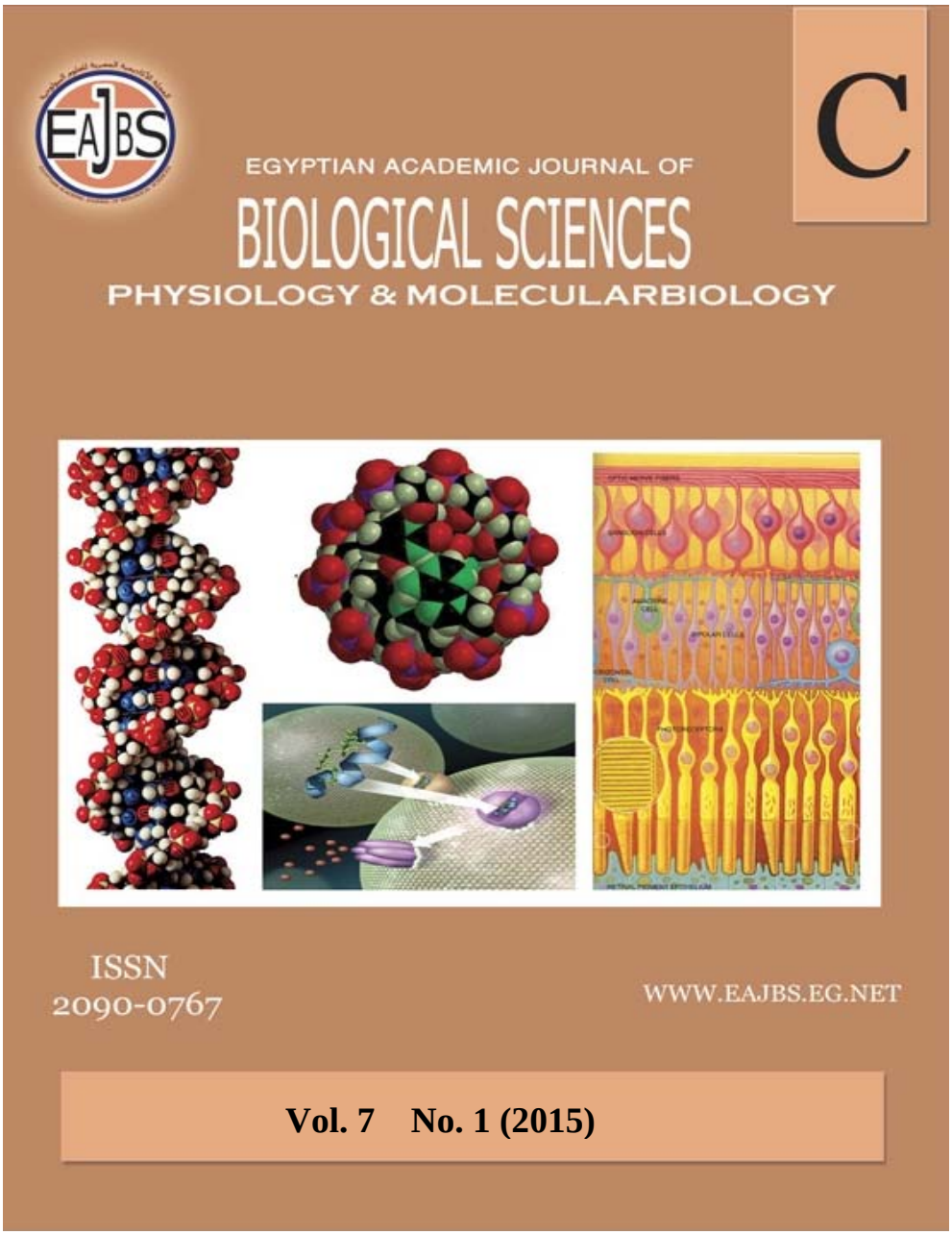

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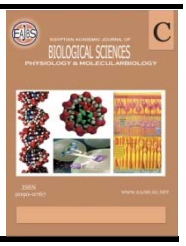

Is vaccine for Hepatitis E virus required at Aseer Region, Saudi Arabia?

