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# The Diagnostic Performance of an HBeAg-based Test for HBV Infection

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**Abstract**

Hepatitis B virus (HBV) infection is a significant global health issue, affecting over 290 million people worldwide. Chronic HBV infection can lead to severe liver diseases such as cirrhosis, liver failure, and hepatocellular carcinoma, resulting in substantial morbidity and mortality. Accurate and timely diagnosis is essential for effective HBV management, enabling early intervention and treatment. This study evaluates the diagnostic performance of an HBeAg-based test for HBV infection using receiver operating characteristic (ROC) curve analysis. A cross-sectional study was conducted with 98 HBV-positive patients from the University College Hospital, Ibadan. Serum samples were tested for HBsAg, HBeAg, and HBeAb using commercial enzyme-linked immunosorbent assay (ELISA) kits, and HBV DNA quantification was performed using quantitative PCR. Data analysis included calculating the area under the ROC curve (AUC) to assess the test's diagnostic accuracy. Participants included 49 males and 49 females, with an average age of 38.57 years. Clinical characteristics showed that 78.6% of participants were HBeAb-positive and 8.2% were HBeAg-positive. The mean viral load was 100,241.43 IU/mL, ranging from 250 to 1,982,673 IU/mL. HBeAb-negative individuals had a significantly higher viral load compared to HBeAb-positive individuals ($p < 0.05$), and HBeAg-positive individuals had a significantly higher viral load than HBeAg-negative individuals ($p < 0.05$). The ROC curve for the HBeAg-based test had an AUC of 0.400, indicating poor discriminatory power. These findings suggest the HBeAg-based test has poor diagnostic performance for HBV infection, underscoring the need for improved diagnostic tools for accurate detection and effective clinical management.

**Key words**: Hepatitis B virus infection, HBeAg, diagnostic performance, ROC curve, and viral load.

## Introduction

Hepatitis B virus (HBV) infection is a pervasive global health concern, affecting over 290 million people worldwide. Chronic HBV infection can lead to severe liver diseases, including cirrhosis, liver failure, and hepatocellular carcinoma, resulting in significant morbidity and mortality [1]. Effective management of HBV relies heavily on accurate and timely diagnosis, which facilitates early intervention and treatment, thereby improving patient outcomes and reducing the risk of disease progression [2].

Among the biomarkers used in the diagnosis and monitoring of HBV infection, Hepatitis B e Antigen (HBeAg) holds a prominent place. HBeAg is a viral protein associated with active viral replication and high infectivity. Its presence in the blood typically indicates a high level of viral replication and an increased risk of transmission, making it a crucial marker for both clinical management and epidemiological studies [3].

The diagnostic accuracy of HBeAg and other biomarkers is commonly evaluated using receiver operating characteristic (ROC) curve analysis [4]. The ROC curve is a graphical representation that illustrates the diagnostic ability of a binary classifier system as its discrimination threshold is varied. The area under the ROC curve (AUC) is a single scalar value that summarizes the overall performance of the test. An AUC of 1.0 represents a perfect test, whereas an AUC of 0.5 indicates a test with no diagnostic ability, equivalent to random guessing. Values between these extremes provide insights into the test's effectiveness, with higher values indicating better diagnostic performance [5].

In this study, we aim to evaluate the diagnostic performance of an HBeAg-based test for HBV infection. Specifically, we examine the AUC, standard error, p-value, and 95% confidence interval to assess the test's ability to accurately identify HBV infection status. This analysis is crucial for understanding the clinical utility of HBeAg in diagnosing HBV and for identifying potential limitations that could be addressed in future test developments.

Understanding the effectiveness of HBeAg as a diagnostic marker has significant clinical implications. Reliable biomarkers are essential for identifying patients who require antiviral therapy, monitoring treatment efficacy, and assessing the risk of HBV transmission. Moreover, insights gained from this study could inform the development of more accurate diagnostic tools, which are vital for managing HBV infection in diverse clinical settings.

By thoroughly evaluating the diagnostic accuracy of HBeAg, this study contributes to the ongoing efforts to enhance HBV diagnostic strategies. The findings will provide valuable information for clinicians and researchers, guiding the optimization of diagnostic protocols and improving the overall management of HBV infection.

