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Significance of RT-PCR over ELISA Technique for the Detection of HCV in Blood Donor.

Hepatitis C virus (HCV) is one of the most important causes of chronic liver disease. This study was undertaken to analyze and compare the sensitivity and specificity between Enzyme-linked immunosorbent assay (ELISA) and Real-time Polymerase Chain Reaction (RT- PCR) in the detection of hepatitis C virus in blood donors. The aim was to adapt published PCR methods for use in routine diagnostic work rather than for research purposes. For this purpose, forty-eight samples from blood donors were randomly recruited from those attending the Transfusion Medicine Unit at the Hospital University Sciences Malaysia, Kelantan. The samples were analyzed using ELISA and RT-PCR techniques. The results indicate that among the suspected HCV cases, 22.92% were found to be anti-HCV Elisa positive. When these samples were retested by using RT-PCR, 33.33% of the total samples were found to be HCV-RNA positive. So it may be concluded from this study that RT-PCR is very important for early diagnosis of HCV infection as well as for monitoring antiviral therapy response.

Keywords: HCV, ELISA, RT- PCR.

INTRODUCTION

Hepatitis C virus (HCV) infection is becoming a global public health problem {1}. The overall prevalence of HCV infection is 1-2% in most countries that have been studied {2}, but the distribution of HCV varies considerably among populations. Hepatitis C is a relatively common disease and the word "hepatitis" means inflammation of the liver. Today, almost 20 years after the identification of the virus, WHO estimates that about 180 million persons, 3% of the world's population, are chronically infected with HCV and 3 to 4 million persons are newly infected each year. In Malaysia the prevalence of hepatitis C is about 1.5% and amongst blood donors 0.3% is positive for anti-HCV. Screening for blood donation has been carried out since 1991 in the National Blood Bank {3}. HCV accounts for approximately 20% of cases of acute hepatitis and 70% of cases of chronic hepatitis. Chronic hepatitis C is a major cause of cirrhosis (40%) and hepatocellular carcinoma (60%). Moreover, HCV related end-stage liver disease is the first cause of liver transplantation (30%) {4, 5}. Chronic hepatitis C can cause cirrhosis, liver failure, and liver cancer. The viral hepatitis C infection had been referred to as parentrally transmitted "non A, non B hepatitis". It has a positive-strand RNA genome with approximately 10,000 nucleotides and encodes a single polyprotein of about 3,000 amino acids {6}. Detection and quantification of hepatitis C virus (HCV) RNA is integral to diagnostic and therapeutic regimens. Hepatitis C virus test looks for proteins (antibodies) or genetic material (RNA) of the virus that causes hepatitis C. Enzyme-linked immunosorbent assay (ELISA) test is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality control check in various industries. In simple terms, in ELISA an unknown amount of antigen is affixed to a surface, and then a specific antibody is washed over the surface so that it can bind to the antigen. Unlike antibody tests, HCV RNA tests directly measure for the presence of the hepatitis C virus. HCV RNA tests may be qualitative or quantitative. Qualitative HCV- RNA tests are used to diagnose hepatitis C. In Hepatitis C Infection RT-PCR test can be used for qualitative and quantitative measurement of viraemeia in diagnosis of acute HCV infection, diagnosis of anti-HCV-negative chronic hepatitis C carriers, evaluation of HCV viraemia in asymptomatic blood donors with normal liver enzymes, assessing virological response to treatment, predicting treatment response to alpha interferon and assessing severity of disease. It is recommended that RT- PCR be used in assessing treatment efficacy. The current research is designed upon rational basis of molecular diagnosis knowledge to compare between ELISA and RT-PCR techniques in detecting HCV in blood donors. The main objectives of the current study are to assert the significance of RT-PCR in the diagnosis of HCV infection over ELISA techniques. It is also aimed to prevent infection transmitted through screening of donor blood and plasma.

MATERIAL AND METHODS

Forty-eight sera samples were collected from randomly recruited blood donors those attending the Transfusion Medicine Unit at the Hospital University Sciences Malaysia, Kelantan. All sera were collected aseptically and stored at -20 oC prior to testing.ELISA test data was collected from Transfusion Medicine Unit at the Hospital University Sciences Malaysia, Kelantan. The viral nucleic acid extraction kit (DNA/ RNA) by Yeastern Biotech was used to extract RNA from sera. Preparation for RT- PCR: Geno-Sen's HCV Real Time PCR Kit was used.

