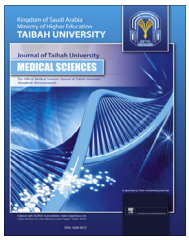
Journal of Taibah University Medical Sciences (2014) 9(2), 166–170

Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com



Student Section

Molecular detection of hepatitis B virus (HBV) among voluntary ELISA positive blood donors in Almadinah Almunawwarah

Mohiadeen Kurdi *, Muath Abughararah, Mohammed Mulike, Omar Yamani, Mohammed Bugdady and Mamdooh Noor

Students, Faculty of Applied Medical Sciences, Taibah University, Almadinah Almunawwarah, Kingdom of Saudi Arabia

Received 8 January 2014; revised 21 January 2014; accepted 22 January 2014

الملخص

أهداف البحث: يعتبر فيروس التهاب الكبد البائي من الفيروسات الأكثر انتقالا عن طريق التبرع بالدم والأعضاء، مما ينتج عنه كثيراً من الاعتلالات المرضية والوفيات. فكان من الضروري إجراء اختبارات لكل المتبرعين بالدم والأعضاء للتأكد من خلوهم من الفيروس. والهدف من هذه الدراسة هو توضيح الصورة السيرولوجية لحاملي هذا الفيروس من المتبرعين بالدم.

طرق البحث: أجريت هذه الدراسة على 17131 عينة أخذت من رجال ونساء (متبرعين للدم تطوعياً ومرضى مشكوك بإصابتهم بالمرض، تم تحويلهم لبنك الدم المركزي في المدينة المنورة – المملكة العربية السعودية). تم الكشف عن الفيروس باستخدام تقنيتين مختلفتين: تقنية تفاعل البوليميريز المتسلسل، وتقنية مقايسة الممتز المناعي المرتبط بالإنزيم. أجريت اختبارات تقنية تفاعل البوليميريز المتسلسل للكشف عن الحمض النووي للفيروس، واستخدمت ثلاثة اختبارات سيرولوجية مختلفة لاختبار مقايسة الممتز المناعي المرتبط بالإنزيم وهي: المستضد السطحي لإلتهاب الكبد البائي، والأجسام المضادة اللبية لإلتهاب الكبد البائي، والأجسام المضادة السطحية لإلتهاب الكبد البائي.

النتائج: كانت نسبة ظهور المستضد السطحي لإلتهاب الكبد البائي هي (9.02%)، ونسبة الأجسام المضادة اللبية لإلتهاب الكبد البائي هي (9.02%)، ونسبة الأجسام المضادة السطحية لإلتهاب الكبد البائي هي (7.93%) وكانت نسبة وجود الحمض النووي لفيروس إلتهاب الكبد البائي (9.29%) هي الأعلى.

الاستنتاجات: عند مقارنة النتائج الإيجابية لتقنية تفاعل البوليميريز المتسلسل مع تقنية مقايسة الممتز المناعي المرتبط بالإنزيم، وجد أن تقنية تفاعل البوليميريز المتسلسل هي أكثر دقة وحساسية لكشف الفيروس، كما أن الاختبارات الإيجابية لتقنية مقايسة الممتز المناعي المرتبط بالإنزيم ليست جميعها دقيقة.

الكلمات المفتاحية: فيروس التهاب الكبد البائي; جزيئي; كشف; المدينة المنورة; متبرعي الدم

Abstract

Objective: Hepatitis B virus is considered as one of the most common viruses spreading through blood transfusion and organ transplants. This usually results in more considerable cases of disease and mortalities; so it is necessary to perform tests for viral infection in all blood donors.

The present study aimed at highlighting the serological picture of blood donors and HBV suspected patients reported to the hospitals in Al-Madina Al-Mounawara; using standard PCR technique.

* Corresponding address: Final Year Medical Laboratory Technology Student, Faculty of Applied Medical Sciences, Taibah University, Almadinah Almunawwarah, Kingdom of Saudi Arabia. Tel.: +966 560050608.

