ORIGINAL RESEARCH

The correlation between polimerase chain reaction and serological assay in the diagnosis of hepatitis c virus infection

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ABSTRACT
Background: Hepatitis C virus (HCV) is a major public health problem globally. Diagnosis of HCV infection is mainly based on the detection of anti-HCV antibodies by the Enzyme Linked Immunosorbent Assay (ELISA) or Rapid Diagnostic Tests (RDTs) and HCV Ribonucleic Acid (RNA) by Polymerase Chain Reaction (PCR) of serum or plasma samples. **Aims & objective:** This study evaluate the efficacy of the RDT/third generation ELISA for detection of antibodies to hepatitis C virus (anti-HCV) in comparison with reverse transcriptase polymerase chain reaction (RT-PCR) to detect HCV RNA for the diagnosis of hepatitis C virus. **Material & method:** This was a comparative observational study. A total of 500 samples received were tested for HCV infection. Different techniques for diagnosis of HCV were used rapid card test (RDT), ELISA, and RT-PCR etc RDT/ELISA positive samples further tested for detection of HCV RNA by RT-PCR. HCV viral load was also measured. **Results:** Out of total positive cases majority of them (54.6%) were 21-30 years age group, predominantly male (77.3%).blood transfusion was the most common risk factor of HCV infection. Out of total 22 (4.4%) samples were reported positive for anti HCV antibody by RDT and ELISA, same number of samples positive for HCV RNA by RT-PCR. **Conclusion:** RT-PCR is very important gold standard test for early diagnosis of HCV infection as well as for monitoring antiviral therapy response.

Keywords: HCV infection, Anti-HCV antibodies, ELISA, RDTs, RT-PCR

INTRODUCTION
Hepatitis C virus (HCV) is a blood borne pathogen that is endemic in most parts of the world, with an estimated overall prevalence of nearly 3% [1]. HCV transmission by blood and blood derivatives can always occur and in viral terms there is no safe blood if not the autologous one. HCV transmission, even by repeat donors is possible due to laboratory mistakes or to the 10-20 week window period separating anti-HCV detectability from infectivity [2-3].

Risk factors of HCV infection are; intravenous drug users, HIV, haemodialysis patients, individuals with high-risk sexual behaviour or requiring multiple blood transfusions [4]. Diagnosis and management of HCV infection depends on serological tests to detect anti-HCV antibodies using RDTs or ELISA and molecular tests to detect and quantitate HCV-RNA or to detect genotypes employing Nucleic Acid Testing (NAT) in the serum or plasma of the patients [5]. The anti-HCV antibodies can be demonstrated in seven to eight weeks after infection and generally persist lifelong [6]. WHO has recommended employing RDTs with a sensitivity of ≥98% and a specificity of ≥97% for HCV serology in plasma or serum specimens [7]. Hence, detection of HCV using the most accurate and sensitive NAT assay like real time RT-PCR will reduce the risk of transmission of HCV and help in the early detection even during serological window period as it can detect the HCV RNA in one to three weeks after infection [8]. Serological assays for detecting anti-HCV antibody cannot distinguish between patients with active infection and those who have cleared the virus, and due to the absence of an efficient in vitro culture system for HCV or assays capable of detecting viral antigens, direct detection of HCV has depended on nucleic acid amplification technology (NAT) techniques [9-10].

AIMS & OBJECTIVES

This study was undertaken to evaluate the diagnostic accuracy of rapid antibody test/third generation ELISA comparing with reverse transcriptase real time PCR, for the detection of HCV.

MATERIALS AND METHODS

This was a comparative observational study carried out in the Department of Microbiology, MGM Medical College in Central India. A total of 500 blood samples were screened for the presence of Anti-HCV antibodies through rapid diagnostic test or third generation ELISA.

The patient socio-demographic data (name, age, sex and address), Inpatient/outpatient number, risk factors, history of blood transfusion and clinical history were noted.

Serum was separated from 5 ml of blood in the disposable tubes. Further process were followed according to the manufacturer's instructions RDT/ELISA

RDT/ELISA positive samples further tested for detection of HCV RNA by reverse transcription and real time polymerase chain reaction (RT-PCR). Rapid test (Oscar HCV test) was used for qualitative detection of antibodies (IgG) to HCV in human serum/plasma by Immuno-chromatography. The HCV ELISA test (VoxEL Bio LTD.) for the qualitative detection of antibodies against HCV in human serum and plasma. Quantitative detection of HCV viral load also measured.

Sensitivity and efficacy of all the diagnostic tests (RDT/ELISA and PCR) were compared.

