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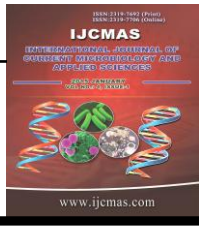

Original Research Article

Seroprevalence of Hepatitis B and C Viruses among Population of Al-Quwayiyah Governorate, Saudi Arabia

Abdel-Ghaffar[1*], Mamdouh H., Al-Harbi[1], Samir A., R. Kadhar Nivas[1] and Khater[2, 3,] Enas SH.

[1]Department of Medical Laboratory Science, College of Applied Medical Sciences, Al-Quwayiyah, Shaqra University, Kingdom of Saudi Arabia
[2]Al-Quwayiyah General Hospital, Al-Quwayiyah, Kingdom of Saudi Arabia
[3]Department of Microbiology & Immunology, Faculty of Medicine, Benha University, Egypt
**Corresponding author*

Keywords

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A B S T R A C T

Transfusion of blood and blood products are a life saving intervention and benefits in numerous patients worldwide. At the same time it could be an ominous mode of infection transmission to recipients. Human Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) are considered the most spread transmitted-blood viruses worldwide. The purpose of this study was to determine the prevalence of HBV and HCV in the blood Saudis persons (including patients, donors) and non-Saudis (including cases of extraction of patients and donors) who came to Al-Quwayiyah General Hospital, Saudi Arabia, over the last 6 month (June 2014 to December 2014). According to the data recorded at Al-Quwayiyah General Hospital, Saudi Arabia, over the last 6 month (from June 2014 to December 2014), 1629 blood samples and 586 blood donors were subjected for serodiagnosis of HBV and HCV using Enzymes Linked Immunosorbent Assay (ELISA). Out of 1629 blood samples, 18 (1.10%) had serological evidence of infection with at least one virus, either HBV or HCV. These included 8 (0.49%) with HBV and 10 (0.61%) with HCV. Out of 586 blood donors, 6 (1%) had serological evidence of infection with at least one virus, either HBV or HCV. These included 4 (0.68%) with HBV and 2 (0.34%) with HCV. The drop in HBV and HCV prevalence rates are likely multifactorial and may have resulted from more diligent donor questioning upon screening, a higher level of public awareness on viral hepatitis as well as the expansion of HBV vaccination coverage in Saudi Arabia.

Introduction

Viral hepatitis is a general name for several forms of liver inflammation that are caused by a virus. Viral hepatitis is the most common type of hepatitis, a group of serious diseases that cause inflammation of the liver and liver damage. Viral hepatitis is often called infectious hepatitis, and the viruses that cause it are contagious and can be passed from person to person (Gallegos-Orozco JF et al., 2010).

Viral hepatitis includes hepatitis A, hepatitis B, hepatitis C, hepatitis D, and hepatitis E. Each of these types of viral hepatitis is caused by the hepatitis virus of the same name, such as the hepatitis A virus. The most common forms of viral hepatitis are hepatitis A, hepatitis B, and hepatitis C (Murray, P. R et al., 2005). All types of viral hepatitis cause inflammation of the liver. The liver is a vital organ, and normal functioning of the liver is crucial to health and life. Viral hepatitis can reduce the liver's ability to do its vital function in helping the body to resist infection, stop bleeding, clear the blood of toxins, store energy, digest food and remove waste (Hepatitis A Symptoms, eMedicine Health.)

Symptoms of viral hepatitis differ between individuals depending on such variables as the specific type of viral hepatitis, age, medical history, the presence of complications and general health. Symptoms common to viral hepatitis include flu-like symptoms, fever, headache, nausea, muscle aches and jaundice. Complications can be serious, even life-threatening, and include the development of chronic hepatitis, cirrhosis, and increased risk of liver cancer, and liver failure (Brundage SC et al., 2006).

Transmission of viral hepatitis may occur by the fecal-oral route often associated with ingestion of contaminated food, depending on the type of hepatitis. Other identified methods of transmission include blood transfusion, tattoos, sexual transmission (through sexual intercourse or through contact with bodily fluids), or parental via mother to child by breast feeding (Brundage SC et al., 2006 and (http://www.cdc.gov/ hepatitis/A/aFAQ.htm).

