



International Journal of
Virology

ISSN 1816-4900



Academic
Journals Inc.

www.academicjournals.com

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

¹B.A.H. Al-Nabehi, ²H. Al-Shamahy, ¹W.S.E. Saeed, ¹A.M. Musa, ¹A.M. El Hassan and ¹E.A.G. Khalil

¹Institute of Endemic Diseases, University of Khartoum, P.O. Box 45235, Khartoum, 11111, Sudan

²Department of Medical Microbiology, Faculty of Medicine and Health Sciences, University of Sana'a, P.O. Box 1950, Sana'a, Yemen

Corresponding Author: E.A.G. Khalil, Institute of Endemic Diseases, University of Khartoum, P.O. Box 45235, Khartoum, 11111, Sudan

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 pegylated 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 pricking 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 ($431 \times 1.5 = 501$). 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 EIAgen, 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'CATAAGAGGACTCTTGGACT'3
 - (PC2) 5'AAAGAATTCAGAAGGCAAAAAGA'3
- **Internal primers:**
 - (PC3) 5'AATGTCAACTACCGACCTTG'3
 - (PC4) 5'TCCACAGAAGCTCCGAATTC'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).

Table 1: Demographic data, HBsAg, HBcAbs and HCV frequencies in study sites

Variables	Sana'a (n = 261)	Taiz (n=171)	Aden (n = 696)	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
HDVAbs	0	0	0	
HBV-DNA reactivity	6/7 (85.7%)	3/3 (100%)	3/4 (75%)	0.009
HCVAbs 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.

ACKNOWLEDGMENT

This study was supported by the Institute of Endemic Diseases, University of Khartoum and Faculty of Medicine and Medical Sciences, Sana'a University. Extended thanks to Dr. Brima Younis Musa, Dr Hatim Mohamed and Miss Kawthar Abd Eljalil of the Institute of Endemic Diseases, University of Khartoum.

REFERENCES

- Abdo, A.A., B.M. Al-Jarallah, F.M. Sanai, A.S. Hersi and K. Al-Swat *et al.*, 2006. Hepatitis B genotypes: Relation to clinical outcome in patients with chronic hepatitis B in Saudi Arabia. *World J. Gastroenterol.*, 12: 7019-7024.

