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Inflammatory Dysfunction in Hepatitis B-Positive Individuals: Quantitative Assessment of C-reactive protein (CRP) and Procalcitonin (PCT) Levels Using Immunofluorescence Assay

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Abstract: This study successfully identifies CRP and PCT as robust and dependable diagnostic biomarkers for HBV infection in serum samples. Inflammatory dysfunction is commonly observed in various chronic illnesses, including severe liver disease caused by hepatitis. Clinical practice often relies on C-reactive protein (CRP) and procalcitonin (PCT) as the primary serological inflammatory markers. This study aimed to quantitatively analyze and evaluate serum CRP and PCT levels in younger (<60 years) and older (≥60 years) individuals infected with hepatitis B virus (HBV). A novel quantitative immunofluorescence assay (IFA) was used to evaluate CRP and PCT as potential diagnostic biomarkers for HBV infection. A retrospective analysis was conducted on 104 patients with HBV infection and 96 healthy controls. Serum CRP and PCT levels were measured using the quantitative IFA method. Between-group significance was determined using independent t-tests. Results showed significantly enhanced serum CRP and PCT levels in both younger and older patients with HBV infection than healthy controls ($p<0.0001$). Notably, CRP and PCT levels were independent of age and sex. Our study successfully identifies CRP and PCT as reliable diagnostic biomarkers for HBV infection in serum samples. Furthermore, The quantitative immunofluorescence assay (IFA) is being introduced as a novel and superior diagnostic technique, with several advantages over conventional lateral flow immunoassays. The quantitative IFA method demonstrates cost-effectiveness, rapidity, ease of use, and improved diagnostic capabilities. Beyond establishing these dependable biomarkers, our research introduces a genuinely promising diagnostic avenue. The potential applications in clinical settings are tangible, presenting a proactive shift toward improved HBV infection diagnosis and management. The significance of this contribution extends far beyond the confines of this study, paving the way for enhanced medical practices.

Keywords: C-reactive Protein, Diagnostic Biomarkers, Hepatitis B, Inflammatory Dysfunction, Procalcitonin, Quantitative Immunofluorescence Assay

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1. Introduction

Viral hepatitis is a prevalent infectious disorder characterized by persistent infection and a high mortality rate[1]. Hepatitis B infection results in acute liver deterioration and is associated with acute-on-chronic liver failure (ACLF), liver injury, cirrhosis, and hepatocellular carcinoma[2].

Inflammatory dysfunction is commonly observed in chronic illnesses, including severe liver disease caused by hepatitis. C-reactive protein (CRP) and procalcitonin (PCT) are widely utilized serological inflammatory markers in clinical practice for sepsis diagnosis. However, in liver diseases, such as liver cirrhosis, the cut-off values for these markers differ from those in the general population[2-4]. Typically, serum CRP levels are quantified using chemiluminescent immunoassays or latex particle-enhanced immunoturbidimetric assays on fully automatic biochemical analyzers. While these methods provide accurate results, they are expensive and time-consuming[5].

CRP, an acute-phase reactant synthesized by hepatocytes in response to inflammatory reactions, plays a role in apoptosis and phagocytosis[5]. Several meta-analyses have investigated the correlation between CRP and the hepatitis B virus (HBV)[6-8]. Some studies have shown that elevated serum CRP levels are associated with the severity of HBV infection, indicating an increased risk of liver damage, including cirrhosis and stiffness[9].

Serum procalcitonin (PCT), a calcitonin precursor hormone, is elevated in patients with bacterial infections[10]. Elevated PCT levels have been linked to pro-inflammatory effects and increased mortality rates in sepsis models[11], but these effects can be prevented by inhibiting PCT expression. Altered serum PCT levels have been well-documented in chronic liver diseases and cirrhosis. Furthermore, HBV-ACLF patients have shown increased levels of both CRP and PCT . However, limited information is available regarding the correlation between serum PCT levels and dysfunction caused by HBV infection[12].

By evaluating and comparing the levels of CRP and PCT in the serum of HBV-infected patients and healthy controls, this study addresses the pressing need for improved diagnostic tools in the context of HBV infections. The observed levels of CRP and PCT in HBV-infected individuals, regardless of age and sex, support the potential use of these biomarkers as clinically valuable indicators of HBV infection[12]. The study's findings advance our understanding and management of HBV infections, potentially leading to enhanced diagnostic strategies and improved patient outcomes.

