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Simultaneous Comparison of Serological and Molecular Results of HBV Test in Patients Referring to the Pathobiology Laboratory

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ABSTRACT

Aims: Hepatitis B Virus (HBV) has infected more than million hundreds of people worldwide. Hence, a high rate of morbidity and mortality caused by liver-related diseases is due to HBV infection. However, a strong and effective treatment should be based on an accurate and correct diagnostic method. Hence, the present research provided a multidimensional study comparing and analyzing patients' molecular and serological tests results.

Materials & Methods: In this research, the HBV DNA molecular tests results were studied by examining patients' gender, age, and HBsAg strip results.

Findings: Among the female patients (29 persons) studied in this research, 55.1% were positive for HBV DNA and HBsAg strip tests, and 17.3% were negative for both tests. Also, among the male patients (44 persons), 65.9% were positive, and 6.8% were negative for both tests.

Conclusion: The present study results shed light on the correlation between the HBV DNA and HBsAg tests. Also, the significance of HBV DNA tests was highlighted for particular diagnostic purposes and for the differentiation and interpretation of the pathophysiological conditions of patients with hepatitis B.

Keywords: Hepatitis B virus, Diagnosis, RT-PCR, Serology, Tehran

CITATION LINKS

[1] Hepatitis B virus taxonomy and hepatitis B virus genotypes. [2] Hepatitis B virus biology. [3] Hepatitis B virus replication. [4] Estimations of worldwide prevalence of chronic Hepatitis B virus infection: A systematic ... [5] Recent advances in understanding and diagnosing... [6] Comparison of the sensitivity of NAT using pooled donor samples for HBV and that of a serologic...[7] Hepatitis B virus core-related antigens as markers for... [8] Laboratory evaluation of a fully automated chemiluminescence immunoassay for rapid detection of HBsAg, antibodies... [9] Diagnosis of Hepatitis...[10] Hepatitis B virus. Geneva.. [11] Hepatitis B virus infection. [12] Hepatitis B virus blood screening: Need for reappraisal of... [13] Identification of a mutation in Hepatitis B virus surface antigen....[14] Overview of Hepatitis B virus mutations and their implications in the management of infection. [15] Viral biomarkers in chronic HBeAg negative HBV infection. [16] HBV virological assessment. [17] Quantitative assay of PCR-amplified Hepatitis B virus DNA using a peroxidase-labelled...[18] Viral Hepatitis B: Clinical and epidemiological characteristics. Cold Spring Harb....[19] EASL international consensus conference on Hepatitis B. 13-14 September, 2002 Geneva... [20] Viral Hepatitis B. Lancet. 2003; 362(9401):2089-94. [21] What is the role of serology for the study of chronic Hepatitis B virus ... [22] Comparing HBV viral load in serum, cerumen, and saliva and aorrelation with HBeAg serum status in patients with chronic... [23] Evaluation of serum HBV viral load, transaminases and histological features in chronic HBeAg-negative Hepatitis B patients. [24] Frequency of Hepatitis B virus DNA in anti-HBc positive, HBsAg negative blood donors in Rasht, northern Iran. [25] Real time polymerase chain reaction for Hepatitis B screening in donor corneas in the central eye bank of Iran.

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Introduction

Hepatitis B Virus (HBV), a partially double-stranded DNA virus, is a member of the family *Hepadnaviridae*. The genome of HBV is a 3.2 kb-length DNA consisting of 4 predominant Open Reading Frames (ORFs) coding S (Pre surface/surface), C (Core), P (Polymerase), and X proteins [1-3]. HBV as one of the main causes of acute and chronic liver diseases has infected more than million hundreds of people worldwide. The mortality rate due to the HBV has been estimated to be over 800,000 patients per year. Regardless of the strategies used in the area of Hepatitis B vaccination, there is an increasing trend for the Hepatitis B virus carriers. But recent epidemiology data have shed light on the fact that in spite of growing world population, the prevalence of the Hepatitis B surface antigen (HBsAg) has reduced [4-5] .

One of the symptoms of the disease progression and conversion into chronic hepatitis is the persistence and presence of HBsAg for more than 6 months [6]. However, in the convalescent phase after recovering infection, heightened levels of HBsAg decrease and anti-HBs antibodies increase [7] .

