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Atypical Serology Profiles in Hepatitis B Cases: A Retrospective Analysis

Hepatit B Olgularında Atipik Seroloji Profilleri: Retrospektif Bir Analiz

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ABSTRACT

Aim: Understanding antigen-antibody profiles in hepatitis B virus (HBV) infection is essential for accurate diagnosis and clinical assessment. Although standard serological patterns are well defined, variations in viral protein expression and host immune responses can result in the loss of expected antibodies and the emergence of atypical serological profiles. Such deviations may complicate the interpretation of diagnostic and follow-up testing. This study retrospectively analyzed cases presenting with atypical HBV serological findings.

Methods: Patient data from HBV serology tests conducted at the Microbiology Laboratory of Bursa City Hospital were reviewed from hospital records. Additional tests, including HBV polymerase chain reaction (PCR), hepatitis C virus (HCV) tests (anti-HCV, HCV PCR), and human immunodeficiency virus (HIV) tests (anti-HIV, HIV confirmation), were evaluated in patients with isolated anti-HBc positivity. Patients were categorized on the basis of their HBeAg and anti-HBe status, and the means and standard deviations of the HBV viral load values were calculated.

Results: Atypical HBV serological profiles were identified in 1404 patients (0.49%). The most common atypical profile was isolated anti-HBc positivity, which was observed in 5.27% (n=1079) of the patients. Among 119 the patients tested with HBV PCR, 12 had detectable HBV DNA. Among the 23 patients whose HCV PCR results were tested, 13 had detectable HCV RNA. Three of the 995 patients tested for anti-HIV were reactive, with two confirmed as HIV-1 positive by confirmatory testing.

Conclusion: This study investigated the prevalence and characteristics of atypical HBV serological profiles among patients at Bursa City Hospital. Such profiles may arise due to false reactivity or reflect different stages of infection. Comprehensive evaluation and appropriate clinical management are essential for patients exhibiting these atypical patterns.

Key Words: Hepatitis B; serology; diagnosis

ÖZ

Amaç: Hepatit B virüsü (HBV) enfeksiyonu sırasında konakçı tarafından üretilen antijen-antikor profillerinin incelenmesi, enfeksiyon tanısı ve klinik seyrin değerlendirilmesinde önemli yere sahiptir. Serolojik profiller büyük ölçüde tanımlanmış olsa da, viral protein ekspresyonundaki varyasyonlar ve antikor immünojenisitesine bağlı farklılıklar, antikor yanıtında beklenmeyen kayıplara ve alışılmadık serolojik sonuçlara yol açabilmektedir. Bu atipik serolojik profiller tanı ve izlem sırasında enfeksiyonun yorumlanmasında zorluklara yol açabilir. Bu çalışmada, HBV enfeksiyonu yönünden incelenen hastalarda görülen atipik HBV seroloji sonuçlarının retrospektif olarak değerlendirilmesi amaçlanmıştır.

Yöntem: Bursa Şehir Hastanesi Mikrobiyoloji Laboratuvarı'nda çeşitli nedenlerle HBV seroloji testleri araştırılan hastaların sonuçları hastane otomasyon sistemi kayıtlarından retrospektif olarak incelendi. Ayrıca izole anti-HBc pozitifliği olan hastalarda HBV Polimeraz Zincir Reaksiyonu (PCR), Hepatit C virüsü (HCV) testleri (anti-HCV, HCV PCR) ve insan immün yetmezlik virüsü (HIV) testleri (anti-HIV, HIV doğrulama) değerlendirildi. Hastalar HBeAg ve anti-HBe durumlarına göre analiz edildi ve HBV viral yük değerlerinin ortalaması ve standart sapması hesaplandı.

Bulgular: Atipik HBV serolojik profili 1404 (%0.49) hastada tespit edilmiştir. En sık saptanan atipik profil izole anti-HBc pozitifliği (n: 1079, %5.27) olmuştur. Bu gruptaki 119 hastada HBV PCR yapılmış, 12 hastada HBV DNA saptanmıştır. HCV PCR çalışılan 23 hastadan 13'ünde HCV RNA pozitifliği tespit edilmiştir. Anti-HIV testi yapılan 995 hastanın üçü reaktif bulunmuş, bunlardan ikisinin HIV-1 doğrulama testi pozitif sonuçlanmıştır.

