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# The Significance of Serum IL-10 Levels in Diagnosing Hepatitis B Virus (HBV) Infection

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## Abstract

**Background:** Cytokine secretion is one of the most immunological affecters that play a major role in the pathogenesis hepatitis B and, a member of these anti-inflammatory cytokines with unique characteristic is interleukin-10 (IL-10). Objective: To evaluate the levels of IL-10 in clients with hepatitis B infections is the primary objective of the present study. Methods: A case–control study design was developed consisting of 62 laboratory-confirmed hepatitis B infection subjects and 58 controls who were apparently healthy. Enzyme-linked immunosorbent assay (ELISA) was performed to assess serum IL-10 levels. Differences between groups were investigated via independent samples t-tests; differences by infection site were assessed with analysis of variance (ANOVA). Furthermore, the diagnostic value of IL-10 was evaluated using receiver operating characteristic (ROC) curve analysis for the diagnosis of infected versus non-infected. Results: The IL-10 was significantly increased in patients than controls [18.8 ± 32 vs. 15.9 ± 3.9 PG/ml (p <30]. Elevation of serum IL-10 levels in relation with vaccination status suggested higher concentrations in non-vaccinated patients with hepatitis B infections (19.2 ± 3.2 pg/ml) than those vaccinated (13.5 ± 2.8 pg/ml). The AUC was 0.78, established an optimal diagnostic threshold at 16 pg/ml (sensitivity: 82%, specificity: 73%). Further, IL-10 was identified as a potential biomarker for hepatitis B by showing the average serum level of it increased significantly in patients with hepatitis B.

**Keywords**: Specificity, Sensitivity, AUC, hepatitis B, Interleukin-10

## Introduction

Hepatitis B virus (HBV), despite being the focus of widespread vaccination efforts and having effective antiviral therapies available for treating active infection, is still a major global public health challenge. Chronic HBV infection is now estimated to impact nearly 296 million people worldwide, and it remains one of the most clinically significant viral causes of liver cirrhosis and hepatocellular carcinoma (HCC). The clinical course of hepatitis B virus (HBV) infection is extremely heterogeneous: while many individuals successfully eliminate the virus after acute infection, others progress to chronic disease that can be silent for decades before advancing to end-stage liver damage and/or clinically evident life-threatening events (Kramvis et al., 2022). This heterogeneity in disease outcome highlights the important need for robust, reliable biomarkers that can refine diagnosis, phenotype different states of the disease and provide prognostic information. Indeed, such biomarkers are designed to complement traditional serological and virological assays, thus aiding clinicians in providing optimal clinical care and more reliably predicting patient outcomes. (Liu et al., 2024).

In recent years, attention has increasingly shifted toward biomarkers derived from host immune responses. Such markers are of special interest because they

represent the complex host–virus interaction rather than being restricted to the detection of viral components alone. During a viral infection and in the context of hepatic inflammation, cytokines, and chemokines which mediate many aspects of innate and adaptive immune responses show distinct expression profiles. As a result, some of these molecules are suggested as promising diagnostic and predictive markers in hepatitis B virus (HBV) infection (Zhong et al., 2021; Zhang et al., 2022). In contrast to direct viral markers (HBV DNA, HBsAg, and HBeAg), host-derived immune biomarkers may give pre-clinical information of the status of immune regulation and onset of liver injury (Kramvis et al., 2022; Xiao et al., 2024).

Of these immune mediators, interleukin-10 (IL-10) has received a lot of attention due to its key mechanism in inhibiting pro-inflammatory signaling. IL-10 is a pleiotropic anti-inflammatory cytokine produced by multiple immune cell populations, including regulatory T cells, monocytes/macrophages and some subsets of B lymphocytes. The main role of it is to inhibit the overproduction of pro-inflammatory cytokines, and thus reduce tissue damage that accompanies inflammation and viral infections. IL-10 is involved in maintenance of immune homeostasis and provides protection against immune-mediated tissue damage by its immunomodulatory action (Dimitriadis et al., 2023).

