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Original Research Article

Indeterminate HCV ELISA and RIBA results versus HCV RNA; A comparative study in premarital couples in Makkah, Saudi Arabia

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Abstract

Hepatitis C virus (HCV) infection is diagnosed by the presence of antibodies and is supplemented by confirmatory testing methods, such as recombinant immunoblot assay (RIBA) and HCV RNA detection. It is mandatory to go for HCV virus screening before marriage in Saudi Arabia, the diagnosis is based on serology and confirmation by RIBA testing. This study aimed to evaluate the efficacy of RIBA testing in diagnosis of HCV infection in pre-marital screening couples positive or indeterminate for anti-HCV antibodies. A total 15.342 subjects were screened for HCV, out of which 98 samples with positive and indeterminate anti-HCV enzyme-linked immunosorbent assay (ELIZA) results were further assessed using RIBA 3 and quantitative HCV RNA Abbott real-time polymerase chain reaction (PCR) assay. A total 98 blood samples with positive and indeterminate results for anti-HCV were further assessed for anti-HCV antibodies using a RIBA 3.0 Strip Immunoblot Assay. The positivity rates for HCV infection by ELISA among premarital screening couples were 0.63 % (98 out of 15342). Using RIBA, 28 out of ELISA +ve cases were RIBA indeterminate, 43 were RIBA +ve and the remaining 27 were RIBA negative. All RIBA indeterminate cases were negative using qualitative HCV-RNA analysis. In this study, individuals with indeterminate RIBA results had no detectable HCV-RNA suggesting that real-time PCR may be unnecessary, particularly in low-risk populations.

Keywords: Hepatitis C virus. ELISA. RIBA. HCV-RNA. Indeterminate

INTRODUCTION

Hepatitis C virus (HCV) is a hepatotropic virus which is a major cause of liver disease and a potential cause of considerable morbidity and mortality worldwide. HCV is estimated to infect about 3% of the world population; this prevalence translates into roughly 130 million HCV carriers (Alter, 2007). HCV causes acute hepatitis which is mostly subclinical, but gradually progresses into chronic hepatitis in about 80% of those infected (Forman et al., 2011). The data on HCV prevalence in Saudi Arabia is both, limited and conflicting in disease estimation. Saudi blood donor screening centers indicate HCV infection rates of 0.4-1.1% (Bashawri et al., 2004; Mehdi et al., 2000; El-Hazmi, 2004; Madani, 2007). Data from the premarital screening program estimates a prevalence of 0.33% (Alswaidi and O'Brien, 2010). A recent systematic review including all published studies on the Saudi populations estimated that the prevalence ranges from 1.0-1.9% (Sievert et al., 2011).

HCV is primarily transmitted via contaminated blood product exposure, healthcare related procedures, sexual

activity or perinatal transmission (Dunkelberg et al., 2014). Furthermore, the virus can be transmitted sexually and prenatally. The CDC recommends that all anti-HCV screening using ELISA be confirmed using supplementary serological testing or Nucleic acid amplification tests (NAT). RIBA (recombinant immunoblot assay) is the preferred supplementary serological testing method due to its robust specificity. RIBA testing requires the identification of false-positive results obtained by ELISA, particularly when considering populations with low HCV prevalence rates (Alvaro Mena et al., 2014; Pereira et al., 2014).

RIBA detects the reactivity of antibodies to antigens that are immobilized on nitrocellulose immunoblot strips. When reactivity is observed against two or more proteins, the result is considered positive. When a reaction is registered against only one protein, it is then considered indeterminate. However, when no reactions are observed against any of the evaluated antigens, a RIBA test is considered negative (Pereira et al., 2014; Tashkandy et al., 2007).

In Saudi Arabia a Premarital Screening and Genetic Counseling (PMSGC) Program was predetermined by law in December 2003 and applied in February 2004. The main program objective was to reduce the prevalence of genetic disorders and infectious diseases by reduction of the number of at-risk marriages. The program infrastructure consisted of 150 health care reception clinics, 70 laboratories, and 78 genetic counseling clinics covering the 13 administrative regions of the kingdom. Comprehensive PMSGC program guidelines were distributed to all workers in the program. According to these guidelines, couples with marriage proposals had to report to the nearest health care clinic to apply for premarital certificates (Saudi Ministry of Health, 2018). AL-Nour hospital (NSH) laboratory is one among the specified laboratories. Viral screening, including hepatitis C virus is pivotal in Premarital Screening. The policy used in the NSH is to start using anti-HCV 3rd generation ELISA, if ELISA results are positive or indeterminate, a confirmatory RIBA is performed. Confirmed positive result by RIBA is reported HCV positive, while indeterminate RIBA results are subjected to NAT. This study aimed to evaluate the efficacy of RIBA testing to diagnose HCV infection in pre-marital screening couples positive or indeterminate for anti-HCV antibodies.

