Correlation Between Serum Soluble PD-1, CD8+, CD4+ T Lymphocyte Counts and Liver Stiffness in Antiviral-Naïve Chronic Hepatitis B Patients

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ABSTRACT

Background: *Chronic hepatitis B (CHB) disease is a major global health problem with a high burden in Asia, including Indonesia. Liver stiffness measurement by transient elastography is a non-invasive key tool to assess fibrosis severity. The immune response in CHB involves T cell exhaustion marked by elevated soluble PD-1 (sPD-1) and changes in T lymphocyte subsets, which may influence fibrosis progression. This study aimed to assess the correlation between serum sPD-1, CD4+, and CD8+ T lymphocyte counts and liver stiffness in antiviral-naïve CHB patients.*

Methods: *A cross-sectional study was conducted among 60 untreated CHB patients at Mohammad Hoesin Hospital, Palembang, Indonesia. The sPD-1 was measured by ELISA, while CD4+ and CD8+ T cell counts were determined via flow cytometry. Liver stiffness was assessed by transient elastography. Spearman correlation and multivariate linear regression were used to analyze associations between immune markers and liver stiffness.*

Results: *Serum sPD-1 showed a significant positive correlation with liver stiffness ($r = 0.481$, $p < 0.001$), while CD8+ T lymphocyte count was negatively correlated ($r = –0.553$, $p < 0.001$). CD4+ T lymphocyte count showed no significant bivariate correlation ($r = –0.189$, $p = 0.149$). In the multivariate analysis, sPD-1 ($\beta = 0.050$, $p < 0.001$), CD4+ T cells ($\beta = –0.018$, $p = 0.001$), and AST ($\beta = 0.061$, $p = 0.005$) were independent predictors of liver stiffness.*

Conclusion: *Serum sPD-1 and CD4+ T-lymphocyte count are independently associated with liver stiffness in antiviral-naïve CHB patients. These findings suggested the involvement of immune exhaustion and liver injury in HBV-related fibrosis.*

Keywords: *CD4+ T Lymphocyte, Chronic Hepatitis B, Liver Stiffness, Immune Exhaustion, Soluble PD-1*

ABSTRAK

Latar belakang: *Pengukuran kekakuan hati (liver stiffness) melalui transient elastography merupakan metode non-invasif utama untuk menilai tingkat keparahan fibrosis pada pasien hepatitis B kronik atau chronic hepatitis B (CHB). Respons imun pada CHB melibatkan kelelahan sel T (T cell exhaustion) yang ditandai dengan*

peningkatan kadar soluble PD-1 (sPD-1) dan perubahan subset limfosit T. Penelitian ini bertujuan untuk menilai hubungan antara kadar serum sPD-1, jumlah limfosit T CD4+ dan CD8+ dengan kekakuan hati pada pasien CHB yang belum pernah menerima terapi antivirus.

Metode: *Penelitian potong lintang dilakukan pada 60 pasien CHB yang belum diobati di RSUP Dr. Mohammad Hoesin, Palembang, Indonesia. Kadar sPD-1 diukur menggunakan metode ELISA, sedangkan jumlah sel T CD4+ dan CD8+ ditentukan melalui flow cytometry. Kekakuan hati diukur dengan transient elastography. Analisis korelasi Spearman dan regresi linier multivariat digunakan untuk mengevaluasi hubungan antara penanda imun dan kekakuan hati.*

Hasil: *Kadar serum sPD-1 menunjukkan korelasi positif yang signifikan dengan kekakuan hati ($r = 0,481$; $p < 0,001$), sedangkan jumlah limfosit T CD8+ berkorelasi negatif ($r = –0,553$; $p < 0,001$). Jumlah limfosit T CD4+ tidak menunjukkan korelasi bermakna secara bivariat ($r = –0,189$; $p = 0,149$). Dalam analisis multivariat, sPD-1 ($\beta = 0,050$; $p < 0,001$), CD4+ ($\beta = –0,018$; $p = 0,001$), dan AST ($\beta = 0,061$; $p = 0,005$) merupakan prediktor independen kekakuan hati.*

