Areej Atiyah Hussein et al /J. Pharm. Sci. & Res. Vol. 10(12), 2018, 3157-3159

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Evaluation of ELISA and HBsAg Rapid Test Cassette Assay in Detection of Hepatitis B Virus

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Abstract

Background: Screening blood is widely used and necessary to prevent several blood-borne viral infections such as hepatitis viruses, immunodeficiency virus, human parvovirus B19 and others. **Objectives:** To compare between enzyme linked immunosorabant assay (ELISA) test and hepatitis B surface antigen rapid test cassette assay in detecting hepatitis B virus among blood donors.

Methods: A cross-sectional study was conducted on 150 blood donors, attending Blood Bank in Baqubah city/ Iraq from October 2016 till March 2017, who were seropositive for hepatitis B virus. Participants were 123 males and 27 females. Their age ranged from 20-60 years. All subjects were tested for the presence of hepatitis B surface antigen (HBsAg) by use of two different diagnostic methods; ELISA and rapid test cassette assay. A questionnaire was used to collect demographic and personal data of each positive subject.

Result: The positivity of hepatitis B surface antigen was 1.44% and 1.35% by the two methods, respectively. Sensitivity and specificity were 100% for ELISA test compared to rapid test cassette assay. High percentage of positive cases was recorded in age group 20-35 years, among patients live in urban area, married and those having secondary school educational level.

Conclusion: ELISA is a highly sensitive and specific technique for detection of viral infections. In addition, there is potentially significant risk of HBV transmission despite HBsAg testing and this is an important message for clinicians deciding to transfuse blood.

Key words: Hepatitis B virus, ELISA, HBsAg, rapid test cassette assay, blood donors.

INTRODUCTION

Liver disease attributed to hepatitis B virus (HBV) has turned into a tremendous issue comprehensively [1]. As per ongoing appraisals, around 2 billion people worldwide are infected with HBV; among whom around 400 million with chronic disease [2] . Roughly, 15% to 40% of chronically infected people will in the long run develop liver cirrhosis, end-stage liver disease, hepatocellular carcinoma or require liver transplantation [3].

Hepatitis B virus has a double-stranded DNA genome of around 3200 base pairs sorted out into four halfway covering open reading frames which encode the envelope, core (precore/core), polymerase and X proteins. The envelope proteins are surface glycoproteins and largely assigned as hepatitis B surface antigen (HBsAg) [4]. Viral DNA is replicated via reverse transcription of an RNA pregenome [5]. Hepatitis B virus has been found in all body secretions and excretions. Notwithstanding, just blood, body fluids containing visible blood, semen and vaginal discharges represent a danger of transmission [6].

Apart from health issue, the weight sparing misfortune because of HBV is high. Surprisingly, the highest incidence rate of HBV infection was recorded among the most beneficial age class 20-39 years of age. Subsequently, it can be envisioned what number of work hour are lost attributed to such infection. In one of the neighboring countries, it was assessed that yearly aggregate cost for chronic hepatitis B, HBV-related liver cirrhosis and hepatocellular carcinoma was 3094.5, 17483 and 32958 US dollars, respectively, for each patient [7].

In blood donation centers, screening for HBsAg is done routinely to distinguish present or past HBV infection. Mysterious HBV infection is characterized by presence of HBV DNA in blood or liver tissues in patients negative for HBsAg, however, who could possibly be sure or not for HBV antibodies [8].

Serological techniques are most common, fast and cost-effective strategies to investigate large number of markers like HBsAg, anti-HBsAg, anti-HBcAg, HBeAg, anti-HBeAg, *etc*. On the other hand, Enzyme Linked Immunosorbent Assay (ELISA) is a type of solid phase immunoassay in which antigens or antibodies are covalently bound with suitable enzymes that can catalyze the change of substrates into dyed products. It is an approved technique to investigate diverse serological markers [9].

Taken together, the objective of this study was to compare between ELISA and hepatitis B surface antigen rapid test cassette assay in detection of hepatitis B virus among blood donors attending blood bank in Baqubah city, Iraq.

METHODS

Study design

A cross-sectional study conducted on blood donors attending Blood Bank in Baqubah city, Iraq, during the period from 1st October 2016 till 30th March 2017. Full information had been taken directly from all participants using specific data sheet.