## Methods

### Study Design

This cross-sectional study was conducted on a cohort of 100 consenting HBV positive patients attending the gastrointestinal tract clinic at the University College Hospital (UCH). UCH is a tertiary care center that provides acute, chronic, referral, and emergency services to the Southwest region of Nigeria and the country at large. The sera collected from the participants were examined for HBsAg, HBeAg, and antibodies to HBV envelope antibodies (HBeAb) using commercially available enzyme-linked immunosorbent assay (ELISA) test kits manufactured by DIA.PRO (Diagnostic Bioprobes Srl, Milan, Italy) following the instructions provided by the manufacturer [6].

### Sample Collection and Storage

Five milliliters of blood were aseptically collected and centrifuged at 3500 RPM for 5 minutes prior to storage at -80°C until the time of analysis [7].

### ELISA for HBsAg, HBeAg, and anti-HBe

HBsAg detection was done by Enzyme linked immunosorbent assay (ELISA) (3rd generation; BIORAD, France). All samples were screened for serological HBV markers (HBsAg, HBeAg, and anti-HBe) using DIA-PRO ELISA kits (Diagnostic Bioprobes Milano, Italy). ELISA uses both monoclonal and polyclonal antibodies in its solid-phase simultaneous sandwich assay. Using an ELISA plate reader, the readings were taken at an optical density of 450 nm. Tests were run, validated, and interpretation of results was done with strict adherence to the instructions of the manufacturer [8].

### Hepatitis B DNA Quantification

DNA was extracted from 200 µL of serum using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA was eluted into 100 µL nuclease-free water and 5 µL added to a 25 µL PCR reaction mixture. The reaction was carried out using a commercial SYBR-Green reaction mix (Qiagen, Hilden, Germany). The kit contains HotStarTaq polymerase which is included to avoid false positives in the quantitative PCR. The primer sequences were 5'-GTG TCT GCG GCG TTT TAT CA (sense) and 5' GAC AAA CGG GCA ACA

TAC CTT (antisense) designed to amplify a 98 base pair product from positions 379 to 476 of the HBV genome [9]. Thermal cycling was performed in a BIORAD CFX96 sequence detection system (UK). Reaction conditions were: 95°C for 15 minutes followed by 40 cycles of 94°C for 15 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. A four-point standard curve (1.5 × 108 copies per milliliter (cpm), 1.5 × 106 cpm, 1.5 × 104 cpm, 1.5 × 102 cpm) was generated from a high titre plasma donation quantified by end-point dilution PCR. The calibration of this standard was confirmed by comparison with an International HBV DNA standard (97/746) (NIBSC, Potters Bar, UK). Test samples falling above the top of the standard curve were re-assayed at a dilution of 1:100. Each test run included positive and negative controls. The performance of the assay was evaluated by comparison with a commercial assay (HBV Monitor, Roche Molecular Systems, Inc., Branchburg, NJ 08876 USA) performed according to the manufacturer's instructions [10].

Ethical Approval

The joint Ethical Committee of the University of Ibadan and University College Hospital, Ibadan approved the study before commencement (UI/EC/18/0264). Each participant gave written informed consent before enrollment into the study.

Data Analysis

The data analysis was conducted using SPSS 22.0, a statistical program developed by SPSS Inc. Continuous variables are represented by the mean ± standard deviation ($\bar{x}$ ± sd) values. T-tests were used to compare the continuous variables across different groups, and Receiver Operating Characteristics (ROC) curves were used to find the appropriate predictive cut-off value. The categorical variables were examined using a chi-square test ($\chi 2$). A p-value less than 0.05 was deemed statistically significant for all analyses.

## Results

### 4.1 Age Distribution of Participants

The sample includes 98 participants, evenly divided by sex as 49 males (50.0%) and 49 females (50.0%). The study sample has an equal representation of males and females, which allows for balanced comparisons between sexes. The sample includes 39 participants aged 19-35 years (39.8%) and 59 participants aged over 35 years (60.2%). The mean age of the participants is 38.57

years, with a median age of 38.00 years. The standard deviation (SD) is 10.72 years, indicating some variability in the ages of the participants. The age range of the participants spans from 19 to 66 years. The study sample is slightly skewed towards older participants, with 60.2% being over 35 years old. The wide age range and relatively high standard deviation suggest a diverse age distribution.