Due to its anti-inflammatory activity, IL-10 may limit hepatocellular damage following infection. Numerous studies have demonstrated that serum IL-10 levels are significantly higher in chronic HBV infection patients compared to healthy controls (Manea et al., 2024). High levels of IL-10 have been linked with ongoing viral replication and immune tolerance, alongside the advance towards late-stage liver diseases like cirrhosis and hepatic failure (Dimitriadis et al., 2023; Jia et al., 2024). Moreover, IL-10 is a potential prognostic biomarker in severe HBV-related complications, especially acute-on-chronic liver failure (ACLF), with higher plasma levels associated with worse clinical outcomes and mortality. These findings refers that IL-10 may be used not only as a marker of immune regulation, but also of HBV immunity and severity.

(Liu et al., 2023; Rybicka et al., 2020).

Although there is a great interest in IL-10 as a biomarker in HBV infection, the clinical application of it faces many challenges. Publications have shown notable discrepancies in cut-off values, analytical methods, and characteristics of the studied populations (Zhong et al., 2021; Manea et al., 2024), rendering comparisons among investigations cumbersome and findings less reproducible. In addition, genetic polymorphisms in the IL10 gene with other coexisting infections and prior antiviral treatments may affect circulating levels of IL-10 that complex the interpretation of serum measurements (Rybicka et al., 2020; Jia et al., 2024).

In this respect, cytokine profiles that include IL-10 with either IL-6, IL-17 or HBV DNA have been shown to better discriminate between immune-active and immune-tolerant patients, suggesting utility in optimizing antiviral therapy timing (Zhang et al., 2022; Wang et al., 2023). Moreover, IL-10 has been integrated into predictive models for predicting the probability of HBeAg seroconversion and HBsAg clearance in treatment (Xiao et al., 2024).

However, the importance of serum IL-10 from a clinical standpoint is still under discussion. Despite persistent reports of increased IL-10 levels in patients with HBV infection as compared to healthy controls, the significant overlap seen between these groups together with a lack of widely standardized cutoff values challenges its diagnostic sensitivity and specificity. Therefore, additional studies using standardized methods and larger well characterized populations are needed to validate the specific role of IL-10 as a potentially useful HBV infection biomarker. Its role in early disease stages is particularly underexplored, though accurate identification of patients at risk of chronic progression would be most valuable in that context (Dimitriadis et al., 2023; Manea et al., 2024).

Accordingly, the present study aimed to clarify the clinical significance of IL-10 in HBV infection. The objectives are threefold: (1) to evaluate differences in IL-10 levels between infected patients and healthy individuals, (2) to determine its diagnostic performance through receiver operating characteristic (ROC) curve analysis, and (3) to explore associations between IL-10 concentrations and established clinical as well as laboratory indices of HBV. Through this approach, the research aims to establish whether IL-10 can be employed as a practical, non-invasive biomarker to strengthen diagnostic precision and assist in the clinical management of HBV infection.

## Patients and Methods

### Study Design and Setting

A cross-sectional case–control study was conducted at the Teaching Hospital in Thi-Qar City, Iraq, over a seven-month period from November 2024 to May 2025. In this study, a total of 62 patients with laboratory confirmed hepatitis B virus (HBV) infection for years and up to 58 apparently healthy individuals (depending on HIV positivity) were investigated as controls. Patients were retained from both inpatient and outpatient units of a hepatitis unit after clinical suspicion followed by confirmation to HBV infection.

### Eligibility Criteria

Inclusion criteria for the patient group included: (1) clinical manifestations consistent with HBV infection, (2) laboratory confirmation of HBV infection based on serological markers including hepatitis B surface antigen (HBsAg) and HBV DNA detection, and (3) voluntary informed consent to participate in the study. Patients were excluded if co-infected with hepatitis D virus (HDV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV); had a past medical history of autoimmune disorder, malignancy; or were taking immunosuppressive therapy as such confounders may affect measurements of cytokines. The exclusion criteria for the control group were likewise applied.

### Sample Collection and Laboratory Procedures

Demographic and clinical data were collected using standardized questionnaires, which were then validated through review of the patients' medical records. A total of 5 mL of peripheral venous blood was drawn from each participant under aseptic conditions and stored in plain tubes. After the clots were allowed to form at room temperature (around 25 °C) for a minimum of one hour, centrifugation was performed at 3,000 rpm for 10 minutes and serum was obtained. They were then aliqoutted into sterile tubes for being frozen at −20 °C until the laboratory analysis.

Hepatitis B virus (HBV) infection was diagnosed by serological detection of hepatitis B surface antigen (HBsAg). The viral replication status was additionally determined in reported cases by real-time polymerase chain reaction (PCR) quantification of HBV DNA (Qiagen, Germany), according to the manufacturer's instructions. We selected only patients who were positive for HBsAg and HBV DNA was detectable.