Detection of hepatitis B virus (HBsAg)

Enzyme immunoassay for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum was done according to manufacturer's instruction (Foresight, Cat. No. 3000-1101, Spain). Briefly, sera from all participants were added to the microwell plate which contains monoclonal antibodies specific to different subtypes of HBsAg, after incubation, the conjugate and substrate were added, then washed off and finally measured by an ELISA reader at 450/630-700 nm or 450 nm.

Chromatographic immunoassay

All samples, which were positive for HBsAg, were compared with onsite HBsAg rapid test cassette (CTK Biotech, Inc, USA, Cat. No.R0040C). Onsite HBsAg rapid test cassette is colored chromatographic immunoassay that was used for detection of HBsAg. Specimens were put in the samples wells then the specimens migrate by capillary action across the test cassette to areas which contain mouse anti HBsAg antibody conjugates with colloid gold HBsAb conjugates. After 10 minutes, the results were read according to color development as described in manufacturer's instructions. So that, in positive results, color development in T and C lines. Sensitivity and specificity were determine as follows:

Sensitivity = Number of true positive / Total number of individual in population.

Specificity = Number of true negative / Total number of individual in population.

Statistical analysis

Data were analyzed using the t- and chi-squared tests to obtain statistically significant differences ($p<0.05$) among different parameters.

RESULTS

Of 10,355 blood donors, 150 (1.44%) showed the presence of HBsAg in the specimens examined by ELISA assay while by onsite HBsAg rapid test cassette was 140 (1.35%). However statistical analysis showed highly significant differences as shown in Table (1).

Table (1): Detection of HBsAg in blood donors by use two laboratory methods.

Test	Total No. of examined cases	No.(%) of positive cases	P value
ELISA	10355	150(100%)	0.00
Rapid test cassette	10355	140(93.33%)	

Significant ($p \leq 0.05$).

According to results presented in Table 2, the sensitivity and specificity were 100% for ELISA test compared to rapid test cassette assay.

Table 2 Sensitivity and specificity of ELISA and onsite HBsAg rapid test cassette for diagnosis of hepatitis B virus in blood samples from blood donors

HBsAg detection technique	Positive	Sensitivity	Specificity
ELISA	150	100%	100%
One-step card test	140	93.33%	100%

All socio-demographic data were listed in Table 3. Among studied group 123(82%) were males while 27(18%) were females. In addition, the age of patients ranged from 20-60 years and high percentage (53.34%) were within the age group 20-35 years. Among the entire population of participants, 86(57.33%) were lived in urban area while 64 (42.67%) in rural area. Also, most positive cases (110(73.33%) were married In terms of educational level, high frequency 60(40%) of participants had secondary school education.

Table (3): Socio-demographic characteristics among studied group

Parameters	No. (%)
Gender type	
Male	123(82.00%)
Female	27(18.00%)
Age groups	
20-35 year	80(53.34%)
36-50 year	44(29.33%)
51-65 years	26(17.33%)
Residence	
Urban	86(57.33%)
Rural	64(42.67%)
Marital status	
Single	37(24.67%)
Married	110(73.33%)
Divorced	3(2%)
Education	
Illiterate	17(11.33%)
Primary school	50(33.34%)
Secondary school	60(40%)
High education	23(15.33%)

DISCUSSION

Hepatitis is one of the most important diseases and is a common cause a wide spectrum of liver diseases ranging from acute hepatitis to chronic hepatitis, cirrhosis and liver cancer. Therefore, correct identification of the disease helps reduce its mortality and morbidity rates.

At present time, hepatitis B surface antigen detection is the only diagnostic screening test for HBV infection identification in blood transfusion centers in different area which is based on finding of persistent HBsAg for at least 6 months [10,11] .

