

## Research Article

# Efficiency of a rapid assay for diagnosis of hepatitis B virus infection in early ages with high levels of bilirubin

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**Received:** 10 June 2016

**Revised:** 11 June 2016

**Accepted:** 25 June 2016

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## ABSTRACT

**Background:** The infection by hepatitis B virus (HBV) considers one of the common types of viral disease that can infect all ages of the human. Babies and children as an early age of the human life are usually infected by such type of virus with high incidence rates all over the world. An evaluation of a rapid assay (RA) in comparison with ELISA for diagnosis of HBV infection in early ages patients with a high level of bilirubin was investigated in the present study.

**Methods:** A total of 86 early age patients with high bilirubin levels (35 babies and 51 children) were involved in this study. Sandwich ELISA and RA were applied to detect HBsAg of HBV in the patient's blood samples.

**Results:** According to the rapid assay, all of our patients were shown a negative result for HBV infection, while the application of sandwich ELISA showed positive results in two of them (child and baby).

**Conclusions:** The RA could be used as a second chose to detect HBV infection after ELISA. The diagnostic device produce by ABNO company which was used as one of the RA was revealed less effective to detect HBV infection.

**Keywords:** HBV, RA, ELISA, Bilirubin

## INTRODUCTION

Hepatitis B virus (HBV) infection is one of the most important viral diseases in the liver that infected different ages of human being. It causes a serious of acute and chronic disease with various symptoms. Annually, a large number of reports described the prevalence of HBV in children worldwide. In some countries with a high endemic level, such as sub-Saharan Africa, Asia (excluding Japan), the pacific basin, parts of the Middle East, and the Amazon basin, the incidence rate is very high among children, while it declined in other countries with intermediate and low endemicity level.<sup>1</sup> In non-Asian children, the number of HBV infection found to be increased from 24 per 100,000 children who were younger than 10 years to 2580 per 100,000 in children of South East Asian immigrant mothers.<sup>2</sup>

Recently, there are many methods used for the diagnosis of HBV infection through detection of viral antigen that found in either of serum (most common) or in biopsy examination.<sup>3-6</sup> Although it is expensive for most of clinical laboratories and needs a time, different types of ELISA techniques revealed more accuracy and suitability to detect HBV antigens. Rapid assay (RA) considers one of the simplest application methods in comparison with ELISA which is depended in most of them on chromatography method.<sup>7-10</sup> It is artificially produced by many companies in different countries. The efficiency of these products was evaluated after comparison with standard methods such as ELISA and PCR.<sup>7,10-15</sup>

Various samples can use in RA such as serum, blood or both.<sup>2,5,11-15</sup> The suitability of RA to detect HBV infection is usually ranked between excellent and failure depending on the efficiency of the product and the production company.<sup>10,13</sup>

In addition to producing company, evaluation of the efficiency of RA in comparing with ELISA to diagnosis HBV infection in children with high level of bilirubin is the main aim of this study.

**METHODS**

**Patients**

A total of 86 patients distributed between 35 babies (4-180 days; males=21, females= 14) and 51 children (1-15 years; males= 21, females= 30) were involved in this study during admitted in AL-Hussein Pediatric teaching hospital at Karbala Province from 1 July to 30 August 2015. Bilirubin level that assay by bilirubin meter (APEL. BR.501, Japan) was high in all of involved patients. The lipomic and hemolytic samples were eliminated from the analysis.

**Reagents**

The HBsAg ELISA test kit was purchased from In Tec products, INC (China). Rapid test device of HBsAg was purchased from ABON Biopharm (Hangzhou) Co., Ltd (China).

**Assay**

The double antibody sandwich ELISA immunoassay was applied for analyzing of the serum of all patients in order to detect HBs Ag. Horseradish peroxidase (HRP) was used as the conjugate solution. The absorbance of the final product was measured at 450 nm within 30 min by a BioTek ELx800 ELISA reader (U.S.A). As mentioned in manufactory instructions, the expected value for HBsAg in healthy individuals is  $\leq 6$  ng/ml.

