Seroprevalence of Hepatitis B and C Genotypes Among Young Apparently Healthy Females of Karachi-Pakistan

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Abstract

Introduction: Although the prevalence of hepatitis virus infections in Pakistan is still unknown, limited data indicate that the exposure rate to HBV is 35-38% with 4% being carriers and 32% having anti-HBV surface antibodies through natural conversion [1,2,3]. Studies in Pakistan have shown that the prevalence rate of HCV is 4.8-14% for, and that it is continuously increasing. Hence there is an urgent need to create awareness about the prevalence of both hepatitis B and C, and to develop preventive measures aimed at minimizing the prevalence of these diseases in the country. **Study Design:** Prospective, descriptive study. The study took place from March 2002 till October 2006 at two university campuses in Karachi. **Materials and methods:** A total of 4000 healthy female students were screened for HBs Ag, anti-HBs antibodies and anti-HCV antibodies by rapid immunochromatography, ELISA and PCR. **Results:** A total of 3820 volunteers (95.5%) were negative by all three methods, 181 (4.5%) tested positive for HB surface antigen and 20 (0.5%) were positive for anti HB surface antibodies; 208 volunteers (5.2%) were positive for HCV. Double infection with HBV and HCV was found in only one patient (0.025%). Out of 180 HBs antigen positive samples 151 (83.89%) were genotype D, 28 (15.56%) showed mixed infection with genotypes B and D, and one patient (0.56%) showed mixed infection with genotypes C and D. Out of 208 samples positive for HCV antibodies, 107 (51.44%) were genotype 3a, 50 (24.04%) were mix of genotype 3a and 3b, 33 (15.87%) were genotype 3b, 10 (4.81%) were genotype 1b while, 8 (3.84%) samples could not be typed. **Conclusion:** Although the presence of these pathogenic viruses was not very high in our young healthy female population, it is still a matter of concern to control the unregulated spread of these deadly infections by promoting increased awareness and regular immunization programs in the community. Local manufacturing of vaccines and related products may reduce these infections.

Key words: HBV, HCV, healthy females, genotypes.

Introduction

In Pakistan, it is estimated that at least nine million people harbor Hepatitis B virus, and over fourteen million are chronically infected with Hepatitis C virus [1,2,11,19,21-27]. The exact prevalence rate of Hepatitis virus infections in Pakistan is unknown, and most studies have been done in different small groups, e.g. blood recipients, paid blood donors, patients suffering from liver disorders, hemodialysis patients, health care workers and voluntary blood donors [24,25,28,29]. Earlier studies done in Pakistan have shown a prevalence rate of 4-10% for HBV and 4.8-14% for HCV [30,31]. Recent data indicate the prevalence of HBV might be declining, probably because of increased awareness and wide acceptance of health care measures, including mass vaccination program undertaken by the government [4,5,6]. On the other hand, the burden of HCV-related chronic liver disease (CLD) in Pakistan has increased [7,8,12-18].

Earlier studies showed that of all patients presenting with CLD, 16.6% were anti-HCV positive [32]. Furthermore, recent data show that 60-70% of patients with CLD tend to be positive for anti-HCV [31, 33,34,35]. It has been demonstrated that nearly 50% of patients with hepatocellular carcinoma (HCC) in Pakistan are anti-HCV positive [36]. The main purpose of this study was to investigate the prevalence of HBV and HCV in young, apparently healthy women of childbearing age in a large city. Women are expected to be the major victims of infections such as HBV, HCV and HIV because of greater exposure to syringes, blood and blood products, especially during pregnancy, delivery and ear piercing. Another purpose was to make these women aware of those infections, which can be prevented easily by practicing simple precautionary measures such as regular health checkups and vaccination [9,10,20,53]. These young women can further educate their families and thereby indirectly play a role in reducing the burden of these kinds of diseases.

Materials and methods

Ethical issues

Informed signed consent was collected from all volunteers who participated in the study, after the purpose, nature and risks of the participation were fully explained to them verbally and in writing. The individual laboratory results were kept confidential and given to the participants at the completion of the project. Also, they were provided with information on limiting the spread of these infections and referred to the nearest government medical facility for guidance.

Subjects

A total of 4000 healthy female students from two universities in Karachi, aged 18-30 years, participated in this study.

Study Plan and Design

To obtain enough volunteers, six screening camps were organized at the Department of Microbiology, University of Karachi, and the Department of Microbiology, Jinnah University for women, Karachi, during March 2002 and October 2006. Every camp followed an awareness lecture on "Hepatitis infections, their consequences, and preventive measures", to create awareness about the importance of regular health checkups and hepatitis

screening. After submitting their signed consents, volunteers were subjected to health checkups by a medical doctor.

Inclusion Criteria

Age 18 to 30 years; weight: >45 kg; body temperature 96 to 98°F; hemoglobin >10 g/dl, blood pressure: systolic 100-180 mm, diastolic: 60-100 mm; pulse rate >65/min.

Clinical history of volunteers was noted, especially jaundice, blood transfusion, exposure to syringes, surgical and dental procedures.

