

Prevalence of Hepatitis B Surface Antigen and its Association with Anti-Hepatitis C Virus Antibodies among Pilgrims

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Abstract

The present study aimed to evaluate the prevalence of the asymptomatic carriers of hepatitis B surface antigen among pilgrims; to demonstrate its distribution in different nationalities, age groups, and gender. The prevalence of hepatitis B surface antigen, among 982 apparently healthy pilgrims was determined using enzyme-linked immunosorbent assay. Positive samples were confirmed by the MiniVIDAS system. Positivity rate was 4.1%, and was higher in males (87.5%), age group of 40-59 years (60%) and in Nigerian pilgrims (8 pilgrims). All samples were also analyzed for antibodies against hepatitis C virus using enzyme-linked immunosorbent assay. Only one sample was positive for both hepatitis B surface antigen and hepatitis C virus antibodies. It was concluded that there was an intermediate endemicity (4.1%) with significant ($P < 0.05$) difference between the rate in the different age groups but not between the rate in males and females nor in nationalities. Nationalities with low frequencies of pilgrims should not be neglected as a source of infection. No association between the presence of hepatitis B surface antigen and hepatitis C virus antibodies. Performance of this study (with genotyping) on a large scale and genotyping of the 40 positive samples is recommended..

Keywords

HBsAg prevalence, Hepatitis B Virus, Pilgrims, Hajj, ELISA.

Introduction

Over two million pilgrims from over 170 countries congregate annually in Makkah (the holiest place in Islam) in Saudi Arabia to perform Hajj. During Hajj, the chances of disease are high; not only from heat exhaustion and sunstroke, but also from infectious agents such as hepatitis B virus (HBV) which can be transmitted via blood and its products, bodily fluids, and the sharing of tools (toothbrushes or razors) that had been used by an infected person^[1-5]. Approximately 350 million people (7%) of the world's population are infected

with HBV^[6], and about 0.6 million people die annually due to this infection^[7]. According to the rate of carriers, the globe has three categories of endemicity: high [$\geq 8\%$ hepatitis B surface antigen (HBsAg) positivity], medium (2–7% HBsAg positive), and low ($< 2\%$ HBsAg positive). Almost 45% of the global population live in areas of high endemicity, 43% live in intermediate endemicity areas and 12% live in low endemicity areas^[8].

Areas of high endemicity in the Middle East include Saudi Arabia, Oman, Yemen, Palestine, Jordan, and Egypt; while the United Arab Emirates, Iraq, and Cyprus

have intermediate endemicity; and Kuwait, Bahrain, and Iran have low endemicity^[9].

“Dane particle” (that is the hepatitis B virion) is infectious (can infect hepatocytes); its outer envelope contains surface proteins^[10]. Non-infectious particles (spherical and filamentous) lacking a core can be present in the serum of infected individuals; they are composed of the protein and lipid that forms part of the surface of the virion (“Dane particle”), and thus are called the surface antigen (HBsAg)^[11]. Antibodies against the hepatitis B core antigen (anti-HBc) and HBsAg are the most diagnostic markers for hepatitis B^[12]. A person that has cleared an infection or has been vaccinated previously will become negative for HBsAg, which will be replaced by antibodies (anti-HBs IgG and anti-HBc IgG)^[13]. Individuals who remain HBsAg positive for six months or more are known as carriers^[14]. Methods for detection of HBsAg include latex agglutination or haemagglutination (HA) and enzyme-linked immunosorbent assay (ELISA); the first two are cheap and fast, but are not as sensitive as ELISA^[15]. In addition to these tests, PCR tests have been developed to detect and measure the amount of HBV DNA (called the viral load) in clinical specimens. These tests are used as confirmatory to assess a person’s infection status and to monitor treatment^[16].

HBV can be transmitted by three categories: horizontal of adult (such as intravenous drug use and sexual contact), horizontal of early life (such as lesions, bites and sanitary habits) and vertical during childbirth^[17]. Infected items and surfaces such as razor blades, blood, stains, and benches are infectious, as the HBV- infectivity is stable for about a week on infected items and surfaces^[18-20]. The pathogenesis of liver disease for both HBV and HCV shares many common features, although the two viruses differ in their virological properties, survival strategies and their immune escape^[21]. Hepatitis B coinciding with HCV infection had been reported^[21-25] and found to be responsible for both the deterioration of the disease and towards its transition into its chronic phase^[22]. In addition, HBV replicative states may influence HCV replication, indicating possible interference between the 2 viruses^[23]. Marusawa et al. provided evidence for the high prevalence of anti-HBc in patients with HCV-related chronic liver disease^[24].

