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Evaluation of hepatitis B surface antigen and hepatitis B virus-DNA results in postmortem plasma specimens

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PEER REVIEW

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ABSTRACT

Objective: To assess the presence of hepatitis B surface antigen, one of the serologic markers of hepatitis B virus (HBV) infection, in postmortem blood samples from autopsy cases using ELISA, and to compare the results with those obtained by PCR, which is the gold standard method in assessing HBV infection.

Methods: The HBV test results of the blood samples from 880 autopsy cases determined in our laboratory, were retrospectively studied.

Results: When compared with the gold standard method PCR, the sensitivity and specificity of postmortem ELISA were 100% and 84.1%, respectively.

Conclusions: The increasingly used molecular diagnostic methods, such as PCR, should be used in cases where serological tests remain insufficient.We think that prospective studies on the comparison of ELISA and PCR assessment of postmortem blood samples with larger material should be carried out.

1. Introduction

Hepatitis B virus (HBV) infection, which is prevalent in the world, is also prevalent in Turkey, with middle endemicity. The seroprevalence studies in Turkey have shown a seropositivity of hepatitis B surface antigen (HBsAg) ranging from 0.8% to 14.3% and of anti-HBs ranging from 20.6% to 52.3%[1-3].

The diagnosis of HBV infection is generally made by assessing the viral antigens (HBsAg and hepatitis B e antigen) and/or antibodies [anti-HBs, hepatitis B e-antibody and hepatitis B core antibody (IgM-IgG)] in the patient serum. HBsAg is the most frequently used basic marker of HBV infection[4]. Although the HBV tests are also used for assessing postmortem sera, the use of these tests for postmortem sera have not been validated until the present time. There is very limited data on the performance of these tests, and hence, false-positive results may be obtained. The kit manufacturers have stated that there is a limited number of studies on the validity of the FDA-approved diagnostic kits used for antigen and antibody assessments[5].

The non-specific positivity of antigen-antibody in postmortem blood samples can be reduced to a minimum by using appropriate screening algorithms. If one single screening test is used for determination of antigen or antibody, a confirmation test should absolutely be made, too[6]. As far as we know, this study is the first in Turkey on the postmortem assessment for HBV infection.

The purpose of this study was to assess the presence of HBsAg, one of the serologic markers of HBV infection in postmortem blood samples from autopsy cases using the ELISA and to compare the results with those obtained by PCR, which is the gold standard method in assessing HBV infection.

2. Materials and methods

The results of postmortem test for HBV in 880 autopsy cases determined in the Postmortem Microbiology Laboratory of the Forensic Medicine Institute, Istanbul, Turkey, between the dates of 23rd December 2010 and 26th May 2011, were retrospectively studied. For serological testing, blood samples were drawn from the big blood vessels (femoral artery, femoral vein, jugular vein, *etc*.) of the autopsy cases. The samples that had been placed in ethylene diamine tetraacetic acid-tubes were promptly transferred to the laboratory. The blood samples were then centrifuged at 10 000 r/min for 10 min and the the obtained plasma samples were assessed

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for the presence of HBsAg with ELISA using the miniVIDAS analyzer (Biomerieux, France). The HBsAg-positive plasma samples were then assessed with the RT-PCR for confirmation. HBV DNA extraction was performed using the Mag 16 Viral Nucleic Acid Extraction Kit (Fluorion, Iontek), and the quantitative HBV DNA was determined using the HBV QNP 2.0 (Iontek) RT-PCR kit, which had an analytic sensitivity of 10 IU/mL and a linear regression range of 2×10^{9}-2×10^{1} IU/mL, according to the recommendations of the manufacturers. When PCR was accepted as the gold standard method in assessing HBV infection, the sensitivity, specificity, and the positive and negative prediction values of ELISA were calculated and evaluated. The advantages and disadvantages of these two methods in determining HBV infection were compared and discussed.

The SPSS Version 16 was used for the analysis of the obtained data. The descriptive statistics were expressed as numbers and percentages.

Normally, the postmortem interval (PMI, the time between death and autopsy) is expressed as 12, 24, 48 h or other hours since the PMIs of the autopsy cases in this study were unknown; 12 h means autopsy on the day of death and 24 h means autopsy on the day after the death.