E-mail: mmkurdi@hotmail.com, mmkurdi2@gmail.com (M. Kurdi)

Peer review under responsibility of Taibah University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jtumed.2014.01.002

Methods: The present study is conducted on 17,131 samples (blood donors and suspected patients reported to the Central Blood Bank the achievement of conformation tests in Al-Madina Al-Mounawara Saudi Arabia). Virus was detected by two techniques: PCR and ELISA. COBAS TagMan V2.0 was used as PCR test to measure HBV-DNA in human plasma or serum. Three different ELISA tests had been used to detect HBV antigen and antibody: HBsAg, HBcAb and HBsAb.

Results: The overall prevalence of hepatitis B surface antigen (HBsAg) was (9.02%), hepatitis B core antibody (HBcAb) was (9.02%), hepatitis B surface antibody (HBsAb) was approximately (7.93%) and hepatitis B DNA (HBVDNA) was the highest one (9.29%).

Conclusion: Comparison of PCR positive results with those of the ELISA positive indicates that the PCR technique is more sensitive and reliable than the ELISA technique, as not all ELISA positive cases confirmed HBV infection.

Keywords: Almadinah Almunawwarah; Blood donor's; Detection; HBV; Molecular

Introduction

Hepatitis B virus (HBV) infects the liver of humans causing an inflammation called hepatitis. The disease was originally known as "serum hepatitis".[1]

Complications such as cirrhosis and hepatocellular carcinoma (HCC) usually occur. It was estimated that approximately 2 billion people have serological evidence of past or present HBV infection. More than 350 million are chronic carriers of HBV.[2]

Approximately 75% of chronic carriers live in Asia and the Western Pacific. It was reported that 15–40% of HBV infected patients would develop cirrhosis, liver failure, or HCC, and 500,000 to 1.2 million people die of HBV infection annually. Because of the high HBV-related morbidity and mortality, the global disease burden of HB is substantial.[3–5]

The average prevalence rate of HBsAg among adults in Saudi population is approximately 8% and 60% of them having evidence of past exposure to (HBV). However, several surveys of voluntary blood donors have shown marked regional variations in the prevalence of (HBV). Arya et al. (1985) reported Jizan region of KSA as an area of hyper-endemic (HBV) infection, with a rate of HBsAg to be 32.2% among 237 blood donors, compared to a rate of 4.7% in a similar population in Riyadh in the Central region of KSA.[6–8]

The viral etiology of hepatitis B was firmly established by electron microscopy and the detection of several viral particles (referred to as Dane particles) that reacted with antisera to Australia antigen. It is a partially double-stranded DNA virus and one of many unrelated viruses that cause viral hepatitis. It was demonstrated that the Dane particle was HBV, and its surface component was designated hepatitis B surface antigen (HBsAg). The core component contained endogenous DNA and hepatitis core antigen (HBcAg). The differential presence of HBsAg, antibodies to HBsAg (anti-HBs), and antibodies to HBcAg (anti-HBc) was used to classify patients as having acute or chronic infections.[9–11]

Hepatitis B viruses cause an acute illness with patients having symptoms of acute jaundice and very high serum ALT (a part of liver function tests). In majority (90%) of adults, acute hepatitis B resolves within 2–3 months with full recovery. The scenario is different in newborn up to 5 years of age where full recovery is seen only in 10% while 90% develop chronic liver disease. In acute hepatitis C full recovery occurs in only 20–30% of cases while 70–80% require treatment for clearance of disease as they may go onto develop chronic liver disease.[12,13]

Majority of hepatitis B cases that we see are in children and neonates who have been exposed to this virus in early life and have carried the virus due to reasons mentioned above.[14]

If a person has two positive reports of HBsAg 6 months apart, then there is almost a 100% chance that this individual will carry the virus throughout his/her life and will always be found positive on tests whenever tested. Natural seroconversion over years occurs in 5–20% cases in each country depending upon the genes and genotypes of HBV.[15]

