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Comparison of Rapid Diagnostic Test with Enzyme-Linked Immunosorbent Assay and PCR for Detection of Hepatitis B Surface Antigen

Hepatit B Yüzey Antijeninin Saptanması için Hızlı Tanı Testinin Enzime Bağlı İmmünosorbent Testi ve PCR ile Karşılaştırılması

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ÖZ

Amaç: Hepatit B virüs (HBV) enfeksiyonu, önemli morbidite ve mortalite oranları ile küresel halk sağlığı sorunu olmaya devam etmektedir ve tanı için HBs Ag'nin tespiti çok önemlidir. İdeal olarak hızlı testlerin duyarlılığının yüksek özgüllüğününde kabul edilebilir yükseklikte olması gerekir, böylelikle yanlış pozitif ve yanlış negatif sonuçların önüne geçilebilir. Bu çalışmanın amacı, ELISA ve PCR ile doğrulanmış vakalarla hızlı tarama testlerinin performansını değerlendirmektir.
Araçlar ve Yöntem: Prospektif olarak planlanan bu çalışma Şubat 2024-Mart 2024 tarihleri arasında Samsun'da üçüncü basamak bir hastanede gerçekleştirilmiştir. HBsAg testi için mikrobiyoloji laboratuvarına çeşitli kliniklerden gönderilen toplam 160 kan örneği çalışmaya dahil edilmiştir. Tüm örnekler ELISA ve PCR yöntemi ile çalışıldıktan sonra hızlı test ile çalışılmıştır.
Bulgular: ELISA ile karşılaştırıldığında hızlı testin özgüllüğü %97.70, duyarlılığı %87.20, pozitif prediktif değeri (PPV) %57.82, negatif prediktif değeri (NPV) %99.53olarak saptanmıştır. Ayrıca HBsAg hızlı testi PCR ile karşılaştırıldığında duyarlılığı %44.50, özgüllüğü ise %47.40 olarak belirlenmiştir.
Sonuç: Çalışmamız ile hızlı testlerin ELISA ile karşılaştırıldığında yüksek özgüllük ve kabul edilebilir duyarlılığa sahip olduğu ancak PCR ile karşılaştırıldığında yeterince uyumlu olmadığı sonucuna varılmıştır.

Anahtar Kelimeler: HBsAg; hızlı test; ELISA; PCR

ABSTRACT

Purpose: Hepatitis B virus (HBV) infection is a global public health problem with significant morbidity and mortality rates and the detection of HBsAg is a very important test for diagnosis. Ideally, rapid tests should have a high sensitivity and an acceptable level of specificity, so that false positive and false negative results can be prevented. The objective of this study was to evaluate the performance of rapid screening tests with confirmed cases with ELISA and PCR.
Materials and Methods: This study was conducted as a prospective study in a tertiary hospital in Samsun between February 2024 and March 2024. A total of 160 blood samples sent to the microbiology laboratory for HBsAg testing from various departments were included in the study. All samples were studied with a rapid test after being studied with ELISA and PCR methods.
Results: Compared to ELISA, the rapid test had a specificity of 97.70%, a sensitivity of 87.20%,a positive predictive value (PPV) of 57.82% and a negative predictive value (NPV) of 99.53%. In addition, when the HBs Ag rapid test was compared with PCR, the sensitivity was 44.50% and the specificity was 47.40%.
Conclusion: Our study concluded that rapid tests have high specificity and acceptable sensitivity compared to ELISA, but they are not sufficiently consistent when compared to PCR.

Keywords: ELISA; HBsAg; PCR; rapid test

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INTRODUCTION

Hepatitis B virus (HBV) is an important public health problem that can cause chronic hepatitis infection, liver cirrhosis and hepatocellular carcinoma.[1,2] The World Health Organization (WHO) estimates that chronic HBV infection affects approximately 350 million people worldwide and plans to eliminate this infection, which is a public health problem, by 2030.[3] If this target is not achieved, annual global deaths due to HBV are predicted to increase by 39% from 2015 to 2030.[4] Laboratory confirmation of the diagnosis is necessary because it's impossible to distinguish hepatitis B from other viruses based on clinical symptoms alone.[4] Because HBV virus often causes asymptomatic infections, accurate detection of viral markers is very important in controlling the transmission of highly infectious HBV virus.[5] HBsAg is a crucial viral antigen that serves as a superior marker for detecting the Hepatitis B virus.[5] Several methods are employed to detect HBsAg, such as enzyme-linked immunosorbent assay (ELISA), enzyme immunoassays (EIA), Nucleic Acid Amplification Test (NAT), polymerase chain reaction (PCR), and immunochromatographic tests (ICT) in patient samples.[6] Enzyme-linked immunosorbent assay, EIA, PCR, and NAT methods are expensive and require technical human support, making them suitable for well-equipped laboratories. In contrast, rapid kits are cost-effective and can be used in basic laboratories.[6] The immunochromatographic method has the advantage of being quick and can be performed by minimally laboratory technician.[6]

HBsAg rapid test is a rapid screening method used for qualitatively detecting HBsAg in whole blood samples, serum, or plasma.[7] The clinical performance of rapid tests is currently limited due to their variable sensitivity and non-quantitative results.[8] The present study evaluated the performance of rapid screening tests with already confirmed cases with ELISA.

