

Prevalence Study of Hepatitis B virus (HBV) Infection by Serological techniques in Jeddah, Saudi ArabiaRedwan N A¹; M M M Ahmed^{1, 2*} and M B I Barnawi¹¹Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.² Nucleic Acids Research Dept., Genetic Engineering and Biotechnology Research Institute (GEBRI), Mubarak City for Scientific Research and Technology Applications. Alexandria, Egypt.mmmahmed6@yahoo.ca

Abstract: Chronic liver disease is an important health challenge in the world, where hepatitis B virus (HBV) infections are the main causes of liver insufficiency. HBV infection is a serious global health problem, with two billion people infected worldwide, and 350 million suffering from chronic HBV infection. The objective of this study was prevalence of Hepatitis B virus on Jeddah populations, Saudi Arabia. (HBV) was screened in serum and plasma by using Elisa test, samples were collected and stratified according to nationality into Saudi and non Saudi and according to gender into male and female and also according to age. Prevalence was calculated separately for each group and for studied population as a whole. **Results** showed that HBsAg were found in 6.11 % seropositive of sample in study populations, the prevalence of HBs Ag was higher in males than females it is 8 % among males and 5.61 % among females with significant difference ($p < 0.05$). Also HBs Ag were found in 6.53 % of Saudi population and 1.79 % of non Saudi patient with no statistically significant difference ($p > 0.05$). According to age results all positive samples were found in adult age while no positive sample at age under 15 years with no significant difference ($p > 0.05$). Finally in this study we tried to draw attention through the study to create future strategies to deal with this virus and limit its spread in Saudi society. This study provides valuable information that can be used to examine the incidence of infection in the community and help focus the administration of a future HBV vaccine to appropriate target populations.

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Key words: HBV; virus; seroprevalence; ELISA; HBsAg

1. Introduction

Hepatitis means inflammation of the liver. The liver is a vital organ that processes nutrients, filters the blood, and fights infections. When the liver is inflamed or damaged, its function can be affected. Heavy alcohol use, toxins, some medications, and certain medical conditions can cause hepatitis. Viral hepatitis is a major global health problem. There are five known viruses that primarily infect the liver and cause hepatitis: hepatitis A; B; C; D and E. Hepatitis B, C, D virus (HBV, HCV, HDV) infections can lead to chronic liver disease with its attendant long term sequelae including cirrhosis and hepatocellular carcinoma (HCC). Viral hepatitis A (HAV), hepatitis B and hepatitis C virus (HCV) are the three most commonly identified worldwide.

This disease represents a major public health problem in Saudi Arabia. According to the Saudi Ministry of Health (MOH) data, viral hepatitis ranked the second most common reportable viral disease after chickenpox in 2007, with almost 9000 new cases diagnosed in that year (52% HBV, 32% HCV, and 16% HAV) (MOH; 2009). In Saudi Arabia, HBV and HCV are major causes of disease requiring liver transplantation and of hepatocellular carcinoma, resulting in the need for considerable healthcare

resources (Al-Sebayel *et al.*, 2007). The epidemiology of viral hepatitis in Saudi Arabia has undergone major changes, concurrent with major socioeconomic developments over the last two to three decades. Since the 1980s, Saudi Arabia has been known as a high HBV endemic area (Andre, 2000).

The prevalence of HBV infection varies widely, with rates ranging from 0.1% to 20% in different parts of the world (Lavanchy, 2004). "High" prevalence (hepatitis B surface antigen [HBsAg] positivity rates > 8%) regions where the viral infection is highly endemic include the Far East, parts of the Middle East, sub-Saharan Africa, and the Amazon basin. In these regions, serologic evidence of prior HBV infection (anti-hepatitis B core antigen [anti-HBc] or anti-HBs positivity) is present in the vast majority of individuals (Berenguer and Wright, 2002).

Hepatitis B virus (HBV) is a public health problem worldwide. The World Health Organization (WHO) estimates that approximately 350 million people are infected chronically with hepatitis B virus (HBV), and that the prevalence of the certain state is more than 20% in certain highly endemic areas of Africa and Asia (EPI-NEWS, No. 25, 2002). Sequelae of HBV infection are serious. Up to 15% of

carriers develop eventually primary hepatocellular carcinoma; the younger a patient is when they acquire chronic infection, the higher the risk of primary hepatocellular carcinoma. Premature mortality from chronic liver disease occurs in 15–25% (**SOM 208 Microbiology Syllabus Viral Hepatitis, 2006**).