Objectives

This study aimed to evaluate the prevalence of the asymptomatic carriers of hepatitis B virus (mainly HBsAg) among pilgrims, to demonstrate their distribution in different nationalities, in various age groups and between both sexes; and to assess their association with anti-hepatitis C viruses (anti-HCV) positivity.

Materials and Methods

Pilgrims and Samples

Subjects (pilgrims) were selected at random from pilgrims seeking medical advice for minor ailments such as headache, heat exhaustion, fatigue, etc., in Hajj hospitals at Makkah and Mina. After excluding overtly ill subjects and those who had refused to give blood samples, the rest (1000) were subjected each to an inquiry, comprising their nationality, age and sex. These data have been compiled on the computer. Venous blood (10 ml) was withdrawn from each subject. Blood was then allowed to clot and centrifuged. The clear serum was isolated, divided into three aliquots and stored at -75°C until used. However, only 982 serum samples were included in this study because 18 samples were found to be lysed and insufficient to perform all tests.

Sampling of the subjects was according to previous approval from KAU, Ministry of Health at Makkah, from the authorities in Hajj hospitals at Makkah and Mina, from the Custodian of the Two Holy Mosques Institute of the Hajj Research at Umm Al-Qura University in Makkah.

Chemicals, Reagents and Kits

Samples (sera that were stored at -75°C) were analyzed for HBsAg by ELISA (Biokit S.A., Lliçà d’Amunt, Barcelona, Spain). The HBsAg-positive samples were confirmed by the MiniVIDAS System (*bioMérieux / Marcy-l’Etoile, Marcy l’Etoile, France*). All samples were also analyzed for anti-HCV antibodies (HCV-Ab) by a third generation ELISA (Bio Eliza HCV- Biokit S.A., Lliçà d’Amunt, Barcelona, Spain). All of these tests were performed according to the instructions provided with the kits. Reverse transcription-polymerase chain reaction (RT-PCR)^[26] for HCV was performed for the sample which was positive for both HBsAg and HCV-Ab by COBAS® ampliscreen HCV test version 2.0 kit from Roche Diagnostics (F. Hoffmann-La Roche Ltd., Indianapolis, IN USA) by using COBAS® Amplicor instrument.

Statistical Analysis

The data were analyzed using Statistical Package for Social Science (SPSS) software, Version 16 (SPSS Inc., Chicago, IL USA).

Results

The total number of pilgrims was 982, 15(1.5%) of them were Saudi while 967 (98.5%) were non-Saudi, from

Table 1. Distribution of HBsAg between males and females.

			HBsAg		Total
			Positive	Positive	
Sex	Females	Count	179	5	184
		% within Sex	97.3%	2.7%	100.0%
		% within HBsAg	19.0%	12.5%	18.7%
	Males	Count	763	35	798
		% within Sex	95.6%	4.4%	100.0%
		% within HBsAg	81.0%	87.5%	81.3%
Total		Count	942	40	982
		% within Sex	95.9%	4.1%	100.0%
		% within HBsAg	100.0%	100.0%	100.0%

$\chi^2 = 1$; $df = 1$; $P > 0.05$; Odds ratio(OR) 1.6; 95% CI 0.6,4.2

Table 2. Distribution of HBsAg between age groups

Age Groups (Years)	HBsAg		Total
	Negative	Positive (%)	
< 20	12	0 (0%)	12 (1.2%)
20-29	159	2 (5%)	161 (16.5%)
30-39	234	7 (17.5%)	241 (24.5%)
40-49	214	12 (30%)	226 (23%)
50-59	167	12 (30%)	179 (18.2%)
60-69	115	6 (15%)	121 (12.3%)
70-79	37	1 (2.5%)	38 (3.9%)
> 80	4	0 (0%)	4 (0.4%)
Total	942	40 (100%)	982 (100%)

thirty-nine different nationalities. The number of males was 798 (81.3%) while female was 184 (18.7%). The total number of positive HBsAg was 40 (4.1%) distributed among 13 nationalities. The mean age of pilgrims that were positive HBsAg was 47.7 years (SD 11.6) among age group 24-75 years. The total number of positive HBsAg and anti-HCV was 1 (.1%). Statistically, there was no significant association between HBsAg and anti-HCV ($P > 0.05$). Distribution of the HBsAg positivity between both sexes revealed that 87.5% were male (M), and 12.5% were female (F) (Table 1). No significant difference was encountered between the rate in gender (males and females) and HBsAg positivity ($X^2 = 1$ - $df = 1$ - $P > 0.05$ -Odds ratio (OR) 1.6; 95% CI 0.6, 4.2).