Measurement of IL-10 Concentrations

Serum concentrations of the pro-inflammatory interleukin (IL) IL-10 were measured by a commercially available enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (R&D Systems, USA). After dilution, 100 µL of the serum from each sample was added to microplate wells precoated with a monoclonal antibody specific for human IL-10. Detection antibodies, biotinylated, and streptavidin in horseradish peroxidase (HRP) were added according to a sequence following the incubation and washing steps. Tetramethylbenzidine (TMB) substrate was used for color development and after sufficiently developing the color, optical density at 450 nm was measured by using a microplate reader. Serum concentrations of IL-10 were determined by using standard curves based on recombinant IL-10 standards.

### Statistical Analysis

Statistical analyses were performed with IBM SPSS Statistics software version 25.0 (IBM Corp., Armonk, NY, USA). Interval and ratio measurements were described as mean ± SD whereas categorical variables were expressed as frequencies and percentages where suitable. Independent-samples t-test analysis was applied to determine differences in serum IL-10 concentrations between patients with hepatitis B virus (HBV) infection and healthy controls. ROC curve analysis was used to ascertain the diagnostic performance of serum IL-10 as a biomarker for HBV infection. We calculated the area under the curve (AUC), sensitivity, specificity, and best cutoff value to estimate the capacity of IL-10 as a discriminative marker. A two-tailed p-value of $<0.05$ was defined as significant.

## Results

Table 1 shows the comparison of age, sex, and smoking habits among control and Hepatitis B infection patients. The majority of subjects in both groups were between 30–49 years of age. In addition, the gender distribution are not differ, as males constituted 58.1% of the patient group and 53.4% of the controls, while females represented 41.9% and 46.6%, correspondingly. Regarding smoking status, most participants are smokers especially observed among patients (35.5%) compared to controls (29.3%).

**Table 1. Comparison for Age, sex and smoking habit among control and Hepatitis B infection patients**

| Items | | `Patients (No. = 62) | | Control (No. = 58) | | Chi Square | P value (Sig.) |
|---|---|---|---|---|---|---|---|
| | | Freq. | % | Freq. | % | | |
| Age/Years | 20-29 | 14 | 22.60 | 13 | 22.40 | 1.43 | 0.23 (NS) |
| | 30-39 | 18 | 29.00 | 15 | 25.90 | | |
| | 40-49 | 16 | 25.8 | 16 | 27.6 | | |
| | ≥ 50 | 14 | 22.6 | 14 | 24.1 | | |
| Sex | Male | 36 | 58.1 | 31 | 53.4 | 2.58 | 0.11 (NS) |
| | Female | 26 | 41.9 | 27 | 46.6 | | |
| Smoking | Smoker | 22 | 35.5 | 17 | 29.3 | 2.89 | 0.09 (NS) |
| | Non-smoker | 40 | 64.5 | 41 | 70.7 | | |

**NS: Non-significant at P>0.05**

The distribution of patients by hepatitis B vaccination status revealed that the majority were non-vaccinated (67.7%), while only a smaller proportion had received prior vaccination (32.3%). (Figure 1).