RESULTS: Out of the forty-eight sera were collected and tested by Enzyme-linked immunosorbent assay (ELISA). The results pointed out that 34 samples were negatives for HCV, 3 samples were indeterminate and 11 samples were ELIZA positives HCV. While, in the same samples RT- PCR technique recorded 32/48 samples negatives and 16/48 samples positives for HCV- RNA. The results are listed in the **table I**. The figure 1 shows the percentages of negative and positive samples using ELIZA and RT-PCR techniques to detect HCV in blood donors. It is noticed that RT-PCR is a powerful technique to detect HCV in the blood samples even the virus is present at extremely low level.

**Table I.** Investigation of HCV in blood donors by ELISA and RT-PCR.

Case of sample	Technique	
	HBV-ELISA	**RT-PCR**
- ve	34 (70.83%)	32 (66.67%)
+ve	11 (22.92%)	16 (33.33%)
Indeterminate	3 (06.25%)	00 (00.00%)

Percentages of false negativity of HCV- ELISA test compared to RT-PCR techniques in detection of HCV. The results are listed in table (II).

Table II. Percentages of false negativity of HCV- ELISA test compared to RT-PCR techniques in detection of HCV among blood donors.

Case of sample	HCV-ELISA	HCV-RT-PCR	Percentage of false negativity
- ve	34	32	5.88%
+ve	11	16	31.15%
Indeterminate	3	00	0.00%

Fig 1. Percentages of total, negative and positive samples of blood donors using
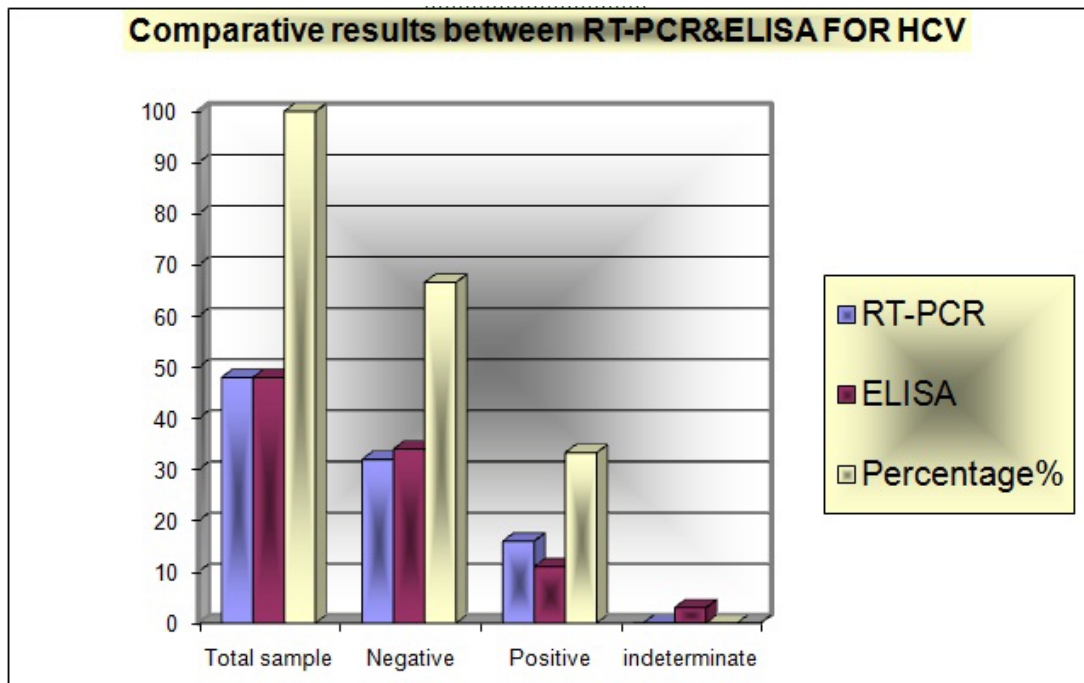
DISCUSSION