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ABSTRACT

Viral hepatitis is one of the major problem worldwide. The detection of hepatitis E virus (HEV) antibodies IgM (HEV-IgM) and HEV-RNA in donated blood in Aseer Region (Southern part of Kingdom of Saudi Arabia) to detect its prevalence was attempted. The study was conducted on random blood samples collected from healthy blood donor volunteers, who were referred to blood transfusion centers found at Aseer region, during the period from March 2012 to January 2013. All the collected blood units were screened for HEV-IgM, hepatitis B surface antigen (HBsAg), anti-HBc, hepatitis C virus (HCV), human immunodeficiency virus (HIV) 1 and 2, human T-cell lymphotropic virus (HTLV) I/II, venereal disease research laboratory (VDRL), and malaria. All donated blood samples were checked for HBV-DNA, HCV-RNA, and HIV-RNA by nucleic acid test (NAT) technology. Of 7267 donors (26 females (0.36%) and 7241 males (99.64%)) blood donors screened, with median age of 28 (female) and 30 years (males), 10 (0.13%) were HEV-IgM near positive of them one was almost positive for HEV-IgM but negative by RT-PCR. In conclusion, prevalence of HEV-IgM in blood donors at Asser region is zero. Vaccination program against HEV is still needed to prevent future outbreaks. Further studies are warranted to determine the true seroprevalence of the virus in the society at large.

INTRODUCTION

Mammalian hepatitis E virus (HEV) is the etiological agent of hepatitis E in humans, a disease of worldwide distribution (Purcell and Emerson, 2008), usually transmitted by the oral-faecal route via contaminated water (Howard *et al*., 2010; Verma and Arankalle, 2010) and blood transfusion (Matsubayashi *et al*, 2011). The infection with HEV leads to acute viral hepatitis. The disease is an important public health problem in many developing countries of Asia and Africa, and is also endemic in many industrialized countries (Ahmad, 2011).

Yearly, about 20 millions of hepatitis E infections occur, of which three millions acute cases of the prevalence of anti-HEV IgG is higher among volunteer blood donors in endemic countries: 7.8% in Iran, 12.1% in Albania, 16.9% in Saudi Arabia and 45.2% in Egypt. In China, a study conducted in 8 rural communities in southern China (where families keep pigs near their home) showed an overall anti-HEV seroprevalence of 43% (Li *et al*., 2006). Although the overall mortality of hepatitis E is less than 1% in the general population, it can reach up to 28% in infected pregnant women (Purcell *et al*., 2001; Bose *et al*., 2010).

In developing countries with poor sanitation conditions, fecal contamination of drinking water is main cause of acute hepatitis E epidemic form. In industrialized countries, acute cases of hepatitis E were reported in travelers returning from endemic regions although sporadic cases have also been reported in patients with no known epidemiological risk factors (Clemente-Casares *et al*., 2003).

Hepatitis E is worldwide distributed and the cause factors include tropical climates, inadequate sanitation and poor personal hygiene. It is most predominant in developing countries near the equator, in both the Eastern and Western hemispheres. Outbreaks are always associated with rainy seasons, floods, and overcrowding. HEV is considered hyperendemic in many developing countries such as India, Bangladesh, Egypt, Mexico and China. Hyperendemic countries carry an HEV prevalence of 25% of all non-A, non-B, acute hepatitis cases or have experienced a major waterborne outbreak of hepatitis E according to the Centers for Disease Control and Prevention.