MATERAILS AND METHODS

Study Population and Design

This study targeted 15342 subjects attending premarital and genetic counseling Al-Noor Specialist Hospital (NSH), Makkah, Saudi Arabia, with marriage proposals between 2015-2016. All samples were collected at the Immunology and Serology Department, NSH. A total of 98 positive and indeterminate HCV ELISA samples are used in this study. All samples were aliquated into two portions; one was kept at 70°C until processing for RT-PCR and the other was subjected to HCV antibody detection by ELISA and recombinant immunoblot assay (RIBA) 3rd generation (INNO-LIA HCV Score; Fujirebio, Europe N.V. Ghent, Belgium). Indeterminate RIBA 3 results were further subjected to PCR.

Methods

Third generation Monaliza ELISA

Monolisa™ Anti-HCV PLUS Version 2 is a direct immunosorbent assay technique that allows detection of antibodies associated with infection by the hepatitis C virus in serum or plasma of the patient. It is based on the use of a solid phase prepared with 3 purified recombinant proteins selected from the nonstructural region (NS3 and NS4) and the structural region of the hepatitis C genome. Detection is performed with goat anti human IgG and attached to the peroxidase. The protocol was followed upon manufacture's advice.

RIBA 3 INNO-LIA HCV Score assay (Fujirebio, Europe N.V. Ghent, Belgium)

We performed the test according to the information provided by the manufacturer. The INNO-LIA HCV Score is a Line Immuno Assay (LIA) for the detection of antibodies to human hepatitis C virus in human serum or plasma. The protocol was followed upon manufacture's advice.

Molecular Diagnosis: Abbott RealTime HCV Quantitative Assay

HCV RNA in serum or plasma can be quantitated using nucleic acid amplification or signal amplification technologies (ref15) The Abbott RealTime HCV assay uses RT-PCR technology combined with homogeneous real time fluorescent detection for the quantitation of HCV RNA. The assay is standardized against the Second WHO International Standard for Hepatitis C Virus RNA (NIBSC Code 96/798) and results are reported in International Units/mL (IU/mL). The Abbott Real Time HCV assay uses RT-PCR to generate amplified product from the RNA genome of HCV in clinical specimens. The protocol was followed upon manufacture's advice.

