FALSE POSITIVITY OF SEROLOGICAL TESTS FOR HEPATITIS C VIRUS

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Background: Hepatitis C virus infection is now one the common infection in Pakistan. Patients are routinely screened by antibody assays. Objective of this study was to assess the viremia in patients labelled as anti-HCV positive by ELISA. **Methods:** It this retrospective study patients labelled as anti HCV positive by ELISA were assessed for HCV RNA by polymerase chain reaction. The 254 HCV positive cases which were analysed by MEIA method on AxSYM System were selected for RNA extraction by RT-PCR upon Cepheid thermal cycler using TaqMan technology and subsequent for amplification. **Results:** Out of 254 anti HCV positive by ELISA, viremia was shown in 211 patients; the remaining 43 (16.92%) samples were false positive. **Conclusion:** Out of a total 254 anti HCV positive patients, 211 had viremia by RT-PCR. The false positivity noted was 16.9%.

Keywords: Anti HCV, RT-PCR, HCV, Blood

INTRODUCTION

The introduction of hepatitis C virus in 1989 illuminated many dark corners in the natural history of the infection formerly known as non-A, non-B hepatitis.[1,2] Hepatitis C virus (HCV) is a single stranded RNA virus and belong to flaviviridae. It is a global health threat.[2] The prevalence range from 2% in the developed countries through 6.5% in the parts of equatorial Africa to as high as 20% in Egypt.

Hepatitis C is a disease with various rates of progression. In general, its course is slowly progressive. About 15% of HCV infected individuals recover spontaneously (viral clearance); an additional 25% have an asymptomatic illness with persistently normal aminotransferases and generally benign histological lesions; hence, about 40% of patients recover or have a benign outcome. In those with biochemical evidence of chronic hepatitis, the majority have only mild to moderate necro-inflammatory lesions and minimal fibrosis: their long-term outcome is unknown and, probably, most of them will not succumb to the liver disease. About 20% of patients with chronic hepatitis C develop cirrhosis in 10–20 years, and may die of complications of cirrhosis in the absence of liver transplantation.[2–4]

Transmission is mostly trough blood and blood products, sexual contact and vertically from mother to newborns. Vertical transmission is seen in less than 6% of neonates.[2,5,6] High-risk population includes patient receiving multiple blood transfusions, patients on haemodialysis, and those with multiple sexual partners.[7–9]

Patients with chronic hepatitis C may come to medical attention because of symptoms of their disease or complication[1]; moreover, they are identified on screening at the time of blood donation or as a part of investigation done for abnormal liver enzymes.[2]

Lab tests generally done are rapid immuno-chromatographic device method, ELISA and subsequently conformed by reverse transcription plymarase chain reaction (RT-PCR).

The rapid immunochromatographic method gives result in 15–20 minutes. The assay starts with a sample applied to the sample well followed by sample diluent. HCV antigen-colloidal gold conjugate embedded in the sample pad reacts with HCV antibodies in patient's serum forming conjugate-HCV antibody complex. This is allowed to migrate laterally along the test strip, the conjugate is captured by an antibody binding immunoglobulin applied on a line A, and thus immobilised forming a coloured band in the test region to be read as positive for anti HCV. Antibody negative sample is not captured and so the test area remains clear.[10]

The ELISA '1' test initially designed in 1989 was detecting antibodies against c100-3 antigen protein. Later on next generation ELISA '2' was introduced in 1992 which recognises epitopes from core (c22), (NS3) and (NS4) viral protein and the chances of false negativity with the first generation ELISA were significantly reduced. Now the latest ELISA '3' recognises and additional (NS5) epitope and chances of missing to detect viral protein are greatly reduced.[11–13] The procedure is further supplemented by coating the antigen on micro-particles by Abbott diagnostics and has increased the sensitivity (designated as microparticulate ELISA method (MEIA).[11,12,14,15]

Unlike ELISA in which antibodies are detected, the RT-PCR identifies the highly conserved antigenic areas of the virus present in the test sample. The method is further improved by the real

time PCR in which TaqMan Chemistry is used. In this method oligonucleotide probe is constructed containing a reporter fluorescent dye on the 5's end and a quencher dye on the 3' end. The proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). If the target sequence is present, the probe anneals downstream from the primer site and is cleaved by the 5' nuclease activity of Taq DNA polymerase as this primer is extended. In this way the quencher dye separates and the primer is increased in length. That completes one cycle. With each cycle, the reporter dye is cleaved and fluorescence is increased with each cycle the intensity of which is proportion to amount of amplicon produced which can be graphically seen on computer screen and then easily interpreted.[14] Different automation with software are available to assemble the dye fluorescence in graphic pattern.