Distribution of the HBsAg positivity according to the age groups (Table 2) showed a peak (30%) in age groups 40-49 and 50-59, while the age groups >80 and < 20 did not have any positive results (0.0%). Thirteen nationalities were found to have positive HBsAg by ELISA and by MiniVIDAS system, among which Guinea showed the highest prevalence 50% (Table 3). Distribution of the 40 HBsAg positive pilgrims according to the nationality showed that Nigerians had the highest percentage 8/40 (20%) (Table 3).

However, only one sample was positive for both HBsAg (by ELISA and MiniVIDAS system) and HCV-Ab (by ELISA). This sample was further tested for HCV RNA by RT-PCR and was negative (RNA level < 60 IU/mL) using COBAS® amplicscreen HCV test version 2.0 kit and

Table 3. Percentage % of nationalities and distribution of HBsAg between nationalities % and within each nationality (%).

Nationality	HBsAg		Total
	Negative	Positive	
Nigerian	20	8 (28.5%) 20%	28 (100%) 2.85%
Ethiopian	134	7 (4.9%) 17.5%	141 (100%) 14.35%
Somali	58	6 (9.3%) 15%	64 (100%) 6.52%
Egyptian	227	4 (1.73%) 10%	231 (100%) 23.53%
Yemeni	63	4 (6%) 10%	67 (100%) 6.82%
Bangladeshi	20	2 (9%) 5%	22 (100%) 2.25%
Syrian	69	2 (2.8%) 5%	71 (100%) 7.24%
Pakistani	88	2 (2.2%) 5%	90 (100%) 9.16%
Afghani	34	1 (2.8%) 2.5%	35 (100%) 3.56%
Jordanian	2	1 (33.3%) 2.5%	3 (100%) 0.3%
Guinean	1	1 (50%) 2.5%	2 (100%) 0.2%
Saudi	14	1 (6.66%) 2.5%	15 (100%) 1.53%
Sudanese	65	1 (1.5%) 2.5%	66 (100%) 6.72%
Others (26)	147	0 (0%) 0.0%	147 (100%) 14.97%
Total	942	40 (4.1%) 100%	982 (100%) 100%

Table 4. Linear Regression Analysis (Stepwise Model) of factors related to HBsAg positivity.

Population	Model	Unstandardized Coefficients		Significance	Excluded	R ²
		B	Std. Error			
Pilgrims No = 982	Age	0.001	0.000	0.04	Sex Nationality Anti-MCV	0.4
	Age-groups	0.009	0.004	0.045		

COBAS® Amplicor instrument (please see Materials and Methods). The sample was related to a 46 year old Egyptian male pilgrim.

Multiple logistic regression analysis (Table 4) was performed using HBsAg positivity as the dependent variable. The following were included as independent variables: Gender, age, age group, HCV positivity, and nationality. Only age or age-group were included ($P < 0.05$) in the regression model.

Discussion

Forty samples were found positive for HBsAg by ELISA and the MiniVIDAS system. Hepatitis B virus (HBV DNA), in clinical specimens, can also be detected and measured by PCR^[16]. This PCR was not performed because HBsAg positivity was confirmed by ELISA and MiniVIDAS system, and due to the fact that individuals who are positive for HBsAg (for six months or more) are considered as carriers^[14]. However, PCR will be essential for future genotyping studies.

Only one sample was positive for both HBsAg and anti-HCV antibodies. This sample was negative for HCV-RNA by RT-PCR. Samples of positive HCV-Ab with negative PCR has also been found in other reports^[27,28]. This status can be attributed to a viral amount below the detection limit of PCR^[29], which may happen during the convalescent period in which the patient may lose HCV-RNA^[30,31]. Other possibilities (that are not applicable to this sample) have been reported: an inhibition of PCR by the presence of heparin in the collected samples^[32]; or improper storing and/or repeated thawing and freezing which may lead to some loss of the RNA^[33]. The sample was stored at -75°C and used only once to avoid repeated thawing and freezing. Indeed, co-infection with the two viruses (HBV and HCV) has been mentioned in several reports, especially in highly prevalent areas and in people at high risk for parenteral infection^[34]. However, the reason why this study only had one pilgrim with dual positivity (HBsAg and HCV-antibodies) may be attributed to the fact that combined HBV and HCV infections may lead to more severe liver disease and carcinoma^[34]. Such ill patients would not be expected to be present in this cohort of pilgrims because of the difficult nature of the Hajj.

In this cohort of pilgrims, positive HBsAg were found among pilgrims from various (13) ecological

regions reflecting a wide range (from low to high) of HBV endemicity. For example, 10.1% in Egypt^[35], keeping in mind that Egyptian pilgrims comprise 23.1% of the cohort of this study, 5.7% in Ethiopia^[36] and 3-5% in Pakistan^[37].

In this study, the overall prevalence (4.1%) of HBsAg was of intermediate endemicity. An estimated 45% of the global population lives in regions where chronic HBV infection is endemic, including the Pacific Islands, Africa, Asia the Middle East - several countries in the Middle East have an intermediate or high endemicity of HBV infection^[38].