Once exposed to hepatitis B virus, antibodies develop naturally in over 90% adults within 6 months of exposure. Vaccination also produces antibodies in over 90%. Antibodies (Anti HBs) once produced either following natural recovery or following vaccination persist throughout their life, though their levels may go down with time. Any exposure to the virus any time after production of antibodies will automatically enhance antibody production during that period to protect the individual.[16]

Materials and Methods

The aim of this study is to diagnose the serological hepatitis B virus picture in ELISA positive voluntary blood donors using PCR techniques, and compare the results with those reported on Jzan (1985).[7]

Seventeen thousands and one hundred thirty-one patients suspected of HBV infected and volunteer blood donors were identified from King Fahd Hospital in Al-Madina Al-Mounawara and Blood Bank during 2012. A blood sample is drawn by a needle from a vein in the arm. Blood is usually collected in EDTA, PPT, ACD, CPD or SST tubes but not heparin. Then, sample was centrifuged (within 1 h of collection to separate plasma or serum from the cells) and the plasma or serum was transferred to a screw-cap cryo tube.

There are several ways to detect (HBV). The most common techniques are PCR and ELISA. The COBAS® Ampliprep/COBAS® TagMan® HBV Test, V2.0 is a nucleic acid amplification test for the quantitation of hepatitis B virus (HBV) DNA in human plasma and serum. Test is based on two major processes: (1) specimen preparation to isolate HBV DNA and (2) simultaneous PCR amplification of target DNA and deception of cleaved dual-labeled oligonucleotide detection probe specific to the target. The COBAS® Ampliprep/COBAS® TagMan® HBV Test, V2.0 utilizes automated specimen preparation on the COBAS instrument by a Generic silica-based capture technique. The procedure processes 500 μL of plasma

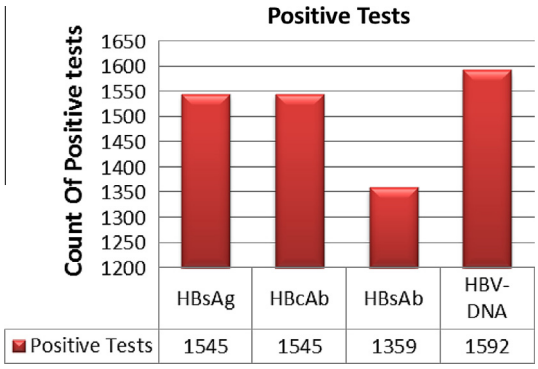


Figure 1: Comparison of positive results between different tests of ELISA and PCR.

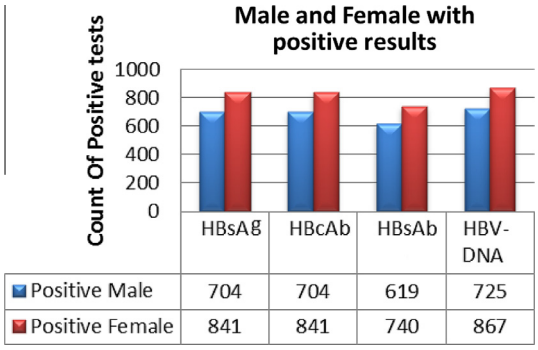


Figure 2: The relation between gender of patients and occurrence of HBsAg, HBcAb, HbsAb or HBV-DNA due to an infection by HBV.

or serum. The HBV virus particles are lysed by incubation at elevated temperature with a protease and chaotropic lysis/binding buffer that release nucleic acids and protects the released HBV DNA from DNases in plasma and serum. Protease and a known number of HBV QS DNA molecules are introduced into each specimen along with the lysis reagent and magnetic glass particle. Subsequently, the mixture is incubated and the HBV DNA and HBV QS DNA are bound to the surface of the glass particles. Unbound substances, such as salts, proteins and other cellular impurities, are removed by washing the magnetic glass particles. After separation the beads and completion the washing steps, the adsorbed nucleic acids are eluted at elevated temperature with an aqueous solution. The processed specimen, containing the magnetic glass particles as well as released HBV DNA and HBV QS DNA, is added to the amplification mixture and transferred to the COBAS® TagMan® Analyzer.[17]