MATERIAL AND METHODS

From the laboratory data 254 patients were selected whose rapid immuno-chromagraphic tests and ELISA were positive. The rapid tests were done with Acon™ test devices. The ELISA for anti HCV was done by MEIA method. The selection was done in continuation with no skipped test result. For all these patients HCV RNA was done subsequently by RT-PCR in the same laboratory.

AxSYM from Abbott Laboratory Diagnostic System was used for the study. This is an automated system for doing ELISA. The kits used were for MEIA assay method from Abbott Diagnostic. The kits were used according to the instructions of the manufacturer, all those cases with cut-off value more than 1 were considered positive.

For RT-PCR, Saccace Isolation and Amplification kits were used. The kits are designed for real time polymerase chain reaction which we ran on Cepheid thermo cycler. The Cepheid thermo cycler is an automation in which the reverse transcription is completed in 90 minutes by reverse transcriptase in the presence of nucleic acid base and other optimum condition. This step is followed by the subsequent steps of annealing, amplification, denaturation in a in computer controlled thermal cycles recommend for annealing, polymerase activity and denaturation of cDNA. The kits used for Real Time PCR were designed by TaqMan Biotechnologies in which the fluorescent dyes used for internal control and test are separate and both procedures are run in the same tube. The emission of flurescence is displayed graphically on the computer screen. In all tests the internal controls were detectible in 32nd to 34th cycles and the unknown if positive showed detectable fluorescence at various cycles corresponding to the viral loads in the specimen. We ran the cycler up to 42nd cycles, up to which if an unknown sample did not show rise of fluorescence that case was labelled as negative according to the instruction manual of Saccace kits.

RESULTS

Table-1 shows PCR positivity of ELISA positive samples.

Table-1: PCR positivity of ELISA positive

Positive samples by ELISA	Positive samples by RT-PCR	Negative samples by RT-PCR	% negativity
254	211	43	16.92

Out of 254 anti-HCV positive by ELISA show viremia in 211 patients, the remaining 43 (16.92%) samples were false positive.

The representation of the test results clearly show a substantial proportion of patients with no viremia.

DISCUSSION

Pakistan is an endemic area for hepatitis C. The routine screening tests for hepatitis C based on antibody detection methods including paper chromatographic methods and ELISA.[16]

ELISA is an antibody dependent test which detects certain protein epitops of viral antigenic protein. After exposure, 90% of the patients show detectable anti HCV in three months and the remaining 10% may take even longer, despite the presence of viremia in acute infections.[2,12,17] The chances of false negativity and false positivity are not uncommon like other antigen-antibody dependent reactions.

In early phase of acute HCV infection there is a window period in which the antibodies have not yet reached the detectable level by ELISA and hence the ELISA tests are falsely negative despite the viremia.[3,4,7,9,15] False negative tests are also frequent in those patient who are immune deficient due to chemotherapy, steroid therapy, HIV infection, patient receiving immunosuppressive therapy for organ transplants, chronic renal failure who are on haemodialysis and neonates being assessed for vertical transmission.[2,3,9,11,13] So in such patients with abnormal liver enzymes RT-PCR should be done to exclude the HCV infection as these patient are high risk patient for HCV infection. Seronegativity in such conditions are reported in 5.5–16.7%.[2,7,18]

False positive ELISA tests are also not uncommon.[18] False positive ELISA for anti HCV can be seen in patients who have cleared the virus after acute infection or by therapy and as such may be positive on ELISA which may indicate past infection.[9,12,17] Patients with autoimmune hepatitis and other hyperglobulinemic states give false positive tests, false positive cases have been noted in 23% of patients in whom these could be due to non-specific antibodies detected by ELISA or more likely due to previously cleared virus after acute attack.[2]

In low-risk population (e.g., healthy blood donors) the false positivity of ELISA has been recorded up to 25% in one study[15] while in another study it was 20.21%.[2]

Some disorders linked to HCV infections, e.g., autoimmune hepatitis, Sjögren's syndrome, Lichen planus, thyroiditis, membranous glomerulonephritis, polyarteritis nodosa and essential mixed cryglobulinaemia may be screened and if positive should be conformed for HCV RNA by RT-PCR.[3,8,20]

We noted 16.92% false positivity in a total of 254 ELISA positive patients. This figure falls somewhere in the middle of different studies performed.

CONCLUSION

We detected false positivity of ELISA test for anti HCV in 16.92% of cases. ELISA should not be considered as the only laboratory tool used for confirmation of HCV infection. RT-PCR should be used in all cases of ELISA positive patient to assess recent or past infection and before initiating antiviral therapy. Keeping in mind the false negativity of the test in certain high-risk population, RT-PCR should be done to exclude the possibility of HCV infection.