Within nationalities there was no significant correlation ($P > 0.05$) between the frequency of pilgrims and HBsAg positivity, as among two samples that were tested from Guinea, one was positive (50%), 1/3 (33.3%) from Jordan, 8/28 (28.5%) from Nigeria and the rest ranged from 0 to 9.3%. Thus, nationalities with low frequencies of pilgrims should not be neglected as a source of infection. When the population was stratified based on age, a peak pattern was obtained, in which there was an increase of HBsAg positivity until the positivity was highest [24/40 (60%)] in the age group of 40-59 years, then decreasing with age; with a significant difference ($P < 0.05$) between the rate in the different age groups. A peak pattern was also reported in other studies. Two recent studies, in Ghana^[39] and Pakistan^[40], reported a peak of HBsAg prevalence at the age 21-34, then the rate of infection declines with increasing age. While children aged 0-10 and the very old (> 60) age groups were much less frequently infected.

In this study, the positivity was highest among males at 35/40 (87.5%). Male pilgrims' frequency was 798 (81.3%), and female pilgrims' frequency was 184 (18.7%). No significant difference (and no correlation) was encountered ($P > 0.05$) between the rate in gender (males and female) and HBsAg positivity. Results from this study showed HBsAg positivity prevalence being seven times more among males than females. Other reports also show that men are more positive for HBsAg than women^[41-45] even when the females' tested samples are more numerous. Some studies have suggested that the plasma clearance rate for HBsAg in males is slower compared to females, and this might be responsible for the ratio^[44,45].

Conclusion and Recommendation

Conclusion

There is an intermediate endemicity (4.1%) of HBsAg in this cohort, with significant difference ($P < 0.05$) between the rate of HBsAg positivity in the different age groups, but not between the rate in gender and in nationalities. No association exists between the presence of HBsAg and anti-HCV antibodies.

Recommendation

Nationalities with low frequencies of pilgrims should not be neglected as a source of infection. Positive HBV cases were found among pilgrims from a range of ecological regions; this may be responsible for the introduction of genotypes not existing in Saudi Arabia. Genotyping of the 40 positive samples is recommended. Performance of this study together with large scale genotyping of pilgrims and further investigations of other hidden infections that may be transmitted during Hajj in the Holy places are recommended.

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معدل انتشار مستضد السطح لفيروس التهاب الكبد (بي) (HBsAg) بين الحجاج؛ وتقييم ارتباطهم بأضداد فيروس التهاب الكبد (سي) (HVC-Ab)

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المستخلص. هدفنا تقييم معدل انتشار مستضد السطح لفيروس التهاب الكبد B (HBsAg) بين الحجاج؛ ولإثبات توزيعه في جنسيات مختلفة، في مختلف الفئات العمرية وبين الجنسين؛ وتقييم ارتباطه بأضداد فيروس التهاب الكبد C. رصدنا معدل إنتشار مستضد السطح عشوائيا بين الحجاج (٩٨٢) بواسطة التحليل المناعي اليماني أليزا (ELISA) وأكدت العينات الإيجابية لمستضد السطح (HBsAg) بواسطة نظام فيداس (MiniVIDAS). نسبة الايجابية كانت ٤,١٪. وكانت الإيجابية أعلى في الذكور في ٨٧,٥٪، وفي الفئة العمرية من ٤٠-٥٩ سنة (٦٠٪). والحجاج من نيجيريا (٨حجاج). وحللنا جميع العينات أيضا لأضداد فيروس التهاب الكبد C (HCV-Ab) بواسطة التحليل المناعي اليماني أليزا (ELISA). وكانت عينة واحدة فقط إيجابية لكلا مستضد السطح وأضداد فيروس التهاب الكبد C (HCV-Ab). نستخلص وجود توطن متوسط لمستضد السطح (HBsAg) بنسبة ٤,١٪ مع فروق معنوية ذات أهمية ($P < ٠,٠٥$) بين المعدل في الفئات العمرية المختلفة، وليس بين المعدل في الذكور والإناث والجنسيات. ويجب عدم إهمال الجنسيات ذات النسب المنخفضة من الحجاج باعتباره مصدرا للعدوى. لا يوجد أي ارتباط بين وجود مستضد السطح (HBsAg) وأضداد فيروس التهاب الكبد C (HCV-Ab). وننصح بإجراء التنميط الجيني للعينات الإيجابية الـ ٤٠، وإجراء هذه الدراسة مع التنميط الجيني على نطاق واسع من الحجاج، وإجراءها على أمراض إنتانية مستورة أخرى التي يمكن أن تنتقل أثناء الحج في الأراضي المقدسة.