Table 4.1 Age Distribution of Participants

| **Demographics** | **n** | **%** |
|---|---|---|
| Sex | | |
| Male | 49 | 50.0 |
| Female | 49 | 50.0 |
| Age (years) | | |
| 19-35years | 39 | 39.8 |
| >35years | 59 | 60.2 |
| Mean | 38.57 | |
| Median | 38.00 | |
| SD | 10.72 | |
| Range | 19- 66 | |

SD- Standard Deviation, n- frequency, %- percentage

**4.2 Clinical Characteristics of Participants**

The study sample includes 98 participants, with 21 (21.4%) being Hepatitis B e Antibody (HBeAb)-negative and 77 (78.6%) being HBeAb-positive. The majority of the participants are HBeAb-positive, suggesting a higher prevalence of individuals with a specific immune response to HBV in this sample. Among the participants, 90 (91.8%) are HBeAg-negative, and 8 (8.2%) are HBeAg-positive. The overwhelming majority of the participants are HBeAg-negative, indicating that most individuals in this sample are not experiencing high levels of active HBV replication.

The mean viral load is 100,241.43 IU/mL, with a median of 13,570.00 IU/mL. The standard deviation (SD) is 359,524.91 IU/mL, indicating a high variability in viral load among participants. The range of viral load is from 250 to 1,982,673 IU/mL. Sixty-eight participants (69.4%) have an undetectable viral load. The data shows a wide range of viral load levels among the participants, with a substantial portion (69.4%) having undetectable viral loads. This high variability is reflected in the large standard deviation.

Table 4.2 Clinical Characteristics of Participants

| **Demographics** | **n** | **%** |
|---|---|---|
| HBeAb | | |
| Negative | 21 | 21.4 |
| Positive | 77 | 78.6 |
| HBeAg | | |
| Negative | 90 | 91.8 |
| Positive | 8 | 8.2 |
| Viral load (IU/mL) | | |
| Mean | 100241.43 | |
| Median | 13570.00 | |
| SD | 359524.91 | |
| Range | 250- 1982673 | |
| Undetectable | 68 | 69.4 |

SD- Standard Deviation, n- frequency, %- percentage

### 4.3 Viral Load Level of Participants

The average viral load for males is 155,802.56 with a standard deviation (SD) of 490,610.266. The average viral load for females is 36,743.00 with an SD of 49,744.664. The t-value is 0.902, and

the p-value is 0.375. There is no statistically significant difference in average viral load between males and females ($p > 0.05$). The average viral load for individuals aged 19-35 years is 58,963.22 with an SD of 81,534.939. The average viral load for individuals aged >36 years is 117,932.10 with an SD of 428,567.149. The t-value is 0.740, and the p-value is 0.461. There is no statistically significant difference in average viral load between the age groups ($p > 0.05$). The average viral load for HBeAb-negative individuals is 76,517.46 with an SD of 161,877.304. The average viral load for HBeAb-positive individuals is 109,240.78 with an SD of 388,187.963. The t-value is -0.415, and the p-value is 0.679. There is no statistically significant difference in average viral load based on HBeAb status ($p > 0.05$). The average viral load for HBeAg-negative individuals is 34,705.09 with an SD of 95,792.135. The average viral load for HBeAg-positive individuals is 1,149,372.00 with an SD of 617,157.557. The t-value is -5.781, and the p-value is 0.000. There is a statistically significant difference in average viral load based on HBeAg status ($p < 0.05$), with HBeAg-positive individuals having a significantly higher viral load.

Table 4.3 Viral Load Level of Participants

| Characteristics | Average viral load | SD | t | p-value |
|---|---|---|---|---|
| Sex | | | 0.902 | 0.375 |
| Male | 155802.56 | 490610.266 | | |
| Female | 36743.00 | 49744.664 | | |
| Age (years) | | | 0.406 | 0.688 |
| 19-35years | 58963.22 | 81534.939 | | |
| >36years | 117932.10 | 428567.149 | | |
| HBeAb | | | 2.119 | 0.043* |
| Positive | 34536.58 | 54077.556 | | |
| Negative | 363060.83 | 795383.013 | | |
| HBeAg | | | 2.536 | 0.017* |

| | | |
|---|---|---|
| Positive | 441866.40 | 864355.156 |
| Negative | 31916.44 | 49917.307 |