3. Results

Of the 880 autopsy cases, 660 were males and 220 females, with a mean age of 42.4±22.08. As already stated before, due to uncertainty, the PMIs of the cases could not be given in detail. As shown in Table 1, 12 h means autopsy on the day of death and 24 h means autopsy on day following the death, and 48, 72, 96, and 144 h stand for the successive autopsy days. It was seen that most of the autopsies had been performed on the day of death or on the day following death (*n*=836, 95%). The other variables have been displayed in Figure 1.

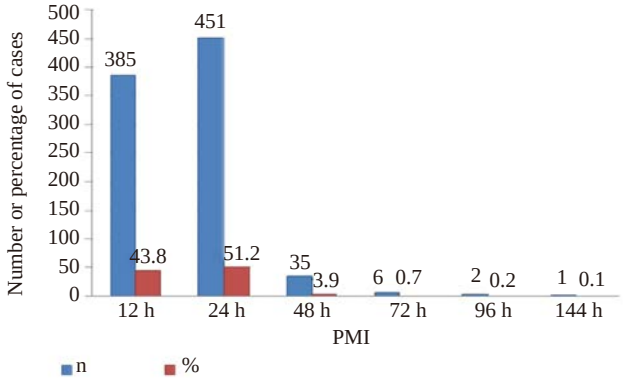

Figure 1. The distribution of number of cases according to the PMI.

Of the 880 plasma samples tested with ELISA, 707 (80.3%) were HBsAg-negative and 173 (19.7%) were HBsAg-positive. In 40 (23.1%) of the 173 HBsAg-positive samples, HBV DNA was positive when tested with PCR (Table 1).

Table 1

The distribution of according to the HBsAg (ELISA) and HBV DNA (PCR) results.

HBsAg (ELISA) [*n* (%)]	HBV DNA (PCR) [*n* (%)]		
	Positive	Negative	Total
Positive	40 (4.5)	133 (15.1)	173 (19.7)
Negative	0 (0.0)	707 (80.3)	707 (80.3)
Total	40 (4.5)	840 (95.5)	880 (100.0)

When PCR was taken as the gold standard method, the sensitivity, specificity, and positive and negative prediction values of postmortem ELISA were calculated (Table 2).

Table 2

The distribution of postmortem ELISA test results according to the gold standard method:

Postmortem ELISA test results	Value
Sensitivity	100.0%
Specificity	84.1%
Positive predictive value	23.0%
Negative predictive value	100.0%
Prevalence (Pre-test probability)	4.5%
Pre-test odds	0.04
Post-test odds	0.30
Post-test probability	23.1%
Likelihood ratio for positive test result	6.30
Likelihood ratio for negative test result	0.00

According to the results obtained, postmortem ELISA can diagnose the presence of HBV in 100% of the cases with true HBV infection and can differentiate 84.1% of the cases with false-positive HBV infection from those with true HBV infection. Among cases determined to have HBV infection by ELISA, only 23% had true HBV infection. A hundred percent of cases determined as HBV-negative by ELISA had in fact no HBV infection. With ELISA, the rate of true positivity of HBV infection is 6.3 times more than the rate of false-positivity. This means that ELISA has more efficiency in detecting true HBV-positivity than false HBV-positivity. With postmortem ELISA, the probability of false-negative results in HBV-infected individuals is equal to the probability of detecting true negative cases. This means that ELISA has equal potency in yielding false-negative results in HBV-infected individuals and true negative results.

If the probability of HBV infection before performing ELISA is 4.5% and the probability of ELISA positiveness is 6.3%, and if findings related to HBV infection are present after ELISA, the real probability of an HBV infection is 23.1%. This shows that the diagnostic value of ELISA is not very high in HBV infection.

4. Discussion

Postmortem serological tests are conducted to determine whether the cause of death is a viral infection or not, as well as to screen organ transplants for the presence of viral infections before performing transplantation surgery[7,8-11]. Studies have shown that there is a significant difference in postmortem samples obtained at the 6th hour and those obtained at the 16th hour following death. It has been reported that the postmortem samples for serological tests should be obtained within 24 h of death[5,12]. In our study, most of the cases had undergone autopsy on the day of death or on the day following death, and therefore, most of the blood samples used for serological tests were obtained between 12 and 24 h after death (*n*=836, 95%).