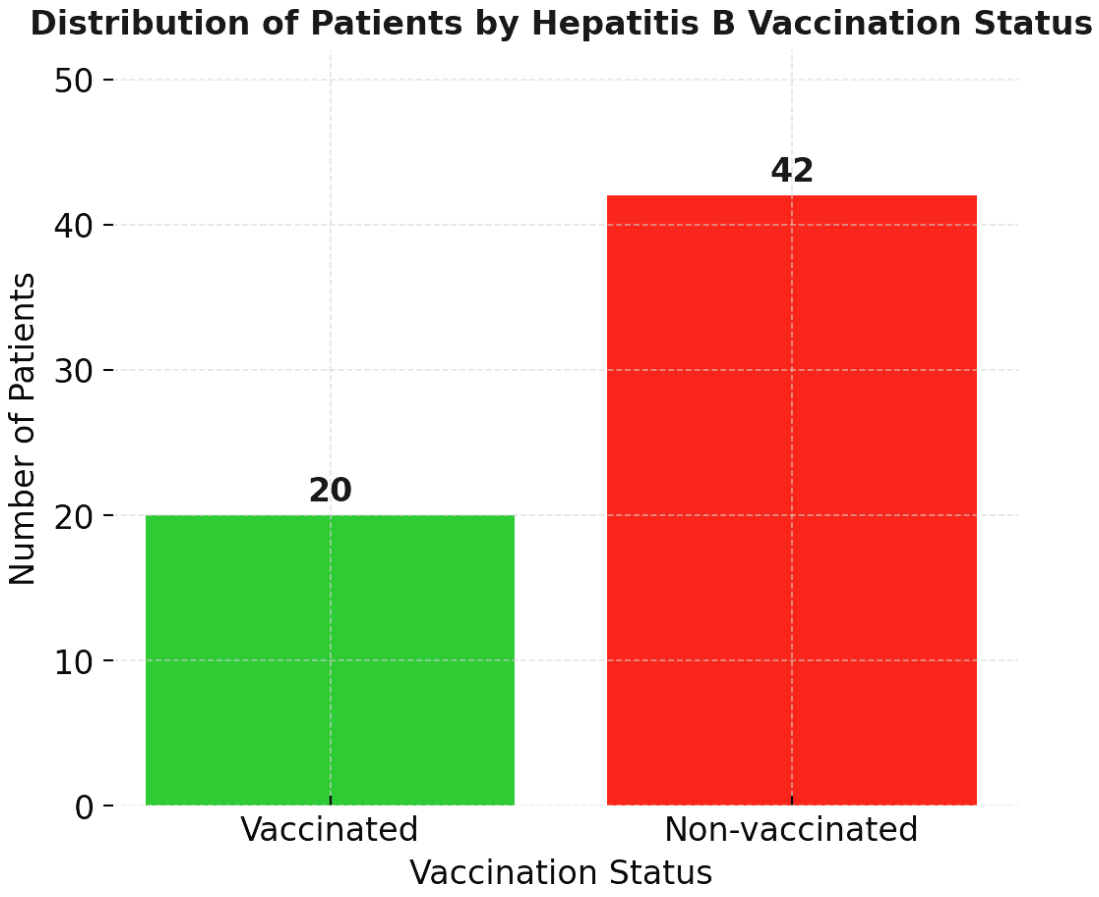


**Figure 1. Distribution of patients according to their vaccination status against hepatitis B**

Table 2 demonstrates that serum IL-10 levels were significantly increased in patients with hepatitis B compared to healthy controls. The mean IL-10 concentration in the patient group was 18.8 ± 4.7 pg/ml, whereas the control group recorded a lower mean value of 15.9 ± 3.8 pg/ml. Statistical analysis using the

independent t-test revealed a significant difference between the two groups ($p < 0.04$).

**Table 2. Assessment of the levels of IL-10 between patients with hepatitis B and control participants**

| Groups | No. | IL-10 (pg/ml) Mean ± SD | (P Value) |
|---|---|---|---|
| Patient | 62 | 18.8 ± 4.7 | P < 0.04 (S) |
| Control | 58 | 15.9 ± 3.8 | |

**S: Significant at P<0.05**

table 3 shows that patients who had received hepatitis B vaccination demonstrated higher mean serum IL-10 levels (19.2 ± 3.2 pg/ml) compared to the non-vaccinated group (13.5 ± 2.8 pg/ml). The difference was statistically significant ($t = 2.54$, $p = 0.02$).

**Table 3. Comparison in IL-10 levels in patients' groups based on vaccination status against hepatitis B**

| Age Sub-groups | Freq. | IL-6 (pg/ml) Mean ± S.D | T test | T test P-value |
|---|---|---|---|---|
| Vaccinated | 20 | 19.2 ± 3.2 | 2.54 | 0.02 (S) |
| Non-vaccinated | 3 | 13.5 ± 2.8 | | |

**S: Significant at P<0.0**

Table 4 reveals the area under the curve (AUC) is about 0.78 ($p < 0.02$). This was determined based on the cutoff value at 15.5 pg/ml, IL-6 resulting in a sensitivity of 82% and a specificity of 73% (figure 2).