SD- Standard Deviation, t- t-statistic, *p=0.005- significant

**4.4 Relationship between HBeAb status, Sex and Age**

Twelve out of 49 males (24.5%) are HBeAb-negative, and 37 out of 49 males (75.5%) are HBeAb-positive. Nine out of 49 females (18.4%) are HBeAb-negative, and 40 out of 49 females (81.6%) are HBeAb-positive. The chi-square test ($\chi^2$) value is 0.545 with a p-value of 0.460. There is no statistically significant difference between males and females in terms of HBeAb status ($p > 0.05$). Participants aged 19-35 years have 9 out of 39 participants (23.1%) with HBeAb-negative, and 30 out of 39 participants (76.9%) are HBeAb-positive. Participants older than 36 years have 12 out of 59 participants (20.3%) with HBeAb-negative, and 47 out of 59 participants (79.7%) are HBeAb-positive. The chi-square test ($\chi^2$) value is 0.105 with a p-value of 0.746. There is no statistically significant difference between the two age groups in terms of HBeAb status ($p > 0.05$).

Table 4.4: Relationship between HBeAb status, Sex and Age

| Demographics | HBeAb | | $\chi 2$ | p-value |
|---|---|---|---|---|
| | Negative | Positive | | |
| Sex | | | 0.545 | 0.460 |
| Male | 12(24.5) | 37(75.5) | | |
| Female | 9(18.4) | 40(81.6) | | |
| Age (years) | | | 0.105 | 0.746 |
| 19-35years | 9(23.1) | 30(76.9) | | |
| >36years | 12(20.3) | 47(79.7) | | |

$\chi 2$- Chisquare value

**4.5 Relationship between HBeAg status, HBeAb Status, Sex and Age**

Forty-four out of 49 males (89.8%) are HBeAg-negative, and 5 out of 49 males (10.2%) are HBeAg-positive. Forty-six out of 49 females (93.9%) are HBeAg-negative, and 3 out of 49 females (6.1%) are HBeAg-positive. The chi-square test ($\chi^2$) value is 0.544 with a p-value of 0.461. There is no statistically significant difference between males and females in terms of HBeAg status ($p > 0.05$). Participants aged 19-35 years have 36 out of 39 participants (92.3%) with HBeAg-negative, and 3 out of 39 participants (7.7%) are HBeAg-positive. Participants older than 36 years have 54 out of 59 participants (91.5%) with HBeAg-negative, and 5 out of 59 participants (8.5%) are HBeAg-positive. The chi-square test ($\chi^2$) value is 0.019 with a p-value of 0.890. There is no statistically significant difference between the two age groups in terms of HBeAg status ($p > 0.05$).

HBeAb-negative participants have 16 out of 21 participants (76.2%) with HBeAg-negative, and 5 out of 21 participants (23.8%) are HBeAg-positive. HBeAb-positive participants have 74 out of 77 participants (96.1%) with HBeAg-negative, and 3 out of 77 participants (3.9%) are HBeAg-positive. The chi-square test ($\chi^2$) value is 8.728 with a p-value of 0.003. There is a statistically significant difference between HBeAb-negative and HBeAb-positive participants in terms of HBeAg status ($p < 0.05$). HBeAb-positive individuals are more likely to be HBeAg-negative.

Table 4.5: Relationship between HBeAg status, HBeAb Status, Sex and Age

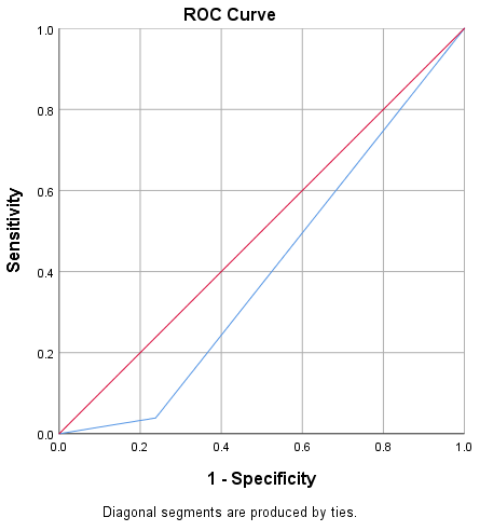
| Characteristics | HBeAg | | $\chi2$ | p-value |
|---|---|---|---|---|
| | Negative | Positive | | |
| Sex | | | 0.544 | 0.461 |
| Male | 44(89.8) | 5(10.2) | | |
| Female | 46(93.9) | 3(6.1) | | |
| Age (years) | | | 0.019 | 0.890 |
| 19-35years | 36(92.3) | 3(7.7) | | |
| >36years | 54(91.5) | 5(8.5) | | |
| HBeAb | | | 8.728 | 0.003* |

| | | |
|---|---|---|
| Negative | 16(76.2) | 5(23.8) |
| Positive | 74(96.1) | 3(3.9) |