Statistical analysis

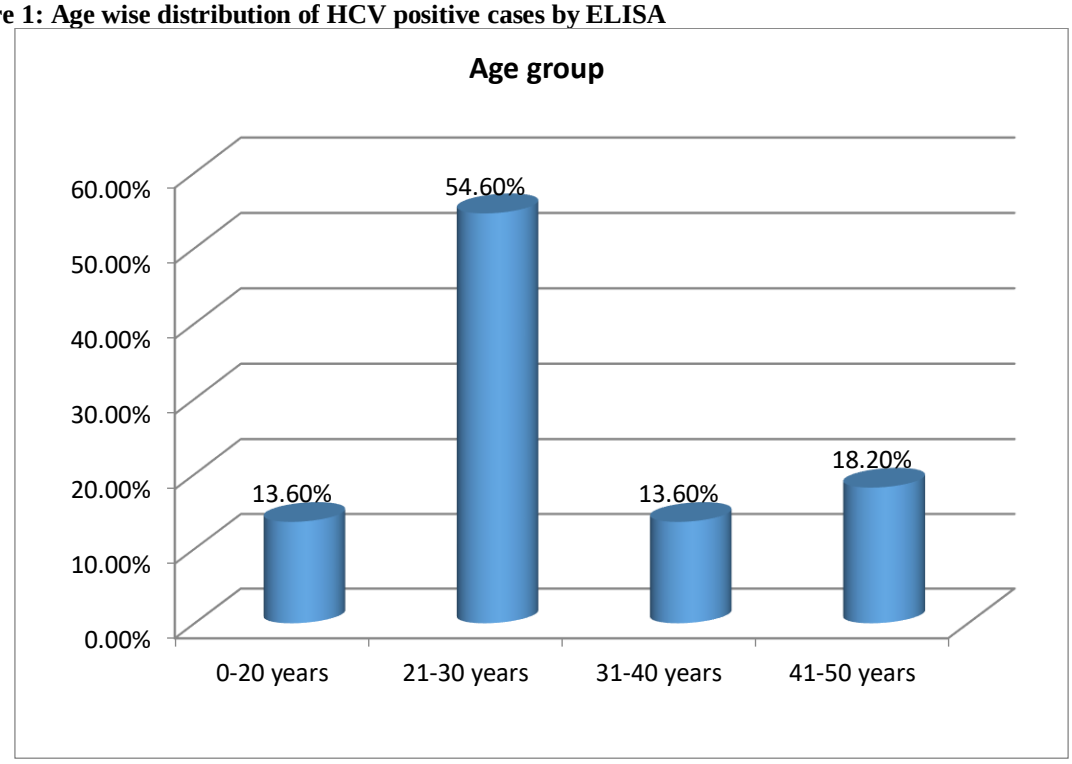
All the collected data were entered and analysed by SPSS (22.0) version. Descriptive statistics such as frequency, percent, mean, and SD were determined. P-value <0.05 was considered as statistically significant in all analyses.

RESULTS

The serological assay was done for detection of Anti-HCV antibodies. Out of 500 samples tested for Anti-HCV antibodies 22 (4.4%) were positive by RAPID and ELISA.

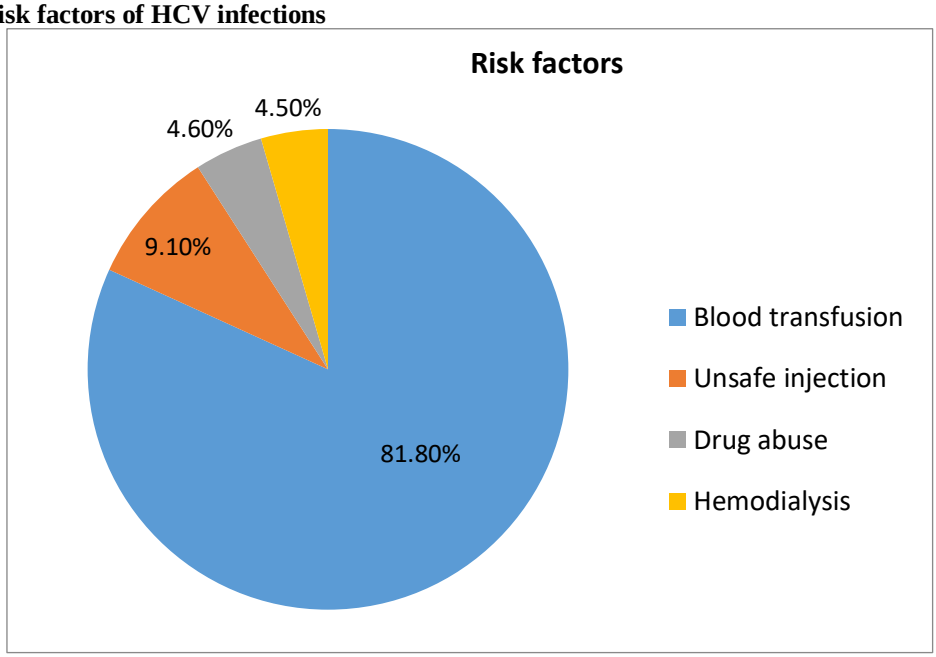
Out of total positive cases majority of them (54.6%) were 21-30 years age group, predominantly male (77.3%).