From clinical point of view, the distinction between the hepatitis caused by Hepatitis B virus and the diseases caused by other viral agents could not be easily achieved unless using laboratory diagnostic methods to determine the acute and chronic infections [8]. The diagnosis of Hepatitis B virus infection is currently based on the detection of serological markers such as HBsAg (Hepatitis B surface Antigen), anti-HBs antibodies, HBcAg (Hepatitis B core Antigen), anti-HBc antibodies (total or IgM), HBeAg (Hepatitis B envelope Antigen), anti- HBe antibodies, and the detection and quantification of HBV DNA in patients' specimens [5, 9] .

Acute HBV infection is characterized by the presence of HBsAg, HBcAg, and immunoglobulin M (IgM) antibody. During the initial phase of infection, patients are also seropositive for Hepatitis B envelope antigen (HBeAg). HBeAg is usually a marker of high levels of virus replication. The presence of HBeAg indicates that the blood and body fluids of infected individual are highly infectious. Chronic infection is characterized by the persistence of HBsAg for at least 6 months (with the presence or absence of HBeAg). Also, the HBV DNA and ALT (Alanine Amino Transferase) levels help in obtaining correct results for patients [5, 10-11].

Due to the partially double-stranded DNA replication system in Hepatitis B virus and the absence of proofreading feature in HBV polymerase enzyme and the presence of transient pre-genomic RNA in HBV replication cycle, mutations in some of the encoding genes of this virus seems to be possible [12-13]. Hence, such viral genes may ultimately code for variant structural proteins. Therefore, in some cases, serological tests based on the detection of these proteins could be falsely negative [14-15]. Thus, due to the introduction of mutations occurring at the viral antigens level [14], HBV DNA molecular diagnosis could be considered as a precise diagnostic method and more effective than serological markers detection not only for infection detection at the early stages but also for monitoring the responses to the treatment methods [16].

Regarding the current events in the diagnosis of Hepatitis B, the accuracy and precision of the selected methods in differentiating between the acute and chronic hepatitis are very important for referring patients [17].

Objectives: For this reason, the present study aimed to provide a better view of

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this truth by comparing the results of the serological and molecular tests.

Material and Methods

Patients: After conducting HBV DNA and serological markers detection tests for 73 patients during 6 months, the results were compared and evaluated.

Sample collection: Of each patient, 5mL of peripheral blood was collected in EDTA containing CBC tube. In the next step, the serum was separated of each sample, and stored at 4˚C.

Serological analysis: HBsAg as a serological marker was determined by Hepatitis B surface antigen test strip (Cat. No. IHBsg-301, Abon, US).

Extraction of HBV DNA: Viral DNA genome was extracted from serum samples using High Pure Viral Nucleic Acid Kit (Roche; Mannheim, Germany). Extraction was performed in accordance with the guidelines and instructions provided by the manufacturer. The quality of extraction was measured by adding HBV detection kit Internal Control (IC) reagent to the serum samples before the extraction steps.

Detection of serum HBV DNA: Qualitative molecular detection assay was performed by Taqman based Real-Time PCR using HBV detection kit (HBV RG Kit, Novin Gene, Iran) applied on the (48well) Step-One Real-Time device (AB Applied Biosystem, Göteborg Sweden). The considered specificity for HBV DNA detection was 150 IU/mL with the probability of 95% according to the Novin Gene kit guidance sheet.

Findings

Serum samples were seperated from blood samples of 73 patients, of whom 44 (60.3%) cases were man, and 29 (39.7%) cases were woman patients with the average age of 49 and 42 years for women and men, respectively. All of the HBV DNA positive specimens were higher than default threshold (0.2) on the amplification curve presented in the Step One® software.

Of 29 samples collected from women, 17 samples (58.6%) were HBV DNA positive, and 12 samples (41.4%) were HBV DNA negative. Also, of 17 HBV DNA positive women samples, 16 (16 of 29, 55.1%) cases were HBsAg positive, and one (1 of 29, 3.4%) cases was HBsAg negative. Of 12 HBV DNA negative women samples, 7 (7 of 29, 24.2%) cases were HBsAg positive, and 5 (5 of 29, 17.3%) cases were HBsAg negative (Table 1).

Of 44 samples collected from men, 31(70.4%) samples were HBV DNA positive, and 13(29.6%) samples were HBV DNA negative. Of 31 HBV DNA positive men samples, 29(29 of 44, 65.9%) cases were HBsAg positive, and 2(4.5%) cases were HBsAg negative. Also, out of 13 HBV DNA negative men samples, 10 (10 of 44, 22.8%) cases were HBsAg positive, and 3 (3 of 44, 6.8%) cases were HBsAg negative (Table1).