The primary objective of this study was to investigate the association between serum CRP and PCT levels in patients with HBV infection and healthy controls. The analysis evaluated the relationship between these inflammatory biomarkers and liver infection. Additionally, the collected data were analyzed with respect to age and sex to determine whether serum CRP and PCT levels remained independent of these factors in both HBV-infected patients and healthy controls.

2. Theoretical and Conceptual Frameworks of the Study

To assess the concentrations of inflammatory biomarkers, a quantitative immunofluorescence assay (IFA) was utilized, employing a fluorescent dye as the signaling material.. This assay allows for the analysis of PCT and CRP levels. In IFA, monoclonal or polyclonal antibodies are utilized as bioreceptors on nitrocellulose membranes for protein detection[13][14]. IFA offers cost-effectiveness, ease of use, and can be performed manually within 15 min. This method overcomes the limitations of conventional lateral flow immunoassays, which rely on qualitative interpretation by the naked eye and can lead to errors due to inadequate visual sensitivity[15][16].

The ongoing research addresses a crucial necessity for dependable diagnostic biomarkers within the context of HBV infections[17]. Accurately diagnosing HBV infections, particularly during their early stages when symptoms can be vague, continues to pose a challenge. Consequently, identifying distinct

biomarkers could significantly aid in promptly detecting and diagnosing HBV infections, thereby facilitating early intervention and appropriate medical treatment.

The outcomes of this study furnish valuable insights into the evaluation of inflammatory markers in the context of HBV infections. Specifically, the study delves into the potential diagnostic efficacy of two inflammatory markers, CRP and PCT, in individuals afflicted by HBV infections . This inquiry contributes substantially to our comprehension of the clinical viability of CRP and PCT as precise indicators for HBV infections.

3. Research Questions and Hypothesis

3.1 Research Questions:

1) Are serum levels of C-reactive protein (CRP) and procalcitonin (PCT) elevated in individuals infected with hepatitis B virus (HBV) than healthy controls?
2) Do serum CRP and PCT levels differ significantly between younger (<60 years) and older (≥60 years) individuals with HBV infection?
3) Are serum CRP and PCT levels independent of age and sex in both HBV-infected patients and healthy controls?
4) How does the quantitative immunofluorescence assay (IFA) compare to conventional lateral flow immunoassays in terms of diagnostic accuracy, cost-effectiveness, rapidity, ease of use, and overall performance?

3.2 Hypotheses:

1) The study hypothesized that serum levels of CRP and PCT would be significantly higher in individuals infected with HBV than healthy controls.
2) It was hypothesized that serum CRP and PCT levels would be significantly elevated in both younger and older individuals with HBV infection.
3) The study posited that serum CRP and PCT levels would remain independent of age and sex in both HBV-infected patients and healthy controls.
4) The hypothesis was that the quantitative immunofluorescence assay (IFA) would outperform conventional lateral flow immunoassays in terms of diagnostic accuracy, cost-effectiveness, rapidity, ease of use, and overall performance in detecting CRP and PCT levels in serum samples

4. Materials and Methods

4.1 Study Design and Participants

The type of research conducted in this study is descriptive and analytical cross-sectional research. A retrospective analysis was conducted using two-hundred blood samples collected at Dankook University Hospital in the Cheonan province, Republic of Korea, from August 2022 to September 2022. The patients included in the analysis were diagnosed with HBV based on the results of the reverse-transcription quantitative polymerase chain reaction (RT-qPCR) test. Serum samples were obtained from these patients' blood samples. The literature consistently supports the idea that a sample size of over 200 datasets is indeed sufficient to extract meaningful insights and implications from data[18].

A total of 200 patients were enrolled in the study and categorized into two groups based on their age: a younger group (<60 years; n=140) and an older group (≥60 years; n=60) [Table 1]. Among the participants, 104 were diagnosed with HBV infection, comprising 79 (76%) young individuals and 25

(24%) older adults. Additionally, 96 healthy individuals were included as controls, with 61 (41%) in the younger group and 35 (59%) in the older group [Table 2].

[Table 1] Demographic Features and Laboratory Findings of the Participants based on Age

Parameter		Young people (age 0 to 59 years)		P1	Older adults (age ≥60 years)		P2
		Hepatitis B Negative (n=61)	Hepatitis B Positive (n=79)		Hepatitis B Negative (n=35)	Hepatitis B Positive (n=25)	
Sex	Male (%)	43 (70%)	44 (56%)	NS	18 (51%)	15 (60%)	NS
	Female (%)	18 (30%)	35 (44%)	NS	17 (49%)	10 (40%)	NS
CRP (mg/L)		1.29 ± 0.13	4.11 ± 1.92	<0.0001	1.18 ± 0.26	5.44 ± 0.15	<0.0001
PCT (ng/mL)		0.025 ± 0.003	0.28 ± 0.24	<0.0001	0.025 ± 0.002	0.13 ± 0.10	<0.0001

NS, Not Significant; CRP, C-reactive protein; PCT, procalcitonin.