Saudi Arabia, as a country of the Middle East, is considered to be an area of the three major types of viral hepatitis A, B and C, mainly A and B. There has been dramatic improvement in the levels of personal and public hygiene and in the proportions of the Saudi population, and the impact of these changes on the prevalence of HAV infection must be encouraging.

The prevalence of hepatitis B virus (HBV) was also found to be relatively high in Saudi Arabia; according to a study (El-Hazmi MA et al., 1989, Al-Faleh FZ et al., 1992).The study revealed that the overall prevalence of hepatitis B based on the detection of surface antigen (HBsAg) was approximately 17%. However, this rate is also believed to be significantly decreased after the considerable precautions taken by Saudi government. Prevalence of >8% is considered high; 2-8% is intermediate, and <2% is low according to WHO (Al-Faleh FZ et al., 1992, Akbar HO (2004).

Transmission of hepatitis C virus (HCV) is mainly parental through blood or blood products transfusion and puncture with infected needles. Accordingly, a survey made by (Akbar HO et al., 2004). Showed that the prevalence of HCV (1993-1997) among Saudi blood donors was reported as 2.74% with more than 500,000 Saudi citizens being already infected with HCV. Such a rate is considered close to the prevalence rate estimated by World Health Organization (WHO) of 3% with over 170 million infections, (Akbar HO et al., 2004, Caruntu FA, Benea L et al., 2006).

Because of the significance of viral infections, and because of its relatively high rate in Saudi Arabia, the government initiated strict measures in order to lower the prevalence rate of viral hepatitis. Amongst the strict measures carried out by the Saudi government is to perform mandatory blood tests for foreign workers and premarital testing in addition to the testing of blood from blood donors in hospitals and health clinics throughout the country.

Al-Quwayiyah Governorate is about 95,000 square kilometres, and located in the heart of the Kingdom in Najd area. Al-Quwayiyah governorate has an estimated population of ~ 200 thousand inhabitants who afford health services mainly by the general hospital in the city of Al-Quwayiyah (http://www.alquwayiyah.gov.sa).

The aim of this study was to determine the prevalence of HBV and HCV in the blood Saudis persons (including patients, donors and marriages) and non-Saudis (including patients and donors) who came to Al-Quwayiyah General Hospital, Saudi Arabia, over the last 6 month (from June 2014 to December 2014).

Materials and Methods

This study was done in Al-Quwayiyah General Hospital, Saudi Arabia, over 6 month (from June 2014 to December 2014). All serum samples obtained were examined for HBsAg of hepatitis B virus and antibodies of HCV. Serum samples were analyzed to detect anti-HCV antibodies by ELISA. All the samples that were found positive by ELISA on initial testing were subjected to test in duplicate with the same sample. Samples that were found to be repeating reactive were considered positive. Tests were performed on fully automated EVOLIS using third generation ELISA kits for HCV antibodies (HCV Ab ELISA, Murex Diagnostics Ltd., UK) and hepatitis B surface antigen (HBsAg) Monolisa HBsAg Ultra (Bio-Rad, Marnes La Coquette, France).

Specimens Collection

Serum, EDTA plasma, citrate plasma or heparin plasma samples were subjected to an examine for detecting HBSAg of HBV and antibodies of HCV. Blood samples collected by venepuncture were allowed to clot naturally. Ensure that the serum samples were fully clotted. The any visible particulate matters were removed from the sample by centrifugation.

Specimen Transport and Storage

Stored samples were at 2 to 8°C. Samples not required for assay within seven days were removed from the clot or cell pellet and stored frozen (-15°C or colder). Avoid multiple freeze-thaw cycles. After thawing, ensure samples were thoroughly mixed before testing.

Detection anti-HCV antibodies by immunoassay:

In Murex Anti-HCV (version 4.0) diluted sample was incubated in microwells coated with highly purified antigens which contain sequences from the core, NS3. NS4 and NS5 regions of HCV. During the course of the first incubation any anti-HCV antibodies in the sample were bind to the immobilized antigens. Following washing to remove unbound material, the captured anti-HCV antibodies were incubated with peroxidise conjugated monoclonal anti-human IgG. During the course of the second incubation, the conjugate was bind to antibody immobilized in the first step. After removal of excess conjugate, bound enzyme was detected by the addition of solution containing 3,3',5,5' tetramethylbenzide (TMB) and hydrogen peroxide. A purple colour was developed in the wells which contained anti HCV positive samples. The enzyme reaction was terminated with sulphuric acid to give an orange colour which was read photo metrically. The amount of Conjugate bound, and hence colour, in the wells, was directly related to the concentration of antibody in the sample.

