Sero-Molecular Epidemiology and Risk Factors of Viral Hepatitis in Urban Yemen

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ABSTRACT
Viral hepatitis is an acute or chronic neglected disease that affects predominantly the liver. This study aimed to determine the sero prevalence and risk factors for viral hepatitis and HCV genotypes in urban areas of Yemen. In a prospective, cross-sectional, analytical and community-based study and following informed consent, 501 volunteers were randomly recruited. Demographic and clinical data was collected. Screening for HBV, HDV and HCV was carried out using ELISA. HBV/HCV viral loads and HCV genotyping were carried out using COBAS® TaqMan® 48 Analyzer (Roche Diagnostics Gmbh, Germany). The mean age of study population was 25.9±10.6 years with a male: female ratio of 1. HBV sero prevalence was similar in Sana’a, Taiz and Aden (p = 0.2). Evidence of past HBV infection was significantly high in Aden and Taiz (14.1 and 18.8%, respectively) compared to Sana’a (5.4%) (p = 0.002). HBV DNA Viral loads were widely variable (1350 and 6320 copies mL\(^{-1}\)). HCV sero prevalence was low and not significantly different in the study cities (p = 0.4). HCV Viral loads were widely variable between samples (657300-1630000 copies mL\(^{-1}\)). HCV Genotypes 4 and 1a were the most predominant genotypes. Blood transfusion (19%), renal dialysis (18.6%), history of cupping (18.1%), surgical operation (17.4%) and dental treatment (15.4%) were the most common risk factors for HBV and HCV sero reactivity. In conclusion, HBV sero prevalence is variable among different geographical areas in Yemen. Past exposure to HBV is high. Blood transfusion and contaminated surgical instruments are important infection risks for viral hepatitis. HCV genotypes 4 and 1a are the most prevalent.

Key words: HBV/HCV sero prevalence, risk factors, Yemen

INTRODUCTION
Viral hepatitis is a major public health problem affecting thousands throughout Yemen. Viral hepatitis due to HBV/HCV is a major cause of morbidity and mortality from acute and chronic infections (Thabit et al., 2012; Murad et al., 2013; Al-Shamahy and Abdu, 2013). About half of the population in cities and main governates have serological evidence of previous HBV infection. Over the past decades HBV prevalence dropped markedly among children (<12 years) and blood donors. HBV vaccination coverage rocketed to 70%. HCV antibodies showed a steady decline, while delta antibodies were reported in a very small percentage of HBsAg reactive sera (Al-Shamahy et al., 2003, 2010; Sallam et al., 2003, 2012; Al Waleedi and Khader, 2012; Scott et al., 1990).
The 8 genotypes (A-H) of HBV have been reported worldwide based on the divergence of domain of α determinant of the genome sequence. HBV genotypes have distinct geographical distributions. In Yemen, 2 major genotypes (A and D) have been reported with genotype A predominating in communities with continuing African links while genotype D remains the dominant genotype in settled populations. The HBV genotypes A and D are most predominant in the Arab World (Al Baqlani et al., 2014; Mahgoub et al., 2011; Yousif and Kramvis, 2013; Al Moslih et al., 2004; Abdo et al., 2006; Alavian et al., 2007). The HBV genotypes differ in their response to peglated IFN-α, genotype A strains respond well, while genotypes D, B and C showed reduced response. In contrast, the response to nucleoside/nucleotide analogues is independent of HBV genotypes (Brunetto et al., 2013; Westland et al., 2003; Cooksley, 2010; Cassidy et al., 2011). Possible risk factors for Hepatitis B and C in Yemen are non-adherence to universal infection control precautions in high risk situations like hemodialysis, blood transfusion, dental clinics, barber shops, circumcision, ear pricing and folk medical practices (Al Waleedi and Khader, 2012). This study was conducted to determine the sero-evidence of past HBV infection, risk factors and HCV genotypes in a changing population in some major cities in Yemen.

MATERIALS AND METHODS

Sample collection: The study protocol was reviewed and approved by the Ethics and Scientific Committees of the Institute of Endemic Diseases, University of Khartoum, Sudan and the research Ethics Committee, Medical College, Sana’a University and the Ministry of Health and Population of Yemen.