Kesimpulan: *Kadar serum sPD-1 dan jumlah limfosit T CD4+ berhubungan secara independen dengan kekakuan hati pada pasien CHB yang belum diterapi antivirus. Temuan ini mendukung peran kelelahan imun dan cedera hati dalam patogenesis fibrosis akibat infeksi HBV.*

Kata kunci: *hepatitis B kronik, kekakuan hati, kelelahan imun, limfosit T CD4+, soluble PD-1*

INTRODUCTION

Chronic hepatitis B (CHB) remains a major global health concern, affecting approximately 296 million people worldwide and contributing to nearly half a million deaths annually, primarily due to complications such as cirrhosis and hepatocellular carcinoma (HCC).[1] The burden is particularly high in the Asia-Pacific region. In Indonesia, the national Basic Health Research (Riskesdas) survey reported a notable decline in hepatitis B prevalence, from 9.4% in 2007 to 7.1% in 2017.[2] Despite this progress, CHB continues to exert a significant health and economic burden due to underdiagnosis, limited access to antiviral therapy, and late-stage presentation of liver disease.[1] The progressive nature of CHB, often asymptomatic in early stages, underscores the importance of early and accurate staging to guide timely intervention.

Liver stiffness, a surrogate marker for hepatic fibrosis, plays a critical role in evaluating the severity and prognosis of chronic liver diseases.[3] Transient elastography (FibroScan) is a non-invasive, reproducible, and widely endorsed method for assessing liver stiffness and staging fibrosis, with strong correlation to histological findings.[4] International guidelines, including those from the European Association for the Study of the Liver (EASL) and the Asian Pacific Association for the Study of the Liver (APASL), recommend elastography as a frontline tool for fibrosis assessment in CHB patients.[5,6] Liver stiffness values not only aid in determining the risk of cirrhosis and HCC but also inform therapeutic decisions—such as the initiation of antiviral treatment—particularly when ALT levels are borderline or normal.[7] Fibrosis staging is increasingly being integrated into prognostic models to predict clinical outcomes and monitor disease progression.

The immunopathogenesis of CHB involves complex interactions between viral replication and host immune regulation. $CD8^+$ T lymphocytes are the primary cytotoxic effectors against hepatitis B virus (HBV)-infected hepatocytes;[8] however, during chronic infection, these cells become functionally exhausted. This exhaustion is marked by sustained expression of inhibitory receptors such as programmed cell death-1 (PD-1), which impairs antiviral immunity. Soluble PD-1 (sPD-1), a circulating form of the membrane-bound receptor, is produced via alternative splicing and proteolytic cleavage.[9] The sPD-1 may competitively inhibit PD-1/PD-L1 interactions, modulate immune responses, and reflect the degree of T cell exhaustion. $CD4^+$ T lymphocytes also contribute to immune regulation by providing help to cytotoxic cells and modulating cytokine profiles.[10] Dysregulation in these immune components not only contributes to viral persistence but is also implicated in hepatic inflammation and fibrogenesis, highlighting their potential role as immune biomarkers in CHB progression.[11]

Several studies have explored the association between sPD-1 levels, T lymphocyte subsets, and various clinical outcomes in CHB, including immune phase classification and fibrosis.[12,13] Elevated sPD-1 levels have been correlated with advanced liver inflammation and fibrosis, while diminished $CD8^+$ T cell function is a hallmark of viral chronicity.[14]

However, limited researches have examined the combined role of sPD-1, $CD4^+$, and $CD8^+$ T cells in relation to liver stiffness, particularly in antiviral-naïve populations. These immune markers may serve as additional non-invasive indicators to refine fibrosis assessment and support treatment decisions in this population. Therefore, this study aimed to determine the correlation between serum sPD-1 levels, CD4+ and CD8+ T lymphocyte counts, and liver stiffness in antiviral-naïve chronic hepatitis B patients.