Sonuç: Bu çalışma ile Bursa Şehir Hastanesi'ne başvuran hastalarda HBV atipik serolojik profillerinin varlığı ve oranları tespit edilmiştir. Atipik serolojik profiller, yalancı reaktiviteden akut-kronik enfeksiyona kadar farklı durumlardan kaynaklı olabilir. İleri tetkik ve tedavi bu profil saptanan hastalarda önemlidir.

Anahtar Sözcükler: Hepatit B; Seroloji; tanı

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Introduction

Hepatitis B virus (HBV) infection represents a significant global public health concern, manifesting in a spectrum of clinical, outcomes ranging from asymptomatic carriage to chronic hepatitis and hepatocellular carcinoma. According to the World Health Organization (WHO), an estimated 254 million individuals were living with chronic HBV infection in 2022, with about 1.2 million new infections occuring annually [1].

The natural course of HBV infection is shaped by the dynamic interaction between viral replication and the host immune response. In clinical settings, diagnosis and monitoring of disease progression rely on the serological detection of HBV derived proteins and host-generated antibodies. Serological markers include hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), hepatitis B e antigen (HBeAg), hepatitis B e antibody (anti-HBe), and hepatitis B core antibodies (anti-HBc IgM and IgG) [2].

During the natural course of infection, the dynamics of viral protein expression and antibody production may change. Factors such as antigenic variants, infections with mutant viruses, co-infections with multiple viruses, variations in the host immune response, and differences in the sensitivity and specificity of diagnostic tests can all contribute to challenges in interpreting HBV infection [3,4]. Atypical serological profiles refer to unusual results observed during the diagnosis and monitoring of HBV infection. These profiles primarily include isolated anti-HBc positivity, simultaneous detection of HBsAg and anti-HBs, concurrent positivity of HBeAg and anti-HBe, and isolated HBsAg positivity [5]. Isolated anti-HBc, in particular, is characterized by the absence of detectable HBV surface antigens and antibodies, with the presence of hepatitis B core antibodies. This serological pattern is frequently observed in individuals co-infected with hepatitis C virus (HCV), human immunodeficiency virus (HIV), or those experiencing other forms of immunosuppression. Screening for isolated anti-HBc in immunocompromised patients is crucial, as HBV replication may reactivate under such conditions, posing a substantial risk of severe disease and increased mortality [6].

This study aimed to retrospectively evaluate atypical HBV serology results in patients tested for HBV infection.

Materials and Methods

The HBV serology results of patients tested for various clinical indications in the Microbiology Laboratory of Bursa City Hospital between July 2019 and October 2022 were retrospectively reviewed. Data were extracted from the hospital's automated information system, and HBV serological screening --comprising HBsAg, anti-HBs, anti-HBc IgG, HBeAg, and anti-HBe-- was independently evaluated by two researchers. Duplicate patient sentries were excluded from the analysis. Due to variability in the combination of parameters tested for each patient, four distinct groups were analyzed: 20,444 patients who underwent simultaneous testing for HBsAg, anti-HBc and anti-HBs were evaluated for isolated anti-HBc positivity; 98,947 patients with concurrent HBsAg and anti-HBs results were assessed for simultaneous HBsAg/anti-HBs positivity; 6,055 patients who had both HBeAg and anti-HBe results were examined for concurrent HBeAg/anti-HBe positivity; and 7,590 patients with HBsAg, anti-HBs, and anti-HBc results were analyzed for isolated HBsAg positivity. In patients with isolated anti-HBc positivity, HBV polymerase chain reaction (PCR), anti-HCV, HCV PCR, and anti-HIV and HIV confirmation results were examined. Serological tests of the patients were performed using the electrochemiluminescence immunoassay (ECLIA) using an Elecsys (Roche Diagnostics, Switzerland) immunoassay analyzer. HBV PCR and HCV PCR were performed using the real-time PCR on a COBAS 6800 system (Roche Diagnostics, Switzerland). The same diagnostic kits and instruments were used consistently throughout the study period, with routine calibration procedures carried out to ensure analytical raliability. Samples exhibiting atypical serological results were subjected to repeat testing for confirmation. Ethical approval fort he study was granted by the Ethics Committee of Bursa City Hospital under decision number 2023-3/2, dated February 01, 2023.

Statistical analysis

Statistical analysis was performed via SPSS version

23.0. Continuous variables are summarized as the means ± standard deviations (SDs) and medians (min-max), whereas categorical variables are presented as numbers and frequencies.

Results

Among the 285122 patients, 117335 (41.2%) were male, and 167787 (58.8%) were female. The mean age was 45.1 ±9.8 years (range, 0-109). Atypical HBV serological profiles were detected in 1404 (0.49%) patients. The most common atypical profile detected is isolated anti-HBc positivity (Table 1).