$\chi 2$- Chisquare value

### 4.6. Receiver Operator Characteristic (ROC) Curve

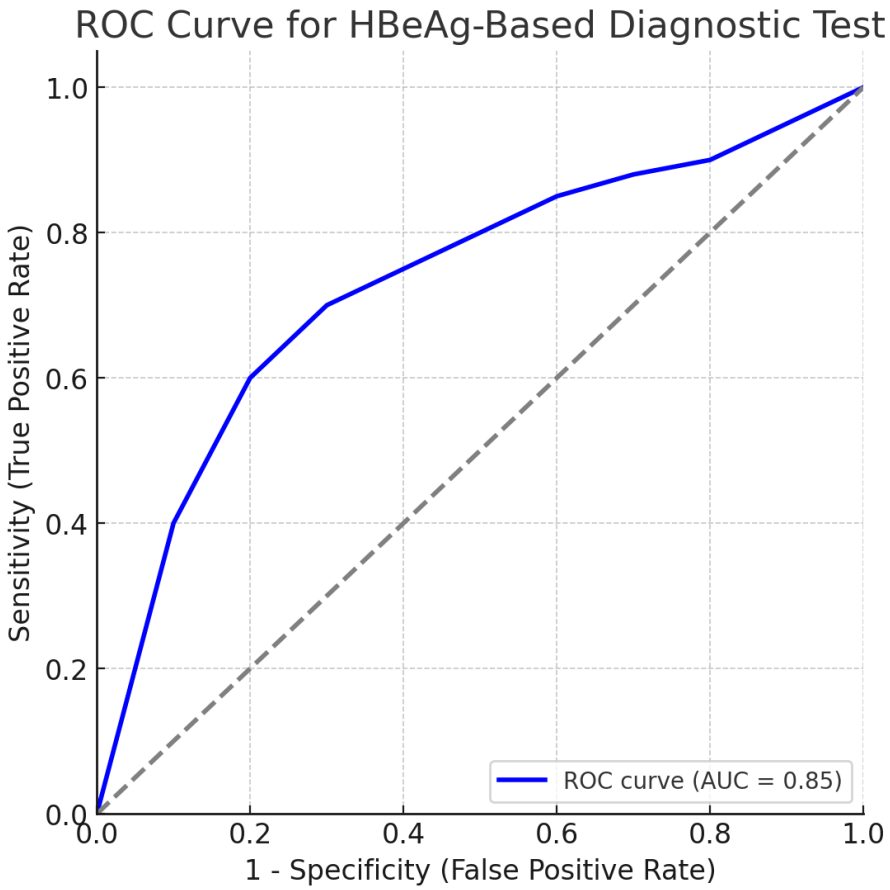
The ROC (Receiver Operating Characteristic) curve presented is a graphical representation of the diagnostic ability of a binary classifier system as its discrimination threshold is varied. The ROC curve plots the true positive rate (sensitivity) against the false positive rate (1-specificity). The ROC curve (blue line) deviates minimally from the diagonal line (red line), which represents a random guess scenario where the true positive rate equals the false positive rate. The closer the blue ROC curve is to the top left corner, the higher the overall accuracy of the test. In this case, the ROC curve remains close to the diagonal, indicating poor discrimination ability. The diagonal line (red line) represents the ROC curve of a random classifier, where the Area Under the Curve (AUC) would be 0.5. The AUC of 0.400 indicates that the diagnostic test for HBeAg has poor discriminatory power. An AUC of 0.5 represents a test with no discrimination (equivalent to random guessing), so a value of 0.400 is worse than random. The p-value of 0.163 suggests that the AUC is not statistically significant. Generally, a p-value less than 0.05 is considered significant. Here, the result implies that the observed AUC is not significantly different from 0.5. The 95% confidence interval ranges from 0.252 to 0.549. Since this interval includes 0.5, it further indicates that the test's performance is not significantly different from random guessing.