- Al Baqlani, S.A., B.T. Sy, B.A. Ratsch, K. Al Naamani and S. Al Awaidy *et al.*, 2014. Molecular epidemiology and genotyping of hepatitis B virus of HBsAg-positive patients in Oman. *PLoS One*, Vol. 9. 10.1371/journal.pone.0097759
- Al Dhahry, S.H.S., J.C. Nograles, S.M.W.W.B. Rajapakse, F.S.S. Al Toqi and G.Z. Kaminski, 2003. Laboratory diagnosis of viral hepatitis C: The Sultan Qaboos University Hospital experience. *J. Sci. Res. Med. Sci.*, 5: 15-20.
- Al Waleedi, A.A. and Y.S. Khader, 2012. Prevalence of hepatitis B and C infections and associated factors among blood donors in Aden City, Yemen. *EMHJ*, 18: 624-629.
- Al Moslih, M.I., R.O. Abuodeh and Y.W. Hu, 2004. Detection and genotyping of TT virus in healthy and subjects with HBV or HCV in different populations in the United Arab Emirates. *J. Med. Virol.*, 72: 502-508.
- Al-Shamahy, H.A., I.A. Rabbad and A. Al-Hababy, 2003. Hepatitis B virus serum markers among pregnant women in Sana'a, Yemen. *Ann. Saudi Med.*, 23: 87-89.
- Al-Shamahy, H.A., T.A. Al-Moyed and A.G. Al-Medhagi, 2010. Evaluation of the immune response to Hepatitis B vaccine in different age groups of the population of Sana'a-Yemen. *Bahrain Med. Soc. J.*, 11: 12-18.
- Al-Shamahy, H.A. and S.S.A. Abdu, 2013. Genotyping of Hepatitis C Virus (HCV) in infected patients from Yemen. *Eur. J. Basic. Med. Sci.*, 3: 78-82.
- Alavian, S.M., F. Fallahian and K.B. Lankarani, 2007. The changing epidemiology of viral hepatitis B in Iran. *J. Gastrointest. Liver Dis.*, 16: 403-406.
- Bajubair, M.A., A.A. Elrub and G. Bather, 2008. Hepatic viral infections in Yemen between 2000--2005. *Saudi. Med. J.*, 29: 871-874.
- Barth, H., T.J. Liang and T.F. Baumert, 2006. Hepatitis C virus entry: Molecular biology and clinical implications. *Hepatology*, 44: 527-535.
- Brunetto, M.R., P. Marcellin, B. Cherubini, C. Yurdaydin and P. Farci *et al.*, 2013. Response to peginterferon alfa-2a (40KD) in HBeAg-negative CHB: On-treatment kinetics of HBsAg serum levels vary by HBV genotype. *J. Hepatol.*, 59: 1153-1159.
- Cassidy, A., S. Mossman, A. Olivieri, M.D. Ridder and G. Leroux-Roels, 2011. Hepatitis B vaccine effectiveness in the face of global HBV genotype diversity. *Exp. Rev. Vaccines*, 10: 1709-1715.
- Cooksley, W.G.E., 2010. Do we need to determine viral genotype in treating chronic hepatitis B? *J. Viral Hepatitis*, 17: 601-610.
- Haidar, N.A., 2002. Prevalence of hepatitis B and hepatitis C in blood donors and high risk groups in Hajjah, Yemen Republic. *Saudi Med. J.*, 23: 1090-1094.
- Mahgoub, S., D. Candotti, M. El Ekiaby and J.P. Allain, 2011. Hepatitis B Virus (HBV) infection and recombination between HBV genotypes D and E in asymptomatic blood donors from Khartoum, Sudan. *J. Clin. Microbiol.*, 49: 298-306.
- Marzouk, D., J. Sass, I. Bakr, M. El Hosseiny and M. Abdel-Hamid *et al.*, 2007. Metabolic and cardiovascular risk profiles and hepatitis C virus infection in rural Egypt. *Gut*, 56: 1105-1110.
- Murad, E.A., S.M. Babiker, G.I. Gasim, D.A. Rayis and I. Adam, 2013. Epidemiology of hepatitis B and hepatitis C virus infections in pregnant women in Sana'a, Yemen. *BMC Pregnancy Childbirth*, Vol. 13. 10.1186/1471-2393-13-127
- Sallam, T.A., C.Y.W. Tong, L.E. Cuevas, Y.A. Raja'a, A.M. Othman and K.R. Al-Kharsa, 2003. Prevalence of blood-borne viral hepatitis in different communities in Yemen. *Epidemiol. Infect.*, 131: 771-775.

- Sallam, T.A., Y.A. Raja'a, S. Bahaj, A.M. Al-Shami, M. Lu, M. Roggendorf and C.Y.W. Tong, 2012. Hepatitis B virus carrier rate, prevalence and susceptibility and impact of immunization program among households in the city of Taiz, Yemen. *Vaccine*, 30: 5564-5568.
- Scott, D.A., J.P. Burans, H.D. Al-Ouzeib, B.K. Arunkumar and M. Al-Fadeel *et al.*, 1990. A seroepidemiological survey of viral hepatitis in the Yemen Arab republic. *Trans. Royal Soc. Trop. Med. Hygiene*, 84: 288-291.
- Thabit, A.M., K.A. Al-Moyed, M.S. Al-Balushi, S.S. Hasson and T.A. Sallam, 2012. Occult hepatitis B virus among chronic liver disease patients in Yemen. *Asian Pacific J. Trop. Dis.*, 2: 4-6.
- Westland, C., W. Delaney, H. Yang, S.S. Chen and P. Marcellin *et al.*, 2003. Hepatitis B virus genotypes and virologic response in 694 patients in phase III studies of adefovir dipivoxil. *Gastroenterology*, 125: 107-116.
- Yousif, M. and A. Kramvis, 2013. Genotype D of hepatitis B virus and its subgenotypes: An update. *Hepato. Res.*, 43: 355-364.
- Yuen, M.F., J.L. Hou and A. Chutaputti, 2009. Hepatocellular carcinoma in the Asia pacific region. *J. Gastroenterol. Hepatol.*, 24: 346-353.
- Zanetti, A.R., P. Van Damme and D. Shouval, 2008. The global impact of vaccination against hepatitis B: A historical overview. *Vaccine*, 26: 6266-6273.