**Table 4. The diagnostic power of IL-10 for the diagnosis of hepatitis B infection**

| Biomarker | (AUC) | p-value | Cut-off Point | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|
| IL-10 | 0.78 | 0.02 | 16 | 0.82 | 0.73 |

**AUC: Area Under the curve**

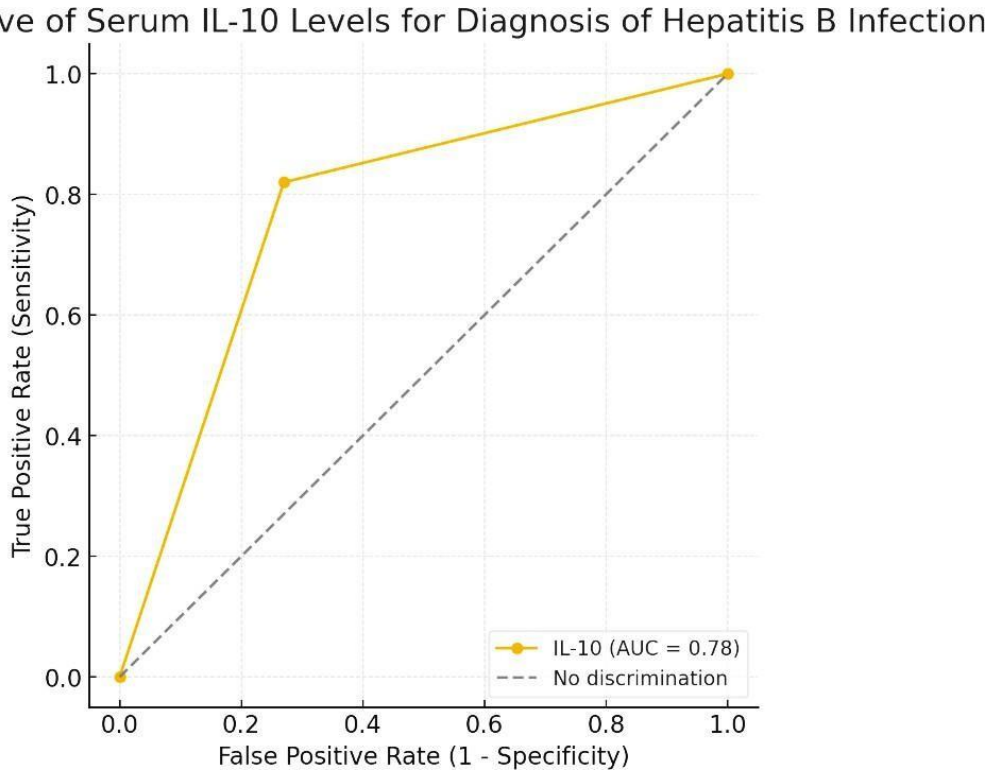


**Figure 2. ROC Curve of Serum IL-10 Levels for diagnosis of hepatitis B infection**

**Discussion**

In this study, serum IL-10 concentrations exhibited a significant increase in patients with hepatitis B infection (n = 62; mean = 18.6 ± 4.7 pg/ml) compared with healthy controls (n = 58; mean = 15.9 ± 3.8 pg/ml; P < 0.04). Subgroup analysis by hepatitis B vaccination status further showed that vaccinated patients had higher IL-10 (19.5 ± 3.2 pg/ml) versus non-vaccinated (13.5 ± 2.8 pg/ml), and ROC analysis reported that IL-10 had moderate diagnostic performance for hepatitis B in this cohort (AUC = 0.78, cut-off ≈ 16.0 pg/ml; sensitivity 82%, specificity 73%). Taken together, these results refer a reproducible association between HBV infection and elevated circulating IL-10 and suggest that IL-10 has potential as a biomarker that reflects disease-associated immunoregulation.