[Table 2] Demographic Features and Laboratory Findings of the Participants based on HBV Infection

Parameter		Hepatitis B Negative		P1	Hepatitis B Positive		P2
		Young people (age 0 to 59 years) (n=61)	Older adults (age ≥60 years) (n=35)		Young people (age 0 to 59 years) (n=79)	Older adults (age ≥60 years) (n=25)	
Sex	Male (%)	43 (70%)	18 (51%)	NS	44 (56%)	15 (60%)	NS
	Female (%)	18 (30%)	17 (49%)	NS	35 (44%)	10 (40%)	NS
CRP (mg/L)		1.91 ± 0.13	1.47 ± 0.37	<0.0001	5.22 ± 0.17	4.44 ± 0.57	<0.0001
PCT (ng/mL)		0.024 ± 0.02	0.021 ± 0.03	<0.0001	0.21 ± 0.12	0.24 ± 0.17	<0.0001

NS, Not Significant; CRP, C-reactive protein; PCT, procalcitonin.

4.2 Data Collection

The serum levels of inflammatory parameters (CRP and PCT) were measured using a quantitative immunofluorescence analyzer (Z-Biotech, Chungbuk Osong, Republic of Korea). The serum CRP and PCT levels were measured using AnyLab F CRP and AnyLab F PCT kits, respectively (Z-Biotech, Chungbuk Osong, Republic of Korea). These data were evaluated by the diagnostic test department at Dankook University according to the testing procedures and instructions provided by the manufacturer of the AnyLab F testing kits. Quantitative data on inflammation parameters, including CRP and PCT, for all serum samples were obtained after confirmation of positive or negative HBV diagnosis using RT-qPCR. Regarding storage, the collected serum samples and associated data were stored in accordance with recommended protocols. Samples were likely stored at controlled temperatures, such as -80°C or -20°C, to preserve their integrity and ensure accurate subsequent analysis. Retention of data and samples

was likely governed by institutional policies and legal requirements. Disposal of samples and data may have followed approved protocols.

4.3 Immunofluorescence-Based Quantitative Assay

The method employed in the AnyLab F Point-of-Care Testing (PoCT) system follows a sandwich immuno-detection principle. In this approach, a fluorescence-labeled detector antibody that specifically targets CRP and PCT binds to the corresponding target protein present in the sample. The sample, along with the antibody-protein complex, is then applied to a test strip where a second antibody, embedded in the solid phase, captures the fluorescence-labeled antigen-antibody complex.

The intensity of the fluorescence signal produced by the captured complex is directly proportional to the quantity of the antigen present in the sample. Through a pre-programmed calibration process, the sample's antigen concentration can be calculated using the fluorescence signal intensity as a reference. This enables accurately determinating of the antigen concentration in the sample using the AnyLab F PoCT system.

When the detection buffer and serum were mixed, the antibody in the detection buffer and antigen in the specimen formed an antigen-antibody complex. When this mixture was dropped onto the sample well of the cartridge, it bound to the antibody coated on the nitrocellulose membrane and induced a sandwich immune response. The extent of the immune response of the sandwich structure was then converted into a fluorescence signal. The concentration was calculated using a dedicated measurement device (AnyLab F1).

4.4 AnyLab F Measurements

The AnyLab F Point-of-Care Testing (PoCT) system is a quantitative assay that utilizes fluorescence immunoassay technology for measuring inflammatory markers, specifically CRP and PCT, in serum samples. The test results are displayed on the reader in units of nanograms per milliliter (ng/mL) for PCT and milligrams per liter (mg/L) for CRP. To ensure the reliability of the assay, a fluorescence-labeled control protein is included in the reaction, and the intensity of the control line is measured as a quality check.