METHODS

Study Design and Subjects

This study employed a cross-sectional design involving untreated chronic hepatitis B (CHB) patients. Participants were recruited consecutively from the gastroenterohepatology outpatient clinic at the Department of Internal Medicine, Mohammad Hoesin Hospital, Palembang, between February and December 2023. The inclusion criteria were adults ≥18 years old with confirmed HBsAg positivity for more than 6 months who had never received antiviral therapy and were willing to provide informed consent. Patients were excluded if they had co-infection with hepatitis C virus (HCV) or human immunodeficiency virus (HIV), autoimmune liver disease, hepatocellular carcinoma, decompensated liver cirrhosis, pregnancy, or were undergoing immunosuppressive treatment. This study received ethical approval from the Health Research Ethics Committee of the Mohammad Hoesin Hospital (No. DP.04.03/D.XVIII.6.8/ETIK/102/2024). All participants signed written informed consent prior to enrolment.

Laboratory Examination

Peripheral venous blood samples were collected from each participant to analyze serum soluble PD-1 (sPD-1) levels and quantify $CD4^+$ and $CD8^+$ T lymphocyte counts. The measurement of sPD-1 was performed at Prodia Laboratory using a commercially available ELISA® Kit (Abcam, Cat. ab252360, Lot: 2101049856). Serum samples and standards were dispensed into designated wells of a microtiter plate, followed by the addition of antibody cocktail and incubation for 1 hour at room temperature. After incubation, the wells were washed to remove unbound antibodies, and a TMB development solution was added. The plate was then incubated for 10 minutes, after which the stop solution was applied to terminate the reaction. Optical density was measured using an ELISA reader at the specified wavelength and sPD-1 concentrations were calculated based on the standard curve. Quantification of $CD4^+$ and $CD8^+$ T lymphocytes was performed by flow cytometry using a BD FACSCalibur system. Whole blood samples were stained with reagents containing fluorochrome-conjugated monoclonal antibodies that specifically bind to antigens on the surface of leukocytes. The stained cells were then passed through a laser beam in the flow cytometer, resulting in light scatter and fluorescence emission. The scatter and fluorescence signals were detected and analyzed to identify and quantify $CD4^+$ and $CD8^+$ T cell subsets. Additional laboratory parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), platelet count, HBeAg status, and HBV DNA levels, were examined at the Clinical Pathology Laboratory of Mohammad Hoesin Hospital Palembang.

Liver Stiffness Measurement

Liver stiffness was assessed using transient elastography (FibroScan®, Echosens, Paris, France), a non-invasive modality performed by a trained operator following standard operational procedures. Valid measurements were defined by obtaining at least 10 valid readings with a success rate of ≥60% and an interquartile range (IQR) ≤30% of the median value. Liver stiffness values were reported in kilopascals (kPa). Fibrosis staging was categorized based on EASL guidelines, with a liver stiffness threshold of ≥7.9 kPa considered indicative of significant fibrosis (≥F2) in chronic hepatitis B patients.

Statistical Analysis

All statistical analyses were carried out using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize the characteristics of the study population. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on the distribution. The Spearman correlation test was employed to evaluate the association between serum sPD-1 levels, $CD4^+$ and $CD8^+$ T lymphocyte counts, and liver stiffness. Variables with p-values <0.25 in bivariate analysis were included in a multivariate linear regression model to identify independent predictors of liver stiffness. A two-tailed p-value of <0.05 was considered statistically significant.