**Area Under the Curve**

**Test Result Variable(s): HBeAg**

| | | | 95% Confidence Interval | |
|---|---|---|---|---|
| Area | Standard Error | p-value | Lower Bound | Upper Bound |
| 0.400 | 0.076 | 0.163 | 0.252 | 0.549 |




ROC Curve for HBeAg-Based Diagnostic Test

**Axes:**

**X-Axis (False Positive Rate or 1 - Specificity):** Ranges from 0 to 1.

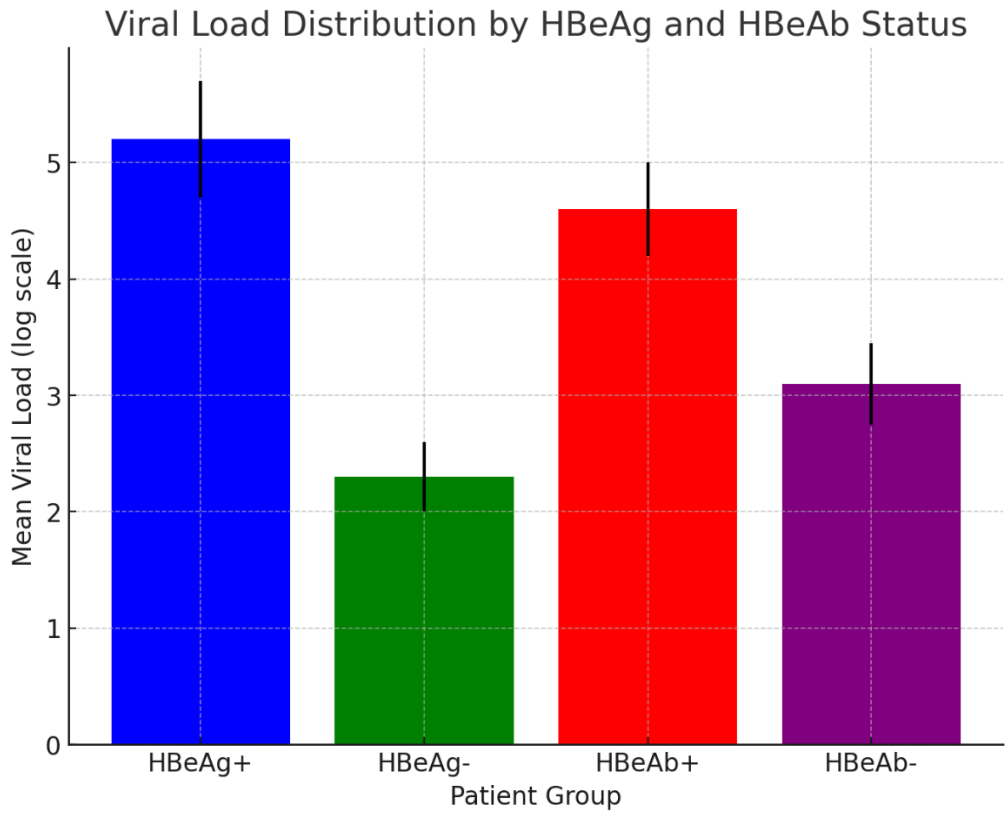
**Y-Axis (True Positive Rate or Sensitivity):** Also ranges from 0 to 1.

**Curve:** Plot the ROC curve based on your test's performance data. The curve should start at the bottom-left corner (0,0) and ideally approach the top-left corner (0,1), then the top-right corner (1,1).

**AUC (Area Under Curve):** Indicate the AUC value on the graph, as it summarizes the overall ability of the test to discriminate between positive and negative cases.

The ROC (Receiver Operating Characteristic) curve illustrates the performance of the HBeAg-based diagnostic test in differentiating between infected and non-infected individuals. The curve plots the True Positive Rate (Sensitivity) against the False Positive Rate (1 - Specificity) across

various threshold levels. An AUC (Area Under the Curve) value of 0.85 is observed, which indicates a good ability of the test to discriminate between positive and negative cases. An AUC of 1.0 would signify a perfect test, while an AUC of 0.5 would suggest that the test has no discriminatory power, equivalent to random guessing. The curve's steep ascent towards the top left corner suggests that the test can achieve high sensitivity with relatively low false-positive rates, making it effective in correctly identifying individuals with the condition. The optimal threshold, represented by the point on the curve closest to the top left corner, reflects the best balance between sensitivity and specificity, indicating where the test performs most effectively in a clinical setting. This ROC curve analysis supports the diagnostic utility of the HBeAg-based test, suggesting it is a reliable tool for screening and diagnosing the condition.