Table 1. Atypical serology profiles detected in hepatitis B patients

	Atypical profile/ Number of samples	%
Isolated anti-HBc positivity (HbsAg -, anti-HBc +, anti-HBs -)	1079/20444	5.27
Concurrent positivity of HBsAg and anti-HBs (HBsAg+, anti-HBs +)	277/98947	0.27
Concurrent positivity of HBeAg and anti-HBe (HBeAg +, anti-HBe +)	45/6055	0.74
Isolated HBsAg positivity (HBsAg +, anti-HBc -, anti-HBs -)	3 /7590	0.03

In 119 patients with isolated anti-HBc positivity, HBV PCR was performed, whereas HBV PCR was not performed in 960 patients. HBV DNA was detected in 12 of the patients and It was not detected in 107 patients. In patients with detectable HBV DNA, HBV DNA was found to be <10 IU/mL in 10 patients, 198 IU/mL in one patient, and 12 x 10^7 IU/mL in another patient. The patient, whose HBV DNA result was 12 x 10^7 IU/mL was positive for anti-HBc IgM. He had been HBsAg -positive and HBeAg -positive one month earlier. Anti-HCV was positive in 32 of the isolated anti-HBc -positive patients. When the HCV PCR results of these patients were examined, PCR was not performed at any time in 9 patients. HCV RNA was not detected in 10 patients, while in 13 patients, HCV RNA levels ranged from <15 to 106 IU/mL. Among the cases with isolated anti-HBc posivity, anti-HIV reactivity was identified in three patients, with confirmatory HIV-1 positivity observed in two of these cases. Descriptive data pertaining to patients with isolated anti-HBc positivity are presented in Table 2.

Table 2. Descriptive data of patients with isolated anti-HBc positivity

Isolated anti-HBc positivity, n:1079	
Sex, n(%)	
Female	478 (44.3)
Male	601 (55.7)
Age, mean ± Standard deviation (min-max)	64.66 ±12.18 (15.0-100.0)
Departments, n(%)	
Medical Oncology and Hematology	292 (27.06)
İntensive Care Unit	195 (18.07)
Orthopedics and Traumatology	181 (16.77)
İnternal Medicine	162 (15.01)
Infectious Diseases and Clinical Microbiology	105 (9.73)
Others	144 (13.36)
HBV PCR, n: 119, n(%)	
HBV DNA not detected	107 (89.91)
HBV DNA detected	12 (10.09)
Anti-HIV, n:995, n(%)	
Non reactive	992 (99.69)
Reactive	3 (0.31)
HIV confirmatory test positivity	2
Anti-HCV, n:1045, n(%)	
Non reactive	1013 (96.93)
Reactive	32 (3.07)
HCV PCR, n:23, n(%)	
HCV RNA not detected	10 (43.47)
HCV RNA detected	13 (56.53)

Analysis based on HBeAg and anti-HBe status revealed distinct differences in mean HBV DNA levels. The HBeAg-positive group exhibited the highest mean viral load at 6.88 $\log_{10}$ IU/mL ±2.28 (7,585,775 ±190 IU/mL), followed by the group with concurrent HBeAg and anti-HBe positivity, which had a mean viral load of 5.02 $\log_{10}$ IU/mL ±2.55 (104,712 ±354 IU/mL). The anti-HBe-positive group showed the lowest mean viral load at 2.92 $\log_{10}$ IU/mL ±1.29 (831 ±19 IU/mL). Patients were further stratified into five subgroups based on HBV DNA levels: undetectable, below the detection limit (<1 $\log_{10}$ IU/mL or <10 IU/mL), ≥1 and <5 $\log_{10}$ IU/mL (10–100,000 IU/mL), ≥5 and <7 $\log_{10}$ IU/mL (100,000–10,000,000 IU/mL), and ≥7 $\log_{10}$ IU/mL (≥10,000,000 IU/mL). As

illustrated in Figure 1, the proportion of patients with HBV DNA levels ≥7 log10 IU/mL in the concurrent positivity group was 15.6%, positioned between the HBeAg-positive group (42%) and the anti-HBe-positive group (1.4%). Similarly, for the ≥1 and <5 log10 IU/mL subgroups, the concurrent subgroup had an intermediate proportion (33.3%) compared with the HBeAg-positive (13.9%) and anti-HBe-positive (53.9%) groups. Notably, the percentage of patients with HBV-DNA levels below the detection limit (<1 log10 IU/mL) was greater in the concurrent group (20.5%) than in the HBeAg-positive (18.6%) or anti-HBe-positive (13%) groups.