For rapid assay (RA) that dependent on a chromatography immunoassay, three drops of non hemolyzed serum were added on specific location of the rapid test device. The results will consider positive when two distinct colored lines appear. One line should be in the control region (C) and another line in the test region

(T). For negative result, one colored line appears in the control region (C) and no apparent colored line in the test region (T).

**Statistical analysis**

Data of all tests were expressed as mean  $\pm$  SD. The values were analyzed statistically with paired t test. The minimum level of (p) value was  $p < 0.01$  which is considered a significant level.

**RESULTS**

Many different methods can apply for a diagnosis of HBV infection in the early ages of the human life. A total of 35 babies and 51 children with high bilirubin level was investigated for the possibility of infection with HBV by using ELISA and RA. Through the application of RA, all of our patients revealed a negative result for HBs Ag, while used of sandwich ELISA showed positive results in two of them. The first positive ELISA for HBV was observed in 7 years child (female) and the second one was observed in 20 days new baby (male) as shown in Table 1. The two positive results of ELISA confirmed that the diagnostic device produced by ABNO company which was used as a rapid assay considered less effective to detect HBV infection.

**DISCUSSION**

Early ages of the human (infants and children) are not excluded from HBV infection as well as old ages. From 1510 Chinese preschool children (mean age 29 months), 15.9% were infected with HBV, while 84.1% were susceptible to such type of viral infection.<sup>4</sup> Whereas in England, the capture-recapture analysis estimated that 448 of the children who aged less than 16 years were infected with HBV.<sup>16</sup> However, the high incidence among adolescent aged (15-19 years) is relatively considered more than in younger age groups as found in 13829 cases of children (aged  $\leq 19$  years) with acute hepatitis B in the USA between 1990-2002.<sup>17</sup> Generally, the state of HBV infection could be taken either of acute or chronic form in children.

**Table 1: The results of HBV measured by RA and ELISA methods in patients with high bilirubin levels**

Patients	Patient's gender	Patient's No. with HBV (%)				Total No.
		RA		ELISA		
		Positive	Negative	Positive	Negative	
Children	Male	0	21 $\pm$ 3 (100)	0	21 $\pm$ 1.4 (100)	51
	Female	0	30 $\pm$ 2 (100)	1 $\pm$ 0.2 (3.3)	29 $\pm$ 0.8 (96.6)	
Babies	Male	0	21 $\pm$ 1.9 (100)	1 $\pm$ 0 (4.7)	20 $\pm$ 1 (95.2)	35
	Female	0	14 $\pm$ 1 (100)	0	14 $\pm$ 0.6 (100)	
<b>Total No.</b>		0	86 (100)	2 (2.3)	84 (97.6)	86

All values are expressed as Mean  $\pm$  SD, \* Significant differences at  $P < 0.01$

Chronic infection of HBV is recorded to have a high incidence rate in children. It's noted in 90% of infants who get infected at birth, 25-50% in 1-5 years of age and about 1-10% of persons infected as older children and adults.<sup>18</sup> Among 129 Egyptian thalassemic children, 59.2% males and 40.8% females (mean age 7.6 years) had infected with HBV.<sup>19</sup> The long term study (29 years) of Caucasian children who infected with chronic HBV showed that 91 children had positive results with HBsAg test.<sup>3</sup>

The human often acquires HBV infection during the first time of its life by different routes. A baby can get hepatitis B from an infected mother as perinatal transmission at childbirth.<sup>13</sup> The virus can persist in baby body for the rest of their lives, can spread the disease, and can develop liver cirrhosis or even cancer.<sup>12</sup> In the absence of vaccination, the newborn whose mother is positive for HBsAg will suspect to develop a chronic HBV infection in 70-90% of them by age 6 months.<sup>20</sup> In addition to early childhood (<5 years), the perinatal transmitted is the primary route of HBV entering in individuals of high endemic areas, while the infection in later childhood or in adulthood is common in intermediate and low endemic areas.<sup>13,21</sup> The growing of an infant with HBV infection doesn't eliminate their infection and can proceed in their body until late childhood ages.<sup>13,22</sup> Approximately 90% of the infants of HBs Ag-positive mothers become HBsAg carriers without they get an HBV immunization.<sup>20</sup>

