Detection of Hepatitis C virus and Human immunodeficiency virus in expatriates in Saudi Arabia by antigen-antibody combination assays

Alhusain J. Alzahrani¹, Obeid E. Obeid¹, Amein Al-Ali², Burhan Imamwardi³

¹Department of Microbiology, College of Medicine, King Faisal University, Dammam, Saudi Arabia
²Department of Biochemistry, College of Medicine, King Faisal University, Dammam, Saudi Arabia
³Department of Medical Laboratory Technology, College of Applied Medical Sciences, King Faisal University, Dammam, Saudi Arabia

Abstract

Background: The simultaneous detection of antigen and antibody was originally described for the early detection of the human immunodeficiency virus (HIV). The same approach was applied to detect the hepatitis C virus (HCV). The aim of this work was to use the antigen and antibody combination assay for the detection of HCV and HIV infections in expatriates in Eastern Saudi Arabia.

Methodology: The study group (N = 875) included expatriate workers of both sexes who were undergoing mandatory pre-employment testing. Detection of anti-HCV antibodies, HCV core antigen, HCV viral RNA, HIV antigens and antibodies was conducted using commercially available kits.

Results: Of the 875 samples that were screened for HCV-specific antibodies, four (0.46%) tested positive (two from Pakistan, one from India, and one from the Philippines) and two (0.23%) were equivocal (one from Egypt and one from Nepal). All four samples that were positive for HCV-specific antibodies also tested positive using HCV RNA assay and the HCV antigen-antibody combination assay. The two samples that were equivocal tested positive using the HCV RNA assay and the HCV antigen-antibody combination assay. Of the 875 samples that were tested for HIV antibodies, only one (0.11%) sample gave repeatedly positive results. The same sample also tested repeatedly positive using the HIV combination assay. These results were subsequently confirmed by HIV western blot assay.

Conclusions: Our study indicates that the addition of antigen detection to the screening of HCV and HIV may lower the risk of transmission of these viruses in the host country and contribute to the overall control of HCV and HIV in Saudi Arabia.

Key words: HCV, HIV, expatriates

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Introduction

Hepatitis C virus (HCV) is a small encapsulated positive strand RNA member of the Flaviviridae family. HCV is known for its aetiological role in chronic non-A, non-B viral hepatitis, liver cirrhosis and hepatocellular carcinoma. In addition, the virus has also been implicated in a number of extra-hepatic "autoimmune" disease manifestations [1]. At present, approximately 200 million people worldwide are infected with HCV [2]. The diagnosis of HCV is based on detecting either anti-HCV antibody by enzyme linked immunosorbent assay (ELISA) or HCV-RNA by polymerase chain reaction (PCR) [3]. More recently, HCV core antigen assays have been developed and they have a comparable sensitivity to that shown by the PCR-based assay, with a mean detection difference of one to two days [4-6]. The simultaneous detection of antigen and antibody was originally described for the early detection of HIV infection [7]. Since then, the same approach was applied to HCV diagnosis [8-9].

HCV seroprevalence in Saudi blood donors is estimated to be 3.5%, whereas its prevalence in patients on dialysis varies between 15% and 80% [10]. The most prevalent genotype of HCV in patients in Saudi Arabia is genotype 4 followed by genotypes 1a and 1b, whereas genotypes 2a/2b, 3, 5, and 6 are rare. Since the introduction of routine hepatitis B virus (HBV) vaccination in Saudi Arabia, the seroprevalence of HBV has significantly decreased [11-13].

Currently there are 40 million individuals in the world infected with the human immunodeficiency virus (HIV) [14-15]. The World Health Organization
estimates that nearly 16,000 new infections occur worldwide each day [16]. HIV-1 has presented several unique challenges which have prevented the effective control of the virus. The incidence of HIV infection among adults in the Middle East is estimated to be 0.3%. However, there was a significant increase of 20% in 2002 [17]. In 2000, the cumulative number of HIV-infected individuals in Saudi Arabia was estimated to be 1,100 with an adult rate of 0.01% [18-19].

Expatriates form the driving workforce in industry in the Gulf region. Expatriate pre-employment testing for infectious diseases is mandatory in many countries, including Saudi Arabia. This requirement applies to all jobs and all age groups. Currently, assessment of HCV and HIV infections in expatriate workers in Saudi Arabia depend on the detection of the virus-specific antibodies. Detection of both virus-specific antigens as well as virus-specific antibodies minimizes the window period (time between acquiring an infection and documentation of positive laboratory results) and therefore reduces the risk of spreading the infection within the community. The aim of this work was to use an antigen-antibody combination assay for the detection of HCV and HIV infections in expatriate workers in Eastern Saudi Arabia.

Materials and Methods
Patients
The study group (N = 875) included expatriate workers of both sexes who were undergoing mandatory pre-employment testing. Jobs included domestic workers (housekeepers), farm workers, labourers, and various other jobs. The expatriate workers included in this study were from India, Bangladesh, Pakistan, Indonesia, Sri Lanka, Nepal, Thailand, Sudan, Egypt, and the Philippines with a mean age of 30.4 years ± 5.

Detection of anti-HCV antibodies
Anti-HCV antibodies were assayed by a third-generation ELISA kit (AxSYM HCV version 3.0, Abbott Diagnostics, Chicago, Ill.) and HCV 3.0 ELISA kit (Ortho-Clinical Diagnostics, Raritan, N.J.). In addition, the recombinant immunoblot assay (RIBA HCV 3.0; Ortho-Clinical Diagnostics) was used as previously described and in accordance with the manufacturer’s instructions [6, 10].

Detection of HCV core Ag
HCV core Ag assay (Ortho-Clinical Diagnostics) was used (including the neutralization protocol) according to the manufacturer’s recommendations and as previously described [6,10].