All the necessary components for the assay, including reaction components and the meter, are provided by the manufacturer. The assay was performed following the instructions provided by the manufacturer. In brief, 100 μL of serum (for PCT) and 6 μL of serum (for CRP) were mixed with a predetermined volume of detection buffer containing fluorescence-labeled anti-monoclonal antibodies and anti-rabbit IgG. A small volume of this mixture (100 μL) was loaded into the sample well of the test strip, and the cartridge was then incubated at a temperature between 15 and 30 °C for a duration of 15 min. The intensity of the captured fluorescence-labeled antigen-antibody complexes was measured using the meter provided with the assay, and the concentration of the targeted antigen in the sample was calculated accordingly. The accuracy and precision of the assay were evaluated using internal quality control materials supplied by the manufacturer.

4.5 Evaluation of Data

Two hundred anonymized samples were utilized in this study. These samples were subjected to routine measurement of total thyroid protein using the immunofluorescence assay. The selected samples covered the analytical range of the AnyLab F assay, with CRP concentrations ranging from 0.1 mg/L to 300.0 mg/L. The PCT concentrations in the samples ranged from 0.1 ng/mL to 100.0 ng/mL. As per the guidelines provided by the Korean Society of Laboratory Medicine, the normal range for CRP is defined

as less than 10 mg/L, and for PCT, it is defined as less than 0.5 ng/mL.

4.6 Statistical Analysis

Continuous data with a normal distribution were presented as mean ± standard deviation. The significance between groups was assessed using the independent t-test. Statistical analysis was conducted using GraphPad Prism (version 7.00.159). Results were considered statistically significant at p-values less than 0.05.

4.7 Ethical Consideration

In terms of securing permission, this study adhered to ethical guidelines and obtained appropriate permissions from relevant institutional review boards or ethics committees, ensuring that the research was conducted in compliance with established ethical standards. The study complied with the approval granted by the Clinical Research Review Committee of Dankook University (Institutional Review Board DKU Certificate No. 2022-10-030).

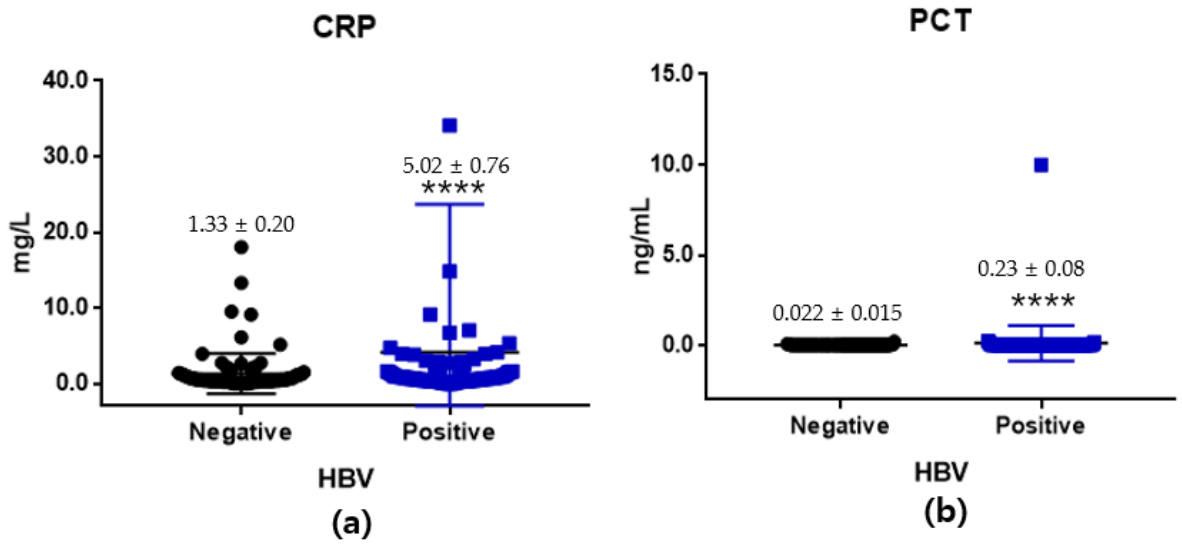
5. Results

5.1 Demographic and Clinical Characteristics of Enrolled Subjects

The study population was divided into two age groups: young individuals (0 to 59 years) and older adults (≥60 years). The mean age in the young group was 48 ± 11 years, while in the older group, it was 67 ± 10 years.

In both the young and older age groups, significant increases in the levels of the two inflammatory markers were observed in patients with HBV infection than healthy individuals ($p < 0.001$). However, there were no significant differences between the young and older individuals in terms of CRP and PCT levels ($p < 0.001$).