Biologically, IL-10 is a central anti-inflammatory and regulatory cytokine produced by multiple cellular sources (regulatory B cells, regulatory T cells, macrophages and other myeloid cells). Increased IL-10 in HBV infection is mechanistically plausible: IL-10 limits antigen-specific effector T cell responses and may thereby contribute to viral persistence while protecting the host from excessive immune-mediated liver damage (Zheng et al., 2023; Dimitriadis, 2023). Our finding of higher IL-10 among patients compared with controls aligns with this view and with several recent clinical reports. For instance, The change in IL-10 was consistent with that reported in this paper, in which a decreasing trend was shown, which seems important to the severity of the disease (Zhang et al., 2022; Su et al., 2022). All available evidence is support of more consistent compensatory regulatory mechanism of the IL-10 expression in hepatitis B virus (HBV) infection than its mere by-product aspect upon HBV-infection. Although, IL-10 declines inflammation, its continuous elevation has clinical significance. Emerging data have repeatedly shown that high levels of IL-10 are often associated with adverse disease phenotypes, including poor antiviral T-cell function, chronic viral persistence and evolution towards severe forms of liver injury compatible with acute-on-chronic liver failure. Moreover, increased IL-10 also has been associated with poor short-term survival in specific cohorts (Liu et al., 2023; Yu et al., 2016). These are in accord with the notion that IL-10 may serve a dual purpose: It protects hepatic tissue from immunopathology but, at the same time, suppresses immune-mediated viral clearance and thereby drives chronicity. Cytokines/chemokines were selected for further exploration of their serum expression in patients with HBV infection-related diseases, periods in HCC patients.

Results of the present study support this interpretation. The higher mean levels of IL-10 and an area under the ROC curve (AUC) of 0.78 refer moderate discriminatory ability for distinguishing infected from uninfected individuals. However, elevated IL-10 levels should be interpreted cautiously, since they may reflect either protective immune regulation or more advanced disease.

The ROC-derived cut-off value (~16 pg/ml), located between the mean values of the two groups, yielded a sensitivity of 82% and specificity of 73%. These findings suggest that IL-10 may serve as an adjunct diagnostic marker rather than a stand-alone test and should be interpreted together with clinical findings and virological parameters. Cytokine levels may be influenced by coexisting infections, vaccination status, treatment history, and host genetic factors. In agreement with previous studies, genetic polymorphisms within the IL10 promoter may affect IL-10 expression and influence the outcome of HBV infection (Rybicka et al., 2020). Interestingly, vaccinated patients exhibited higher IL-10 levels than non-vaccinated patients. This observation may reflect vaccine-induced immune activation and enhanced regulatory responses or differences in immune priming among vaccinated individuals who subsequently acquired HBV infection (Rybicka et al., 2020).

One salient finding from our data is the elevated IL-10 in vaccinated patients vs non-vaccinated. At first glance counterintuitive, several explanations are offered. The protective effects of IL-10 elicited by HBV vaccination in patients with both HBV and HCC could occur for one (or more) of the following general reasons: first, anti-HBV vaccination causes antigen exposure and immune stimulation that temporarily increase regulatory responders including IL-10 as part of normal homeostatic recovery. Third, given the small size of the non-vaccinated subgroup (n = 3), this difference could be unstable and susceptible to sampling error. Published literature on vaccination and IL-10 in the setting of breakthrough or natural HBV infection is limited and heterogeneous, so this finding should be treated as hypothesis-generating and tested in larger cohorts.

When compared with recent literature, our principal findings are concordant with broader trends. Systematic and narrative reviews emphasize IL-10's pivotal role in HBV immunopathogenesis and its correlation with viral persistence and disease severity (Dimitriadis, 2023; Zheng et al., 2023). Observational cohort studies and biomarker analyses published since 2016 commonly report elevated IL-10 in CHB and in advanced HBV-related syndromes; some studies also show post-treatment modulation of IL-10 (increases or decreases depending on therapy and disease stage) and associations between IL-10 and virological markers (Zhang et al., 2022; Su et al., 2022; Liu et al., 2023).

This study has important limitations. Although the patient sample (n = 62) provided power to detect group differences, subgroup analyses—particularly in non-vaccinated patients (n = 3)—are underpowered and may not generalizable. Second, the cross-sectional design is limiting for causal inference because elevated IL-10 could be a consequence of disease severity, a factor in viral persistence, or both. Third, not all of the potential confounders (such as concurrent infections, medication use, timing since vaccination or exposure, viral load, HBeAg status, liver fibrosis stage and IL10 genotypes) were controlled for in this analysis. Last, different types of assays and preanalytical handling can lead to variation in cytokine measurements replication using standardized assays and longitudinal sampling would be ideal.

**Conclusion**

Serum IL-10 levels were significantly higher in hepatitis B patients versus healthy controls, with moderate diagnostic accuracy (AUC 0.73). This finding reinforces the immunosuppressive role of IL-10 during HBV infection and its potential for use as a diagnostic and prognostic biomarker. Additional investigation with larger cohorts, longitudinal evaluation, and incorporation of virological and immunological correlates will improve its clinical utility.

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