For ELISA no special preparation of the patient is required prior to blood collection and either serum or plasma specimens can be used with this test kit. We used three different Elisa tests. HBsAg ELISA Kit is a fast test for the qualitative detection of the presence of HBsAg in serum or plasma. HBsAb ELISA Kit micro titer wells of the plate are covered with the HBsAg antigen (solid phase). Specimens of serum or plasma containing HBsAb antibodies are added to the wells together with an HBsAg conjugated with peroxidase. After incubation, it will form an antigen–antibody–antigen complex represented by the HBsAg conjugated to peroxidase, by the HBsAb antibody from the specimen, and by the HBsAg antigen bound to the micro titer well. HBcAb ELISA Kit micro titer wells are covered with recombinant antigens of the core of hepatitis B virus (solid phase). Serum or plasma specimens containing Anti-HBcAg antibodies (HBcAb) and anti-HBcAg (HBcAb) – peroxidase conjugate compete to bind to the limited number of solid phase sites (Table 1).[18–21]

Results

In total, 17,131 patients enrolled and among them males were 45.55% and females were 55.45% with different ages and nationality. Most of the patients are suspected to have HBV with different symptoms. Tests were processed upon three different antigen–antibody reactions (HBsAg, HBcAb and HBsAb) and virus DNA. The data obtained in this study

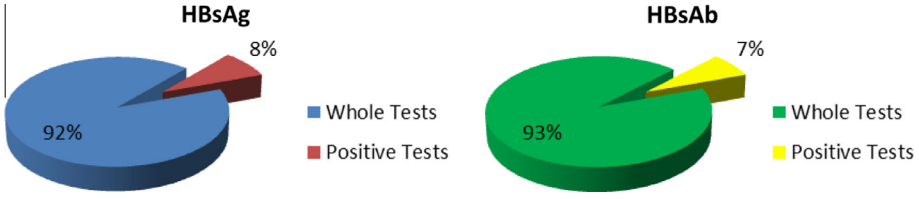


Figure 3: Comparison of detection of HBsAg and HBsAb using ELISA and positive result percentage.

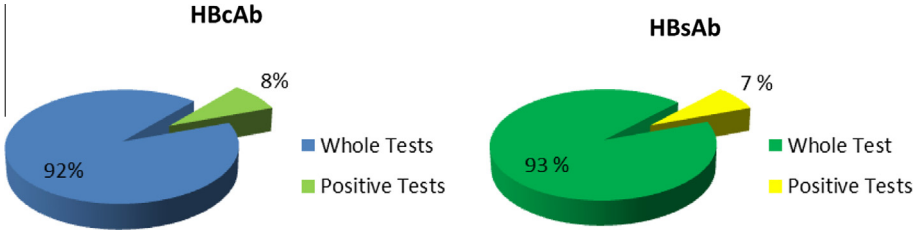


Figure 4: Comparison of detection of HBsAg and HBcAb using ELISA and positive result percentage.

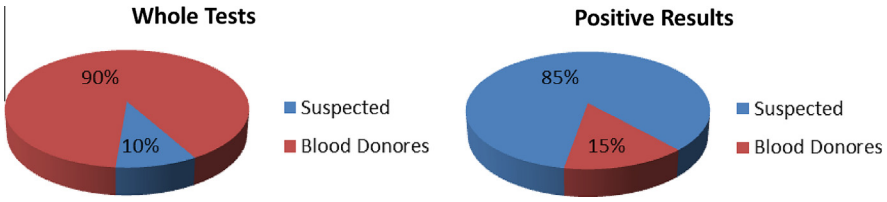


Figure 5: Comparison of positive result percentage between suspected patients and volunteer blood donors.

