

Seroprevalence and Comparative Screening of HBsAg in Pediatric Patients

ZAIGHAM ABBAS, GHAZALA JAFFERY AND MUHAMAD SAEED ANWAR

Department of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan (ZA) and Department of Pathology, Services Institute of Medical Sciences, 6, Birdwood Road. Lahore. Pakistan (GJ, MSA))

Abstract.- The present study was a cross sectional descriptive study to check the prevalence of Hepatitis B surface Antigen (HBsAg) among pediatric patients which included a total of 2,152 children both male and female who reported to Department of Immunology in Children's Hospital Lahore, from September 2004 to August 2005. The prevalence of HBsAg among the pediatric patients was found to be 5.1%, during the specified course of study. Further, the detailed history of 109 HBsAg reactive subjects was collected to assess the prevalence of HBsAg in different age and sex groups, the distribution of different signs and symptoms among HBsAg positive patients, risk factors among HBsAg positive patients and the prevalence of HBsAg on the basis of socio-economic status. Finally, to evaluate the immunochromatographic method (Biorad), using Enzyme Linked Immunosorbent Assay (ELISA, Biorad) as golden standard for the detection of HBsAg, a sub-cohort of 200 subjects was analyzed. The specificity and sensitivity of the immunochromatographic method was found to be 99.5% and 84.6% respectively. From this evaluation it was concluded that immunochromatographic technique is less reliable for screening than the ELISA.

Key words: HBsAg, pediatric patients, immunochromatographic method, ELISA.

INTRODUCTION

About 2 billion people worldwide have been infected with HBV and approximately 300 million individuals have chronic liver disease due to Infection with HBV (WHO, 2000). Hepatitis B virus (HBV) is a widespread human pathogen, and chronic hepatitis B is a serious clinical problem with worldwide distribution and has been well documented in Pakistan. Hepatitis B (HBV) is an etiological agent for major form of hepatitis belonging to Hepadnaviruses, which multiply preferentially in liver, resulting in a chronic infection to decimate the health of its host (Marion, 1988; Korba *et al.*, 1988).

HBV is a partially double-stranded, enveloped DNA virus of the Hepadnaviridae family, which replicates in the liver and causes hepatic dysfunction (Xiaodong *et al.*, 2006). HBsAg is found on the surface of the virus and is also produced in excess amounts, circulating in the blood as 22-nm spherical and tubular particles. HBsAg acts as a marker for the diagnosis of HBV infection (Marion, 1988; Korba *et al.*, 1988), can be identified

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in serum 30 to 60 days after exposure to HBV and persists for variable periods (Klingmüller and Schaller, 1993). HBV is transmitted by percutaneous or permucosal exposure to infectious body fluids, by sexual contact with an infected person, and perinatally from an infected mother to her infant (Wright, 2006). The frequency of HBV infection and patterns of HBV transmission vary markedly in different parts of the world.

Virological markers can be used to detect the presence of virus and these can be direct markers such as viral antigens or indirect markers such as specific antibodies, produced by immune cells in response to viral antigenic stimulation which can be detected in plasma or serum by means of sensitive and specific EIA tests (Neumann *et al.*, 1998).

The detection of HBsAg has become necessary to find out antigen carrier rate among healthy individuals. A number of methods for detection of HBsAg have been reported in the diagnostics, which vary in their sensitivity as well as specificity. Immunochromatographic method is being used in Pakistan for the screening of HBsAg which has a very low sensitivity as compared to ELISA. This study was performed to check the

* Corresponding author: e-mail zaighum09@yahoo.com

prevalence of HBsAg in pediatric patients and to evaluate the immunochromatographic method which is used for the screening of HBsAg.

MATERIALS AND METHODS

Study population and settings

The study included a total of 2,152 children both male and female, referred for screening for HBsAg, to the Department of Immunology in Children's Hospital and Institute of Child Health, Lahore, from September 2004 to August 2005.

Study design

Initially in the study, the prevalence of HBsAg among the children, was assessed by non-probability sampling technique. Further, the detailed history of 109 HBsAg reactive subjects was collected with following objectives: To assess the prevalence of HBsAg in different age and sex groups, to assess the distribution of different signs and symptoms among HBsAg positive patients, to assess risk factors among HBsAg positive patients, and to check the prevalence of HBsAg on the basis of socio-economic status. Children of either sex with age range of 1 day to 14 years are included in the present study. In order to avoid the risks, the standard procedure was adapted for the collection of blood samples.