A correlation coefficient test conducted for the whole studied samples (n=73) in Excel (Microsoft office 2013) showed a significant relationship between the HBV DNA and

Table 1) Evaluated parameters in 73 serum samples.

Gender	**Female**				**Male**			
No. of Sera	**29 (39.8%)**				**44 (60.2%)**			
HBV DNA	>150 IU/mL		<150 IU/mL		>150 IU/mL		<150 IU/mL	
No.	17		12		31		13	
HBsAg Strip*	+	-	+	-	+	-	+	-
No.	16	1	7	5	29	2	10	3

* Cut-off > 0.5 ng/mL

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HBsAg tests (r = .9526, p-value = .0474).

Discussion

The aim of this study was to estimate a meaningful correlation between the molecular and serological markers detection tests for HBV diagnosis. It is evidenced that HBV is a major global burden of liver disease caused by chronic Hepatitis B. It is over 50 years since HBV outbreak; however, the diseases caused by this virus (hepatitis, liver failure, and hepatocellular carcinoma (HCC)) have remained as major public health challenges [18]. The range of clinical symptoms of acute hepatitis infection is very wide and contagious, while the clinical symptoms of chronic hepatitis infection range from inactive HBsAg carriers to chronic hepatitis leading to cirrhosis and hepatocellular carcinoma [19-20]. Quantitation of HBV DNA in patients with chronic HBV hepatitis is the most important marker for virus replication [21].

The present study results highlighted the effectiveness of HBV DNA test as studied elsewhere. In a study by Parizad et al. (2016) conducted on 50 patients, it was shown that saliva and cerumen samples were also suitable for hepatitis molecular testing; in addition, the blood of all patients was HBsAg positive [22]. Esmaeelzadeh et al. (2017) studied 125 HBV DNA positive serum samples for viral loads; a group of these patients was identifies as anti-HBe negative with a series of multi-faceted observations [23]. In a study conducted on 2041 persons, it was reported that 3.6% of the blood donors were negative for HBsAg test and positive for anti-HBc; also, positive results were reported for HBV DNA test on serum samples [24]. In another study in 2018, out of 122 patients with anti-HBc negative results, 11 patients were positive for HBV DNA molecular test [25]. Also, the present study showed that among the women samples, 55.1% were positive for both HBV DNA and HBsAg tests, and 17.3% were negative for both tests. Also, out of 44 samples collected from men, 65.9% showed positive, and 6.8% showed negative results for both aforementioned detection methods. Although the statistical analysis showed a significant correlation between the HBV DNA and HBsAg tests results, there are considerable exceptions depending on the pathophysiological conditions of the sampled patients. Furthermore, controversies between the results of HBV DNA and HBsAg tests show the complementary nature of the molecular and serological tests which should be interpreted simultaneously to decipher the pathophysiological conditions of patients with Hepatitis B infections. Such a complementary function of those tests could be explained in clinical features like occult Hepatitis B virus (HBV) infection (OBI) in which HBsAg and HBV DNA are both negative, but anti-HBc could be positive. In another OBI cases, HBsAg is negative, but HBV DNA and anti-HBc are positive [21]. However, such findings highlight the necessity of HBV DNA test at least in defined conditions, for example, for blood donors.

Conclusion

This research was an effective investigation in determining the accuracy and adequacy of HBV DNA testing for the diagnosis of Hepatitis B infection. The present study results are in line with those of the previous studies; therefore, the HBV DNA testing seems to be necessary for the differentiation and interpretation of the pathophysiological conditions of patients.

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was stated by the authors.

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Authors' Contribution: RSB & MP conceived and designed the study, MT & MRP extracted and analyzed the data. RSB & MP wrote the manuscript. RSB & MRP revised the paper. RSB, MT, MP and MRP had full access to all of the data in the study.

