ANALYSIS OF THE INFLUENCE OF HBSAG DETECTION RESULTS ON THE INTERPRETATION OF ANTI-HBC RESULTS IN THE DIAGNOSIS OF HEPATITIS B INFECTION

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ABSTRACT

This study aims to analyze the relationship between Hepatitis C Virus Antibody (anti-HCV) test results using the ELISA method and Hepatitis B surface Antigen (HBsAg) test results using the rapid test method. HBV and HCV co-infection is a clinically relevant issue as it can accelerate the progression of chronic liver disease. Data from 62 patient samples were analyzed using the Chi-Square test to examine the significance of the relationship between these two categorical variables. The statistical analysis revealed a Chi-Square value of 3.89 with a P-value of 0.048, which is below the 0.05 significance level. This finding indicates a statistically significant association between the HBsAg and anti-HCV test results. This study concludes that in the sampled population, a reactive result on one test is associated with reactivity on the other, highlighting the importance of dual screening for both viruses in high-risk populations.

KEYWORDS

HBsAg, anti-HCV, Hepatitis B, Hepatitis C, Co-infection, Chi-Square

INTRODUCTION

Hepatitis B is a serious liver infection caused by the Hepatitis B Virus (HBV). This virus can cause both acute and chronic diseases, ranging from asymptomatic infections to liver cirrhosis, liver failure, and hepatocellular carcinoma (liver cancer). (Chen, 2022). Worldwide, Hepatitis B is a significant public health issue, with millions of people chronically infected. Detecting Acute and Chronic Infections: If HBsAg is found in the blood, it indicates the presence of the hepatitis B virus, either in the acute phase (new infection) or chronic phase (long-term infection lasting more than 6 months). Identifying Transmission Status: A positive HBsAg test result indicates that the individual can transmit the virus to others through blood or body fluids. Health Screening: This test is often part of

routine health examinations, especially for high-risk groups such as pregnant women, medical personnel, or individuals with a history of contact with infected persons.

Reactive/Positive HBsAg Results: This means that the surface antigen of the hepatitis B virus has been detected in the blood. The presence of this antigen is evidence of an active hepatitis B virus infection. Doctors usually recommend further tests to determine whether the infection is acute or chronic. (Liu, 2024) Non-Reactive/Negative HBsAg Results: This means that the surface antigen of the hepatitis B virus is not detected in the blood. This could mean that a person is not infected with the virus, or they were previously infected and have completely recovered. To confirm whether the body has developed immunity, doctors will recommend further testing, specifically Anti-HBs testing. In patients with chronic hepatitis B, the HBsAg test can be used to monitor the body's response to antiviral treatment. A decrease or disappearance of HBsAg may indicate treatment success.(Lok, 2024).

Unlike HBsAg (which indicates the presence of an active virus) or Anti-HBs (which indicates immunity), the presence of Anti-HBc shows that a person has been previously or is currently infected with hepatitis B virus. Anti-HBc is an antibody produced by the immune system in response to HBcAg (Hepatitis B core antigen). HBcAg is a protein that forms the core of the hepatitis B virus (HBV). The Anti-HBc test is very useful because it is not influenced by vaccination. This means that a person who has only received the hepatitis B vaccine will not have this antibody. Anti-HBc is only formed if the body has been truly exposed to the hepatitis B virus.(Zang, 2024). The Anti-HBc test is divided into two main types, providing different information regarding the phase of infection: Total Anti-HBc is used to detect all types of Anti-HBc antibodies, both IgM and IgG; a positive result indicates that a person has been previously infected with HBV or is currently experiencing an active infection. This antibody appears in the early phase of acute infection and can last for a lifetime. Therefore, this test is a very reliable marker for indicating a history of exposure to the hepatitis B virus. Meanwhile, Anti-HBc IgM is used to detect IgM-type antibodies, which are the first antibodies produced by the body when a new infection occurs. A positive result indicates an acute hepatitis B infection (a recent infection, usually within the last 6 months).(Martin, 2025).