All HEV strains belong to a single serotype, but based on genetic diversity of HEV sequences, four phylogenetically distinct HEV genotypes (Emerson *et al*., 2005) and 24 subtypes (Lu *et al*., 2006) have been defined. However, due to the recent identification in various animal species (wild Norway rats, Rex rabbits, wild boars and bats) of HEV strains genetically distinct to the four recognized HEV genotypes, the genotypic classification of HEV might evolve (Drexler *et al*., 2012). For example, the HEV recovered from rats and bats in studies conducted in Germany appear to belong to two new mammalian HEV genotypes based on phylogenetic analysis (Drexler *et al*., 2012). The geographical distribution of HEV genotypes is complex and changing. Genotypes 1 and 2 only infect humans and are responsible for both epidemic and sporadic hepatitis E cases occurring in tropical and sub-tropical countries (Teshale *et al*., 2010; Vivek *et al*., 2010; Delarocque-Astagneau *et al*., 2012). Genotypes 3 and 4 have been shown to infect not only humans, but also domestic animals throughout the world, especially pigs and wild boars (Kaba *et al*., 2011; Wilhelm *et al*., 2011; Colson *et al*., 2012). Genotypes 3 or 4 are responsible for autochthonous sporadic hepatitis E cases in America, Europe, Oceania and Asia (Lewis *et al*., 2010; Miyamura, 2010; Wilhelm *et al*., 2011). The genotype 4 of HEV, which is indigenous to Asia, was described in swine in Belgium and in travel-unrelated hepatitis E cases in Germany and France (Wichmann *et al*., 2008; Colson *et al*., 2012). Trends throughout the World point to a continued high anti-HEV seroprevalence and HEV infection likely due to increases in interest, awareness and surveillance efforts as well as increased spread among known animal reservoirs and hosts (Pavio, 2010; Mansuy *et al*., 2011). Seroprevalence reports vary dramatically from country to country and study to study with some studies reporting overall declines in seroprevalence over time,

while other yield continued high levels of seroprevalence (Purdy *et al*., 2010).

Prevalence of anti-HEV IgG tends to increase with age, especially in men (Danielle *et al*., 2013). Humans and other animals excrete a considerable amount of virus early in the acute phase of HEV infection and likely contribute to maintain the cycle of endemicity.

Transmission of HEV occurs primarily by the fecal-oral route through fecal contamination of drinking water in developing countries. HEV may also be transmitted parenterally as well as vertically particularly in endemic areas, but person to person transmission is uncommon (Ataei *et al*., 2009).

The aim of this work was to study the prevalence of HEV-IgM antibodies in donations of blood donors who were referred to blood banks at Aseer region, KSA.

MATERIALS AND METHODS

Collection of Samples

Accepting donors for blood donation was done according to routine practice and roles of Saudi Ministry of Health. Blood samples were obtained from healthy blood donor volunteers, who were referred to blood transfusion centers found at Aseer region (Southern part of KSA), during the period from March 2012 to January 2013 after singing of informed consent.

Blood Bank Routine Serological Tests

All Sera of accepted donations were tested for HBsAg, anti-HBc antibodies (Abs), anti-HCV-Abs, anti-HIV-1/2-Abs, anti-HTLV-I/II-Abs, Malaria, and Treponema Abs according to predefined protocol of blood banking safety requirements by Saudi Ministry of Health.

Nucleic Acid Test (NAT)

All samples were tested for the presence of HBV, HCV, and HIV nucleic acids by NAT using Roche COBAS® TaqScreen MPX Test which is a qualitative multiplex test that enables simultaneous screening of HIV-1 Group M and Group O RNA, HIV-2 RNA, HCV RNA, and HBV DNA in pooled and individual plasma donations.

Detection of Antibodies Type M against HEV (HEV-IgM)

Detection of HEV IgM was done using commercially available enzyme immunoassay for the determination of IgM antibodies to hepatitis E virus in human serum or plasma (DIA.PRO, Italy). Confirmatory test to HEV-Ab was done using HEV IgM Antibody ELISA Kit (ANOGEN, Canada).

Detection of HEV RNA by RT-PCR

Anti-HEV IgM suspected positive samples were tested for the presence of HEV RNA. Total RNA was purified from sera using QIAamp Viral RNA Mini Kits (Qiagen, Germany). In detection of HEV-RNA, primers had been previously confirmed to detect all 4 known mammalian HEV genotypes were used (Inoue *et al*., 2006, Huang *et al*., 2010, Ibrahim *et al*, 2011). Reverse transcription and PCR amplification was performed using ready to go RT-PCR beads (GE Health care) according to the manufacturer's directions.

Statistical Analysis

The biochemical data recorded were expressed as mean±SD and statistical and correlation analyses were undertaken using the one-way ANOVA followed by a post-hoc LSD (Least Significant Difference) test. A P value < 0.05 was statistically significant. A statistical analysis was performed with the Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA).