Reactive Results:

Samples giving an absorbance equal to or greater than the Cut-off value were

considered initially reactive in the assay. Such samples were subjected to retest in duplicate using the original source. Samples that were reactive in at least one of the duplicate retests were considered repeatedly reactive and were presumed to contain antibody to HCV antigens. These samples were further investigated by RIBA by sending samples to regional laboratory in Riyadh.

Non Reactive Results

Samples giving an absorbance less than the cut off value were considered negative in the assay.

Detection of HBs Ag

Detection of HBsAg Monolisa™ HBsAg 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 subtypes of HBsAg now recognized by the WHO and the most part of variant HBV strains. The Monolisa™ HBsAg ULTRA solid phase was coated with monoclonal antibodies. The Monolisa™ HBsAg ULTRA conjugates were based upon the use of monoclonal antibodies from mouse and polyclonal antibody from goat against the HBsAg. These antibodies were bound to the peroxidise.

Non Reactive Results

Samples with ratio values lower than 1 were 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 advisable to retest in duplicate the corresponding samples when the systems and laboratory procedures permit.

Reactive Results

Samples with ratio values equal to or greater than 1 were considered to be initially positive by the Monolisa™ HBsAg ULTRA. They were subjected to retest in duplicate before final interpretation. If after retesting of a sample, the ratio values of the 2 duplicates were less than 1, the initial result was non repeatable and the sample was declared to be negative with the Monolisa™ HBsAg ULTRA. For initial reactive or doubtful ($0.9 < \text{ratio} < 1$) samples, if after retesting the ratio values of at least one of the 2 duplicates were equal to or greater than 1, the initial result was repeatable and the sample was declared to be positive with the Monolisa™ HBsAg ULTRA test, subject to the limitations of the procedure, described below. The samples which had been retested twice and found negative with Monolisa™ HBsAg ULTRA test, but with one value near the cut-off value (ratio between 0.9 and 1) were considered with care. It is advised to retest the patient with another method or on another sample.

Results and Discussion

According to the data recorded in Al-Quwayiyah General Hospital, Saudi Arabia, over the last 6 months (from June 2014 to December 2014), 1629 blood samples (Table 1& Table 2) were subjected for serological detection and 586 blood donors (Table 3& Table 4) also were subjected for serological detection .These samples were serodiagnosed for antigen and antibodies of HBV and antibodies of HCV using Enzymes Linked Immunosorbent Assay (ELISA) according to the Lab methods of Al-Quwayiyah General Hospital.

According to the data recorded in Al-Quwayiyah General Hospital, Saudi Arabia, over the last 6 months (from June 2014 to

December 2014) showed considerably low prevalence rates for both HBV and HCV.

Out of 1629 blood samples, 18 (1.10%) had serological evidence of infection with at least one pathogen, either HBV or HCV. These included 8 (0.49%) with HBV and 10 (0.61%) with HCV (Table 1).

Out of 586 blood donors, 6 (1%) had serological evidence of infection with at least one virus, either HBV or HCV. These included 4 (0.68%) with HBV and 2 (0.34%) with HCV.

The results also showed that 44.44% of the total positive results belonged to HBV, while 55.55% belonged to HCV (Figure 1).

Saudis: non-Saudis ratio was 7:1 for HBV and 2.33:1 for HCV. Male: Female ratio was 3:1 for HBV and 3:2 for HCV.

The results indicate an increased seroprevalence of HBV in Saudis males and females compare with that found in non-Saudis persons (Figure 2). The data represented in Table 1 and illustrated by Figure 3 demonstrate that seroprevalence of HCV in Saudis and non-Saudis males was equally determined, while was totally different in Saudis and Non-Saudis female. The significant improvement in the socioeconomic status within the last two decades is demonstrated by a well-developed road network, communication, water supply, distribution of electricity, as well as a comprehensive health care delivery system in the country, which has dramatically lessened the prevalence of many infectious diseases in the Kingdom wholly and in Al-Quwayiyah Governorate in particular.