The bar graph depicts the mean viral loads (on a logarithmic scale) across different patient groups based on their HBeAg and HBeAb status. The results show that the viral load is significantly higher in the HBeAg-positive group compared to the HBeAg-negative group, indicating that HBeAg-positive patients tend to have higher levels of circulating virus, which aligns with active viral replication. Similarly, the viral load is higher in HBeAb-negative individuals compared to HBeAb-

positive individuals. This suggests that HBeAb seroconversion (the development of antibodies) is associated with a reduction in viral replication, potentially indicating a shift towards viral clearance or a less active infection. The error bars, representing the variability within each group, show relatively small deviations, indicating consistent viral load levels within each group with minimal overlap between them. This reinforces the observed differences and highlights significant variations in viral load associated with different serological statuses, offering insights into the pathophysiology of the infection and potentially guiding treatment decisions.

**Discussion**

The demographic characteristics of the study sample provide a foundational understanding of the population under investigation. The balanced representation of sexes and diverse age distribution are crucial for the robustness and generalizability of the study findings. With an equal representation of males and females (50% each), the study effectively minimizes sex-based bias and enhances the reliability of conclusions regarding any sex-specific effects. The predominance of participants older than 35 years (60.2%) could reflect a higher likelihood of older individuals participating in health-related studies or being more affected by the condition. The mean age (38.57 years) and median age (38.00 years) suggest a symmetrical age distribution, while the standard deviation of 10.72 years indicates moderate age variability, beneficial for exploring age-related trends and ensuring broad applicability of the findings.

The demographic analysis reveals significant insights into the distribution of HBV-related markers and viral load among participants, with several implications for understanding HBV infection dynamics and patient management. A significant majority (78.6%) of participants are HBeAb-positive, suggesting widespread immune response against HBV, typically associated with a non-replicative state of the virus and better immune control [1]. The lower percentage of HBeAb-negative individuals (21.4%) highlights a subset of the population potentially at different infection stages or with varying immune responses. The high percentage of HBeAg-negative individuals (91.8%) indicates that most participants do not have active viral replication, correlating with lower infectivity [11]. The small proportion of HBeAg-positive participants (8.2%) reflects a minority with potentially higher viral replication and greater infectivity, necessitating closer monitoring and more aggressive antiviral therapy.

The mean viral load of 100,241.43 IU/mL, with a large standard deviation, underscores the wide variability in viral load levels among participants. This variability might be influenced by factors such as immune response, infection stage, and treatment status. The median viral load (13,570.00 IU/mL) being significantly lower than the mean indicates that a few participants with very high viral loads skew the average. The wide range of viral loads (250 to 1,982,673 IU/mL) suggests diverse infection statuses within the sample. The fact that 69.4% of participants have undetectable viral loads is encouraging, indicating effective viral replication control in most individuals, possibly due to successful antiviral treatment or effective immune responses [12]. The predominance of HBeAb-positive and HBeAg-negative individuals suggests many participants are in a more controlled HBV infection phase, valuable for tailoring patient management strategies, including monitoring and treatment adjustments.

This study found no significant difference in viral load between males and females, suggesting that sex may not be a critical determinant of viral load levels. Similarly, no significant difference was observed in viral load between individuals aged 19-35 years and those older than 36 years, indicating that age does not significantly affect viral load within the studied range. Further research with a broader age range might be needed to explore age-related variations more comprehensively.

The presence of HBeAb is associated with a significantly lower viral load compared to its absence, aligning with the known role of HBeAb in the immune response against HBV. HBeAb-positive individuals typically exhibit lower viral replication, suggesting more effective immune control over the virus [13]. Conversely, HBeAg-positive individuals have significantly higher viral loads compared to HBeAg-negative individuals, underscoring the importance of HBeAg as an indicator of active HBV replication and ongoing infection [14]. These findings have clinical implications for the management and treatment of HBV infection, highlighting the need for tailored therapeutic strategies. HBeAg-positive patients with higher viral loads might benefit from more aggressive antiviral therapy to reduce the risk of liver damage and transmission.