Table 1: Different types of antigen, antibody and DNA of HBV used to diagnose HBV among ELISA and PCR with different own leads for each one.

HBsAg	Surface protein contained within the lipoprotein coat
HBsAb	Antibody to HBsAg, indicator of recovery/immunity to HBV infection
HBeAg	Viral product secreted in blood, marker of infectivity, active replication (though absent in precore mutants)
HBeAb	Antibody to HBeAg, denoting decreased infectivity
HBcAg	Core antigen (viral capsid), intracellular and not detected in serum
HBcAb IgM	Antibody denoting recent HBV infection or exacerbation
HBcAb IgG	Contamination marker, positive after HBV contact
HBV DNA	Quantitative indicator of virus in blood

Table 2: Summarization of the results of the whole study.

Tests	HBsAg	HBcAb	HBsAb	HBV-DNA
Positive tests	1545 (9.02%)	1545 (9.02%)	1359 (7.93%)	1592 (9.29%)
Negative tests	15,586	15,586	15,772	15,539
Rate	10:1	10:1	12:1	9:1
Positive male	704	704	619	725
Positive female	841	841	740	867
Negative male	7099	7099	7184	7078
Negative female	8487	8487	8588	8461
Total tests	17,131	17,131	17,131	17,131

Table 3: The results of the whole study of suspected patients and blood donors by HBVDNA.

HBVDNA	Whole tests count	Positive tests	Percentage %
Suspected	1653	1359	82.2
Blood donors	15,478	233	1.51

clearly confirm the existence of HBV in 9.29% of cases by molecular detection (DNA), while 9.02% cases were positive to HBV on ELISA detection (Table 2, Figures 1–4) .

Samples were collected from 1653 suspected patients, and 15,478 blood donors. All samples were tested for HBV-DNA and presented positive for suspected patient up to 82.2%, while only 1.51% for blood donors (Table 3, Figure 5).

Discussion

This study reflect the prevalence of hepatitis B using the ELISA and PCR techniques for the determination HBV values, and indicate the presence of viral infection between male and female Saudi blood donors. Meanwhile, compare the efficiency of PCR technique as an alternative to ELISA technique.

Our study confirmed that the spread of HBV infection in Al-Madina Al-Mounawara is less than that reported in Jizan 1985[8]; That was confirmed when we conducted 17,131 sample collected from male and female population (blood donors and suspected patient reported to the central blood bank (the achievement of conformation tests)) in Al-Madina Al-Mounawara Saudi Arabia. The overall prevalence of hepatitis B surface antigen (HBsAg) was (9.02%), hepatitis B core antibody (HBcAb) was (9.02%), hepatitis B surface antibody (HBsAb) was approximately (7.93%) and hepatitis B DNA (HBVDNA) was the highest one (9.29%). The comparison of the PCR positive results with those of the ELISA positive, indicates that the PCR technique is more sensitive and reliable than the ELISA technique (Table 3).

The hepatitis B surface antigen (HBsAg) is most frequently used to screen for the presence of this infection. It is the first detectable viral antigen to appear during infection. However, early in an infection, this antigen may not be present and it may be undetectable later in the infection as it is being cleared by the host.[22] The obtained results confirm the great importance of the PCR technique in accuracy and reliability of detection and diagnosis of hepatitis viral infection during the cancelation of the antigen "window period" of hepatitis B infection.

Recommendations

According to our obtained result, we can strongly recommend the use of PCR as the reliable most accurate test for detecting (HBV) rather than used as confirmatory test to promote the health of the community.

Conflict of interest

We have no conflict of interest to declare.

Acknowledgments

Many thanks go to our supervisor in this research Prof. Khalil Al-Ali – Professor of virology in Taibah University, Mr. Waeal Alnozha – the Supervisor of Molecular Biology Lab. on Blood Bank in Al-Madena Al-Monawara – Prof. Atef Diab, Professor of Molecular Microbiology in Taibah University and Prof. Mohammad Abdel Razik, Professor of Medical (Mycology and Microbiology) in Taibah University for building our interest about this study and gave us all help to make it successful.