MATERIALS and METHODS

This study was conducted in a tertiary care hospital in Samsun in compliance with the Declaration of Helsinki from February 2024 to March 2024. Approval for this study was obtained from Samsun University Non-Interventional Research Ethics Committee (dated 28.02.2024 and numbered 2024/5/18).

Serum samples of the microbiology laboratory for HBsAg testing from various departments were included in the study.

Sample procedure: One hundred and sixty blood samples were included in this study; firstly all samples were centrifuged, and serum was separated. The samples were tested for HBsAg with Elisa then rapid test and PCR. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of rapid tests were compared with ELISA and PCR.

Procedure: The HBsAg rapid test device (ABON^{Tm} Abbott ABD) is a chromatographic immunoassay that detects HBsAg in blood, plasma, or serum. The working procedure was carried out as specified in the kits; add 3 drops of serum (approximately 75 µlt) into the sample wells of the test device, wait for 15 minutes for the colored line(s) to appear, and then read the test results visually without any instrument. The sensitivity and specificity of these assays were reported (by the manufacturer) as 99.3% (95%Cl:98.3%-99.68%) and 99.84% (95% Cl:99.63%-99.95%), respectively.

The ELISA technique was used for comparative evaluation. HBsAg detection was performed using the Architect i2000SR immunoassay analyzer (Abbott Laboratories, North Chicago, USA). The HBsAg Qualitative II test is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of Hepatitis B surface antigen (HBsAg) in human serum or plasma. The system calculates the result for the using the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen. Results were interpreted as <1.00 nonreactive and ≥1.00 reactive.

HBV DNA quantitation was performed using the Abbott RealTime HBV PCR assay (Wiesbaden, GERMANY) using nucleic acid amplification or signal amplification technologies to detect A-H genotypes according to the manufacturer's instructions. A highly conserved Surface gene was used to detect these genotypes. The HBV DNA level detected by this test with 95% probability was 10 IU/mL for a 0.5 mL sample.

Statistical Analysis

Statistical analysis were performed using SPSS v22 for Windows (IBM, IL, USA). The data for continuous variables were shown as mean±standard deviation, and categorical variables were shown with percent differences. To compare categorical values, the chi-square test was used. Additionally, to evaluate the sensitivity and specifity of the HBs Ag rapid test we performed Receiver operating characteristic (ROC) curve analysis.

Sample size calculation was determined using the G*Power 3.1.9.7 power analysis program. As a result of the analysis, it was determined that the total sample size was at least 134. (Test family: Chi-square test, Stastical test: Variance: Difference from constant (one sample case), Type of power analysis: Apriori: Compute required sample size- given α, power and effect size).

RESULTS

A total of 160 patients aged between 20 and 82 were included in our study. The average age of these patients was found to be 57.28±14.72. Of these patients, 65 (40.63%) were female and 95 (59.38%) were male. Blood samples of these patients were tested for HbsAg using ELISA and rapid test method. PCR was performed on 129 of the 141 patients whose HBs Ag were positive with Elisa. The HbsAg results with ELISA and rapid test and HBV DNA results with PCR are presented in Table 1. The highest HbsAg positivity rate was detected in the test performed according to the PCR method. The HbsAg positivity rate detected by the ELISA method was found to be statistically higher than the rate detected by the rapid diagnostic test ($p<0.001$). Since patients whose HbsAg test was negative by the ELISA method were not tested with the PCR method, no comparison could be made between the two methods. The HbsAg positivity rate detected by the PCR method was found to be statistically higher than the rate detected by the rapid diagnostic test ($p<0.001$) (Table 1).

We compared rapid antigen tests with ELISA for the detection of HBsAg from patient serum. The comparison of rapid tests with ELISA is shown in Table 2.

Table 1. Test results obtained with 3 different methods.

Methods	Positive n (%)	Negative n (%)
ELISA n=160	141 (88.13)	19 (11.88)
PCR n=129	122 (94.57)	7 (05.43)
Rapid test n=160	117 (73.13)	43 (26.88)

ELISA: Enzyme-Linked ImmunoSorbent Assay, PCR: Polymerase Chain Reaction

Table 2. Comparison of rapid test with ELISA.

