association of HBV serological markers with host antiviral immune response relevant hepatic inflammatory damage in chronic HBV infection," *Journal of Medical Virology*, 2024, vol. 96, no.(4), p. e29569, DOI: <https://doi.org/10.1002/jmv.29569>
- [43] M. Manea, I. Mărunțelu, and I. Constantinescu, "Extended analysis on peripheral blood cytokines correlated with hepatitis B virus viral load in chronically infected patients—a systematic review and meta-analysis," *Frontiers in Medicine*, 2024, vol. 11, p. 1429926, DOI: <https://doi.org/10.3389/fmed.2024.1429926>
- [44] L. Xiao, K. Tang, T. Fu, X. Yuan, S. Seery, W. Zhang, Z. Ji, Z. He, Y. Yang, and W. Zhang, "Cytokine profiles and virological markers highlight distinctive immune statuses, and effectiveness and limitations of NAs across different courses of chronic HBV infection," *Cytokine*, 2024, vol. 173, p. 156442, DOI: <https://doi.org/10.1016/j.cyto.2023.156442>
- [45] S. Lens, A. R. Burton, J. Davies, M. Locatelli, M. García-López, A. Pocurull, A. Jeffery-Smith, N. Novikov, S. P. Fletcher, and X. Forns, "Tracking B cell immunity during perturbation of hepatitis B infection induced by treatment withdrawal," *Gut*, 2025, p. 1–10, DOI: <https://doi.org/10.1136/gutjnl-2024-333309>
- [46] Y. Wang, J. Gu, G. Chen, Y. Jiang, Y. Xu, X. Huang, W. Sun, and J. Gan, "Roles of inflammatory cytokines in the pathogenesis of hepatitis B virus-related acute-on-chronic liver failure and CAR-T therapy," *Virology Journal*, 2025, vol. 22, no.(1), p. 281, DOI: <https://doi.org/10.1186/s12985-025-02868-7>
- [47] L. Bègré, A. Boyd, M.-L. Plissonnier, B. Testoni, C. Béguélin, F. Suter-Riniker, C. Scholtès, J. K. Rockstroh, K. Lacombe, and L. Peters, "Circulating HBV RNA and hepatitis B core-related antigen as determinants of HBsAg loss in persons with HIV in Europe," *JHEP Reports*, 2025, vol. 8, no.(2), p. 101671, DOI: <https://doi.org/10.1016/j.jhepr.2025.101671>
- [48] U. S. Gill, V. J. Appleby, and S. Lens, "What is new in hepatitis B," *Frontline Gastroenterology*, 2026, vol. 17, no.(2), p. 161–171, DOI: [10.1136/flgastro-2024-102850](https://doi.org/10.1136/flgastro-2024-102850)
- [49] M. G. Ghany, M. Buti, P. Lampertico, and H. M. Lee, "Guidance on treatment endpoints and study design for clinical trials aiming to achieve cure in chronic hepatitis B and D: Report from the 2022 AASLD-EASL HBV-HDV Treatment Endpoints Conference," *Hepatology*, 2023, vol. 78, no.(5), p. 1654–1673, DOI: <https://doi.org/10.1016/j.jhep.2023.06.002>
- [50] H. Ma, Q.-Z. Yan, J.-R. Ma, D.-F. Li, and J.-L. Yang, "Overview of the immunological mechanisms in hepatitis B virus reactivation: Implications for disease progression and management strategies," *World Journal of*

- Gastroenterology, 2024, vol. 30, no.(10), p. 1295, DOI: <https://doi.org/10.3748/wjg.v30.i10.1295>
- [51] A. Schuch, A. Hoh, and R. Thimme, "The role of natural killer cells and CD8+ T cells in hepatitis B virus infection," *Frontiers in Immunology*, 2014, vol. 5, p. 258, DOI: <https://doi.org/10.3389/fimmu.2014.00258>
- [52] J. P. Costa, A. de Carvalho, A. Paiva, and O. Borges, "Insights into immune exhaustion in chronic hepatitis B: A review of checkpoint receptor expression," *Pharmaceuticals*, 2024, vol. 17, no.(7), p. 964, DOI: <https://www.mdpi.com/1424-8247/17/7/964#>
- [53] J. Lin and A. J. Gehring, "Role of Immune Cells in Hepatitis B Virus and Associated Sequelae," *Annual Review of Pathology: Mechanisms of Disease*, 2026, vol. 21, p. 399–421, DOI: <https://doi.org/10.1146/annurev-path-mechdis-042524-030646>