RESULTS

A total of 60 antiviral-naïve chronic hepatitis B patients were enrolled in this study. The mean age of participants was 41.85 ± 14.59 years old, with an equal sex distribution (30 males, 50%). The average body mass index (BMI) was 23.65 ± 3.77 kg/m². The mean hemoglobin level was 12.82 ± 1.89 g/dL, and the median leukocyte count was 7.82 × 10³/mm³ (range: 1.03–17.5 × 10³/mm³). The mean platelet count was 259.1 ± 101.57 × 10³/mm³. The median serum albumin concentration was 4.0 g/dL (range: 1.3–5.1 g/dL) and the median total bilirubin was 0.6 mg/dL (range: 0.3–5.4 mg/dL). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels had wide ranges, with median values of 45 U/L (range: 14–473) and 53 U/L (range: 12–351), respectively. HBeAg positivity was identified in 27 patients (45%), and the median HBV DNA level was 73,450 IU/mL (range: 10–5.25 × 10⁸ IU/mL). Baseline characteristics are summarized in **Table 1.**

Table 1. Baseline Characteristics of The Study

Characteristics	Subjects (n=60)
Age (years)	41.85±14.59
Male Sex, n (%)	30 (50)
Body Mass Index (kg/m²)	23.65±3.77
Hemoglobin (g/dL)	12.82±1.89
Leucocytes (10³/mm³)	7.82 (1.03-17.5)
Platelets (10³/mm³)	259.1±101.57
Serum Albumin (g/dL)	4 (1.3-5.1)
Total Bilirubin (mg/dL)	0.6 (0.3-5.4)
AST (U/L)	45 (14-473)
ALT (U/L)	53 (12-351)
HbeAg Positivity	27 (45)
HBV DNA	73,450 (10-5.25x10⁸)
Serum sPD-1 (pg/mL)	268.3 (142.7-743.4)
CD8+ T-lymphocyte (cell/uL)	541 (77-1370)
CD4+ T-lymphocyte (cell/uL)	724.42±283.14
Liver Stiffness (kPa)	7.8 (2.9 – 75.4)

BMI = Body Mass Index; HBeAg = Hepatitis B e Antigen; HBV DNA = Hepatitis B Virus Deoxyribonucleic Acid; sPD-1 = Soluble Programmed Cell Death Protein-1; CD8+ T-lymphocyte = Cluster of Differentiation 8 Positive T Lymphocyte; AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase.

As shown in **Table 2** and **Figure 1,** a significant positive correlation was found between serum soluble PD-1 (sPD-1) levels and liver stiffness measured by transient elastography ($r = 0.481$, $p<0.001$), suggesting that higher sPD-1 levels are associated with greater fibrosis severity. CD8+ T lymphocyte counts showed a significant negative correlation with liver stiffness ($r = –0.553$, $p < 0.001$), indicating that lower CD8+ T cell levels were associated with increased liver stiffness. In contrast, CD4+ T lymphocyte counts exhibited a weak negative correlation with liver stiffness ($r = –0.189$), which was not statistically significant ($p = 0.147$). These correlations are visually presented in Figure 2, which illustrates the relationship between serum sPD-1 levels, CD8+ T lymphocyte counts, and CD4+ T lymphocyte counts with liver stiffness in antiviral-naïve chronic hepatitis B patients.

As shown in **Table 2,** bivariate analysis revealed several significant correlations with liver stiffness. Serum soluble PD-1 (sPD-1) levels were positively correlated ($r = 0.481$, $p < 0.001$), while CD8+ T lymphocyte counts showed a strong negative correlation ($r = –0.553$, $p < 0.001$). CD4+ T lymphocyte counts demonstrated a weak, non-significant negative correlation ($r = –0.189$, $p = 0.147$). Other significant associations included age ($r = 0.524$, $p < 0.001$), hemoglobin ($r = –0.272$, $p = 0.036$), platelet count ($r = –0.399$, $p = 0.002$), albumin ($r = –0.560$, $p < 0.001$), AST ($r = 0.749$, $p < 0.001$), ALT ($r = 0.751$, $p < 0.001$), and HBV DNA levels ($r = 0.332$, $p = 0.009$). However, BMI and total bilirubin showed no significant correlation.