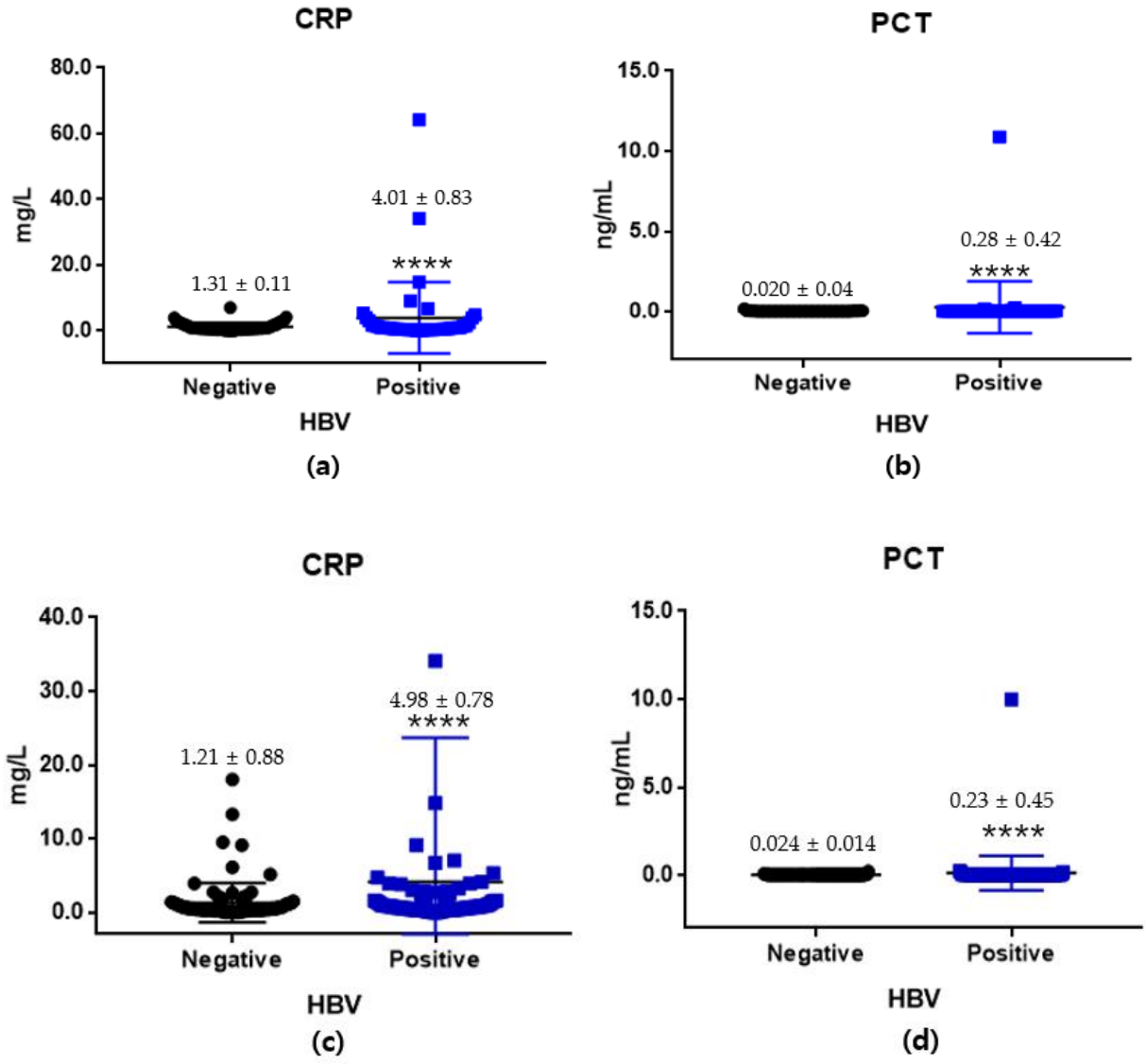
5.2 Serum CRP and PCT Levels in Patients with HBV Infection



[Fig. 1] A Comparison of Inflammation Marker Levels (CRP and PCT) based on HBV Infection
(a) CRP levels ($p < 0.0001$). (b) PCT levels ($p < 0.0001$).

In patients with HBV infection, the CRP and PCT levels were 5.02 ± 0.76 mg/L and 0.23 ± 0.08 ng/mL, respectively, whereas in healthy individuals, the respective values were 1.91 ± 0.13 mg/L and 0.022 ± 0.015 ng/mL [Fig. 1]. The average CRP level in patients with HBV infection is notably higher than in healthy individuals . Similarly, the average PCT level in patients with HBV infection is significantly higher than in healthy individuals. As with CRP, the relatively small standard deviations within each group suggest consistent PCT levels within those groups.