In the present study, the infection rate of HBV was found to be 1.44% by ELISA test and 1.35% by onsite HBsAg rapid test cassette. This finding is comparable that reported by Hussein (2018) who found a 1.14% infection rate in Duhok city (North of Iraq) among potential blood donors [12], however, it is slightly higher than that reported by Al-Rubaye et al. (2016) in Basra city (South of Iraq) and Al-Juboury et al. (2010) in Babylon city (Middle of Iraq) who reported HBV infection rates of 0.2% and 0.7%, respectively, [13.,14]. This is related to a fact that is in Iraq, vaccination against HBV is part of an expanded program of vaccination targeting new born babies, yet all other at-risk groups are urged to take the vaccination [15,16]. Furthermore, results of current study revealed that ELISA test is more sensitive than onsite HBsAg rapid test and this finding is consistent with that reported by Maity et al. (2012) who evaluated three ELISA kits (Span diagnostics Ltd., J. Mitra and Co. Pvt. Ltd., and Transasia Biomedicals Ltd.) in 300 samples. All the kits were found to be good for screening as they exhibited higher specificity, positive predictive value (PPV) and negative predictive value (NPV) were 100% when panels were tested by kits of J. Mitra and Co. Pvt Ltd. and Transasia Biomedicals Ltd, though little less in case [17]. Recombinant HBcAg is expressed in Escherichia coli and *Pichia pastoris* by Li et al. [18]. Also, the high sensitivity and specificity of ELISA found in current study was reported by Yazdani et al. (2010) who used novel monoclonal antibodies as capture layer and a polyclonal biotinylated antibody as detector phase to develop one new ELISA system. The mentioned study reported sensitivity and specificity of the assay of up to 98.98% [19]. In comparative studies with PCR, the sensitivity of chemiluminescent enzyme immunoassay (CLEIA/ CLIA) was 96% [20]. Its sensitivity is even more enhanced by different modifications by researchers [21].

Concerning gender, this study revealed that the majority of participants who have HBV infection were males (Table 3). This finding is in agreement with that reported Wang et al. (2015) who found that the sex disparity of HBV-related liver diseases has been noticed for a long time. These findings could be attributed to sex hormone effects other than gender behaviors or environmental impact. This difference is experimentally confirmed in HBV transgenic mice as well as in immunocompetent mice receiving hydrodynamic delivery of HBV. Androgen and estrogen pathways were identified to play opposite regulations of HBV transcription by targeting viral enhancer I at molecular level. Moreover, in addition to the direct effects on HBV life cycle, sex hormones may also be involved in the immune response to hepatitis B virus infection and the progression of associated liver diseases though the detailed mechanisms are still unclear. Besides, several unaddressed issues such as hepatitis B virus entry, microRNA profiles, viral integration and adaptability in which androgen and estrogen axes might be involved are warranted to be delineated [22]. In the study conducted in Basra [13], the authors found no significant gender-wise difference in the rate of HBV infection ($p=0.28$). The findings of current study support the fact that male gender can be a risk factor for viral hepatitis due to more activity than females, spending most of the time outside the home and come into contact with different pathogens given the fact that HBV can survive outside the body for at least 7 days [23].

In terms of participants age, current study revealed that higher rates of HBV infection were seen among participants within the 20-35 years age group (Table 3). These observations were similar to those documented in other studies [24,25].

In keeping with demographic criteria of participants, current study revealed that 86 (57.33%) of HBV-infected participants were living urban residents. This finding was similar to that reported in a southeastern region of Turkey [26],yet contrasts that documented in another study where the prevalence of HBV infection was higher in rural areas [27].

Concerning the relation between HBV infection and marital status, current study found that the rate of infection was higher in married patients (Table 3). This finding agrees with that reported by a previous study which showed high frequency of HBV

infection among married patients [28,29]. These data can be explained by the fact that sexual intercourse is a major mode of HBV transmission [30].

Furthermore, current study revealed that most patients with HBV infection were having secondary school educational level (Table 3). This information was in agreement with that revealed by another study which stated that lower educational level was also an important risk factor for HBV seropositivity in urban areas but not in rural ones [27].

In conclusion, there is potentially substantial risk of hepatitis B virus transmission despite HBsAg testing and this is an important message for clinicians deciding to transfuse blood. Furthermore, several studies should be conducted in different areas to determine the efficiency of screening techniques for HBV detection in our population in addition to raise public awareness about HBV infection.

Ethical Clearance: It was obtained from the Scientific Research Committee at the Health Directorate in Diyala Province, Iraq.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

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