RESULTS

Following the donor selection criteria, 26 females (0.36%, median age of 28) and 7241 males (99.64%, median age of 30) were accepted to donate their blood. Donors of ages between 21 and 30 years constituted the largest proportion

(50.52%, P ≤ 0.001) with a median age of 26 years (Table 1). The blood donors were from 15 countries. The majority of donors were Saudis (95.13%) followed by Yemenis (1.58%) and then Egyptians (1.3%) (Fig. 1).

Table 1: Age ranges of accepted volunteers for donation.

Age range	Number	Median
18-20	429	20
21-30	3676	26
31-40	2203	35
41-50	786	45
51-60	173	55
Total	**7267**	**30**

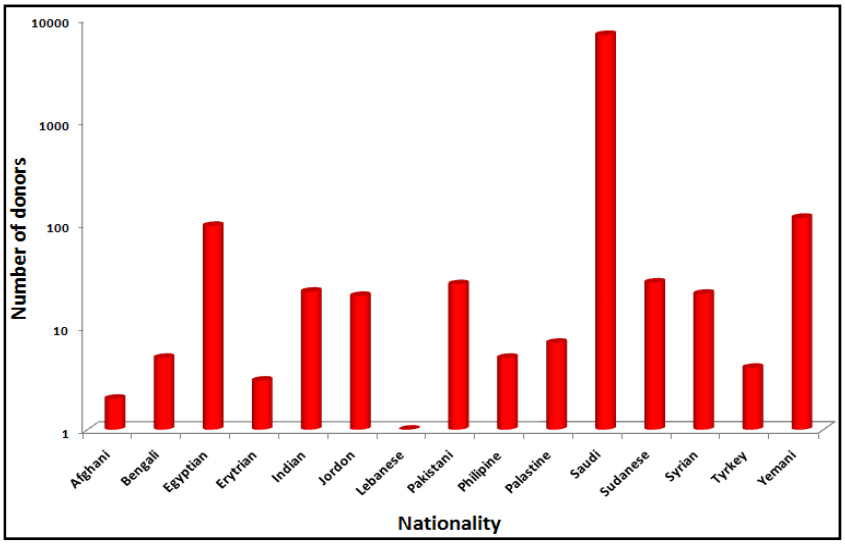

Fig. 1: Numbers and nationalities of accepted blood donors for donation.

Serological screening of samples resulted in positivity to many different markers (Table 2). Two (0.028%) positive cases to HIV-1/2, one is 22 years old with positive markers for HBsAg and HBcAb and the second is 33 years old with no other associated markers. Detection of HIV-RNA by PCR showed that there were no RNA for HIV in samples confirmed positive by ELISA. Two (0.028%) positive cases to anti-*Treponema pallidum* antibodies, one is 33 years old and the second is 36 years old both with positive markers for HBcAb. There were 5 (0.069%) positive cases to HCV-Ab, 2 of them (0.028%, 36 and 45 years) were positive for HCV-RNA as confirmed by PCR. Only one (0.014%) case, in addition to positivity to HCV-Ab, showed positivity to HBcAb. Detection of HBsAg resulted in 71 positive cases. There were 70 cases positive to HBcAb and one case positive for HIV. Cases positive for HBV-DNA by PCR were 66. Cases positive for HBcAb and HBV-PCR were 65 and negative for HBV-DNA by PCR were 5. There were no coinfection with either HCV or HTLV viruses. Screening of samples for HBcAb resulted in 449 positive cases. Of these cases there were 69 positive to HBsAg and one positive for HIV. Cases positive for HBcAb and HBV-DNA were 78 and negative for HBV-DNA by PCR were 371. There were no HBcAb cases companied with HCV, syphilis and HTLV markers. No positive samples for HTLV-1/2 antibodies were found.

Table 2: Serological marker after screening of accepted donors for donation.

Marker	Syphilis	HBsAg	HBcAb	HCV	HIV	HTLV	NAT
HCV	0	0	1	5	0	0	2
HBsAg	0	71	70	0	1	0	66
HBcAb	0	69	449	0	1	0	78
HIV	0	1	1	0	2	0	0
Syphilis	2	0	2	0	0	0	0
HTLV	0	0	0	0	0	0	0

Screening for HBV, HCV and HIV nucleic acid by NAT resulted in 88 positive cases. Cases positive for NAT and associated with other positive markers were as follow; 66 cases were positive to HBsAg, one case was positive to HCV-Ab and 79 cases positive to HBcAb. Mixed infections were one positive case with positive markers for HCV-Ab and one HBcAb with HIV-ab and 63 positive cases with positive markers for HBsAg and HBcAb (Table 3).