According to the results in this study, we can clearly notice the dramatic drop in the prevalence of HBV from the study of 1980 (El-Hazmi MA et al., 1989). From 17% to o.49 %. Moreover, the prevalence of HCV shown in this study (0.61%) was relatively lower than that in 1990s (2.74%) (El-Hazmi MA et al., 1989, Akbar HO et al., 2004**).**

Both figures we obtained from the study showed that the prevalence of HBV and HCV in Al-Quwayiyah Governorate is moderate according to WHO scales. The significant drop of prevalence of viral hepatitis is a proof for the success of the controlling measure by the Saudi government so far. It is expected that the prevalence will drop even more in the becoming few years since the current prevalence rates are very close to the 'low' category of WHO classification.

The outcomes of this study have shown that the diagnosed viral hepatitis cases in Al-Quwayiyah Governorate of B and C types are almost the same.

The male: female ratio for HBV and HCV shows an apparent higher incidence in male blood samples. It is possible that male potential persons are three to four times more than female. This possibility can be supported, for example, by the fact that male non-Saudis workers are more in number than females. However, there is evidence that males are more susceptible to viral hepatitis than females shown in a study performed in Libya where the male: female ratio was 2:1(Qatrani H, et al 2007).

Nevertheless, the higher incidence of HBV and HCV in males in this study is likely to be attributed to the fact that more samples were collected from males. It was not applicable to identify the nationalities of non-Saudis infected patients.

In this Study, hospital lab diagnosed HIV through ELISA Technique but further confirmation they are sending sample to Riyadh General Hospital for doing PCR and Western Blotting Technique, because of here less reputable and less instruments are available in laboratory. Furthermore, negative samples are not considered negative, after the confirmation of negative report which was send the blood samples for doing above test to Riyadh.

Viral Infection is one of the main contagious diseases worldwide. Its prevalence in the Saudi Arabia was considered high two to three decades ago but its prevalence seems to be noticeably declined nowadays because of the successful and responsible way the Saudi government dealt with it through adopting strict controlling measure by improving the quality of health care and performing continuous and comprehensive screening for all potential blood-transmitted viruses. we believe that the drop in HBV and HCV prevalence rates are likely multifactorial and may have resulted from more diligent donor questioning upon screening, a higher level of public awareness on viral hepatitis as well as the expansion of HBV vaccination coverage in Saudi Arabia.

Table.1 Number of collected blood samples according to origin and gender

Origin	**Male**	**Female**	**Total No. of collected blood samples**
Saudis	268	194	462
Non Saudi	781	386	1167
Saudis & Non Saudi	Total =		1629

Table.2 Distribution of HBV- and HCV- seropositive blood samples

Origin	**Male**		**Female**		**Total HBV**	**Total HCV**	**Total No. of collected blood sample**
	HBV	HCV	HBV	HCV			
Saudi	5/268 (1.86 %)	3/268 (1.11%)	2/194 (1.03%)	4/194 (2.06%)	7/462 (1.29%)	7/462 (1.29%)	14/462 (3.03%)
Non - Saudi	1/781 (0.12%)	3/781 (0.38%)	0/386 (0%)	0/386 (0%)	1/1167 (0.08%)	3/1167 (0.25%)	4/1167 (0.34%)
HBV & HCV TOTAL =					8/1629 (0.49 %)	10/1629 (0.61%)	18/1629 (1.10%)

Table.3 Number of collected blood donors according to origin and gender

Origin	**Male**	**Female**	**Total No. of collected blood samples**
Saudis	380	140	520
Non Saudi	46	20	66
Saudis & Non Saudi	Total =		586

Table.4 Distribution of HBV- and HCV- seropositive blood donors

Origin	Male		Female		Total HBV	Total HCV	Total No. of collected blood donors
	HBV	HCV	HBV	HCV			
Saudi	3/380 (0.78%)	1/380 (0.26%)	0/140 (0%)	0/140 (0%)	3/520 (0.57%)	1/520 (0.19%)	4/520 (0.76%)
Non - Saudi	2/46 (4.34%)	0/46 (0%)	0/20 (0%)	0/20 (0%)	2/66 (3%)	0/66 (0%)	2/66 (3%)
HBV & HCV TOTAL =					5/586 (0.85%)	1/586 (0.17%)	6/586 (1%)