5.3 Levels of CRP and PCT in Males and Females with HBV Infection



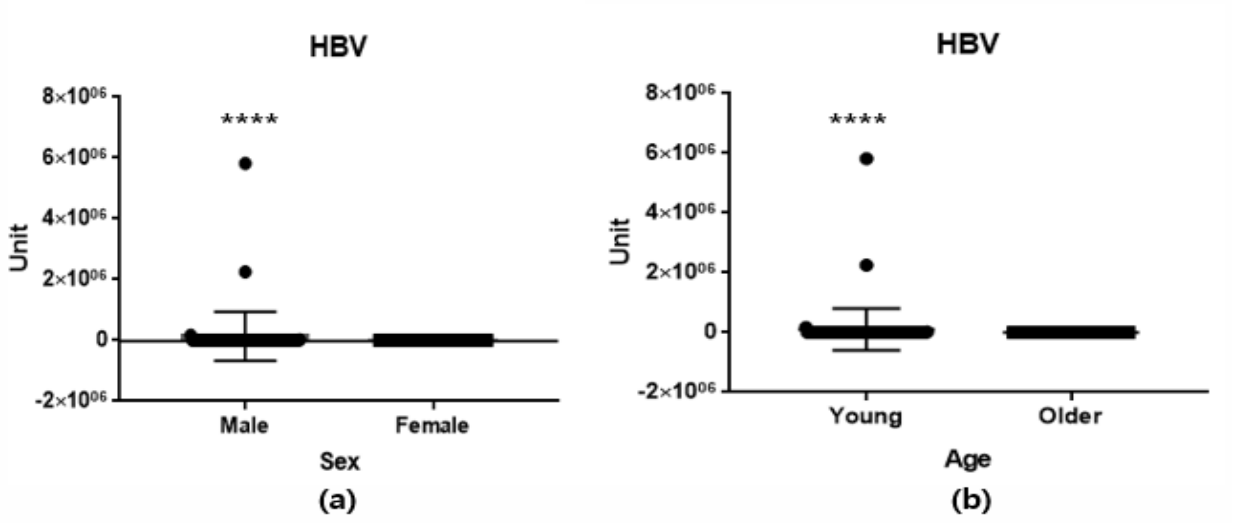
[Fig. 2] Comparison of CRP and PCT Levels based on Sex
(a) CRP levels in the HBV-positive group (male and female). (b) CRP levels in the HBV-negative group (male and female). (c) PCT levels in the HBV-positive group (male and female). (d) PCT levels in the HBV-negative group (male and female)

As shown in [Fig. 2], in males with HBV infection, the CRP and PCT values were 4.01 ± 0.83 mg/L and 0.28 ± 0.42 ng/mL respectively, whereas in healthy males, the respective values were 1.31 ± 0.11 mg/L and 0.020 ± 0.04 ng/mL. In females with HBV infection, the CRP and PCT values were 4.98 ±

0.78 mg/L and 0.23 ± 0.45 ng/mL, respectively, whereas in healthy females, the values were 1.21 ± 0.88 mg/L and 0.028 ± 0.014 ng/mL, respectively. Both HBV-infected and healthy males show a clear difference in mean CRP levels. Males with HBV infection have substantially elevated CRP levels than healthy males. This suggests a significant inflammatory response in males with HBV infection. Similar to males, females with HBV infection also exhibit significantly higher CRP levels than healthy females. This suggests that the inflammatory response in females with HBV infection is also notably heightened. Interestingly, males with HBV infection show an increase in PCT levels than healthy males. However, the standard deviation for PCT in males with HBV infection is relatively high, indicating a degree of variability within the group. In females with HBV infection, the PCT levels are again slightly higher than in healthy females. Similar to males, there is notable variability in PCT levels within the HBV-infected group.