Following written informed consent, 501 volunteer were enrolled in this study. A multistage random sampling design was employed. The number of population in districts was based on the General Population Census of 2008. Volunteers were randomly selected from 3 provinces (Sana’a, Aden and Taiz), 3 districts in each city, high secondary schools, health facilities (hospitals and health centers) and Universities. The obtained sample size was multiplied by 1.5 to correct for design effects (431×1.5 = 501). A standard questionnaire was used for demographic, HBV/HCV/HDV risk factors and laboratory data collection.

The 5 mL of venous blood were collected for HBsAg, anti-HB core antibodies and anti-HCV antibodies measurements using Enzyme-linked immunosorbent assay (Biokit, Barcelona, Spain; ADALTIS EIAgem, Italy).

COBAS® TaqMan® HBV test: This test involved manual specimen preparation to extract HBV DNA (High pure system viral nucleic acid kit; Roche Diagnostics Gmbh, Germany). Automated PCR amplification and quantification of the target DNA using specific complementary primers and simultaneous detection of cleaved dual fluorescent dye-labeled oligonucleotide probes that permit quantification of the amplified target HBV DNA.

Primers: The following primers were used:

- **External primers:**
  - (PC1) 5’CATAAGAGGACTCTTGGGACT’3
  - (PC2) 5’AAAGAATTCAGAAGGCAAAAAAGA’3

- **Internal primers:**
  - (PC3) 5’AATGTCAACTACCGACCTTG’3
  - (PC4) 5’TCCACAGAAGCTCCGAATTCT’3
Quantification of HCV RNA: The quantification of HCV RNA in human serum or plasma was carried out using COBAS AmpliPrep/COBAS TaqMan HCV test that permits automated specimen preparation followed by automated reverse transcription. PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide detection probe specific to the target. The highly conserved 5' UTR of the HCV has been used for genotyping based on hybridization using subtype-specific probes.

Statistical analysis: The data were analyzed by EpiInfo version 7 (CDC, Atlanta, USA) for statistical significance. Quantitative data was expressed as Mean±SD. Means were compared using t-test and significance levels were taken as <0.05.

RESULTS
The 501 apparently healthy volunteers with a mean age of 25.9±10.6 years and a male:female ratios of 1 were enrolled. The study age groups were as follows: <15 (n = 67/501), 16-40 (n = 391/501) and ≥41 (n = 43/501). In Sana’a (n = 261/501) the mean age was 23.3±10.1 and the male:female ratio was 1. The age groups were as follows: <15 (n = 45/261), 16-40 (n = 200/261) and ≥41 (n = 16). In Taiz (n = 171) the mean age was 27.6±10.5 with a male:female ratio of 2. Age groups were as follows: <15 (n = 14), 16-40 (n = 137) and ≥41 (n = 20). In Aden (n = 69) the mean age was 31.2±9.7 and a male:female ratio of 1. The age groups were as follows: <15 (n = 7), 16-40 (n = 54) and ≥41 (n = 8). It is noteworthy that Aden city volunteer’s were older than Taiz and Sana’a volunteers where the mean age was 31.2±10.5 years compared to 23.3±10.1 years and 27.6±10.5 years, respectively (p = 0.00) (Table 1).

HBV DNA reactivity was comparable to HBsAg ELISA testing in the 3 study cities (p = 0.95) (Table 1). The HBV-DNA Viral loads were variable between samples (1350-6320 copies mL⁻¹). The most prevalent HCV genotypes among HCV positive volunteers were genotype 4 was found exclusively (4/4, 100%) in Sana’a, while genotype 1a was seen exclusively in Taiz (1/1, 100%).