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References

1. Schaefer S. Hepatitis B virus taxonomy and hepatitis B virus genotypes. World J Gastroenterol. 2007; 13(1):14-21.
2. Seeger C, Mason WS. Hepatitis B virus biology. Microbiol Mol Biol Rev. 2000; 64(1):51-68.
3. Nassal M, Schaller H. Hepatitis B virus replication. Trends Microbiol. 1993; 1(6):221-8.
4. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic Hepatitis B virus infection: A systematic review of data published between 1965 and 2013. Lancet. 2015; 386(10003):1546-55.
5. Fourati S, Pawlotsky J-M. Recent advances in understanding and diagnosing Hepatitis B virus infection. F1000 Res. 2016;5.
6. Sato S, Ohhashi W, Ihara H, Sakaya S, Kato T, Ikeda H. Comparison of the sensitivity of NAT using pooled donor samples for HBV and that of a serologic HBsAg assay. Transfusion. 2001; 41(9):1107-13.
7. Wong DK-H, Tanaka Y, Lai C-L, Mizokami M, Fung J, Yuen M-F. Hepatitis B virus core-related antigens as markers for monitoring chronic Hepatitis B infection. J Clin Microbiol. 2007; 45(12):3942-7.
8. Ismail N, Fish GE, Smith MB. Laboratory evaluation of a fully automated chemiluminescence immunoassay for rapid detection of HBsAg, antibodies to HBsAg, and antibodies to Hepatitis C virus. J Clin Microbiol. 2004; 42(2):610-7.
9. Song JE, Kim DY. Diagnosis of Hepatitis B. Ann Transl Med. 2016;4(18):338.
10. WHO. Hepatitis B virus. Geneva: WHO; 2018.
11. Trépo C, Chan HLY, Lok A. Hepatitis B virus infection. Lancet. 2014; 384(9959):2053-63.
12. Candotti D, Laperche S. Hepatitis B virus blood screening: Need for reappraisal of blood safety measures? Front Med. 2018; 5:29.
13. Cui Y, Zhang T, Yan Y, Liu K. Identification of a mutation in Hepatitis B virus surface antigen capable of evading ELISA screening. Genet Mol Res. 2016; 15(3).
14. Caligiuri P, Cerruti R, Icardi G, Bruzzone B. Overview of Hepatitis B virus mutations and their implications in the management of infection. World J Gastroenterol. 2016; 22(1):145-54.
15. Hadziyannis E, Laras A. Viral biomarkers in chronic HBeAg negative HBV infection. Genes. 2018; 9(10):469.
16. Hatzakis A, Magiorkinis E, Haida C. HBV virological assessment. J Hepatol.2006;44(1 Suppl):S71-6.
17. Erhardt A, Schaefer S, Athanassiou N, Kann M, Gerlich WH. Quantitative assay of PCR-amplified Hepatitis B virus DNA using a peroxidase-labelled DNA probe and enhanced chemiluminescence. J Clin Microbiol. 1996; 34(8):1885-91.
18. Burns GS, Thompson AJ. Viral Hepatitis B: Clinical and epidemiological characteristics. Cold Spring Harb Perspect Medi. 2014; 4(12):a024935.
19. de Franchis R, Hadengue A, Lau G, Lavanchy D, Lok A, McIntyre N, et al. EASL international consensus conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). J Hepatol. 2003;39 (Suppl 1):S3-25.

[DOR: 20.1001.1.25884107.2019.5.2.2.5]

20. Lai CL, Ratziu V, Yuen MF, Poynard T. Viral Hepatitis B. Lancet. 2003; 362(9401):2089-94.
21. Galli C, Orlandini E, Penzo L, Badiale R, Caltran G, Valverde S, et al. What is the role of serology for the study of chronic Hepatitis B virus infection in the age of molecular biology? J Med Virol. 2008; 80(6):974-9.
22. Parizad EG, Parizad EG, Khosravi A, Amraei M, Valizadeh A, Davoudian A. Comparing HBV viral load in serum, cerumen, and saliva and aorrelation with HBeAg serum status in patients with chronic Hepatitis B infection. Hepatitis monthly. 2016; 16(5):e30385.
23. Esmaeelzadeh A, Saadatnia H, Memar B, Mokhtari Amirmajdi E, Ganji A, Goshayeshi L, et al. Evaluation of serum HBV viral load, transaminases and histological features in chronic HBeAg-negative Hepatitis B patients. Gastroenterol Hepatol Bed Bench. 2017; 10(1):39-43.
24. Khamesipour A, Amiri ZM, Kafiabad SA, Saadat F, Mansour-ghanaei F, Esteghamati AR, et al. Frequency of Hepatitis B virus DNA in anti-HBc positive, HBsAg negative blood donors in Rasht, northern Iran. Transfus

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