Method		ELISA		Total
	Result	Positive n	Negative n	
Rapid test	**Positive n**	117	0	117
	Negative n	24	19	43
Total		141	19	160

ELISA: Enzyme-Linked ImmunoSorbent Assay

A total of 160 serums were performed with ELISA; 141 were HBsAg positive and 19 were HBsAg negative. Among the 141 samples with HBsAg seropositivity by ELISA, 117 showed positive results and 24 (17.02%) showed negative results by HBsAg rapid test, these are evaluated as false negative group. In the HbsAg positive group by ELISA, the HbsAg values ranged from 6 s/co to 7426 s/co. HBs Ag values of 141 positive samples are shown in Table 3.

Table 3. HBs Ag values of 141 positive samples.

HBsAg values (s/co) by ELISA	Number of patients positive with ELISA	Number of patients negative with rapid test
1-100 s/co	14 (9.93%)	13 (54.17%)
101-500 s/co	26 (18.44%)	10 (41.67%)
501-1000 s/co	10 (7.1%)	1 (4.16%)
≥ 1000 s/co	91 (64.53%)	-
Total	141 (100%)	24 (100%)

Upon examination of the ELISA values of 24 samples that tested negative with the rapid test, it was found that only one sample had a value of more than 500 s/co. The receiver-operating characteristic (ROC) curve analysis was used to evaluate the performance of the rapid test (Figure1)

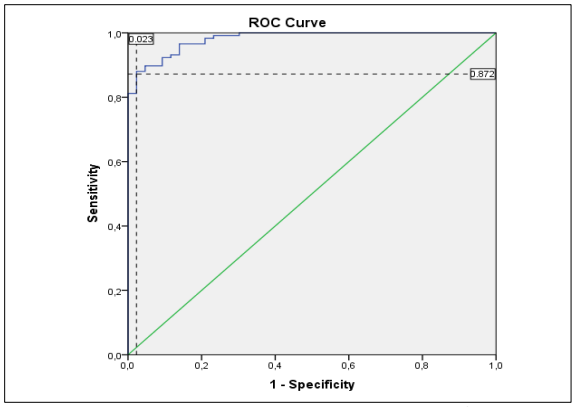

Figure 1. Receiver operating characteristic curve for HbsAg rapid test compared with ELISA.

According to the ROC analysis, the cut-off value of HbsAg positivity obtained according to the rapid test and the HbsAg test levels obtained according to the ELISA was determined to be 449.00 s/co. In addition as a result of ROC analysis, the area under the curve was found to be 0.981 (95% CI 0.966-0.997), the sensitivity was 87.20% and the specificity was 97.70%. However, the negative predictive value (NPV) of the rapid test was 99.53% (95%CI 99.27-99.70), and the positive predictive value (PPV) was 57.82% (95%CI 06.81-96.26).

Among the 141 samples with HBsAg seropositivity by ELISA, 129 were tested with PCR. PCR and rapid test results of these patients are shown in Table 4.

Table 4. Comparison of rapid test with PCR.

Method		PCR		Total
	Result	Positive n	Negative n	
Rapid test	**Positive n**	105	5	110
	Negative n	17	2	19
Total		122	7	129

PCR: Polymerase Chain Reaction

PCR was performed on 129 samples with positive Hbs Ag ELISA test. 122 were detected as positive and 7 as negative. 17 of 122 positive samples were detected as negative by rapid test. When these samples were examined, it was determined that the HBV DNA level of 15 of them were <100 IU/ml and 2 of them were between 100-200 IU/ml.

The correlation of HBV DNA level and HBsAg rapid test was evaluated using ROC analysis.

According to the ROC analysis, the cut-off value of HbsAg positivity obtained according to the rapid test and the HBV DNA levels obtained according to the PCR method was determined to be 21.50 IU/L. In addition, as a result of ROC analysis, the area under the curve was found to be 0.418 (95% CI 0.280-0.56), the sensitivity was 44.50% and the specificity was 47.40% (Figure 2).

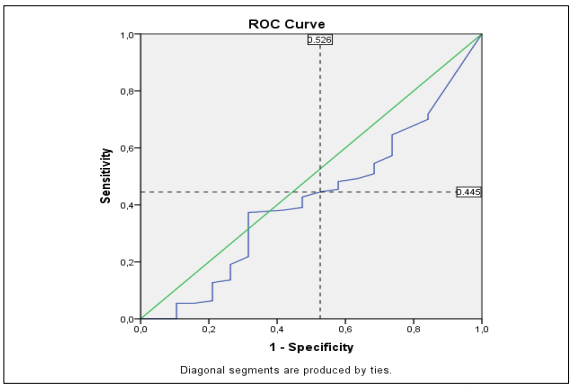

Figure 2. Receiver operating characteristic curve for HbsAg rapid test compared with PCR.