The HBV infection revealed many clinical symptoms which are very considerable. The acute HBV infection of children can be either asymptomatic in which it's common in infant and children or symptomatic.<sup>13</sup> Jaundice that can monitor by measuring of bilirubin level in the blood stream is one of these symptoms.<sup>23</sup> Some of the scientists have undoubtedly that Hippocrates referred to persons infected with acute hepatitis B virus when he described epidemic jaundice.<sup>24</sup> However, Jaundice found to be represented 10% of children (5 years of age) with HBV infection or manifested in 50% of older children and adults.<sup>25</sup> Otherwise, hepatitis B in children has frequently less severe, but more likely to be prolonged.<sup>12</sup> Soliman found that 96% of Egyptian thalassemic children were jaundiced.<sup>19</sup> Generally, newborn infected with HBV often have no clinical signs or symptoms.<sup>12,25</sup>

The hepatitis B virus possesses various antigens that can detect during its life cycle in the human body. These antigens are very helpful to diagnosis the different stages of viral infection. HBsAg is regarded the first serological marker of HBV infection, which can detect from 2 to 12 weeks after infection.<sup>25</sup> The presence of HBsAg indicates either acute or chronic infection.<sup>18</sup> Based on detection of antibodies that produce against HBs Ag, 10.6% of 924 children gave positive for HBV markers.<sup>4</sup>

Rapid methods become more common assay for diagnosis of HBV infection. It's widely used these days

because of its have more advantages than other methods. It's inexpensive, easy to complete in a short time and doesn't need large amounts of sample, as well as suitable for case-finding and epidemiological surveillance. The principle of RA depends on a rapid chromatographic immunoassay for the quantitative detection of HBsAg in serum, plasma or whole blood.<sup>2,12</sup>

Although all of our patients revealed negative results with RA, two of them (female child and male baby) were exhibited a positive result for HBsAg when we used ELISA test. Thus, more known sensitive and specific assays such as ELISA and PCR are required to evaluate the suitability of RA for diagnosis of HBV infection. Some of RA improved its efficiency for diagnosis HBV with a high accuracy rate as confirmed by other more specific methods.<sup>10</sup> Hyder found that three of rapid immunochromatography tests were revealed equally sensitive and specific for detection of HBsAg after comparison with the ELISA method.<sup>8</sup> Moreover, hepacard as one of the RA was also shown a high sensitivity (79%) and specificity (98.9%).<sup>14</sup>

According to our results, the negative results showed by RA that produced by ABON after comparison with the results of ELISA indicated that the product is less effective to use for diagnosis of HBV infection and needs more evaluation before it using in our laboratory. Many companies specialize nowadays to produce such type of diagnostic kit over all the world. The accuracy of RA that based on sensitivity and specificity to diagnosis HBV infection is usually differed from one product to another. Strip from Intec and Blue Cross Chinese companies had shown higher sensitivity for detecting HBsAg in serum, while Cortez, Acon (U.S.A), Atlas (UK), and DIMA (Germany) devices were less sensitive.<sup>7</sup> The high sensitivity of Intec device compared to Acon was also proved.<sup>8</sup>

On the other hand, failure of RA to diagnosis of HBV antigens as proved by ELISA is also expected. This failure may be related to many factors such as; inadequate coating of the antigens, the nature of the antigens used, and genetic heterogeneity of the virus.<sup>26</sup> Nucleic acid testing can justify the variable results obtaining from RA and ELISA for support one of them. However, RA rarely gives false positive results, although it's given false negative results.<sup>27</sup>

## CONCLUSION

Although RA has many advantages than other methods for diagnosis of HBV infections, it's still the second chose to apply due to its variable results and the high percentage of the false negative results. Therefore, rapid assay can use as a back up to other standard testing methods. Moreover, diagnostic device produce by ABNO company was revealed less effective to detect HBV infection.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the institutional ethics committee*

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**Cite this article as:** AL-Janabi AAHS, Musa RA, Hussein ZZA. Efficiency of a rapid assay for diagnosis of hepatitis B virus infection in early ages with high levels of bilirubin. *Int J Sci Rep* 2016;2(7):155-8.