5.4 Quantification of Hepatitis B DNA Levels Based on Sex and Age Among Patients with HBV

[Fig. 3] shows the hepatitis B quantitative unit (HBV unit) measured at diagnosis by RT-qPCR in patients with HBV. Notably, the HBV units in male patients (140256 ± 105119 units) and female patients (55.93 ± 30.58 units) were remarkably different. Furthermore, there was a marked difference between the young (104697 ± 78641 units) and older adult (262.3 ± 217.2 units) groups. As [Fig. 3] illustrates, there was not a significant correlation between the respective trends. Regarding sex and age in HBV patients, the CRP and PCT values were not significant. The distinct difference in HBV unit measurements between male and female patients is noteworthy. Male patients showed a significantly higher average HBV unit measurement than female patients. This suggests that, on average, male patients have a higher viral load of HBV than females. There is also a marked difference in HBV unit measurements between the younger and older adult groups. The older adult group had a notably lower average HBV unit measurement than the younger adult group. This difference could indicate that older adults in this context tend to have lower HBV viral loads on average. The lack of significant correlation between HBV units and CRP/PCT values indicates that other factors, apart from viral load and age, might contribute more substantially to the observed inflammation in HBV patients. This suggests that the relationship between viral load and inflammation might be complex and influenced by various factors beyond gender and age.



[Fig. 3] Comparison of hepatitis B DNA quantitative units (HBV units) based on sex and age. (a)The HBV units based on sex in patients with HBV. (b) The HBV units based on age in patients with HBV.

6. Discussion

This study focuses on evaluating the analytical and diagnostic performance of two inflammatory markers, CRP and PCT, at the serological level using an immunofluorescence assay (IFA) platform. The results reveal a marked increase in serum CRP and PCT levels following HBV infection. The use of IFA as a simpler, faster, and cost-effective modality for clinical evaluation than conventional methods such as enzyme-linked immunosorbent assay or chemiluminescence immunoassay is noteworthy. Furthermore, the findings support previous studies suggesting a remarkable association between inflammatory markers and hepatitis B.

[Fig. 1] demonstrates that CRP and PCT are promising biomarkers for detecting HBV infection, regardless of age. The lack of significant difference in CRP and PCT levels between the two age groups suggests that the association between these biomarkers and HBV infection is not influenced by age. These findings have important implications for the diagnosis and management of HBV infection. The elevated levels of CRP and PCT in both younger and older individuals with HBV infection highlight their potential as diagnostic markers for identifying HBV-infected patients. Measurement of these biomarkers may aid healthcare providers in distinguishing between individuals with HBV infection and those without, enabling early detection and appropriate treatment initiation. Moreover, the independence of CRP and PCT levels from age suggests their utility across different age groups, enhancing their clinical applicability. However, it should be noted that although CRP and PCT levels can indicate HBV infection, they may not be specific to HBV and could potentially be elevated in other liver diseases or inflammatory conditions.

Results from [Fig. 2] indicate that HBV infection is associated with an enhanced inflammatory response, as evidenced by elevated CRP and PCT levels. CRP is an acute-phase protein produced by the liver in response to inflammation[19], while PCT is a precursor hormone released during bacterial infections and inflammatory processes[20]. The extensive increase in both CRP and PCT levels in patients with HBV infection suggests the presence of ongoing inflammatory activity within the liver.

[Fig. 2] findings also reveal that both males and females experience elevated CRP and PCT levels following HBV infection. This indicates that the inflammatory response triggered by HBV affects both sexes, resulting in higher biomarker levels in individuals with HBV infection than those without. The observed differences in CRP and PCT levels between HBV-infected and uninfected individuals further underscore the potential diagnostic value of these biomarkers in identifying HBV infection. Sex-specific observations suggest that females with HBV infection tend to exhibit higher CRP and PCT levels than males with HBV infection, implying a potentially stronger inflammatory response in females. However, it is important to note that this study does not provide insights into the underlying reasons for these sex differences. Further research is needed to explore the mechanisms contributing to the observed sex-specific variations in CRP and PCT levels in the context of HBV infection[21][22].

The findings in [Fig. 3] suggest a potential correlation between the inflammatory response (indicated by elevated CRP and PCT levels) and the viral replication activity of HBV (represented by HBV unit measurements). The differences in HBV unit levels between males and females may be attributed to various factors, including hormonal influences, genetic variations, or differences in the immune response to HBV infection. Further research must elucidate the underlying mechanisms responsible for these sex-related disparities in HBV viral load. The remarkable correlation between inflammatory biomarkers and HBV unit levels implies an association between the severity of HBV infection and the extent of liver inflammation. Elevated CRP and PCT levels could potentially serve as indicators of increased viral replication activity and liver inflammation in patients with HBV infection. However, it is important to note that the exact cause-and-effect relationship between inflammatory biomarkers and HBV replication or liver inflammation cannot be determined solely based on these findings. Further studies are warranted to establish a more comprehensive understanding of the interplay between viral

replication, inflammation, and the host immune response in HBV infection[23].