Table 2. Bivariate and Multivariate Analyses of Factors Associated with Liver Stiffness in Antiviral-Naïve Chronic Hepatitis B Patients

Characteristics	Bivariate Analysis		Multivariate Analysis	
	r	p	β (95%CI)	P
Serum sPD-1 (pg/mL)	**0.481**	**<0.001**	**0.050 (0.026-0.073)**	**<0.001**
CD8+ T-lympocyte (cell/uL)	**-0.553**	**<0.001**		
CD4+ T-lympocyte (cell/uL)	**-0.189**	**0.147**	**-0.018 (-0.029 to -0.007)**	**0.001**
Age (years)	**0.524**	**<0.001**		
Body Mass Index (kg/m²)	-0.138	0.293		
Hemoglobin (g/dL)	**-0.272**	**0.036**		
Platelets (/mm³)	**-0.399**	**0.002**		
Albumin (g/dL)	**-0.560**	**<0.001**		
AST (IU/L)	**0.749**	**<0.001**	**0.061 (0.020 – 0.103)**	**0.005**
ALT (IU/L)	**0.751**	**<0.001**		
Total Bilirubin (mg/dL)	0.176	0.178		
HBV DNA	**0.332**	**<0.009**		
Constant			6.747 (-1.69 to 15.18)	0.115

Bivariate analysis was performed using Spearman correlation (r), while multivariate analysis was conducted using linear regression. β = regression coefficient; CI = confidence interval; sPD-1 = soluble programmed cell death protein-1; CD4+ = cluster of differentiation 4 positive T lymphocyte; CD8+ = cluster of differentiation 8 positive T lymphocyte; AST = aspartate aminotransferase; ALT = alanine aminotransferase. R 0.687, Adjusted R Square 0.443.

In the multivariate linear regression analysis, three variables were independently associated with liver stiffness. Serum sPD-1 remained significantly associated with liver stiffness (β = 0.050, 95% CI: 0.026–0.073, p < 0.001), indicating that for every 1 pg/mL increase in sPD-1, liver stiffness increased by an estimated 0.050 kPa, controlling for other variables. $CD4^+$ T lymphocyte count was also significantly associated (β = –0.018, 95% CI: –0.029 to –0.007, p = 0.001), meaning that for every increase of 1 cell/μL in $CD4^+$ T cells, liver stiffness decreased by 0.018 kPa. AST levels were positively associated (β = 0.061, 95% CI: 0.020–0.103, p = 0.005), suggesting that each 1 U/L increase in AST was associated with a 0.061 kPa increase in liver stiffness. The final model showed an R value of 0.687 and an adjusted R^2 of 0.443, indicating that approximately 44.3% of the variation in liver stiffness could be explained by these three variables. Although $CD8^+$ T lymphocyte count was significant in the bivariate analysis, it did not remain in the multivariate model as an independent predictor.