DISCUSSION

In our study, HBsAg rapid test was compared with ELISA and PCR fort the screening of HBs Ag. Since the 1990s, rapid tests have been available for the detection of anti-HIV, HBsAg, and anti-HCV.[10] The biggest problem for HBs Ag rapid test is to detect the low levels of the target antigen that are present in a relatively high proportion of asymptomatic carriers.[11] Therefore rapid tests are particularly required to have a high degree of sensitivity and acceptable level of specifity to reduce false results.

In this study, when the rapid test was compared with ELISA the sensitivity of the rapid test was 87.20% and the specificity was 97.70%. Several previous studies conducted globally have reported similar findings.[12,5] Lau et al. declared that although rapid immunochromatographic testing has several advantages EIA testing is the standard method for detecting HBsAg and HBe-Ag.[13] Similar to our study, Prabha P et al., have reported 83.4% sensitivity and 100% specificity of rapid screening test.[5] Yogendra T et al. reported that sensitivity was 95.12% and specificity was 99.82%.[14] Another study showed 96.8% sensitivity of rapid test kit with a specificity of 99.7% for HBsAg as compared to ELISA and PPV was calculated to be 98.41% and NPV was 99.56%.[15] PPV refers to the ability of an assay to accurately detect infected individuals among all those who test positive with

the kit. NPV measures the ability of an assay to accurately identify non-infected individuals who test negative with the kit being used.[16] In our study, the rapid test had a negative predictive value of 99.53% and a positive predictive value of 57.82%. Due to the high number of HBsAg-positive samples in this study, we observed a higher rate of false negatives which resulted in a lower positive predictive value (PPV) when compared to other studies. In our study, 17.02% of HBsAg-positive samples had negative results in rapid tests, and these false negatives can lead to misdiagnosis and delay in treatment, which can have serious consequences. According to ROC analysis, HBsAg positivity may not be detected by the rapid test if the HBs Ag value is less than 449 s/co with ELISA. It is important to be aware of the limitations of rapid tests and to confirm any negative results with follow-up testing to ensure accurate diagnosis and timely treatment.

Comparisons of the sensitivity and specificity of rapid tests and quantitative immunoassays to detect HBsAg have been performed by many researchers, but few comparative studies are using the quantitative PCR method as the gold standard.[6] In a study conducted by Navvabi et al., the results of two tests, the HBs-Ag rapid test, and the ELISA test were compared with the PCR test. The HBs Ag rapid test showed a sensitivity of 97% and a specificity of 91%, while the ELISA test showed a sensitivity of 78% and a specificity of 76%.[17] In a study by Mohammad Hassan Khadem Ansari et al., six commonly used rapid diagnostic tests were evaluated for their sensitivity and specificity, compared with PCR methods and they reported that immunochromatographic results must be interpreted with caution because samples with low reactivity in quantitative PCR may show negative HBsAg results.[6] In our study, we also compared the rapid test with the PCR method and the rapid test has a sensitivity of 44.50% and a specificity of 47.40%. While these figures provide some indication of the test's reliability, they also suggest that rapid tests may fail to detect of HBsAg in cases where the HBV DNA level is below 21.50 IU/L.

When we compared the results of ELISA and PCR tests, we found that out of the 141 sera that were detected positive by ELISA, 129 were retested with PCR and 7 of them were negative with PCR. Due to these results, sensitivity and specificity were lower when we compared the rapid test with PCR.

However, this study had some limitations. Firstly, due to patients being unreachable or missing hospital appointments, HBV DNA quantification was not performed on all patients, resulting in a relatively small sample size. Secondly, there was a lack of knowledge regarding the patients' treatments and follow-up.

Conclusion

Our study concluded that rapid tests have high specificity and acceptable sensitivity compared to ELISA, but are not compatible enough when compared to PCR. Although in some situations where large groups of people are being screened, false positives are often preferred over false negatives, it is crucial to conduct further studies that can demonstrate the effectiveness of rapid tests in various populations and geographic locations. Only by ensuring the reliability and accuracy of these tests can we use them confidently to diagnose medical conditions. Rapid tests are simple and rapid screening methods that offer comparable sensitivity and specificity to the ELISA. But it should not be forgotten that false negatives can lead to misdiagnosis and delay in treatment.

Conflict of Interest

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

Ethics Committee Permission

Approval for this study was obtained from Samsun University Non-Interventional Research Ethics Committee (dated 28.02.2024 and numbered 2024/5/18).

Authors' Contributions

Concept/Design: MB, MHT, EMY, CÇC. Data Collection and/or Processing: MB, MHT, EMY, CÇC. Data analysis and interpretation: MB, MHT, EMY, CÇC. Literature Search: MB, CÇC. Drafting manuscript: MB, MHT. Critical revision of manuscript: CÇC, EMY.

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