Figure.1 The distribution of HBV and HCV seropositive samples

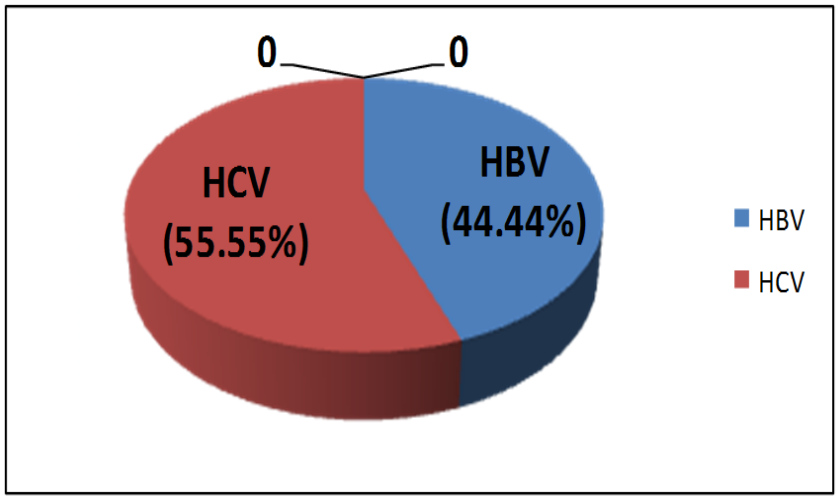


Figure.2 The distribution of HBV seropositive samples according to origin and gender

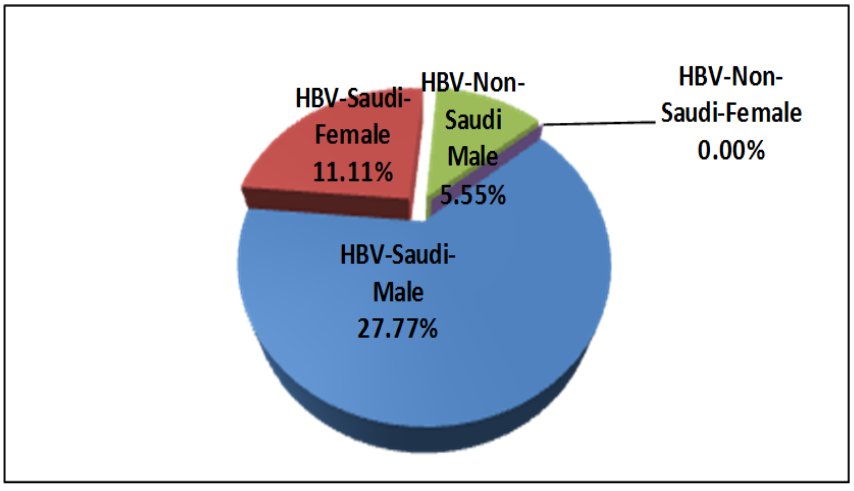
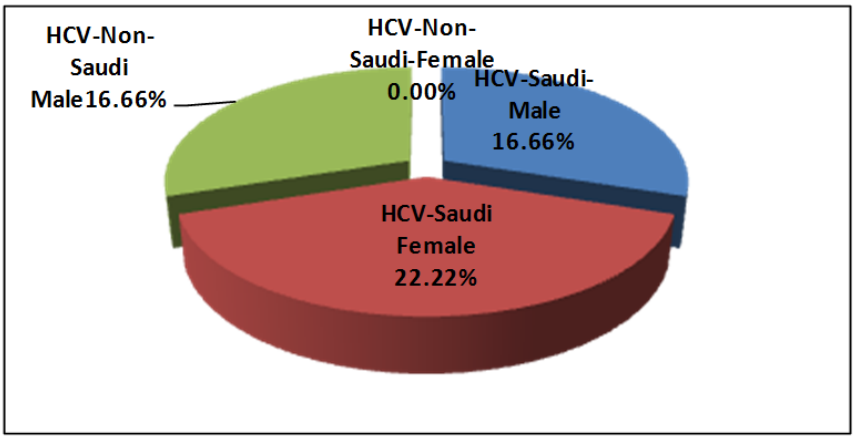


Figure.3 The distribution of HCV seropositive samples according to the Origin and gender



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