Detection of serological markers is the mainstay of diagnosis of HBV infection and the most reliable marker of HBV carriage is HBV surface antigen (HBsAg) in serum. HBV e antigen (HBeAg) is generally used as secondary marker to indicate high levels of virus in the blood. The minority of chronic HBV carriers in whom HBeAg can be detected have a particularly high risk of progressive liver disease and end stage liver failure (**Fattovich, et al., 1997**). The monitoring of hepatitis B virus DNA in serum is as important as serological markers in predicting the clinical outcome of infection. More recently molecular diagnostic methods have been used to quantify the levels of HBV DNA in serum as a marker of viral replicate activity (**Baker et al., 1991**).

The aim of this study is detection the prevalence of HBV infection by Serological techniques in Jeddah province "Saudi Arabia" and Statistical analysis of results and assessment of the health hazard, and provides valuable information that can be used to examine the incidence of infection in the community and help focus the administration.

2. Materials and Methods

Serum samples collection

Blood samples were collected for 638 patients in different ages, they were selected randomly from AL-Thager general hospital Jeddah and from Jeddah regional laboratory, between November 2011 to April 2012, and all samples were collected from OPD at Plain tube or EDTA tube. Then separated the sample into serum or plasma; samples were transported to molecular virology laboratory in King Abdul AL-Aziz University. All serum samples were stored at -20°C until use.

Serological testing

All samples were screening by Monolisa HBs Ag ULTRA using (ELISA) Technique Cat No.72346 (BIO-RAD). This kits contents of 1 plate – 96 tests.

Principle of assay

Monolisa HBs Ag ULTRA assay is a one step enzyme immunoassay based on the principle of the "sandwich" type using monoclonal antibodies and polyclonal antibodies selected for their ability to bind themselves to the various subtype of HBs- Ag now recognized by the WHO and the most part of variant HBV strains. The Monolisa HBs Ag ULTRA solid phase is coated with monoclonal antibodies. The Monolisa HBs Ag ULTRA conjugates are based upon the use of monoclonal antibodies from mouse and

polyclonal antibodies from goat against the HBs Ag. These antibodies are bound to the peroxides'

Materials for Enzyme Linked Immunosorbent Assay (ELISA):

Monolisa HBsAg ULTRA assay is a one step enzyme immunoassay technique of the "sandwich" type for the detection of the surface antigen of the Hepatitis B virus (HBsAg) in the human serum or plasma.

Preparation of the reagents:

NOTE: before use allow reagents to reach room temperature (18-30 °C).

Reagents to reconstitute:

- **Concentrated washing solution (20x): Reagent 2 (R2):**

Diluted 1:20 in distilled water to obtain the ready-for-use washing solution.

Prepare 800 ml for one plate of 12 strips.

- **Conjugate working solution (R6 + R7):**

Gently tap the vial of the lyophilized conjugate (R7) on the work- bench to remove any substance from the rubber cap. Carefully remove the cap and pour the content of a conjugate diluent vial (6) into the lyophilized conjugate vial (R7). put the cap on and let stand for 10 minutes while gently shaking and inverting from time to time to ease dissolution. Enzyme development solution: reagent 8 (R8) + Reagent 9 (R9) Dilute 1:11 the chromogen (R9) in the substrate buffer (R8) (ex: 1ml reagent R9 + 10 ml reagent R8). Stability is for 6 hours in the dark once prepared.

Interpretation of the result

Samples with ratio values lower than 1 are considered to be negative by the Monolisa HBs Ag ULTRA. Results just below the cut-off value (sample ratio between 0.9 and 1) should however, be interpreted with caution. It is advisable to retest in duplicate the corresponding samples when the systems and laboratory procedures permit. Samples with ratio values equal to or greater than 1 are considered to be initially positive by the Monolisa HBs Ag ULTRA. They should be retested in duplicate before final interpretation.

Statistical analysis

The percentages of individuals with positive, negative, and equivocal results were determined for studied population as a whole. chi square test was used for the analysis and comparison of sero-statuses among age groups, male and female and each nationality. Ninety-five-percent confidence intervals were calculated where appropriate, and *P* values of < 0.05 were considered statistically significant.