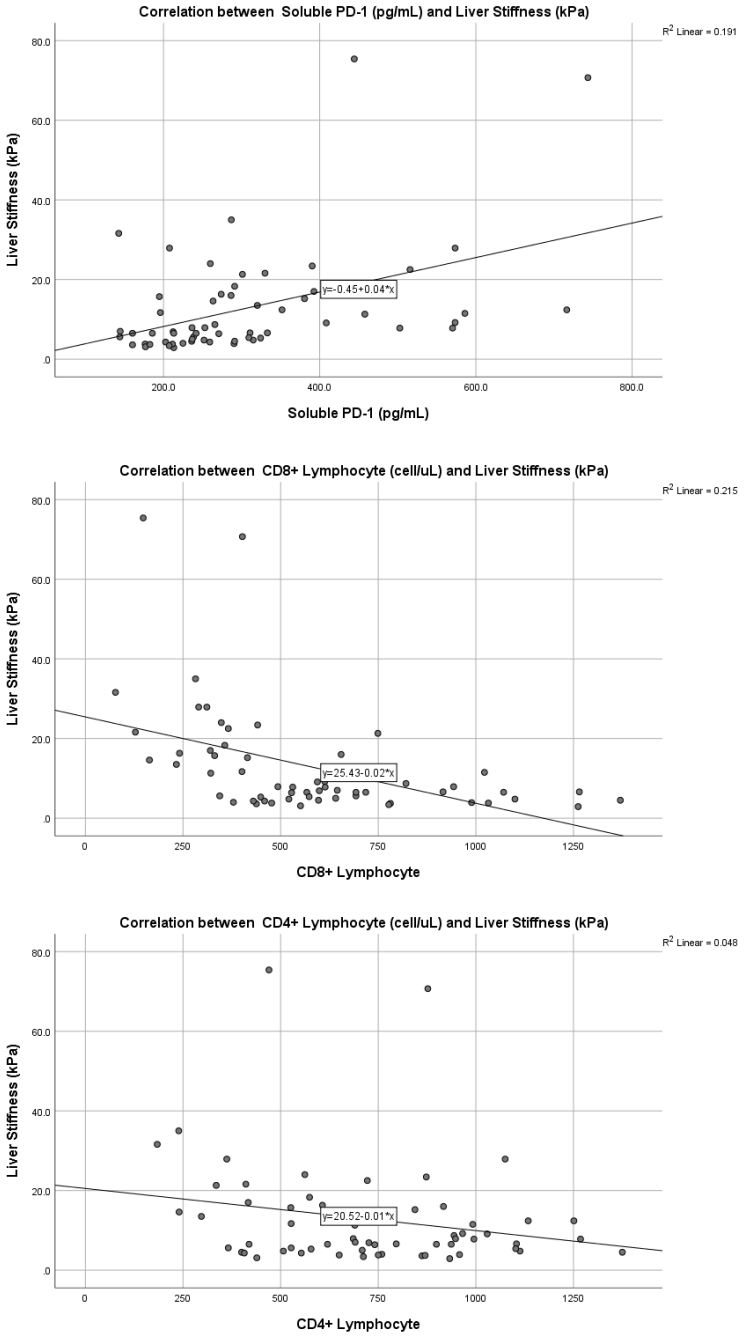

Figure 1. Correlation Between Immune Markers and Liver Stiffness in Antiviral-Naïve Chronic Hepatitis B Patients

Upper panel: Soluble PD-1 (pg/mL) shows a moderate positive correlation with liver stiffness (r = 0.481, p < 0.001). Middle panel: $CD8^+$ T lymphocyte count (cells/μL) shows a moderate negative correlation (r = –0.553, p < 0.001). Lower panel: $CD4^+$ T lymphocyte count (cells/μL) shows a weak, non-significant negative correlation (r = –0.189, p = 0.147).

DISCUSSION

This study identified serum soluble PD-1 (sPD-1), $CD4^+$ T lymphocyte count, and AST as independent factors associated with liver stiffness in antiviral-naïve chronic hepatitis B patients. The analysis revealed that higher levels of sPD-1 and AST were significantly associated with increased liver stiffness, while higher $CD4^+$ T lymphocyte counts were associated with lower stiffness. In contrast, although $CD8^+$ T cell count showed a strong negative correlation in bivariate analysis, it did not remain an independent predictor after adjustment for covariates. These findings suggested that both immunological markers of T cell dysfunction and biochemical markers of liver injury contributed to fibrosis development. Moreover, these results indicated that markers like sPD-1 may offer non-invasive insights into disease progression beyond traditional clinical parameters. The integration of immune status with liver stiffness evaluation provides a more comprehensive understanding of fibrosis in chronic HBV infection. These results may have implications for future fibrosis staging and therapeutic monitoring strategies.