Preparation of samples and reagents

No special preparation, other than sterile technique, was used. Serum was separated by the clotted blood by centrifugation. All reagents were prepared according to the manufacturer's instruction. Two techniques were used for the detection of HBsAg; immunochromatographic method (Bioline) and ELISA (Biorad).

Statistical analysis

Finally, for comparative evaluation of results obtained with Immunochromatographic method and ELISA method, a sub-cohort of 200 subjects was analyzed. Sensitivity, specificity and predictive value of immunochromatographic technique using ELISA technique as golden standard were calculated according to the standard formulas

(WHO, 1992).

RESULTS

Among the 2,152 subjects 2,043 (94.90%) were non-reactive and 109 (5.1%) were reactive for the HBsAg by immunochromatographic method. The prevalence of HBsAg among the children reporting to Children Hospital Lahore was found to be 5.1% (Fig. 1). Further, the detailed history of 109 HBsAg reactive subjects was analyzed with following objectives:

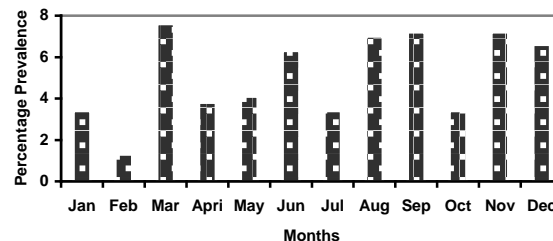


Fig. 1. Prevalence of HBsAg among children from Sep. 2004 to Aug. 2005.

Prevalence of HBsAg in different sex groups

Among the HBsAg reactive patients (n=109): 85 (77.9%) male and 24 (22.1%) female showed seropositivity as screened and confirmed by immunochromatographic and ELISA method respectively.

Prevalence of HBsAg in different age groups

The prevalence of HBsAg on the basis of age was assessed. The patients were divided into four groups A (up to 2 yrs.), B (2-<4), C (4-<9), and D (9-<15), on the basis of age. The prevalence of HBsAg was 13 (12%) in group-A, 13 (12%) in group-B, 54 (49.5%) in group-C and 31 (28.4%) in group-D (Fig. 2).

Risk Factors among HBsAg positive patients

The common risk factors for the prevalence of HBsAg found were hospitalization 49 (44.95%), blood transfusion 44 (40.4%), history of injections 35 (32.11%), chronic illness 27 (24.8%), surgery 17

(15.6%), contact 16 (14.7) and dialysis 3 (2.8%) (Fig. 3).

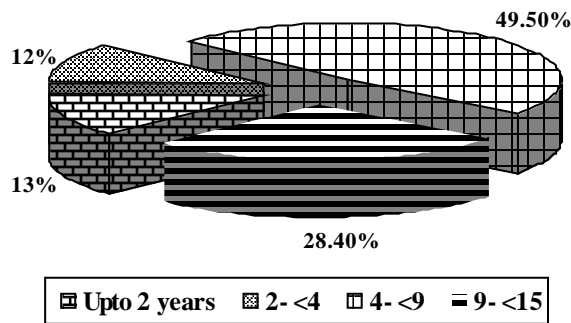


Fig. 2. Prevalence of HBsAg in different age groups.

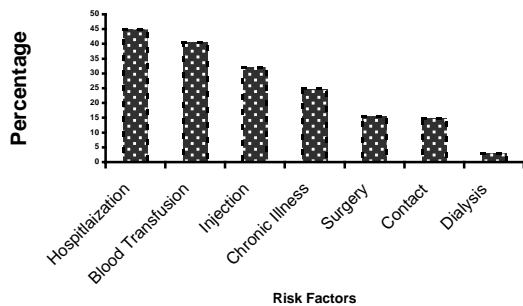


Fig. 3. Distribution of risk factors among pediatric patients.