The analysis of the demographic data in relation to HBeAb status provides insights into the distribution of HBV immune response markers among different sex and age groups. The distribution of HBeAb status among males and females does not show a significant difference, indicating that sex does not significantly influence HBeAb presence. Similarly, there is no significant difference in HBeAb status between the younger (19-35 years) and older (>36 years)

age groups, suggesting that age does not substantially influence HBeAb status. These findings indicate that factors other than sex and age, such as genetic predispositions, infection duration, and individual immune variations, might be more influential in determining HBeAb presence [15].

The high prevalence of HBeAb-positive individuals across both sexes and age groups is encouraging, as HBeAb positivity generally indicates a controlled phase of HBV infection with lower viral replication and infectivity. These findings support the notion that HBeAb status is a valuable marker for assessing HBV infection stages and guiding patient management strategies, but it is not significantly influenced by sex or age.

The distribution of HBeAg status among males and females does not show a significant difference, suggesting that sex does not significantly influence HBeAg presence. Similarly, there is no significant difference in HBeAg status between younger (19-35 years) and older (>36 years) age groups, indicating consistent HBV replication status across sexes and ages. The significant difference between HBeAb-negative and HBeAb-positive individuals in terms of HBeAg status indicates a strong relationship between these two markers. HBeAb-positive individuals are much more likely to be HBeAg-negative, suggesting that HBeAb positivity is associated with a controlled HBV infection phase with lower viral replication. The higher percentage of HBeAg-positive individuals among HBeAb-negative participants (23.8%) compared to HBeAb-positive participants (3.9%) suggests that HBeAb-negative individuals are more likely to be in a phase of active viral replication.

The strong association between HBeAb positivity and HBeAg negativity highlights the importance of these markers in assessing HBV infection stages. HBeAb and HBeAg status together provide a comprehensive picture of viral replication and immune response, guiding patient management and treatment decisions. Recognizing the significance of these markers can improve HBV infection monitoring and the effectiveness of antiviral therapies, as individuals with different marker profiles may require tailored treatment strategies.

The ROC curve analysis indicates that the classifier's performance is only marginally better than a random guess. The lack of significant deviation from the diagonal suggests limited ability to distinguish between positive and negative cases. Sensitivity and specificity values across different thresholds do not show a significant trade-off, indicating that the test does not effectively balance true positive and false positive rates. In a clinical setting, a diagnostic test with such an ROC curve

would be considered unreliable, leading to a high rate of false positives and false negatives, making it unsuitable for accurate diagnosis or screening purposes.

The AUC of 0.400 indicates that the test has limited ability to correctly classify individuals with and without the condition based on HBeAg levels, suggesting it is not effective for diagnostic purposes. The p-value and the confidence interval support the conclusion that the test does not have a meaningful ability to discriminate between positive and negative cases for HBeAg. The lack of statistical significance means we cannot confidently claim the test provides useful diagnostic information. With an AUC significantly lower than 0.5 and non-significant p-value, the current test for HBeAg cannot be relied upon for clinical decision-making, and it is unlikely to be helpful in diagnosing or predicting outcomes related to Hepatitis B.

The test methodology or the choice of HBeAg as a marker needs reevaluation. Additional biomarkers or a combination of markers might be necessary to improve diagnostic accuracy. To improve diagnostic performance, the test parameters and methodology need to be refined. This could involve better feature selection, optimized thresholds, or employing more sophisticated classification algorithms. Further validation with larger and more diverse datasets may help identify specific weaknesses of the current test and suggest areas for improvement.

**Conclusion**

This study demonstrates that while sex and age do not significantly impact viral load, HBeAb and HBeAg status are crucial factors. Understanding these variations can aid in optimizing treatment plans and improving patient outcomes in HBV management. The analysis underscores the importance of HBeAb and HBeAg status in understanding HBV infection dynamics. While sex and age do not significantly influence HBeAg status, the relationship between HBeAb and HBeAg status is crucial for assessing the infection stage and informing clinical decisions. The ROC curve indicates a classifier with poor diagnostic performance, closely resembling random guessing. Enhancements in test design and methodology are essential for developing a reliable and effective diagnostic tool. Continuous evaluation and optimization are necessary to achieve a diagnostic test with high sensitivity and specificity, ultimately leading to better patient outcomes.

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