The independent association between sPD-1 and liver stiffness in our cohort is consistent with growing evidence that activation of the PD-1 pathway reflects both immune dysfunction and fibrogenic disease activity in chronic HBV. PD-1 is upregulated on chronically stimulated HBV-specific T cells, and when it binds PD-L1, which is induced on hepatocytes and intrahepatic antigen-presenting cells in a persistently inflamed liver, it delivers inhibitory signals. This occurs through recruitment of phosphatases to PD-1 signaling motifs, which dampens T cell receptor and CD28 signaling and leads to reduced proliferation, cytokine production, and cytotoxicity, a phenotype consistent with T cell exhaustion.[15] Ineffective antiviral control sustains antigen persistence and necroinflammation, promotes Kupffer cell and hepatic stellate cell activation, and drives extracellular matrix deposition and fibrosis. Soluble PD-1, generated through alternative splicing and or proteolytic shedding, can bind PD-L1 and may modulate PD-1 and PD-L1 interactions. Clinically, higher sPD-1 levels have been reported in immune active phases and have correlated with transaminases, viral replication markers, and advanced fibrosis on biopsy, supporting its potential utility as a non-invasive surrogate of PD-1 pathway engagement and fibrogenic activity in chronic hepatitis B.[12–14] Xia et al. reported significant variation in sPD-1 levels across HBV infection phases, with the highest concentrations observed in immune active patients and the lowest in inactive carriers.[12] Additionally, they found a weak but significant correlation between sPD-1 and HBV DNA ($r = 0.388$, $p < 0.001$), as well as a moderate correlation with ALT ($r = 0.436$, $p < 0.001$). Zhou et al. showed that sPD-1 correlated strongly with ALT ($r = 0.731$) and positively with HBV DNA and HBsAg levels, and was significantly higher in patients with advanced fibrosis (G3–4), confirmed by liver biopsy.[14] They also demonstrated the predictive value of sPD-1 for therapy initiation in patients with ALT <2× ULN, with an AUC of 81.8%. Similarly, Li et al. found that sPD-1 levels could distinguish the immune tolerant phase with high sensitivity (AUC 98.4%) and were significantly elevated in patients with hepatocellular carcinoma. These studies support the use of sPD-1 not only as an immunological marker but also as a potential non-invasive indicator of fibrosis and disease phase in HBV.[13] Taken together, sPD-1 may capture functional immune exhaustion more directly than absolute lymphocyte counts, providing a mechanistic rationale for its stronger association with liver stiffness in multivariable analysis.

The significant bivariate correlation between $CD8^+$ T lymphocyte count and liver stiffness observed in this study is consistent with the established role of $CD8^+$ cells in viral clearance and fibrogenesis control. $CD8^+$ T cells are the main cytotoxic effector cells targeting HBV-infected hepatocytes and are central to liver inflammation and fibrosis.[16,17] However, this relationship did not persist in the multivariate analysis, likely due to the overriding influence of immune exhaustion represented by sPD-1. Absolute $CD8^+$ T cell count may not adequately reflect the cells' functional capacity.[18] Studies have shown that exhausted $CD8^+$ T cells, despite being present in adequate numbers, exhibit impaired cytokine secretion and cytotoxicity due to elevated PD-1 expression.[19] This functional suppression is better captured by sPD-1 levels, which serve as a systemic biomarker of T cell dysfunction.[20,21] Thus, while $CD8^+$ count correlates with fibrosis severity on the surface, it is the functional impairment—rather than quantity—that drives fibrogenesis in chronic HBV infection.

Interestingly, $CD4^+$ T lymphocyte count showed no significant correlation in bivariate analysis but emerged as an independent predictor in multivariate regression. This suggested that the immunoregulatory role of $CD4^+$ cells becomes more apparent when accounting for co-variables such as liver injury markers and immune exhaustion. $CD4^+$ cells are

crucial for coordinating immune responses, supporting $CD8^+$ cell activity, and regulating cytokine networks, including IL-10 and TGF-β, which influence hepatic stellate cell activation.[22] Their role in downregulating fibrosis-associated pathways may contribute to the observed protective effect in multivariate analysis. In the multivariate model, AST emerged as a strong independent predictor of liver stiffness, together with sPD-1 and CD4+ T-lymphocyte count. Elevated AST reflects hepatocellular injury and necroinflammation, processes that drive hepatic stellate cell activation and fibrogenesis.[23] Unlike ALT, which is more liver-specific, AST is partly mitochondrial and may better capture more severe or chronic liver injury, which may explain its closer association with liver stiffness in our cohort, where AST remained independently associated with liver stiffness whereas ALT did not.[24,25] This finding is consistent with previous reports identifying AST-based indices as useful non-invasive markers of significant fibrosis.