References

1. Ascherio A, Zhang SM, Hernan MA, et al. Hepatitis B vaccination and the risk of multiple sclerosis. **N Engl J Med 2001**; 344: 327–332.
2. Yuen MF, Kato T, Mizokami M, Chan AO, Yuen JC, Yuan HJ, et al. Clinical outcome and virologic profiles of severe hepatitis B exacerbation due to YMDD mutations. **J Hepatol 2003**; 39: 850–855.
3. Gust ID. Epidemiology of hepatitis B infection in the Western Pacific and South East Asia. **Gut. 1996**; 38(Suppl 2): S18–S23.
4. Lok AS. Chronic hepatitis B. **N Engl J Med 2002**; 346: 1682–1683; Li JWTX, Zhong WW, Xin LZ, Jin, Fu HC. Mechanisms of inactivation of hepatitis A virus in water by chlorine dioxide. **Water Res 2004**; 38: 1514–1519.
5. Mahoney FJ. Update on diagnosis, management, and prevention of hepatitis B virus infection. **Clin Microbiol Rev 1999**; 12: 351–366.
6. Parande CM, Arya SC, Ashraf SJ. Hepatitis B virus among Saudi children in Jizan, Saudi Arabia. **Infection 1986**; 14: 233–235.
7. Arya SC, Ashraf SJ, Parande CM, et al. Hepatitis B virus in Jizan, Saudi Arabia. **J Med Virol 1985**; 17: 267–274.
8. Al-Faleh FZ. Changing pattern of Hepatitis viral infection in Saudi Arabia in the last two decades. **Ann Saudi Med 2003**; 23: 364–371.
9. MacCallum FO, Bauer DJ. Homologous serum hepatitis. **Lancet 1947**; ii: 691–692.
10. Dane DS, Cameron CH, Briggs. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. **Lancet 1970**; i: 695–698.
11. Magnius LO, Espmark JA. New specificities in Australia antigen-positive sera distinct from the Le Bouvier determinants. **J Immunol 1972**; 109: 1017–1021.
12. Villeneuve JP. The natural history of chronic hepatitis B virus infection. **J ClinVirol 2005**; 34(Suppl 1): S139–S142.
13. Central of disease control and prevention. **The ABCs of Hepatitis 2012**; 21-1076.
14. Hepatitis B guidelines for pregnant women. **Hepatitis B Foundation 2012**; 215-489-4900.
15. Yuen MF, Wong DK, Zheng BJ, Chan CC, Yuen JC, Wong BC, Lai CL. "Difference in T helper responses during hepatitis flares in hepatitis B (e) antigen (HBeAg)-positive patients with genotypes B and C" implication for early HBeAg seroconversion. **J Viral Hepat 2007**; 14: 269–275.
16. H.B.V. Infected Health Care Workers July 12. Recommendations for preventing transmission of human immunodeficiency virus and hepatitis b virus to patients during exposure-prone invasive procedures. **MMWR 1991**; 40(RR08): 1–9.
17. The COBAS® Ampliprep/COBAS® TagMan®. **HBV Test V2.0 Manual book**, Roche Molecular Systems, Inc.; **2011**. Doc Rev. 7.0.
18. National Center for Biotechnology Information; March **2006**. At: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1079522.
19. Bi-x-act of Finland, HBsAg ELISA 96 & 480 well blat kit (TMB). At: http://www.ekoweb.fi/images/pdf/HbsAg_ELISA_96_well_plate.pdf.
20. HBsAb ELISA Kit creative-diagnostics. At: http://www.creative-diagnostics.com/description_4020_148.htm.
21. Anti-HBcAg ELISA Kit creative-diagnostics. At: http://www.creative-diagnostics.com/description_4003_148.htm.
22. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. **N Engl J Med 2004**; 350: 1118–1129.