RESULTS

A total 98 blood samples with positive and indeterminate

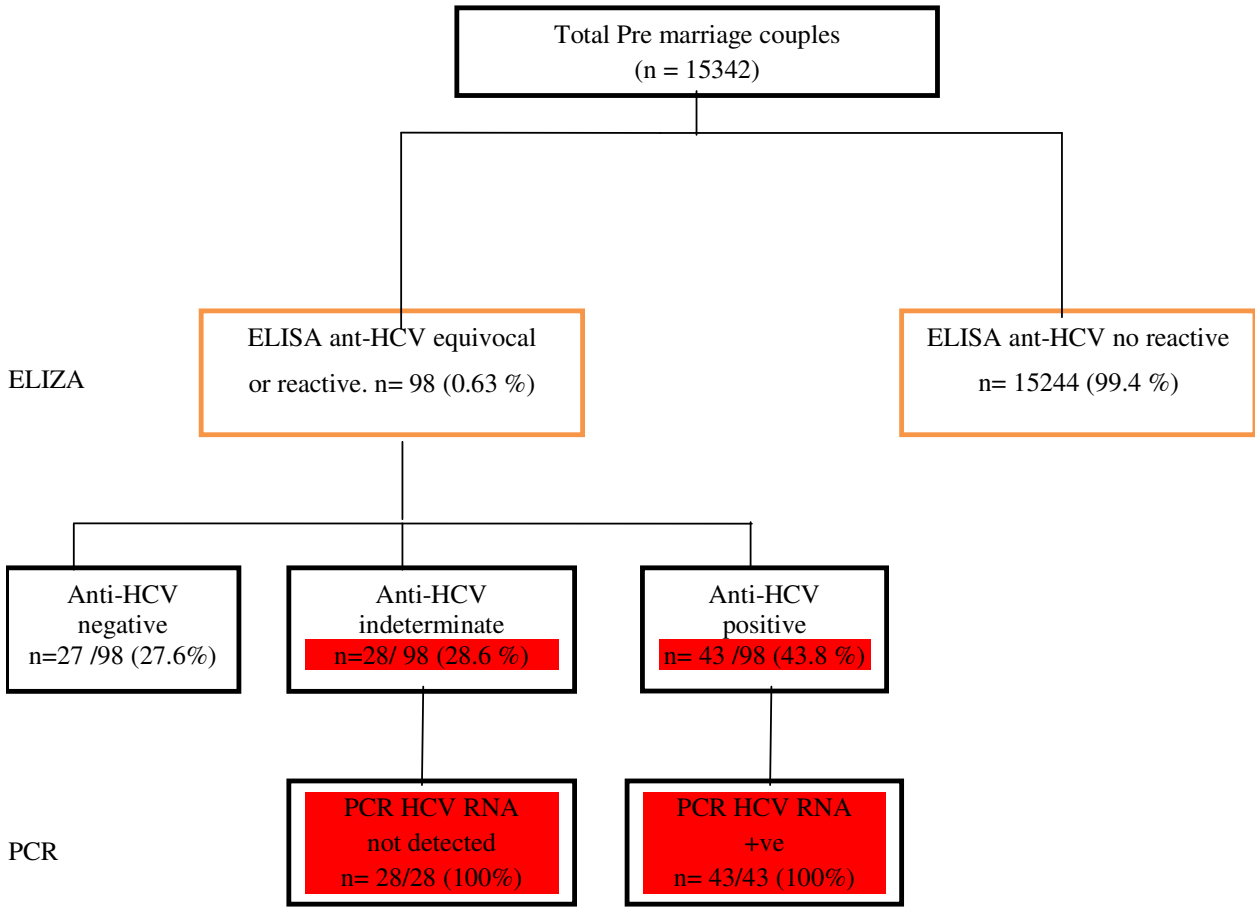

Figure 1. Flowchart of prevalence of anti-HCV pre-marriage couples

Table 1. Comparison of RIBA results, anti-HCV ELIZA screening and HCV-RNA PCR

	ELISA Screening		RIBA			PCR RNA	
	+ ve	-ve	+ ve	Indeterminate	-ve	+ ve	-ve
Pre-marriage couples (n= 15342)	98	15244	43	28	27	0	28
% of positivity	0.63 %		0.28 %			0 %	

Table 2. Frequency and distribution of detected antigens observed in RIBA test

Number of positive RIBA bands		RIBA test antigens					
		C1	C2	E2	NS3	NS4	NS5
Indeterminate (28)	1*	9	3	4	12	0	0
Positive Samples (43)	2 (7)	2	2	0	5	5	0
	3 (7)	7	7	3	2	2	0
	4 (8)	8	8	3	8	5	0
	5 (9)	9	9	7	9	9	2
	6 (12)	12	12	12	12	12	12
Total		47	41	29	48	33	14