Infection with the Hepatitis B virus (HBV) and Hepatitis C virus (HCV) is a serious public health issue globally, affecting millions of people and being a leading cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma. Both viruses have similar transmission routes, particularly through blood, leading to a high risk of co-infection (dual infection) in vulnerable populations. Accurate and timely diagnosis of both infections is crucial for effective clinical management and preventing further transmission. Early diagnosis of HBV and HCV often relies on serological tests, such as the detection of Hepatitis B surface Antigen (HBsAg) for active HBV infection and antibodies against the Hepatitis C virus (anti-HCV) to indicate exposure to HCV. Although these tests are effective screening tools, there is still debate about whether a positive result on one marker has a significant correlation with a positive result on the other marker. Understanding this relationship is important for optimizing screening strategies and interpreting laboratory results. The HBsAg test can be performed using various types of specimens, including whole blood, serum, and plasma. Each specimen has different characteristics that can affect test results. Therefore, it is important to evaluate the sensitivity and specificity of each specimen type to determine the most effective method for diagnosing hepatitis B. In the Rapid Test (Immunochromatography Assay/ICA), gold colloid particles on the conjugate antibody will accumulate and form a colored line (usually pink/purple) in the test area (T) if HBsAg is present. This test line is coated with stationary Anti-HBs antibodies. If the patient's blood sample contains HBsAg, this antigen will "bridge" the labeled antibody in

the conjugate particle and the stationary antibody on the test line. (Pramonodjati, 2025). This reaction creates an immune complex that accumulates on the test line, causing colored particles to gather and generate a colored line. The test line is coated with stationary Anti-HBc antibodies. The mechanism is competitive. If the patient's sample contains Anti-HBc, this antibody will "compete" with the labeled antibody that has bound to the antigen. As a result, the labeled antibody will not be able to bind to the test line, thus no colored line will form. The absence of this line indicates a positive result, while the appearance of this line indicates a negative result. Additionally, a control line (C) will always appear to show that the test is functioning properly. Thus, this research aims to analyze whether there is a statistically significant relationship between HBsAg test results and anti-HCV results (Rahma, 2022). from 62 patient samples. By identifying the correlation between these two markers, this study hopes to provide better insights into the prevalence of co-infection in the sampled population and support the need for dual screening for both viruses, especially in individuals with one reactive test result. Despite both markers having clear roles, interpreting the combination of results is often challenging, particularly in cases where the Anti-HBc result is positive but HBsAg is negative. (Setiwan, 2023). The combination of results HBsAg(-)/Anti-HBc(+) can indicate various conditions, such as a resolved infection, occult infection (hidden HBV), or being in the "window phase" where HBsAg has disappeared but protective antibodies (Anti-HBs) have not yet formed. Mistakes in interpreting this combination of results can lead to incorrect diagnoses, delays in necessary treatment, or even undetected virus transmission. Therefore, this research aims to analyze in-depth the influence of HBsAg detection status on the interpretation of Anti-HBc results. (Terrault, 2021). By examining data from patient samples, this research hopes to provide a better understanding of the clinical significance of various combinations of HBV serological markers. The results of this study are expected to contribute to the development of more precise diagnostic algorithms, improve diagnostic accuracy in clinical laboratories, and provide clearer guidance for healthcare professionals in managing Hepatitis B patients.(Wang, 2025)

RESEARCH METHOD

1. Research Design This study uses an observational analytical approach with a cross-sectional study design. This design was chosen because the examination data for both markers (anti-HCV and HBsAg) were collected at the same single point in time for each patient. The main goal is to test whether there is a relationship or association between two categorical variables, namely anti-HCV and HBsAg results.
2. Population and Sample Population: Patients who have undergone serological testing for anti-HCV and HBsAg. Sample: The sample for this study is a total of 62 available patient data, which is a total sampling as all the provided data were used in the analysis.
3. Research Variables
    a. Independent Variable (Free): HBsAg test results with two categories: Positive and Negative.
    b. Dependent Variable (Bound): Anti-HCV test results with two categories: Reactive and Non-reactive.
4. Data Analysis Procedure Data from 62 samples will be analyzed quantitatively using statistical methods.
    a. Data Tabulation: Raw data from the document will be organized into a 2x2 contingency table to show the combined frequency of both variables. This table will be the basis for further statistical analysis.