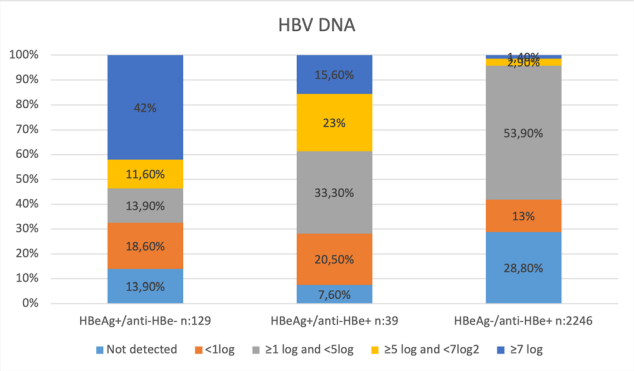

Figure 1. The distribution of patients with serum HBV-DNA levels was stratified into five subgroups (1 $\log_{10}$ IU/mL: 10 IU/mL, 5 $\log_{10}$ IU/mL: 100000 IU/mL, and 7 $\log_{10}$ IU/mL: 10000000 IU/mL).

Discussion

Hepatitis B remains a significant global and national public health concern. Despite the availability of effective vaccines and antiviral therapies, the virüs continues to cause acute and chronic liver failure, as well as hepatocellular carcinoma, resulting in considerable morbidity and mortality [7,8]. The complexity and variability of antigens and antibodies involved in serological testing can give rise to atypical serological profiles, which may complicate diagnosis and clinical management. This may lead to difficulties in interpreting the results. When the atypical HBV serological profile results are interpreted, patients should be assessed for immunosuppression, vaccination history, multi-strain infection, and mutation risk. If necessary, the tests should be repeated to rule out false-positive results, and additional analyses such as HBV PCR and mutation testing may be considered [9].

Anti-HBc is a serological marker indicative of exposure to HBV. Concurrent positivity for anti-HBc and anti-HBs typically reflects a resolved past infection whereas the presence of both HBsAg and anti-HBc suggeste either an acute or chronic infection. These findings warrant careful interpretation, particularly in immunosuppressed individuals or those coinfected with HIV or HCV, due to an elevated risk of HBV reactivation [6]. The isolated anti-HBc pattern is notably more common in individuals with HIV or HCV co-infection, likely reflecting shared transmission routes and complex viral interactions during replication. Among HIV-positive individuals, the prevalence of isolated anti-HBc positivity ranges from 17% to 40%, and this has been linked to impaired T-cell responses [10]. Higher prevalence is observed in individuals with multiple comorbid infections, a history of intravenous drug use, or alcohol abuse, as well as in older patients with low CD4 T-cell counts and detectable HIV RNA levels. In those with HCV infection, isolated anti-HBc positivity can reach up to 37%, a phenomenon attributed more to HCV-mediated inhibition of HBV replication than to immunsuppression [11]. It is also important to recognize that isolated anti-HBc positivity may result from false positivites, particularly in low-prevalence populations, with estimated false positive rates ranging from 10% to 50%, depending on overall prevalence. Therefore, the last generation of enzyme immunoassays should be used, and the test should be repeated with another biological assay. Other possible causes of isolated anti-HBc positivity are the window period during recovery from acute infection when HbsAg disappears, and anti-HBs do not appear, a loss of anti-HBs because of waning immunity or the effect of treatments such as immunosuppressive drugs, mutation in the ‘‘a’’ determinant of HBsAg and co-infection with HCV or HIV agents [6].

In regions with low HBV prevalence, such as Europe and the United States, the rate of isolated anti-HBc positivity has been reported to range from 1% to 4% [12]. Similarly, a study conducted in Korea reported a prevalence of 8.9% for isolated anti-HBc positivity in the general population [13]. In our country, previous studies have reported the prevalence of isolated anti-HBc positivity to range between 1% and 5%, aligning with the findings of our study [14-16]. Notably, one patient with isolated anti-HBc positivity exhibited

an HBV DNA level of 12 × 10^7 IU/mL. When considered alongside serial serological testing, the results were indicative of the window period of acute HBV infection. This finding highlights the critical need for supplementary serological and molecular testing—particularly HBV PCR—in cases presenting with an isolated anti-HBc profile. Thirteen of the patients were also coinfected with HCV, and two with HIV. In such instances, isolated anti-HBc positivity may be associated with HCV or HIV co-infection. When isolated anti-HBc positivity is identified, it is essential to retest using a new sample, preferably with an alternative assay system, to rule out false positivity. Additional testing should include anti-HBc IgM and HBV PCR to exclude the window period, as well as follow-up anti-HBc and anti-HBs testing after 1–3 months. Concurrent screening for HCV and HIV is also recommended to assess potential co-infection.