3. Results

Blood samples were collected for 638 OPD in different ages, they were selected randomly from AL-Thager general hospital Jeddah and from Jeddah

regional laboratory , between November 2011 to April 2012, all samples were transported to molecular virology laboratory in king Abdul-Aziz University and this sample were tested using enzyme linked immunosorbent assay (ELISA) and calculated separately for seroprevalence, gender, nationality, age and studied population as a whole using chi square test.

Results showed that HBsAg were found in 6.11 % seropositive of sample in study populations , the

prevalence of HBs Ag was higher in males than females it is 8 % among males and 5.61 % among females with significant difference ($p < 0.05$). Also HBs Ag were found in 6.53 % of Saudi population and 1.79 % of non Saudi patient with no statistically significant difference ($p > 0.05$). According to age results all positive samples were found in adult age while no positive sample at age under 15 years with no significant difference ($p > 0.05$) .

Table1. Distribution of sociodemographic (Nationality; gender and age) characteristics

Variable		Cases no (N=638)	
		Freq.	%
Nationality	Saudi	582	91.20
	Non-saudi	56	8.80
Gender	Male	139	21.90
	Female	499	78.21
Age	New born	19	3.00
	Adult	619	97.00
Result	ve +	39	6.11
	ve -	599	93.9

Demographic characteristics of studied population

Table2. Show *P*-value results within Nationality; gender and age.

Variable		Results		<i>P</i> -value
		ve +	ve -	
Nationality	Saudi	38	544	0.32
	Non-saudi	1	55	
Gender	Male	11	128	0.02
	Female	28	471	
Age	New born	0	19	0.34
	Adult	39	580	

p-value < 0.05, 0.01 and > 0.05, significant, highly significant and Non significant respectively

. Seroprevalence study of HBV among studied population in relation to demographic variables.

Prevalence of Hepatitis B Virus according Age:

The overall prevalence of HBV among adult population was 6.11% from the total of studied

population while there is no positive sample at age from new born to 15 years. There was no significant difference between age ($p > 0.05$).

Table 3. Prevalence of Hepatitis B Virus according Age

Total Samples	Age	Sample type	Elisa - Result	Ratio
638	Adult	+ ve	39	6.11 %
	Adult	- ve	580	90.91 %
	N.B- 15 Y	- ve	19	2.98 %

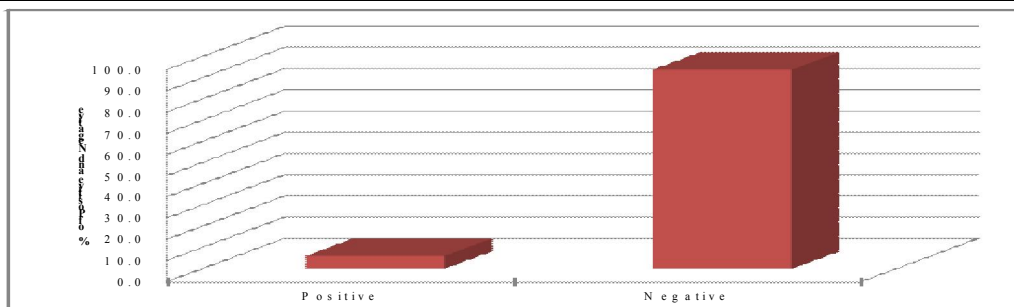
Prevalence of HBV by Samples Type:

The studied populations according to seroprevalence stratified into two groups (positive samples & negative samples). The detection of HBsAg was tested using enzyme linked

immunosorbent assay and calculated separately for each group. The results for HBs Ag were found in 6.11 % seropositive of populations while 93.9% seronegative. These was shown at Table 4.3. and Figure 4.1.

Table 4. Prevalence of Hepatitis B Virus

Total Samples	Samples type	Elisa - Result	Ratio
638	+ve	39	6.11 %
	-ve	599	93.9 %