Table 3: Other markers associated with NAT positive samples.

Marker	HCV	HBcAb	HIV	HBsAg
HCV	2	1	0	0
HBV	0	65	0	1
HIV	0	0	0	0
HBcAb	1	78	0	65

Primary screening of samples for HEV-IgM resulted in one sample (male, 28 years) at borderline. Confirmatory test to that sample also was 0.95 % of cut off value. Detection of HEV-RNA showed that there was no HEV-RNA present in the sample. The sample was also negative for all other markers tested.

DISCUSSION

Hepatitis E virus (HEV) is a non-enveloped, single stranded RNA virus belonging to the family Hepeviridae (Hoofnagle *et al*., 2012; Kamar *et al*., 2012). In developing countries, HEV is a major cause of acute hepatitis, transmitted by the fecal-oral route and associated with contamination of drinking water. In industrialized countries, reports of HEV infection have been uncommon but are being reported more frequently (Ijaz *et al*., 2009). Prospects for control of HEV infection are encouraged by recent efforts in vaccine development (Shrestha *et al*., 2007; Zhu *et al*., 2010).

Zoonotic transmission of HEV genotypes 3 and 4 to humans can occur by consumption of contaminated meat or meat products or by contact with infected animals (Colson *et al*., 2010; Wenzel *et al*., 2011). Shellfish, such as bivalve mollusks, have also been shown to act as reservoirs for HEV (Crossan *et al*., 2012).

The fact that an alternate route of transmission of HEV by transfusion of blood components has been reported in Japan (Matsubayashi *et al*., 2004; Matsubayashi *et al*., 2008), the United Kingdom (Boxall *et al*., 2006), and France (Colson *et al*., 2007; Haim-Boukobza *et al*., 2012) pushed us to study the probability of the presence of HEV in blood of blood donors in Saudi Arabia. Also, where there are only a few epidemiological reports on the prevalence of acute hepatitis E antibodies in Saudi blood donors have been published, this study, therefore, aimed to assess acute HEV infection through detection of HEV-IgM prevalence in Aser Region, Saudi Arabia.

Studies in Japan (Sakata *et al*., 2008) and the People's Republic of China (Guo *et al*., 2010) have identified acute HEV infections in blood donors, confirmed by the detection of HEV RNA. Analysis of blood and plasma donors in Europe has identified HEV-infected donors in Germany (Baylis *et al*., 2012; Vollmer *et al*., 2012; Corman *et al*., 2013), Sweden (Baylis *et al*., 2012a) and England (Ijaz *et al*., 2012). Transmission of HEV by solid organ transplantation has also been reported (Schlosser *et al*., 2012). Rates of HEV infection may be underreported in some countries, and misdiagnosis of HEV infection also occurs. For example, in some cases of suspected drug-induced liver injury, HEV has been determined as the cause (Davern *et al.,* 2011). In one such recent case, HEV was shown to have been transmitted by blood transfusion (Haim-Boukobza *et al.,* 2012).

Parenteral exposure to blood is the most frequent mode of transmission, especially related to blood transfusion before the systematic practice of testing for HBV and HCV in blood donations, in 1986 and 1992, respectively (Rivera-López *et al*., 2004). Since then, laboratory screening of potential blood donors has relied on the use of evolving immunoassays to detect viral antibodies or antigens.

The availability of a reliable source of safe blood and blood products is essential for medical practice. Blood donors in Saudi Arabia and other countries such as Australia are screened for syphilis, hepatitis B surface antigen, and antibodies for HIV types 1 and 2, hepatitis C virus (HCV) and human T cell lymphotropic virus (HTLV) types I and II. These screenings of blood donors and modern blood product manufacturing techniques have greatly reduced the risk of transmission of serious disease by transfusion (Schreiber *et al*., 1996; Ibrahim *et al*., 2014).