RECOMMENDATION

The diagnosis of 'HCV positive' has a deep impact on the life of affected person. Therefore, it must be reached as reliably as possible. Our data show 16.92% false positivity and we strongly recommend RT-PCR for diagnosis of HCV infection. HCV infection clearance/persistence should be assessed by RT-PCR, as the antibody persists for longer duration after the virus is cleared. Similarly, immune suppressed patient should be tested by RT-PCR at some regular intervals, as such high risk patient may have co-infection of HCV.

REFERENCES

1. D'Souza R, Foster GR. Diagnosis and treatment of hepatitis C. J R Soc Med 2004;97:223–5.
2. Tashkandy MA, Khodari YA, Ibrahim AM, Dhafar KO, Gazzaz ZJ, Azab BA. Evaluation of the available anti-HCV antibody detection tests and RT-PCR assay in the diagnosis of Hepatitis C Virus Infection. Saudi J Kidney Dis Transpl 2007;18:4:523–31.
3. EASL International Consensus Conference on Hepatitis C. Paris, 26–28 February 1999, Consensus Statement. European Association for the Study of the Liver. J Hepatol 1999;30:956–61.
4. Quiroga JA, Llorente S, Castillo I, Rodríguez-Iñigo E, Pardo M, Carreño V. Cellular immune responses associated with occult hepatitis C virus infection of the liver. J Virol 2006;80:10972–9.
5. Zylberberg H, Pol S. Reciprocal interaction between HIV and hepatitis C virus infection. Clin Infect Dis 1996;23:1117–25.
6. Wejstal R. Sexual transmission of hepatitis C virus. J Hepatol 1999;31(Suppl 1):92–5.
7. Sánchez-Tapias JM. Nosocomial transmission of hepatitis C. J Hepatol 1999;31(Suppl 1):107–12.
8. Thomas DL, Astemborski J, Rai RM, Anania FA, Schaeffer M, Galai N. The natural history of hepatitis C viral Infection. JAMA 2000;284:450–6.
9. Al-Ghamdi SM, Al-Harbi AS. Hepatitis C Virus serostatus in hemodialysis patient returning from holidays: another risk factor for transmission. Saudi J Kidney Dis Transpl 2001;12(1):14–20.
10. Health-Chem Diagnostics. The accuracy of rapid test for the diagnosis of hepatitis C virus. Available at: www.healthchemdiagnostics.com. Retrieved on 13 Jan 2010.
11. Daniel HD, Abraham P, Raghuraman S, Vivekanandan P, Subramaniam T, Sridharan G. Evaluation of rapid assay as alternative to conventional immunoassays for the detection hepatitis C virus antibodies. J Clin Microbial 2005;43:1977–8.
12. Martinot-Peignoux M, Boyer N, Le Breton V, Le Guludec G, Castelnau C, Akremi R. A new step toward standardization of serum hepatitis C virus-RNA quantification in patients with chronic hepatitis C. Hepatology 2000;31:726–9.
13. Abdel-Nasser MA, Mostafa MM, El-Deeb AM, Khafagy EE, Mahros KE. Correlation between the Serological markers used in the screening for antibodies to hepatitis C virus (HCV). Egyptian J Immunol 1998;5(1):45–51.
14. Barbeau JM, Goforth J, Caliendo AM, Nolte FS. Performance Characteristics of a Quantitative TaqMan Hepatitis C Virus Analyte-Specific Reagent. J Clin Microbiol 2004;42:3739–46.
15. Punungwe P. HCV-RNA PCR is more sensitive test than anti HCV antibody test at detecting HCV. Available at: http://www.bestbets.org/bets/bet.php?id=1223
16. Khattak ST, Ali Marwat M, Khattak ID, Khan TM, Naheed T. Comparison of frequency of hepatitis B and hepatitis C in pregnant women in urban and rural area of district Swat. J Ayub Med Coll Abbottabad 2009;21(2):12–5.
17. Hinrichsen H. Leimenstoll G, Schrader H, Foisch UR, Schmidt WE. Prevalence and risk factors of hepatitis C virus infection in haemodialysis patients: a multicentre study in 2796 patients. Gut 2002;51:429–33.
18. Lee YJ, Chang CT, Wu MS. Acute hepatitis C infection without Anti-HCV antibody in Chronic haemodialysis patient. Chang Gung Med J 2006;29(4 suppl):35–8.
19. Schröter M, Feucht HH, Schäfer P, Zöllner B, Polywka S, Laufs R. Definition of false positive reactions in screening for hepatitis C virus antibodies. J Clin Microbiol 1999;37(1):233–4
20. Chevaliez S, Pawlotsky JM. Hepatitis C virus serologic and virologic tests and clinical diagnosis of HCV related liver disease. Int J Med Sci 2006;3:35–40.

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