Figure 1: Age wise distribution of HCV positive cases by ELISA



Blood transfusion was the most common risk factors of HCV infection

Figure 2: Risk factors of HCV infections



Out of 22 samples tested for the presence of HCV RNA by real time only RT-PCR, 22 (4.4%) were detected to have HCV RNA. 22 (4.4%) samples had been found to be positive by both ELISA and PCR as well as RAPID

Table 1: Quantified results of PCR on positive immunochromatography/ELISA samples

Result	RAPID/ELISA positive cases		PCR +ve cases	
	No	%	No	%
Positive	22	4.4%	22	4.4%
Negative	478	95.6%	478	95.6%

On comparison with values of viral load we found 20 cases in which HCV RNA detected, and quantitative result is within the linear range of the assay and 2 cases in which HCV RNA detected, but quantitation not possible since quantitative result is above the linear range of the assay.

Table 2: Quantified HCV viral load among RT-PCR positive cases

HCV viral load (IU/ml)	PCR Positive cases (n=22)	Interpretation
<15	0	HCV RNA detected but is below the Lower Limit of Quantitation (LLOQ) of the assay
>15 and <=1.0* 10^8	20	HCV RNA detected, and quantitative result is within the linear range of the assay
>1.0*10^8	2	HCV RNA detected, but quantitation not possible since quantitative result is above the linear range of the assay

DISCUSSION

The prevalence of HCV varies in different parts of the country. The complex and uncertain nature of HCV infection and its chronicity emphasizes the difficulties in prevention and control of HCV [11].

The proportion of sero-prevalence positive for Anti-HCV antibodies was 4.4% in the present study, this was comparable with the Trickey A et al [12] and Bahadar et al [13], reported prevalence of HCV infection were 3.6% and 4.6% respectively. M. Tahir et al [14] reported higher prevalence (19.7%), whereas Patil SR et al [15] reported much lower prevalence (0.38%) than our study. In relation to variation of HCV sero-prevalence, the reasons cannot be completely discerned. However, the difference in demographic characteristics of the study population, the difference in Hepatitis epidemiology, awareness of the routes HCV transmission, efforts made to implement universal precautions by health professionals and preliminary benefits due to the mandatory HCV screening while blood donation and prior to any surgical procedure might explain these discrepancies

Majority of the HCV positive cases were male and mostly belong to 21-30 years age group, our results constant with the Chakraborty et al [16] and Soin.D.et al [17]. HCV infection was higher proportion in males than females due to more social mobility in males than females and thus greater vulnerability to be infected.

Most frequent risk factor for HCV transmission in this study was blood transfusion (81.2%) followed by

unsafe drug injections, haemodialysis and drug abuse, similar finding also reported by El-Sokkary et al [18] and Amarapurkar et al [19].

The antibody protection is poor in patients on chronic haemodialysis and after renal transplantation due to immunosuppression. The ELISA alone may fail to detect the HCV infection in these cases. HCV RNA testing should be made mandatory for these patients [20].

In our study Anti HCV antibody positive by ELISA, all were confirmed by real time RT-PCR indicating the HCV infection is active, concordance to the Swellam et al [21].

A study done by R M Singh et al [22], RDTs detected HCV in 13.92% samples, ELISA in 12.69% while Real Time PCR was able to confirm the infection in 11.28% samples only.

Another study conducted by Zinab OA, et al [23], found 22.92% 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.

CONCLUSIONS

Serological assays are simple, reliable, user friendly specially RDTs, easily available and more cost effective for the detection of HCV infection, yet they cannot be employed in case of acute infection. Although, HCV-RNA detection by PCR is the only sensitive, reliable and a gold standard test for HCV infection, it may not be possible to adopt it as a screening test due to its high cost, high technical skill requirement.

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