Hepatitis E virus (HEV) has been recognized since 2004 as a transfusion transmissible infectious agent and recent epidemiological data suggest that it may pose a safety threat to the human blood supply. Although there have been no cases of transfusion transmitted infection reported in the U.S., there are documented cases from Japan, the United Kingdom, Saudi Arabia and France (Boxall *et al*., 2006; Colson *et al.,* 2007; Tamura *et al.*, 2007; Matsubayashi *et al*., 2008). Although documented cases of HEV infection are uncommon in the KSA some studies have detected a high prevalence of antibodies to HEV in blood donors suggesting the possibility of transmission by blood transfusion (Meng *et al*., 2002; Xu *et al*., 2012).

In the present study, routine screening of donated blood included detection of HBsAg, HBc-Ab, HCV-Ab, HIV-1/2 Ab, HTLV-I/II-Abs, malaria, and syphilis-Ab as well as, nucleic acid test technology for HBV-DNA, HCV-RNA and HIV-RNA. Non-routine screening included test for anti-HEV IgM and HEV-RNA. In this work all ELISA tests were valid as it met the criteria indicated by the manufacturers.

Only voluntary non-remunerated blood donors were used. All donors were selected according to recommendations of WHO (TRS No.840, 1994). Professional donors were avoided as they are not used for donation in Saudi Arabian blood donation centers. Health check and Pre-donation counseling were applied in the present study. The questionnaire prepared by Saudi MOH is quite enough to cover required data. Many nationalities were included in the study to cover the majority of residents. The majority of the donors were Saudi Arabian which facilitated the judgment on Saudi Arabia population. Majority of blood donation was among men than in women with average age of 24 years old. This may be due to social considerations.

The age range 21-30 years old constituted the largest population among blood donors. The percentage of non-Saudi (expatriates) donors was low. A similar study conducted on blood donors in Saudi Arabia by El-Hazmi (2004) also showed that the largest group of donors was those at age range 20-29 years old and female donors were as low as 1.2% at the year 2000 and declined to reach 0.7% by the year 2002. Also El-Hazmi (2004) showed that the percentage of non-Saudi donors declined from 17.2% at the year 2000 to reach 14.8 by the year 2002. Also Ankra-Badu *et al*. (2001) previously showed that the proportion of Saudi blood donors increased with the decrease in the non-Saudis blood donors.

In the present study, blood donors were screened for the presence of HBsAg, anti-HBc, and HBV-DNA. It was found that large number of anti-HBc carrier with or without HBsAg positivity. According to De Villa and coworkers (2003), HBcAb positivity with HBsAg (−) status can reflect a number of situations: (1) it may indicate a false-positive result, so in the present study positive cases were confirmed using different detection kit; (2) it may represent past and currently healed infection, and this is why we in the present study screened units for HBsAg positivity; and (3) it may constitute the sole marker of occult HBV infection, which is thus potentially transmissible, as has been demonstrated by contagion occurring through blood transfusion from donors who are only HBcAb (+) (Hoofnagle *et al*. 1978).

In the present study, it was found that HBsAg positive cases were low while HBc-Ab positive cases were relatively high. Similar work done by Panhotra and coworkers (2005) on blood donors showed that 1.9% were HBsAg positive alone, 3.2% were anti-HBc positive alone, and 10.1% were both anti-HBc and anti-HBsAg positive. In the current study, we did find only one case positive for HBsAg alone which means that the presence of HBsAg infection alone in Aseer region is low. It was previously shown that there is a decline in hepatitis B viral infection in South-Western Saudi Arabia and it was attributed to the effectiveness and efficacy of the integration of hepatitis B vaccination into the extended program of immunization in KSA. The significant decline of HBV markers among unvaccinated Saudi adults indicated an indirect effect of other factors (for example, health education and socio-economic progress) on the prevalence and transmission of HBV. In areas of high endemicity, the epidemiological characteristics HBV are modified significantly by the combination of HBV vaccination and other complimentary control strategies (Ayoola *et al*. 2002).