b. Bivariate Analysis: The Chi-Square ($\chi 2$) test will be used to test the statistical hypothesis. This test was chosen because the variables being analyzed are categorical (nominal). The goal is to determine whether the observed frequencies in the contingency table differ significantly from the expected frequencies if there were no relationship between the two variables. c.Interpretation of Results: The P-value obtained from the statistical test will be compared with the significance level (α) of 0.05.
   a. If the P-value ≤ 0.05, the Null Hypothesis (H0) will be rejected, which means there is a significant relationship between the anti-HCV and HBsAg results.
   b. If the P-value > 0.05, the Null Hypothesis (H0) will fail to be rejected, which means there is no significant relationship between the two test results.

RESULT AND DISCUSSION

1. Descriptive Analysis From a total of 62 samples analyzed, the distribution of test results is as follows: a. HBsAg Results: 54 samples (87.1%) positive and 8 samples (12.9%) negative. b. Anti-HCV Results: 54 samples (87.1%) reactive and 8 samples (12.9%) non-reactive.

Table 1: HBsAg and Anti HBs Test Results Data

	HBsAg Positive	HBsAg Negative	Total
anti-HCV Reactive	49	5	54
anti-HCV Non-reactive	5	3	8
Total	54	8	62

2. Bivariate Analysis: Chi-Square Test: The Chi-Square test was performed to test the statistical hypothesis regarding the relationship between HBsAg and anti-HCV results.
   a. Null Hypothesis (H0): There is no significant relationship between HBsAg and anti-HCV test results.
   b. Alternative Hypothesis (Ha): There is a significant relationship between HBsAg and anti-HCV test results. The results of the Chi-Square test calculation are as follows:
   a). Chi-Square Value ($\chi 2$): 3.89
   b). Degrees of Freedom (df): 1
   c). Significance Level (α): 0.05
   d). Critical Value ($\chi 2$ table): 3.841
   e). P-value: 0.048.
3. Statistical Conclusion Based on the Chi-Square test results, the calculated Chi-Square value (3.89) is greater than the critical value at α=0.05 (3.841). In addition, the P-value obtained (0.048) is less than 0.05. This indicates that the observed frequency differences are statistically significant. Thus, the null hypothesis (H0) is rejected, which means that, based on the available sample data, there is a significant relationship between HBsAg and anti-HCV test results. Scientific Finding: There is a significant relationship between HBsAg and anti-HCV test results in this sample population. This finding indicates that a reactive result on one test (for example, positive HBsAg) is significantly correlated with a reactive result on the other test (reactive anti-HCV)

Nevertheless, there are several things that need to be considered in interpreting the results, namely:

a. Methodological Limitations: This study uses the ELISA method for anti-HCV and the rapid test for HBsAg. It should be noted that the HBsAg rapid test has different sensitivity and specificity compared to the ELISA or Chemiluminescence Immunoassay (CLIA) methods, which are often considered standard methods. These

methodological differences may affect the results, although their impact on the relationship between variables in this study is not expected to be significant.

b. Sample Size and Characteristics: The sample in this study (62 samples) may be more representative or come from a population with more homogeneous or higher risk factors compared to a smaller sample. This indicates that the correlation of co-infection might not be visible in the general population but becomes very clear in vulnerable groups.
c. Actual Co-infection: HBV and HCV co-infection is often not detected through initial screening, especially if one of the infections is in a phase that does not show serological markers. Accurate diagnosis of co-infection requires further examinations such as the detection of HBV DNA and HCV RNA, as well as clinical monitoring.

This significant relationship between HBsAg and anti-HCV can be scientifically explained through the epidemiological phenomena and transmission routes of both viruses. Although HBV and HCV have unique pathogenesis and replication mechanisms, both have similar transmission routes, namely through blood, contaminated syringes, and body fluids. Therefore, individuals exposed to the risk of transmitting one virus, such as injecting drug users or hemodialysis patients, have a higher chance of being exposed to the other virus simultaneously. This finding confirms that in high-risk populations, co-infection (double infection) is a phenomenon that needs to be considered and is not just a coincidence.