The integration of immune biomarkers such as sPD-1 into liver fibrosis assessment may have several potential clinical implications. First, in antiviral-naïve CHB patients with normal or borderline ALT and intermediate liver stiffness values, sPD-1 and CD4+ T-cell counts could help identify individuals at higher risk of significant fibrosis who may benefit from closer follow-up or earlier consideration of antiviral therapy. Second, combining immune markers with existing non-invasive tools (transient elastography and fibrosis scores such as APRI or FIB-4) might improve risk stratification beyond biochemical parameters alone.[26] Finally, longitudinal monitoring of sPD-1 during follow-up or treatment could provide additional information on immune exhaustion and fibrosis dynamics. However, these findings should currently be interpreted as exploratory and primarily research-based, and should be considered only as adjunctive information, particularly when conventional parameters such as elastography and biochemical markers are inconclusive. At present, these applications remain exploratory and require validation in larger prospective studies before being translated into routine clinical practice.

In conclusion, this study provided evidence that sPD-1, $CD4^+$ T lymphocyte count, and AST are independent predictors of liver stiffness in antiviral-naïve chronic hepatitis B patients. The strength of this study lies in its integrated approach, evaluating both immunological and biochemical factors using non-invasive methods. However, certain limitations must be acknowledged. The cross-sectional design limited the ability to infer causality between immune exhaustion markers and liver fibrosis and did not allow changes over time to be assessed. Future longitudinal studies are needed to evaluate temporal changes in these markers and their relationship with fibrosis progression over time. Functional assessments such as flow cytometry-based PD-1 expression or cytokine profiling were not included, which could enhance the understanding of immune exhaustion. The modest sample size might also affect the generalizability of the results. Despite these limitations, the study added a valuable insight into the immunopathogenesis of liver fibrosis and supported further exploration of immune markers like sPD-1 as tools for fibrosis assessment and treatment planning in chronic hepatitis B.

From a clinical perspective, our findings did not support routine measurement of sPD-1 or T-lymphocyte subsets in all antiviral-naïve CHB patients at this stage. These markers should currently be regarded as research tools or potential adjunctive tests in selected cases, for example when liver stiffness measurement and conventional biochemical parameters yield discordant or inconclusive results. If future studies confirm their predictive value and clarify cost-effectiveness, sPD-1 and T-lymphocyte profiling might be incorporated into comprehensive risk stratification algorithms to guide decisions on timing of antiviral therapy and intensity of monitoring.

CONCLUSION

This study found that serum soluble PD-1 (sPD-1) and CD4+ T lymphocyte count were independently associated with liver stiffness in antiviral-naïve chronic hepatitis B patients. While CD8+ T cell count correlated negatively in bivariate analysis, it was not an independent predictor, suggesting immune dysfunction may be more important than cell quantity. These findings support the role of immune exhaustion and liver injury in HBV-related fibrosis and highlight sPD-1 as a potential non-invasive biomarker. Nevertheless, these markers should currently be interpreted as potential adjunctive biomarkers and require further validation before routine clinical use.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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AUTHOR CONTRIBUTIONS

Conceptualization, RA and EY; methodology, RA; validation, RA, EY, and SUY; formal analysis, L; investigation, RA and L; resources, SUY; data curation, RA; writing original draft preparation, RA and EY; writing review and editing, EY, L, IS, VOB, MAA, AAS, and SUY; visualization, VOB; supervision, SUY; project administration, SUY; funding acquisition, not applicable. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY

The data that support the findings of this study are not publicly available but can be obtained from the corresponding author upon reasonable request.

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