Occurrence of HBsAg positivity among subpopulation was the lowest in 50-60 years old group, while it was zero in young population (group 18-20 years old). Vaccination against HBV was introduced in 1989 for all infants at birth and in 1990 for school children (Al-Faleh 2003). This may be the most important factor responsible for the decline in HBV infection (Al-Faleh 2003).

A study done by El-Hazmi (El-Hazmi 1989) was conducted on male and female population in different provinces of Saudi Arabia. The overall prevalence of hepatitis B surface antigen (HBsAg) was high (16.7%) and no significant difference was encountered between the rate in males and females. 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. The antibodies anti-HBs and anti-HBc were encountered in 30-67% of the individuals in different provinces, suggesting that a significant number of Saudis were

already immune to HBsAg before they reached adulthood.

The presence of HBV-DNA in HBsAg positive samples was in nearly all cases except one. This indicates to how much these blood units are highly infective. Also, HBV-DNA was found in high percentage (17.76%) of HBc-Ab positive cases. This indicated that positive blood units for HBc-Ab should be discarded as it carries high possibility of infectivity. Current infection showing HBsAg and HBc-Ab in the same time was high (16.55%). In the time where HBc-Ab positive cases with HBV-DNA negative is more than those cases with HBV-DNA positive, it still not secure to use these units in blood transfusion. The virus may be found in polymorhonuclear cells or other places other than serum or plasma used for the detection of HBV-DNA (Catterall *et al*. 1994).

Hepatitis C is a contagious liver disease that results from infection with the hepatitis C virus. It can range in severity from a mild illness lasting a few weeks to a serious, lifelong illness. The hepatitis C virus is usually spread when blood from an infected person enters the body of a susceptible person. About 150 million people are chronically infected and at risk of developing liver cirrhosis and/or liver cancer. More than 350 000 people die from hepatitis C-related liver diseases every year (WHO, Fact sheet N°164, 2014).

The current study showed that HCV infection is very low with complete absence of infection in age ranges 18-20 and 51-60 years old with only 2 cases having HCV-RNA positivity. Since the discovery of HCV in 1988 and introduction of diagnostic tests for this virus, data on HCV prevalence has accumulated in Saudi Arabia. In 1989, a baseline community randomized study in Jizan was done on children from the age of 1 to 10 years for prevalence of HCV. The average prevalence was calculated to be 0.87% (Al-Faleh *et al*., 1991). Another study in the same region in 1991 but on the adult population over the age of 10 years was done. The average prevalence was only 1.8%, which increased with age, reaching 3.5% in persons over 50 years (Al-Faleh *et al.,* 1995). A study done on children aged 1-12 years in 13 regions of KSA in 1997, showed that the prevalence of HCV was only 0.04%. Al-Faleh (2003) also analyzed Saudi blood donors from 1996 to 2001 at KKUH and demonstrated that the prevalence of HCV decreased steadily from 0.58% in 1996 to 0.28% in 2001, which is a decline among blood donors of more than 50% over 4 years. In the present study, prevalence of HCV-Ab was shown to be very low which is agreements with the above study done by Al-Faleh (2003).

Sexually transmitted diseases such as HIV and syphilis showed very low prevalence. This low prevalence may be attributed to conservative social and religion behaviors in Saudi Arabia. Fageeh (2010) showed that the prevalence of HIV among the blood bank donors is similar in KAUH and KFH, 0.01% vs. 0.089%. This is found to be much lower than the incidence in pre-operative cases. The fact that those groups of people knew ahead they were being tested accounted for these low figures in comparison to unsuspecting individuals.

It was reported that adult T-cell leukemia (ATL) is a distinct clinical entity (Takatsuki *et. al.,* 1977; Uchiyama *et. al.,* 1977). The disease which is characterized by its aggressive clinical course, infiltrations into liver, skin, lung and gastrointestinal tract, hypercalcemia and the presence of leukemic cells with multilobulated nuclei. In 1980, the human retrovirus was discovered by Poiesz *et al*. in a cell line derived from a patient with ATL, and designated it human T-cell leukemia virus type I (HTLV-I) (Poiesz *et. al.,* 1980; Gallo, 2005). Later, Hinuma *et al*. proved the

linkage between ATL and HTLV-I by demonstrating the presence of an antibody against HTLV-I in patient sera (Hinuma *et al.,* 1981). The whole sequence of HTLV-I was then determined by Seiki *et al*. (1983) revealed the presence of a unique region, designated pX. The pX region encodes several accessory genes, which control viral replication and the proliferation of infected cells (Yoshida *et al.,* 2001).