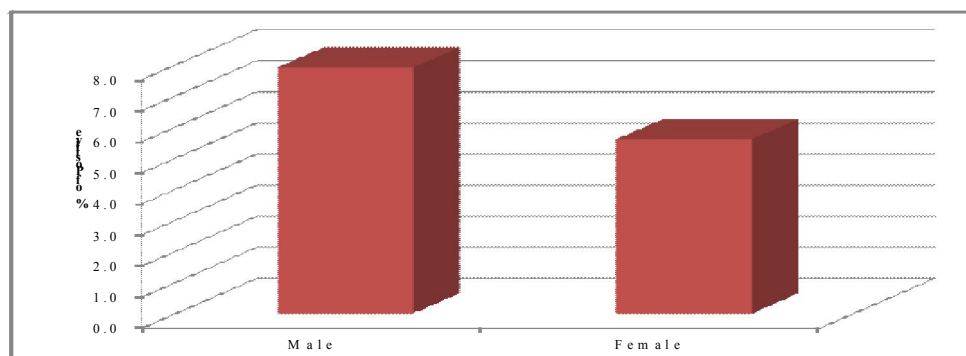
**Figure 1.** Prevalence of Hepatitis B Virus**Prevalence of Hepatitis B Virus by Gender:**

A total of 638 OPD samples were enrolled in the study (139 male and 499 female). The prevalence

of HBs Ag was higher in males than females it is 8 % among males and 5.61 % among females with significant difference ($p < 0.05$).

Table 5. Prevalence of Hepatitis B Virus by Gender

Total Samples	Gender	Sample type	Elisa - Result	Ratio
139	Male	+ ve	11	8 %
	Male	- ve	128	92 %
499	Female	+ ve	28	5.61 %
	Female	- ve	471	94.39 %

**Figure 2.** Prevalence of HBV by Gender**Prevalence of Hepatitis B Virus by Nationality:**

According to nationalities HBs Ag were found in 6.53 % of Saudi population in studied

samples and 1.79 % of non Saudi patients with no statistically significant difference ($p > 0.05$).

Table 6. Prevalence of Hepatitis B Virus according to Nationality

Total Samples	Nationality	Samples type	Elisa - Result	Ratio
582	Saudi	+ve	38	6.53 %
	Saudi	- ve	544	93.47 %
56	Non-Saudi	+ve	1	1.79 %
	Non-Saudi	- ve	55	98.2 %

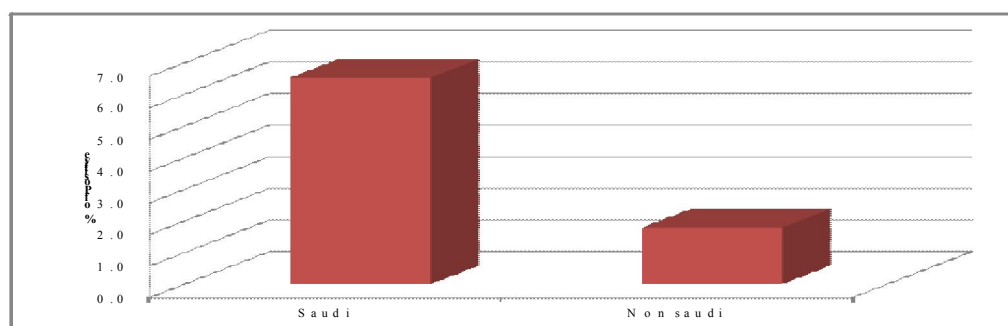


Figure 3. Prevalence of HBV by Nationality

4. Discussion

The human hepatitis B virus (HBV) is a small enveloped DNA virus causing acute and chronic hepatitis. Although a safe and effective vaccine has been available for the last two decades, HBV infection still represents a major global health burden, with about 350 million people chronically infected worldwide and more than 1 million deaths per year due to HBV-associated liver pathologies (Block, *et al.*, 2007). Many epidemiological and molecular studies have shown that chronic HBV infection represents the main risk factor for hepatocellular carcinoma development (Lok, 2004; Shepard, *et al.*, 2006; Pollicino, *et al.*, 2011). The rate for chronicity is approximately 5% in adult infections, but it reaches 90% in neonatal infections. HBV transmission occurs vertically and horizontally via exchange of body fluids. In serum, up to 1012 HBV genome equivalents per ml serum can be found. Although HBV does not induce direct cytopathic effects under normal infection conditions (Thimme, *et al.*, 2003 and Wieland, *et al.*, 2004), liver damage (fibrosis, cirrhosis, and eventually hepatocellular carcinoma) is believed to be induced by the ongoing immune reaction and a consistent inflammation of the liver (Chisari, *et al.*, 2007 and McMahon, 2009).