Nucleic acid amplification technologies such as PCR allow earlier and more specific detection of active infections in donated blood than earlier generation serology tests, helping to ensure a safer blood supply and retention of donors who would otherwise be deferred. The TaqMan HCV quantitative test provides a rapid, extremely sensitive, specific method for measuring HCV viral load over a very broad dynamic range. Results are available within 2.5 hours of initiating amplification instead of the 4 to 6 hours for heterogeneous format tests, in which products are quantified in a separate detection assay performed on completion of amplification.To aid in decision-making, two diagnostic blood tests are available for HCV infection; the anti-body tests ELISA and recombinant immunoblot assay (RIBA) and the polymerase chain reaction (PCR) for RNA detection. The presence of HCV antibodies suggests prior exposure to HCV but does not confer immunity, while PCR-RNA detection indicates that the patient has detectable levels of HCV in his blood.In the present study, data comparing the results of ELISA and RT-PCR in diagnosis of HCV infection, as the levels of HCV-RNA fluctuate above and below the threshold for detection by PCR as pointed out by Testing (1999), showed marked discrepancy in detection percentage between the two techniques. Data in the present study showed that 33.3% of total blood samples (16 out of 48) had detectable HCV-RNA positive by using RT-PCR corresponding to 22.9% (11 out of 48) ELISA positive. This is can be explained as the acute infected patient produce antibodies within three months of exposure and sometimes more. The present study give some support to the finding of Hanuka et al {7},who found that 9% of seronegative dialysis patients were HCV-RNA positive with low viral load. These data question the isolation criteria that are used presently in some chronic hepatitis delta (HD) units to prevent HCV transmission. Although periodic anti-HCV determinations remain mandatory, their negativity does not discard the possibility of infection. So, the increased false negativity for antibody tests ELISA compared with RT- PCR detection in HCV samples may be due to weaker and delayed response of these immunocompromized infected people to produce antibodies. In a study performed in India by Baheti et al {8}, the percentage of seroprevalence of anti-HCV antibodies was studied among healthy blood donor volunteers and high-risk individuals. The present data showed that the percentage of false negativity of HCV- ELISA test compared to RT-PCR technique among Malaysian blood donors was 14.4% (5 out of 34). This false negativity may be due to the presence of low levels of antibodies in blood during the HCV-window period. This has been documented by McCoy et al {9}, who reported that 90% of infected people would produce antibodies within three months of exposure and 10% take an even longer time to produce the antibodies and thus, the test needed to be repeated six months after exposure.The current study shows an interesting and important data on comparing results for false negativity among volunteer with HCV (5.88%). This may be due to the higher prevalence of acute HCV infection among blood donors. These results are documented by Kolker {10}, who reported that third generation ELISA and RIBA assays pick up only half of HCV-RNA positive patients co-infected with HIV. When ELISA was compared with RTPCR, the false positivity was 15.625% among blood donors. This suggests a high specificity of RT-PCR to exclude HCV-viremia inspite of presence of non-specific antibodies. The results of the present study are in agreement with Martins et al {11}, using second generation ELISA, reported that HCV-viremia was present in 76.6% of the anti-HCV positive blood donors, (23.4% false positive) in central Brazil. The presence of HCV-Ab in the absence of viremia in blood donors may be due to a previous infection and that the person is immune at the time of examination {12}. Among immune compromised patients, the percentage of false antibody positivity is less since synthesis of antibodies in these cases indicates the presence high viremia to stimulate their depressed immune system. However, the presence of false positivity by RT-RNA may be due to the presence of non-specific antibodies detected by ELISA screening test {13, 14}. The high percentage of false negativity for HCV-antibody detection by ELISA may be due to the immunosuppression that characterizes HD and HIV patients or it may be due to the absence of detectable anti-HCV antibody levels during the window stage of infection. The high degree of false negativity associated with ELISA screening for HCV among blood donors is a big problem and leads to increased risk of transfusing contaminated blood. On the other hand, false positivity leads to increased wastage of otherwise the safe units of blood, especially those belonging to rare blood groups, apart from causing anxiety to the donor.

CONCLUSION

The TaqMan HCV RT-PCR is an almost completely automated, highly sensitive, specific, and rapid method that is reliable for HCV RNA screening of blood donations. It allows closed-tube HCV RNA detection without risk of contamination by PCR products. Thus, it is very important to screen blood donors, by using the RT-PCR for HCV RNA to avoid the occurrence of false negative results of HCV antibody detection tests. To avoid false positive results, we should repeat RT-PCR twice every year and if it remains negative, this means that the patient is free from viremia. So, we recommend the use of highly specific and sensitive tests RT-PCR for detection of HCV in the blood to control and prevent infection with the HCV. The preliminary study suggested the important and needs of the implementation of nucleic acid test in blood transfusion services.

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