Simultaneous detection of HCV antibodies and antigen (HCV Ag/Ab Combination assay)
Simultaneous detection of HCV antigen and HCV antibodies for all specimens was conducted using Murex HCV Ag/Ab combination assay (Abbott Murex, USA) according to the manufacturer’s instructions. In brief, test specimens and control sera were incubated in wells for 60 minutes to allow for the binding of HCV core antigen and HCV antibodies. After washing, peroxidase labeled conjugate was added and the wells were further incubated for 60 minutes. Unbound conjugate was washed and the substrate was incubated for 30 minutes after which the reaction was stopped and the plate was read at 450 nm [6].

HCV RNA assays (Qualitative)
To detect HCV RNA, the STRP Hepatitis C Virus Genome Detection Kit (CinnaGen Inc., Tehran, Iran) was used according to the manufacturer’s instructions. The steps involved RNA extraction, cDNA synthesis, and nested PCR techniques. The assay has a limit of detection of 100 copies of HCV genome per ml of serum.

Detection of HIV antigen and antibodies
Detection of HIV antibody and the simultaneous detection of HIV-specific antigens and antibodies were performed using Abbott AxSym MIEA and Combo assay (Abbott Laboratories, USA) according to the manufacturer’s recommendations. Positive results were confirmed by HIV western blot assay (DuPont Company, Wilmington, DE, USA) as recommended by the manufacturer.

Results
Detection of HCV antigen, antibodies and viral RNA
Of the 875 samples that were screened for HCV-specific antibodies, four (0.46%) tested positive (two from Pakistan, one from India and one from the Philippines) and two (0.23%) were equivocal (one from Egypt and one from Nepal). All four samples that were positive for HCV-specific antibodies also tested positive using HCV RNA assay and the HCV antigen-antibody combination assay. The two samples that were equivocal tested positive using the HCV RNA assay and the HCV antigen-antibody combination assay.
Detection of HIV antigen and antibodies

Of the 875 samples that were tested for HIV antibodies, only one (0.11%) sample gave repeatedly positive results. The same sample also tested repeatedly positive using the HIV combination assay. These results were subsequently confirmed by HIV western blot assay.

Discussion

The globalization of business activity can lead to the movement of key employees and their dependants from country to country. Expatriates comprise an important but rarely studied subset of international travelers. Adult expatriates that come from countries where HCV and HIV are highly endemic may spread these infections in their host countries. Therefore, pre-employment testing is widely applied to expatriates.

Initial testing for HCV should be performed using sensitive third-generation enzyme immunoassays (EIA) licensed for detection of anti-HCV. False negative anti-HCV EIA results may occur in HIV-infected persons with advanced immune suppression (CD4 < 100/mm³) and true negative EIA are common in the setting of acute HCV infection (< 12 weeks following acquisition) prior to seroconversion [20-21]. If serologic test results are negative and HCV infection is suspected due to elevated liver enzyme levels or risk factors such as intravenous drug usage or high-risk sex, HCV RNA testing should be performed. While a single detectable HCV RNA result is sufficient to confirm the diagnosis of active HCV infection, a single negative result cannot exclude active viremia because RNA levels might transiently decline below the limit of detection. Therefore, it is of paramount importance that testing should be repeated in the host country.

This report presents data on the use of an antigen-antibody combination assay for the detection of HCV and HIV infections in a cohort of expatriates in the Eastern Province of Saudi Arabia. Because antigenemia usually precedes specific formation of antibodies, detection of virus-specific antigen will narrow the window period, hence reducing the risk of spreading the infection within the community [22]. Currently, there is no published data on the use of an antigen-antibody combination assay in expatriate pre-employment testing.

Of the 875 expatriate samples that were screened for HCV antibodies, four were positive (By ELISA and RIBA). This may indicate past infection by HCV. Because of the high propensity of HCV to persist (in up to 80% of cases), these individuals may spread HCV by the various modes mentioned earlier. Two samples were equivocal using the HCV antibody assay, but they tested positive using the HCV RNA assay and the HCV antigen-antibody combination assay. These two subjects may be in the early stages of HCV infection, where there is marked viremia and antigenemia or alternatively, they might have a chronic HCV infection. The antibody production to a given antigen is influenced by genetic factors that determine whether the individual is a high responder or low responder to the given antigen [23].

Mahaba et al. [24] described the prevalence of HCV in 8862 subjects from the Hail region, Saudi Arabia. The overall prevalence was found to be 5.1%, with a very high prevalence among Egyptian expatriates (26%). The relatively low prevalence of HCV in our study group may well be due to an improvement in the control the testing for infectious diseases in the home countries of the expatriates.

One subject had detectable HIV antibodies. Both conventional antibody assay and antigen-antibody combination assay gave similar results. The advantage of the combination assay is the detection of early infection. Hamdi & Ibrahim [25] analyzed the prevalence of sexually transmitted diseases (STDs) in domestic workers in Jeddah, Saudi Arabia. They HIV seroprevalence in their study group was 19%. Consequently, they concluded that pre-employment screening is a viable means of identifying major STDs and communicable diseases. It was recommended that stringent measures should be adopted to prevent fraudulent reporting from laboratories and health care providers locally and from the home country of the expatriates.

Based on the data presented here and on data from previous publications [26-27], the addition of antigen detection to the screening of HCV and HIV may lower the risk of transmission of these viruses in the host country and contribute to the overall control of HCV and HIV in Saudi Arabia.

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References


Corresponding Author

Dr. Obeid E. Obeid, Department of Microbiology, College of Medicine, King Faisal University, P.O. Box 2114, Dammam # 31451, Saudi Arabia
Phone: 0096638577000-2005. Fax: 0096638575329
E mail: obeid@yahoo.com

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