Exclusion Criteria

Apparently unhealthy or malnourished individuals were excluded.

Pre study screening

From every volunteer 15 ml of blood was taken. Serum was separated by centrifugation and stored at –20ºC.

1. Hematologic screening (hemoglobin, red cell count, total and differential white cell count, hematocrit, platelet count, mean cell volume, mean cell hemoglobin concentration) was done using a Sysmex-KX-21 hematology analyzer (S. Ejazuddin & Company, Pakistan).
2. Biochemical screening (total bilirubin, direct bilirubin, ALT, AST and alkaline phosphatase) was done using a Microlab 200 analyzer (Merck, Germany).
3. HBV and HCV screening: On the spot screening for HBs Ag, anti HBs antibodies and HCV antibodies was done by using rapid immunochromatography kits (ICT, Australia and Abbott, USA).

Confirmatory tests for HBs Ag and HCV antibodies were done by using ELISA (IMX, Abbott, USA).

1. HBV DNA and HCV RNA were extracted from 200 µl of serum using Purelink viral RNA/DNA Mini Kit (Invitrogen, USA; for procedure refer to the manufacturer's instructions).
2. All positive cases were further confirmed by PCR using puReTaq Ready-To-Go PCR Beads (Amersham, UK/ USA; for procedure refer to the manufacturer's instructions).

Results

Before screening, all volunteers were subjected to routine physical checkups for exclusion criteria. A total of 4000 healthy women were screened by immunochromatography for the presence of HBsAg, anti HBs antibodies and HCV antibodies. Positive results were confirmed by ELISA. We found 3820 (95.5%) subjects negative by both immunochromatography and ELISA, 181 (4.5%) tested positive for HB surface antigen, 20 (0.5%) were positive for anti-HB surface antibodies, and 208 (5.2%) were positive for anti-HCV antibodies. Only two volunteers (0.05%) were infected with both HBV and HCV (Table1).

WBC count, RBC count, hematocrit and platelet count in the 181 HbsAg-positive individuals were within normal range, while mean Hb was 9.8±1.6 g/dl. Direct bilirubin, indirect bilirubin, ALT, AST and alkaline phosphatase were within normal range in all tested individuals, except for the raised ALT and AST levels in 10 participants with a previous history of jaundice who were positive HBsAg. Of 208 participants who scored positive for HCV antibodies, 13 showed elevated amino transferase activity (AST) and raised ALT levels (Table 2).

All samples that were positive for HBs antigen or HCV antibodies were later on confirmed by PCR. Out of 181 HBs antigen positive samples, 151 (83.42%) were genotype D, 28 (15.46%) showed mixed infection with genotypes B and D, while one (0.025%) showed mixed infection of genotypes C and D (Table 3). Out of 208 samples positive for HCV antibodies, 107 (51.44%) were genotype 3a, 50 (24.03%) were a mix of genotype 3a and 3b, 33 (15.86%) were genotype 3b, 10 (4.80%) were genotype 1b while, and 8 (3.84%) samples could not be typed (Table 4).

Table 1 Prevalence of HbsAg, and ANTI-HBs and anti-HCV antibodies in 4000 volunteers. Mean age was 21.5 years (SEM +3.7 years). The number of volunteers in each column is followed by the percentage parentheses.

HbsAg-positive	Anti-HBs antibodies positive	Anti-HCV antibodies positive	Anti-HBc IgM antibodies	Negative	Mixed infection: HBV & HCV
181 (4.5%)	20 (0.5%)	208 (5.2%)	00 (0.0%)	3819 (95.5%)	01 (0.025%)

Table 2 Biochemical/ Hematological & Serological profiles of HBV Genotypes. Values are given as mean ± SEM

	HbsAg Positive (n=181)	**HBcAg Positive (n=7)**	**HBcAg Negative (n=7)**
Sex (F)	181	07	173
Age (yrs.)	20.5± 3.2	21.0±3.1	20.9±3.0
Total serum bilirubin (mg/dl)	0.91±0.8	1.0±0.3	0.85±0.2
ALT (U/L)	50± 3	67± 9	52±2
Alkaline phosphatase (U/L)	210±35	209± 15	196±20
Serum Albumin (g/dl)	4.1±0.8	5.0±0.5	4.0±0.5
Platelet count (10^9/L)	407±60	425±80	410±30
Genotype D	181	7	173
Coinfection with genotype B	28	2	26
Coinfection with genotype C	1	0	1

Table 3 Genotypic Distribution of HBV With respects To Single & Mixed Infection; the number of volunteers in each column is followed by the percentage within parentheses.

Genotype	D	B & D mixed infection	C & D mixed infection	Total
Number	151 (83.88%)	28 (15.56%)	1 (0.56%)	180 (100%)

Table 4 Genotypic Distribution of HCV with Respect To Single & Mixed Infection; The number of volunteers in each column is followed by the percentage within parentheses.