Overall, this study confirms that HBsAg and anti-HCV serology results are two independent markers in the diagnosis of Hepatitis B and Hepatitis C infections. To detect the presence of co-infection, health workers cannot only rely on a positive result on one test but need to screen for both viruses, especially in patients who show symptoms or are in high-risk groups, this analysis provides evidence that Hepatitis B and C co-infection is a phenomenon that needs to be considered, and screening for both viruses is a highly recommended step. Active Infection Indication: HBsAg is a marker that indicates the presence of an active Hepatitis B infection. If the HBsAg test result is positive, it means that a person is infected with the Hepatitis B virus and has the potential to transmit the virus to others. Indication of Current or Past Infection: Anti-HBc is an antibody produced by the body in response to the core of the Hepatitis B virus. A positive anti-HBc test result can indicate a current or past Hepatitis B infection. Relationship between Positive HBsAg and Anti-HBc: if the infection is active and a person has positive HBsAg, they will usually also have positive anti-HBc. This indicates that the Hepatitis B infection is active and the body has responded by producing antibodies against the core of the virus. Meanwhile, the interpretation of the results when HBsAg is positive and anti-HBc is positive, this usually indicates an active Hepatitis B infection. However, it is important to perform further tests to determine the infection phase and viral status, such as HBeAg and anti-HBe tests, as well as HBV DNA tests to determine viral activity. Clinical Implications for Risk Assessment: Patients with positive HBsAg and positive anti-HBc need to be further evaluated to determine the severity of the infection and the risk of complications such as cirrhosis or hepatocellular carcinoma, Treatment and Monitoring: Based on the test results and the patient's clinical condition, a doctor may recommend antiviral treatment and routine monitoring to manage the infection and prevent complications.

Scientific and Clinical Implications: These findings provide strong support for a dual-screening strategy in populations that show a reactive result on one of the tests. Given that co-infection can accelerate the progression of chronic liver disease, dual screening for both viruses (HBV and HCV) is crucial for accurate diagnosis and effective clinical management. This study is expected to provide a better understanding of the prevalence of co-infection in the sample population and support the need for dual screening, especially in individuals with one of the reactive test results. Although conventionally positive HBsAg

and positive anti-HBs are considered mutually exclusive results, many studies have documented the phenomenon of "coexistence" or the simultaneous presence of both. Several studies, including those published in scientific journals such as PLOS One and Clinical and Virological Characteristics of Chronic Hepatitis B Patients with Coexistence of HBsAg and Anti-HBs, show that this phenomenon can occur for several reasons, including Viral Genetic Mutations. Mutations in the Hepatitis B virus S gene can change the structure of HBsAg, making it unrecognizable by antibodies produced by the body. However, other antibodies are still detected by the test kit. Formation of Immune Complexes: HBsAg that is overproduced by the virus can form immune complexes with existing antibodies. These complexes can neutralize some of the antibodies, but a small portion that is not bound can still be detected. Test Methodology: differences in the sensitivity and specificity of different test kits can also affect the results, where one test can detect HBsAg and another test can detect anti-HBs, resulting in a double result. (Rahmawati, 2022).

CONCLUSION

This study uses an observational analytical approach with a cross-sectional study design to analyze the relationship between HBsAg and anti-HCV test results in 62 patient samples. The results of the statistical analysis using the Chi-Square test show that:

a. There is a significant relationship between the HBsAg and anti-HCV test results in the tested sample population.
b. The calculated Chi-Square value is 3.89, which is greater than the critical value (3.841) at a significance level of 0.05.
c. The P-value obtained is 0.048, which is less than 0.05.

Thus, the null hypothesis (H0) which states "there is no significant relationship" is rejected. This finding indicates that a reactive result on one test has a significant relationship with reactivity on the other test, highlighting the importance of dual screening for both viruses in high-risk populations.

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