Prevalence of HBsAg on the basis of socio-economic status

To assess the prevalence of HBsAg on the basis of socio-economic status, we divided the patients into three groups: poor (<Rs.5000), middle class (Rs.5000-10000) and high class (>Rs.10,000) groups. The prevalence of HBsAg was 38 (34.9%) in poor, 54 (49.5%) in middle and 16 (14.7%) in high class subjects.

Distribution of signs and symptoms among HBsAg reactive patients

As far as the prevalence of signs and symptoms among HBsAg reactive patients (n=109) is concerned, 66 (60.5%) had fever, 41 (37.6%) had jaundice, and 50 (45.9%) had abdominal distension.

Comparative evaluation

By the comparative evaluation the sensitivity, specificity, positive predictive value, and negative predictive value of immunochromatographic technique was found to be 84.6%, 99.5%, 91.6% and 98.9% respectively as calculated by the standard formulas (WHO, 1992) (Table I).

Table I.- Sensitivity, specificity and predictive value of results obtained with immunochromatographic technique using ELISA technique as golden standard. (n=200)

Device test results	A = 11 (True positives)	B = 1 (False positives)	A+B = 12
	C = 2 (False negatives)	D = 186 (True negatives)	C+D = 188
	A+C = 13	B+D = 187	

Sensitivity = $A/A + C \times 100 = 11/13 \times 100 = 84.6\%$
 Specificity = $D/B + D \times 100 = 186/187 \times 100 = 99.5\%$
 Positive predictive value = $A/A+B \times 100 = 11/12 \times 100 = 91.6\%$
 Negative predictive value = $D/C+D \times 100 = 188/186 \times 100 = 98.9\%$

DISCUSSION

The prevalence of HBsAg among the children in our study was found to be 5.1%. But Malik *et al.* (2000) have reported that the carrier rate of HBsAg is 10-14% and that the rate of HBV infection is increasing in many parts of Pakistan. In the present study the prevalence of HBsAg was found lesser because the hepatitis B occurs more often in adults but our study included only children who are relatively less susceptible to HBV (MacCallum and Bauer, 1947). The common risk factors for the prevalence of HBsAg found were hospitalization 49 (45%), blood transfusion 44 (40.4%), needle prick 35 (32%), chronic illness 27 (24.8%), surgery 17 (15.6%), contact 16 (14.7) and dialysis 3 (2.8%). For HBV infection, the risk factors also depend upon geographic location, with area of high HBV such as Asia and sub-Saharan Africa, having perinatal transmission and unsafe injection as major cause of new disease (Kane, 1995). In areas of lower HBV seroprevalence such as North America and Western Europe, transmission is primarily through intravenous illicit drug abuse and through unsafe sexual contact with infected partner (WHO, 2000). People at high risk of infection include those requiring frequent transfusions or hemodialysis,

physicians, dentists, nurses and other health care workers, laboratory technicians, intravenous drug users, police firemen, laundry workers and others who are likely to come into contact with potentially infected blood and blood products. The prevalence of HBsAg on the basis of socioeconomic status was found highest in the middle class (49.5%) and least in the high class (14.7%) because middle and poor class has less access to good medical facilities as compared to high class pediatric patients. An HBV infection shares important diagnostic features with other hepatitis viruses. Symptoms of chronic viral hepatitis are variable: they might include fatigue, mild abdominal pain, nausea, poor appetite, muscle and joint pains, weight loss, and occasional bouts of jaundice (Beers and Merck, 2004). In the present study as far as the prevalence of signs and symptoms among HBsAg reactive patients (n=109) is concerned, 66 (60.5%) showed fever, 41 (37.6%) showed jaundice, and 50 (45.9%) showed abdominal distension.

At the end the immunochromatographic method which is used for the screening of HBsAg in the hospitals of Pakistan, was evaluated. The minimum sensitivity of the technique used for the screening of HBsAg should be 90% but the sensitivity of the immunochromatographic method found in the present study was 84.6% which is less than the ideal value. So, this study revealed that immunochromatographic method is less reliable for the screening of HBsAg. The study of Shanmugam *et al.*, (1983) clearly showed that ELISA is the most sensitive technique. So, more sensitive test like ELISA should be used to screen donors to prevent transmission of hepatitis to patients and the hospital personnel (Kuhns and Busch, 2006).

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