Genotype	3a	3a & 3b mixed infection	3b	1b	Could not be typed
Number	107 (51.44%)	50 (24.04%)	33 (15.87%)	10 (4.81%)	8 (3.84%)

Discussion

Confirmation of a diagnosis of hepatitis B and assessment of prognosis rely on examination of liver biopsy. Most people who are chronic inactive carriers (no symptoms, HbsAg-positive, normal serum aminotransferase activity) generally have little or no inflammation on biopsy. An important feature in such patients is the presence of "ground glass cells" in liver biopsy, i.e. liver cells producing large amounts of HbsAg. Individuals with chronic hepatitis B have various degrees of liver inflammation, and some have fibrosis or cirrhosis. Substantial inflammation and the presence of fibrosis or cirrhosis correlate with a worse prognosis. A major problem estimating prognosis of patients with chronic hepatitis C is that it is difficult to predict who will have a relatively benign course and who will go on to develop cirrhosis or cancer. One clear factor for progression to cirrhosis is concurrent alcohol abuse. Viral genotype may also play a role.

There is a wide variation in the prevalence of anti-HCV antibodies and HBsAg worldwide. The global prevalence of HCV is 3% [37]. The prevalence of HCV is high in Africa, especially in some Egyptian cities where more than 15% of the population is infected [38]. The carrier rate of HBs Ag varies from 0.1% to 0.2% in Britain and USA, more than 3% in Greece and southern Italy, and up to 15% in Africa and the Far East [39]. Pakistan is highly endemic with hepatitis B and hepatitis C [40]. Studies are too limited to give a clear picture of the prevalence of hepatitis B and hepatitis C at the national level, especially among apparently healthy individuals. Most previous studies targeted different small groups of individuals with some clinical indications, so they do not accurately reflect the overall prevalence in Pakistan.

For example, the prevalence of HBsAg has been variously reported as 9.97%, 10%, 3.1%, 0.99%, 1.11%, 4%, 3%, 3.2%, 3%, 4.3% and 6.5%, respectively, in different groups of individuals [28,39,41-49]. On the other hand, seroprevalence of HCV antibodies has been variously reported as 4%, 16.3%, 4.8%, 2.2%, 3.3%, 16% and 11.3%, respectively [46,47,49,50-53] (Table 5).

Available data indicates that HBV has been classified into eight genotypes (A -H), according to the criterion of 8% differences in the complete nucleotide sequence of the viral genome [54,55,56,57]. HCV is divided into six genotypes with numerous subtypes. These genotypes can differ by up to 30% from each other in nucleotide sequence. These genotypes have different geographic distributions. In addition to the epidemiological importance, these genotypes may influence the disease pattern and response to treatment [58], and so it is important to identify the virus genotype.

In our study we selected subjects who were apparently healthy and fit our basic inclusion criteria based on age, weight, body temperature, pulse rate, blood pressure and previous clinical history. For that reason, almost all participants had normal hematological biochemical values (Table 2). Noteworthy, however, is that 10 HBsAg-positive volunteers had elevated levels of AST (>31 U/L) and ALT (>36 U/L), and 13 volunteers who were positive for HCV antibodies had elevated alkaline phosphatase level (>270 U/L).

The prevalence rate of HBsAg we observed among apparently healthy women (4.5%) is close to previously reported rates [43,45,47-66]. Prevalence of HCV antibodies (5.2%) is also similar to rates previously reported in healthy individuals [39,46,47]. Genotyping results showed that all HBV-positive individuals had genotype D, either alone or in a mixed infection (Table 3). In HCV-positive individuals, genotype 3a was the most common (51.44%), followed by 3b (15.87%) (Table 4).

Over 350 reports, papers and presentations estimate the combined prevalence of hepatitis B and C in various parts of Pakistan at 8-10%. A substantial decline in hepatitis B has been observed due to mass scale immunization program started with government support against HBV infection. However, the prevalence of hepatitis C has risen due to improper screening of blood, unavailability of vaccine and other modes of transmission.

Due to the high cost of treatment of hepatitis B and C virus infection and the unavailability of a vaccine against HCV, the main focus should be on preventive aspects. Here comes the importance of identifying the genotype of the virus infecting a person, which is not a common practice in Pakistan. Mostly treatment is given without knowing the genotype, which may result in no response or emergence of mutant strains of the virus.

In Pakistan there is an urgent need to raise public awareness, which can be accomplished through programs in schools, colleges, and universities, and through information media about the value of immunization and preventive measures. Women are considered to play the main role in the families. Infected women can spread infection among their family members, especially infections such as HBV and HCV. Their health and education in health related issues could have a broad impact on the overall health status of the country. Besides awareness programs and improved treatment strategies, we also need to evaluate the available HBV vaccines in the market for their proper storage, their efficacy, side effects and immunogenicity.

Conclusion

Genotype D was the predominant type of HBV (100%), and types 3a (51.44%) and 3b (15.86%) were the predominant types of HCV among the apparently healthy women participating in this study. Prevalence of HBV and HCV was 4.5% and 5.2%, respectively. Attempts should be made to reduce the incidence of HCV and HBV and their unregulated spread. This can be done by increasing public awareness of simple preventive measures. We also need to have similar studies at a national level to determine the overall prevalence and incidence of hepatitis infections in Pakistan.

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