The prevalence of chronic HBV infection varies geographically, from high (>8%), intermediate (2-7%) to low (<2%) prevalence (Margolis *et al.*, 1991). In the Middle East, HBV prevalence has altered from high to intermediate or low prevalence, but HBV infection is a problem of public health, and a major cause of mortality and morbidity particularly in developing countries. Most countries in the Middle East region are still in intermediate to high endemicity for HBV infection. Insufficient coverage of HBV vaccination, blood-contaminated equipment sharing between injection drug users, unsafe blood transfusion, and inadequate health precautions are major risk factors of HBV infection in this region (Ander, 2000).

In our study, serology was the method of choice as it permits a direct evaluation of the prevalence of HBV infections. In this study all blood samples were collected for 638 randomly patients in different ages, nationalities, sexes they were screened by Elisa test and molecular techniques (PCR) for detection of HBV in studied population in Jeddah province that located in west of Saudi Arabia. Most samples were found for females patients (n=499, 78.2%) and for males were found (n=139, 21.9%). In our study the seroprevalence of Hepatitis B Virus was 6.11 % in studied population's samples at age over 15 years. The prevalence rates were suggestive of history of past exposure to high risk procedure or behavior. The results of our study are generally consistent with the international reports indicate a prevalence ranging from 5.88 % in Bahrain and Saudi Arabia (Toosi *et al.*, 2008).

The average prevalence rate of HBsAg in the Saudi adult population is approximately 8% and 60% have evidence of past exposure to HBV (Faleh, 1988 and Shobokshi *et al.*, 1999). The different regions of Saudi Arabia showed a significantly variable prevalence of HBsAg. The eastern province had a prevalence of about 9% compared to the southwestern province where the prevalence was 25% in Jizan. Another studies indicate to form healthy male Saudi voluntary blood donors in Tabuk region 3.0% were positive for HBsAg while in the eastern region 6.7% and in the southwestern region 5.4%(Fatahalla, *et al.*, 2000; Ayoola, *et al.*, 2003). Also the prevalence of HBsAg from western countries (0.1 - 0.5%) (Sobeslavsky, 1980; Papaevangelou, 1994). However several survey of voluntary blood donors have shown marked regional variation in the prevalence of HBV in KSA (Arya *et al.*, 1985; El- Hazmi, 1989). The Jizan region of KSA is a focus of hyper-endemic HBV infection and its sequelae of chronic liver diseases and hepatocellular carcinoma (Ashraf *et al.*, 1986 and Tandon *et al.*, 1995). A survey carried out in 1985 demonstrated an overall HBV exposure rate

of 46.5% and HBsAg prevalence of 12.7% among 724 Saudi adults residing in the region (Arya *et al.*, 1985). In 1986, a comparative study of different regions of KSA, reported a rate of HBsAg to be 32.2% among 237 blood donors in Jizan, compared to a rate of 4.7% in a similar population in Riyadh in the Central region of KSA (El- Hazmi, 1989).

The present study found the prevalence of HBs Ag was higher in males than females it is 8 % among males and 5.61 % among females with significant difference ($p < 0.05$). Also HBs Ag were found in 6.53 % of Saudi population and 1.79 % of non Saudi patient with no statistically significant difference ($p > 0.05$) this result are consistent with the result of other reports Memish *et al.*, (2010) reported that HBV incidence was significantly higher in males than females , Saudis than non- Saudis and in central and western region than the eastern region .

In our result according to age results all positive samples were found in adult age while no positive sample at age under 15 years with no significant difference ($p > 0.05$) this results consistent with other reported that the incidence of HBV seropositivity was almost 30-fold higher in those who were aged >15 years compared to those who were <15 years (annual average incidence of 169.8 vs. 5.6 per 100 000; $p < 0.001$) (Memish *et al.*, 2010).

Between 2000 and 2007 the incidence of viral hepatitis seropositivity for all three virus types (HAV, HBV, and HCV) showed 20—30% declining trends. Similarly, a comprehensive review of prevalence studies of viral hepatitis conducted in Saudi Arabia in the 1980s and 1990s showed a more than 50% decline in all viral hepatitis types (Al-Faleh, 2003). The age group that included children <15 years was that which showed the greatest HBV incidence decline and this results consistent with our results and it is probably due to the universal infant/child immunization program started in 1990 in Saudi Arabia (Al-Faleh, *et al.*, 1999). Despite the declines in incidence, the current report confirms that seropositive viral hepatitis especially that caused by HBV and HCV, remains a major public health problem in Saudi Arabia, and was probably underestimated by the national surveillance system.

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