The HCV antibodies reactivity was low and not statistically different in the 3 cities, Sana’a (4/261, 1.5%), Taiz (1/171, 0.6%) and was zero in Aden (p = 0.4). The HCV Viral loads were widely variable between samples (657300-1630000 copies mL⁻¹). The HDV antibodies were not detected in the study volunteers (Table 1). Blood transfusion (19%), renal dialysis (18.6%), history of cupping (18.1%), surgical operations (17.4%) and dental treatment (15.4%) were the most common risk factors for HBV and HCV sero-reactivity (Table 2).

| Variables            | Sana’a (n = 261) | Taiz (n = 171) | Aden (n = 69) | p-value |
|----------------------|-----------------|----------------|---------------|---------|
| Mean age±SD          | 23.3±10.1       | 27.6±10.5      | 31.2±9.7      | 0.000   |
| M:F                  | 1:1             | 2:1            | 1:1           |         |
| HBs Ag               | 7/261 (2.7%)    | 3/171 (1.8%)   | 4/69 (5.8%)   | 0.22    |
| HBcAbs               | 14/261 (5.4%)   | 24/171 (14.1%) | 13/69 (18.8%) | 0.001   |
| HDV Abs              | 0               | 0              | 0             |         |
| HBV-DNA reactivity   | 6/7 (85.7%)     | 3/3 (100%)     | 3/4 (75%)     | 0.009   |
| HCV Abs reactivity    | 4/261 (1.5%)    | 1/171 (0.6%)   | 0/69 (0%)     | 0.4     |
| HCV genotype         | Type 4 (4/4)    | Type 1a (1/1)  | 0             |         |

p-value<0.05 indicates significant difference
Table 2: Potential risk factors for contracting HCV among subjects

| Potential risk factors | HCV | Sana’a | Taiz  | Aden |
|------------------------|-----|--------|-------|------|
| Surgical operations    | 2/98 (2%) | 0 | 0 |
| Dental procedures      | 2/98 (2%) | 7/96 (7.2%) | 0 |
| Sharps injuries        | 2/22 (9.0%) | 1/21 (4.7%) | 0 |
| History of hepatitis   | 2/93 (2.1%) | 1/53 (1.9%) | 0 |
| Travel abroad          | 1/41 (2.4%) | 0 | 0 |

DISCUSSION

Viral hepatitis and its long term complications, cirrhosis and primary liver cancer (HCC) put a massive strain on the struggling economies of developing countries. In Yemen, HBV prevalence is high reaching 20% in some areas and about half of the population has evidence of past HBV infection. Over the past decades, HBV prevalence dropped markedly as shown by the results of our recent study that reported an overall HBV prevalence of 1.8-5.8%. A similar reduction was also seen HCV with a recent prevalence of 0.6-1.5%. The reported HBV prevalence reflected a reduction among some geographical areas compared to that reported more than a decade ago (Al-Shamahy et al., 2003; Sallam et al., 2003; Al Waleedi and Khader, 2012). The reduction was very significant in Taiz City compared to that reported in 2012 by Sallam and colleagues (4.2-1.8%) (Sallam et al., 2012). The reported HBV prevalence is still higher than that reported in Europe, North America and Australia (0.2-0.5%), but is similar to that reported in Eastern Europe, the Mediterranean area, Russia, Russian Federation, Southwest Asia, Central and South America (Zanetti et al., 2008). HBV prevalence is still lower than those reported in Parts of China, Southeast Asia and Tropical Africa (Yuen et al., 2009).

Hepatitis C virus (HCV) prevalence although low did not change significantly since 2002 as reported by others. The reported HCV prevalence in Yemen is lower to that in surrounding countries in the region. HCV prevalence increased with age, indicating a accumulative pattern and chronicity (Haidar, 2002; Bajubair et al., 2008; Al Dhahry et al., 2003; Marzouk et al., 2007). The appearance of HCV genotype 1a could probably be due to waves of migration from Asia, Europe or North America (Barth et al., 2006). Absence of Hepatitis D Virus in our report is probably due to reduction in HBV prevalence.

CONCLUSION

In conclusion, HBV sero prevalence is variable between different geographical regions in Yemen with marked reduction over the last few years. Evidence of past exposure to HBV is alarmingly high. Blood transfusion, hemodialysis and contaminated surgical instruments are important infection risks for viral hepatitis. HCV genotypes 4 and 1a are the most prevalent.

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