*RIBA indeterminate

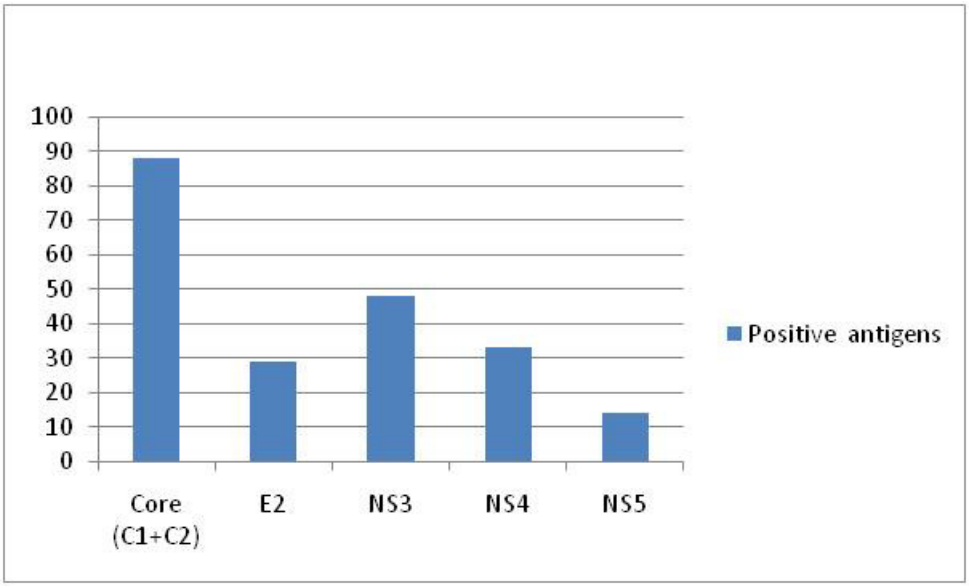

Figure 2. Frequency of positive antigens

results for anti-HCV were further assessed for anti-HCV antibodies using a RIBA 3.0 Strip Immunoblot Assay. The positivity rates for HCV infection by ELISA among premarital screening couples were 0.63 % (98 out of 15342). Using RIBA, 28 out of ELISA +ve cases were RIBA indeterminate, 43 were RIBA +ve and the remaining 27 were RIBA -ve. All RIBA indeterminate cases were negative using qualitative HCV-RNA analysis (results shown and summarized in Fig 1 and 2 & Tables 1 and 2).

DISSCUSSION

Anti-HCV antibodies' testing is the main stage to diagnose the infection with hepatitis C virus. Although anti-HCV assay performance had better-quality during the last 2 decades, higher sensitivity required for population screening may lead to limitations in specificity. Thus, there remains an uncertainty how to interpret anti-HCV test results with a borderline values. The presence of anti-HCV antibodies indicates prior exposure to HCV, while PCR-RNA detection suggests that the person has detectable levels of HCV in his blood (Colin et al., 2001).

The Monolisa was best to identify negative samples (100%) (Maasoumy et al., 2014). False-reactive HCV test results are of great concern in premarital screening because it may lead to psychological adverse effects in the screened couples. So efforts should be made to report correct accurate results. In the present study, third generation ELISA for the anti-HCV antibody detection test is used at Al-Nour hospital laboratory. ELISA is easy to use and inexpensive. Furthermore, this assay could be fully automated and adapted to large volume testing. Therefore, ELISA to detect anti-HCV antibody are generally recommended for screening the HCV infections (Colin et al., 2001; Maasoumy et al., 2014).

In the current study, HCV was detected in (0.6%, 98/15342) of the couples who were tested positive or indeterminate for anti-HCV antibodies by ELIZA. They were submitted to RIBA, which in contrast with the lower percentage of indeterminate RIBA samples that was previously reported (Saudi Ministry of Health, 2018). False positive results were (27/98, 27.6%). In low-risk populations, such as blood donors, HCV screening lacks specificity, as approximately 60% of reagent samples return false-positive results (Bes et al., 2009).

Twenty eight samples had RIBA-indeterminate results. HCV-RNA was not detectable in any of these samples, which is consistent with results that have been previously reported (Sievert et al., 2011; Pereira et al., 2014; Tashkandy et al., 2007). However, in other studies (Kiely et al., 2004), the virus was successfully detected in just two indeterminate RIBA samples. The presence of all six bands in most RIBA +ve samples (28%, 12/43) may be explained by the increased prevalence of a specific genotype in the present study and the fact that the proteins considered by the RIBA nitrocellulose test strips relate to this genotype.

Indeterminate RIBA results were reported in 28.6% (28/98) of the samples obtained from the individuals included in this study, indicating the need for molecular testing as a confirmatory tool in the diagnosis of HCV infection. However, most individuals who had positive RIBA results had strong band intensity (2+ or 3+). In the present study, antibodies directed to core region (c1 and C2) encoded antigens were found to be of higher frequency in most of the studied persons.

Three types of assays (ELISA, RIBA and PCR) were used at AL-Nour hospital to screen for HCV infection. Does premarital screening of couples need to use all three? Diverse diagnostic algorithms have been considered which reflects different opinions on this subject (El-Hazmi, 2004; Madani, 2007; Alswaidi and O'Brien, 2010). There is no question on the utility of ELISA as a screening test for all subjects.

CONCLUSION

In conclusion, those individuals with indeterminate RIBA results had no detectable HCV-RNA suggesting that real-time PCR may be unnecessary, particularly in low-risk populations.

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