The limitations in postmortem screening for infectious diseases include insufficient quantity or bad quality of blood samples, or samples obtained at inappropriate time intervals. The most important problem of postmortem serological samples is their controversial specificity. Some studies on the subject have shown higher positive results than expected[7,9,10]. Heim *et al*. compared the premortem and postmortem sera of 33 cornea donors by ELISA in terms of HBsAg and determined false-positive results in 16 out of 33 serum samples[7]. This study has shown that ELISA is not a reliable method for screening postmortem sera, and that another screening method such as determining the viral nucleic acids with PCR is required. Another study has also suggested that serological kits used for postmortem screening should be highly sensitive[6]. Most of the serological kits used for postmortem studies have not been validated. Due to loss of specific reactions, the postmortem samples show

decreased sensitivity and increased false-negative results. Hence, the use of appropriate screening algorithms is important in reducing nonspecific positivity[6]. The negative result by a test used in a standard algorithm is the evidence of the absence of infection in postmortem cases. If the result is positive, it is suggested that the test be repeated twice. At least, screening should be repeated with a different antigen or antibody test of equal sensitivity or with a test of different principle. A negative result shows that there is no evidence of infection. However, if repeated tests are positive once or twice, this positivity should then be confirmed with a reference sample[6]. Kitchen *et al*. reported that this serological screening program was effectively used in their center[6]. Furthermore, the authors in their study on 1 659 postmortem samples found 1 566 (94.4%) samples negative by assessing 6 different markers and determined 12 samples out of 93 positive samples to be true positive by utilizing more than one screening test[6]. The performance of a screening test depends mainly on its sensitivity and specificity. It has been reported that the tests used for assessing postmortem samples show a decreased sensitivity, but the important factor in screening programs is the specificity of the test used, and that specificity of the test depends on the quality of the experiment and the samples used. The test specificity in postmortem screening programs is even expected to be lower than that in blood donor screening programs[6]. Among 6 different serologic tests used, a postmortem study has found the specificity of human T-lymphotropic virus antibody test to be 87.8%, and with repeated tests, 44 negative samples out of the formerly-determined 55 positive samples. Likewise, out of 19 HBsAg-positive samples, 13 have been found to be negative when confirmed[6]. Moreover, in our study, we found that, out of 173 HBsAg-positive samples, 133 (76%) proved to be HBsAg-negative with advanced analysis, and when compared with the gold standard method PCR, the specificity of postmortem ELISA was found to be 84.1%.

In some countries, the serological tests used for screening the postmortem blood samples from organ transplant donors have been validated by their manufacturers, but currently, these tests are not used worldwide. Former studies have obtained higher rates of positivity than expected in screening postmortem samples of organ transplant donors, and this situation has been attributed to the low specificity of the tests used[7,9-11,13,14]. On one hand, the specificity of postmortem screening tests has been claimed to be the existing problem, and on the other hand, the sensitivity of these tests has been claimed to be a more important problem than their specificity. Another study by Kitchen and Newham has screened the postmortem blood samples of organ transplant donors in a broader spectrum[5]. They have confirmed the results by titrating the serologically positive samples (to detect inhibition in postmortem samples) with the postmortem blood samples of organ transplant donors or with pooled negative serum samples of normal individuals. They have found no statistically significant difference between the dilution curves of the normal pooled sera and the diluted positive postmortem serum samples[5]. No marked difference has been determined between the sensitivities of screening tests used in live and dead organ transplant donors. Since the serological tests used for screening autopsy cases have not been validated, postmortem samples still cause an inhibition in reactions due to their nature (false positivity) and decrease the sensitivity of the tests in limited degrees.

Recent studies have concluded that the specificity of serological screening tests is markedly lower than that of molecular tests. Many investigators have accepted molecular screening tests as valid tests especially for HBV, hepatitis C virus, and HIV infections[6,12,15]. We are of the opinion that molecular tests such as PCR should be used when serological tests remain insufficient. As far as we know, this study is the first study in Turkey which compares the results of ELISA and PCR assessment of postmortem blood samples. Prospective and more comprehensive studies on the subject are required.

Conflict of interest statement

We declare that we have no conflict of interest.

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