However, it is important to acknowledge some limitations of the study. The study design and retrospective data collection excluded other inflammatory biomarkers, such as D-dimer and interleukin-6, which may provide additional insights into the inflammatory activity associated with HBV infection . Exploring the serum levels of these excluded biomarkers in future studies would be of interest. Additionally, future research should investigate other potential biomarkers, including cardiac and inflammatory markers, to understand their relationship with hepatitis B . It would also be valuable to determine whether the observed elevation of inflammatory markers in serum is specific to hepatitis B or if it occurs in other liver diseases such as liver cirrhosis and cancer.

Furthermore, in the context of decompensated liver cirrhosis, a high PCT concentration has shown high sensitivity and specificity for bacterial infections[23]. However, since the biomarkers in this study were measured after patients were diagnosed with hepatitis B and hospitalized, they are considered useful for monitoring and predicting hematologic activity rather than diagnosing respiratory illnesses or determining the severity of hepatitis B based on symptoms.

Nevertheless, Our study is significant as it provides biomarker data using a quantitative immunofluorescence method, which offers a distinct approach than commonly used Chemiluminescence Assay (CLIA) or RT-PCR methods in hospital settings. While Point-of-Care Testing (PoCT) methods have historically been considered less reliable due to lower sensitivity in predicting quantitative biomarker data in human samples, recent advancements in detection technology have greatly improved the immunofluorescence quantitative assay, making it highly advantageous with enhanced sensitivity and specificity[24]. Thus, the PoCT platform can serve as a viable alternative to RT-PCR and CLIA methods. One of the advantages of the PoCT platform is its cost-effectiveness and time efficiency. PoC tests are designed to be economically feasible, providing rapid and accessible results at the point of care. They often involve low upfront equipment costs and may not require specialized laboratory infrastructure. Additionally, the PoCT platform has the potential to reduce overall healthcare costs by enabling fast diagnosis and appropriate treatment decisions, ultimately leading to improved patient outcomes and optimized resource utilization. However, it is important to consider various factors when comparing the competitiveness of direct RT-qPCR and the PoCT platform. Direct RT-qPCR of serum samples, if successfully implemented, may offer similar advantages in terms of rapid and sensitive detection of target nucleic acids directly from the samples[24]. Factors such as equipment requirements, expertise, cost-effectiveness, and immediate availability need to be evaluated to assess the feasibility and competitiveness of direct RT-qPCR and the PoCT platform[25]. The immunofluorescence quantitative assay employed in our study utilizes fluorescently labeled antibodies to identify and quantify specific antigens in a sample. This labeling feature enables visualization of the antigen-antibody complex, making it a highly precise and sensitive method for detecting and measuring antigen levels in a given sample[25]. Such tests have proven useful in diagnosing and monitoring various diseases, including autoimmune conditions, cancer, and metabolic and infectious diseases. Furthermore, immunofluorescence quantitative assays are relatively easy to perform and provide results in a relatively short amount of time.

In conclusion, this study emphasizes the analytical and diagnostic performance of two inflammatory markers, CRP and PCT, at the serological level using an immunofluorescence assay (IFA) platform. The findings highlight the marked increase in serum CRP and PCT levels following HBV infection. Moreover, the study underscores the potential of IFA as a simpler, faster, and cost-effective modality for clinical evaluation. Although the study has limitations, including the exclusion of other inflammatory biomarkers and the need to explore their relationship with hepatitis B, it provides valuable biomarker data. Furthermore, the study showcases the potential of point-of-care testing (PoCT) using the quantitative immunofluorescence method, which holds promise for enhancing diagnostic processes. Overall, this study contributes to the understanding of CRP and PCT as diagnostic biomarkers for HBV

infection and highlights the potential of the PoCT method in simplifying and improving diagnostic approaches.

7. Conclusion

In conclusion, this study advances our understanding of CRP and PCT as potential diagnostic biomarkers for HBV infection, highlighting their diagnostic value across different age groups and genders. The study's innovative use of the immunofluorescence assay and its exploration of the PoCT method underscore the potential for improved diagnostic processes. Although the study has certain limitations, such as excluding other inflammatory biomarkers and not explaining underlying sex-related disparities, its contribution remains valuable. Particularly, the study underscores the potential of the quantitative immunofluorescence method in point-of-care testing (PoCT), offering an alternative approach to conventional methods for enhancing diagnostic efficiency. While further research is warranted, this study adds to the body of knowledge concerning the role of inflammatory markers in HBV infection and paves the way for more efficient and effective diagnostic strategies.

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