In the current study, 7267 blood donors were screened for the presence of HTLV-I/II.

We did not get any seropositive case in screened donated blood either in Saudi or non-Saudi blood donors.

All positive cases for HBV, HCV, HIV-Ab and Syphilis were disqualified and rejected from testing for HEV-IgM.

HEV infection is diagnosed on the basis of detection of specific antibodies (IgM and IgG), but the sensitivity and specificity of these assays is not optimal (Bendall *et al*., 2010; Drobeniuc *et al*., 2010; Rossi-Tamisier *et al.,* 2013). Analysis of HEV RNA by using nucleic acid amplification techniques (NATs) is also used for diagnosis; this method can identify active infection and help confirm serologic results (Huang *et al*., 2010). Several NAT assays have been reported for the detection of HEV RNA in serum and plasma or fecal samples: conventional reverse transcription PCR (RT-PCR) and nested protocols (Meng *et al.,* 2001), real-time RT-PCR, and reverse transcription loop-mediated isothermal amplification (Lan *et al.,* 2009). The NATs include generic assays designed for the detection of HEV genotypes 1-4 (Jothikumar *et al.,* 2006).

In the present study HEV-IgM was screened in donated blood showed negativity to all tested virus and bacteria. All samples were negative for HEV-IgM. Similar study was done by Al-Knawy *et al.* (1997) in southern part of Saudi Arabia and demonstrated that clinically apparent HEV infection does not appear to be common in the population studied at that time, despite the fact that a large sector of expatriates from endemic areas work in this region. These data seem to confirm those reported from Spain (Buti *et al*., 1995) and France (Pham *et al.,* 1994). In the Spanish study, none of the examined patients with acute sporadic hepatitis had evidence of acute HEV infection, while in the French study, only one case was documented with a recent history of travel to Pakistan. Another study done by Ayoola *et al.* (2002) to determine the prevalence of HEV-IgM among haemodialysis patients and showed that none of the ward (control group) patients was positive for IgM anti-HEV.

In the time our results showed 0% HEV-IgM, other workers in Saudi Arabia showed 4.3% in Makka (Johargy *et al.,* 2013) and 0.3% in healthy control in Gizan (Ayoola *et al.,* 2002). Worldwide reports stated that prevalence rates of 0.94% in China to 3.6% in Hong Kong (Guo *et al.,* 2010). However, the presence of anti-HEV is not a measure of infectivity, and no tests are available that would be appropriate for hepatitis E.

In the present study it was found that all suspected HEV-IgM cases are in age rage 24-36 with one case at 47 years old. Similar results were obtained by Johargy_*et al.* (2013) on study done in Gizan, Saudi Arabia and found that the greatest number of positive individuals for IgG antibodies in the age group 24-35 years, followed by a decline in the older age groups but in contrast to another in which seroprevalence increased significantly with age (Mathur *et al.,* 2001; Ayoola *et al.,* 2002). A significant proportion of acute viral hepatitis occurring in young to middle-aged adults in Africa, Asia and the Indian subcontinent is caused by hepatitis E virus (Bradley, 1995).

Healthy blood donors in the pre-icteric phase of the disease and presumably infectious, could transmit

HEV to recipients of their blood. The importance of HEV in relation to blood transfusion practices stems from the possibility that there is evidence that the virus could be transmitted parenterally. The blood products that theoretically carry the risk of transmission of HEV are packed red cells, whole blood or platelet concentrate collected from an asymptomatic donor during the viraemic phase (Ding *et al*., 2003; Johargy *et al.*, 2013). Therefore, it is imperative to select tests with good sensitivity, specificity and accuracy in carrying out seroprevalence studies.

CONCLUSION

The results emphasize the need to initiate more studies on the prevalence of HEV in other parts of Saudi Arabia. Besides, they also highlight the dangers of using blood donor data in lieu of epidemiologically sound, population-based observations. Vaccination program against HEV is still needed to prevent future outbreaks.

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