Concurrent positivity of HBsAg and anti-HBs represent one of the atypical profiles seen in hepatitis B infection. This pattern may transiently ocur during the seroconversion phase from HBsAg to anti-HBs. In cases of chronic HBV infection, it may also be observed if vaccination or administration of prophylactic immunoglobulin is carried out without prior knowledge of an individual's HBsAg status. Another important factor that requires careful investigation in cases is the presence of immune escape mutations in the S gene region of the viral genome. These mutations affect the structure of the "a" determinant, resulting in significant antigenic alterations. When infection is caused by a mutated HBV strain targeting this region—recognized by neutralizing antibodies—the anti-HBs antibodies may fail to neutralize the virüs, thereby allowing persistent viral replication [17]. Studies conducted in Türkiye have reported that the rate of concurrent HBsAg and anti-HBs positivity ranges from 0.2% to 3.6% [9,14]. In our study, this rate was 0.27%; however, mutation analysis could not be performed, and information regarding prior vaccination or prophylactic immunoglobulin administration was unavailable.

One atypical serological profile observed in hepatitis B infection is the concurrent positivity of HBeAg and anti-HBe. This pattern may appear transiently during the seroconversion phase of HBeAg to anti-HBe. With the advent of highly sensitive antigen and antibody detection methods, simultaneous detection of HBeAg and anti-HBe has occasionally been documented in clinical cases of chronic hepatitis B. Furthermore, this profile may arise in co-infections involving both wild-type and mutant (hepatitis B viruses, particularly those with precore or basal core promoter mutations) [18,19]. Aydin et al. [9] reported a 1.4% prevalence of concurrent HBeAg and anti-HBe positivity. In the present study, concurrent positivity of HBeAg and anti-HBe was detected at a rate of 0.74%. When the HBV viral load values of the concurrent patients were examined, the majority of them were at values suggesting that they were probably in the transition phase between HBeAg and anti-HBe seroconversion. However, this finding was difficult to interpret because mutation analysis was not performed on these patients.

Isolated HBsAg positivity, when accompanied by detectable HBV DNA, typically indicates an acute infection and is often confirmed by the subsequent appearance of additional serological markers. Persistent isolated HBsAg positivity may result from an inability to produce anti-HBc or from its production at undetectable levels, particularly in individuals with immune tolerance to HBcAg. This immune tolerance is notably observed in infants born to HBeAg positive carrier mothers through vertical transmission, characterized by an absence of an antibody response to HBcAg. Mutations within the core gene region, particularly core deletion mutations, may also lead to isolated HBsAg positivity, commonly observed in patients with longstanding HBV infection and low viral load. Additionally, transient weakly positive HBsAg reactions can ocur following Hepatitis B vaccination, typically lasting 5–18 days in adults and potentially longer in infants [20]. Isolated HBsAg positivity may also reflect false positivity or nonspecific reactivity. In such cases, retesting with a new sample and, if feasible, using a different analytical system is advisable. Further diagnostic clarification may involve additional testing, such as HBsAg neutralization assays and HBV PCR testing [20,21]. In our study, other examinations of three patients with isolated HBsAg positivity revealed negative results for anti-HBs, negative results for anti-HBc IgG, positive results for HBeAg, and HBV DNA <10 IU/mL. Detailed clinical information of the patients could not be obtained

and mutation analysis could not be performed.

In our retrospective study, interpretation challenges arose in certain cases with atypical serological profiles because of missing serological and PCR test results. Furthermore, these patients could not be evaluated for potential mutations as mutation analysis was not performed. Another limitation of our study was the lack of access to detailed clinical histories for these patients, which hindered optimal interpretation of the findings.

Conclusion: In conclusion, this study identified the presence and prevalence of atypical HBV serological profiles among patients presenting to Bursa City Hospital. These atypical profiles arise from various conditions, including false reactivity or stages of acute and chronic infection. For effective patient management, it is essential to carefully evaluate such cases through close laboratory-clinician collaboration, repeat testing with a new blood sample preferably using a different analytical system and monitör patients over time with serial sampling. When appropriate, advanced diagnostic approaches such as PCR and mutation analysis should also be considered.

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