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ORIGINAL ARTICLE

Hepatitis C diagnostic management gap in Pakistan—Clinicians' knowledge impacting public health

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Abstract
Aim Hepatitis C virus (HCV) infects 10 million people in Pakistan with a prevalence rate of 4.9 %. Methods commonly used to diagnose HCV include anti-HCV and HCV RNA testing. However, HCV RNA is a molecular test that aims to identify active infection and is a test indicated for monitoring disease progression during therapy, while anti-HCV is a screening tool for detecting the presence of a virus in the body. Furthermore, liver function tests are part of routine testing in cases to assess the extent of liver damage. However, many clinicians in Pakistan are utilizing molecular tests to screen and diagnose HCV. Furthermore, elevated liver function test values are being justified for direct utilization of molecular tests instead of opting for anti-HCV tests. As HCV RNA tests detect only active infection and LFT values can be elevated or decreased because of other infections, using the HCV RNA test as a screening tool may have implications for patient management. Therefore, the main objective of this study was to evaluate clinicians' perspectives for HCV test-based diagnostic management and identify the quality gap with respect to the utility of molecular tests.
Subjects and Methods A retrospective, cross-sectional study was conducted analyzing medical records of patients coming to the gastroenterology outpatient department (OPD) of The Indus Hospital Laboratory, Karachi, Pakistan, during January 2014–2015. The study included patients in whom HCV RNA testing was performed. Both positive and negative HCV RNA groups were further analyzed to determine whether HCV antibody and liver function testing had been performed for the respective patients. The diagnostic management quality gap was identified on the basis of clinicians' perspectives with respect to the International Centers for Disease Control and Prevention (CDC) diagnostic guidelines.
Results A total of 1758 patients with HCV RNA testing were included in the study. Of these, 17.5 % of the patients had been tested for LFT only with direct testing for HCV RNA. In both the HCV RNA-positive (56 %) and -negative groups (44 %), the following discrepancies in diagnostic management were observed—the anti-HCV test was not performed in 51 and 62 % of the patients in the positive and negative HCV RNA group, respectively. Furthermore, 9.6 and 36 % of the HCV RNA-negative and -positive groups had not been evaluated for liver function tests and anti-HCV prior to molecular testing.
Conclusion Diagnostic management of HCV in Pakistan is compromised as clinicians utilize molecular testing as a screening tool rather than using the international guideline for utilizing anti-HCV for the diagnosis of HCV. Furthermore, the results of the liver function tests are being used to justify the generation of molecular tests. This practice is an important parameter in the diagnostic and disease management of HCV in Pakistan and requires robust clinician awareness at the national level.

Contribution FN conducted the study, analyzed the data and wrote the manuscript; AM conducted the study; AR analyzed the data and reviewed the final draft of the manuscript; AA designed the study and made revisions of the final manuscript.

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Keywords Molecular tests · HCV RNA · Liver function tests · HCV antibody · Diagnostic management gap · Pakistan

Introduction

Hepatitis C virus (HCV) infection is a major health problem worldwide. It causes acute and chronic liver disease leading to hepatocellular carcinoma and cirrhosis (Higuchi et al. 2002, Perz et al. 2006, Zeisel et al. 2015).

Globally, 130–170 million people have chronic HCV infection. More than 350,000–500,000 deaths are attributed to HCV infections each year, most of which are caused by liver cirrhosis and hepatocellular carcinoma (Averhoff et al. 2012). In the acute phase, about 20 % of infected subjects clear the virus within 6 months, while the remaining 80 % will progress to developing chronic infection (Liu et al. 2014).

Studies have documented that about 10 million people in Pakistan are chronically infected with hepatitis C virus (Hamid et al. 2004, Raja and Janjua 2008). The prevalence rate of HCV in Pakistan is the highest in the world, i.e., approximately 4.9 % (Arif et al. 2012, Bibi et al. 2013). As the prevalence rate is increasing annually, there is growing interest in the disease transmission and management. The World Health Organization (WHO) revealed that HCV is primarily transmitted through injection drug users (IDUs). In Pakistan >80 % of IDUs are positive for the HCV antibody (Nelson et al. 2011). In developing countries, the common modes of HCV transmission are through health care-associated risks such as unsafe transfusion and injection practices, receiving blood and blood products, blood transfusions and poor infection control practices in hospitals (Nelson et al. 2011). Pakistan has the highest documented rate of injections in the world, while other exposures include tattoo parlors, unsafe nose/ear piercings, barber shops and unhygienic hair treatments. Such practices are common in Pakistan, leading to the increasing incidence of HCV and making the country highly endemic for this infection (Ahmad et al. 2004, Qureshi et al. 2010).

According to the International Centers for Disease Control and Prevention (CDC) recommendations, making the diagnosis of hepatitis C virus is a two-step process: initially, HCV antibody (anti-HCV) testing is recommended. If the antibody is positive,then the viral nucleic acid is detected to determine active infection. If HCV RNA is not detected or negative, then it signifies that there is no HCV infection, while if HCV RNA is detected, there is a current or active infection, and treatment and further diagnostic steps should be followed as per the clinical guidelines (CDC 2009) (Fig. 1). The guidelines for LFT are that in low-moderate risk patients, elevated serum alanine aminotransferase (ALT) levels should be correlated with anti-HCV results. If the anti-HCV test is negative, it should be repeated after a few weeks. However, molecular testing is not recommended in such cases. However, for high-risk/immunocompromised patients, elevated serum ALT levels with a negative anti-HCV result justify molecular testing. HCV RNA testing should be primarily used for candidates for antiviral therapy and for monitoring disease progression.

The liver function test (LFT) is the first line of tests requested by clinicians to determine changes in the liver profile. Based on the LFT results, further specific tests are generated to determine the causative agent responsible for elevated LFT values. Several studies have shown that alanine amino transferase (ALT), total bilirubin, direct bilirubin, alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT) and serum albumin are useful for evaluating disease severity (Ijaz et al. 2011, Lee et al. 2013). However, many factors can cause the elevation of LFTs in the host, such as damage to the hepatocytes, fatty liver, autoimmune hepatitis and alcoholic liver disease (Jamal et al. 1999, Giboney 2005). Similarly, ALT elevation is significant in acute infection but may be normal in any stage of chronic infection (Helsper et al. 2012), while elevation of ALP and GGT is indicative of cholestatic liver disease, primary biliary cirrhosis, primary sclerosing cholangitis and gall stone disease. Serum albumin evaluates the functional integrity of the liver, and abnormality in the serum albumin levels is indicative of extensive liver damage (Bernstein 2002, Kang 2013). Thus, it is apparent that the cause of LFT elevation is multifactorial. Therefore, in case of suspicion of HCV infection, utilizing LFTs may serve as a step-wise test prior to HCV antibody testing. Furthermore, requesting molecular testing directly on the basis of the LFT result without an anti-HCV test is not justified. Thus, unless a causative agent is specifically identified via more appropriate tests such as antigen or antibody testing, molecular tests should be withheld as the HCV RNA test determines the active state of infection and may be negative if infection is in the latent state in the host (Limdi and Hyde 2003).

Laboratory management and personnel have observed that clinicians in Pakistan request molecular tests without prior testing for anti-HCV, i.e., the HCV RNA test is being used as a screening tool to establish an HCV diagnosis. International guidelines indicate that the diagnosis of HCV should be based on anti-HCV testing as this test is positive throughout a person's lifetime and, if tested after LFTs, can serve a robust tool for detecting HCV as an infectious agent. The guidelines also take into consideration that antibodies may not develop until 4–6 weeks after exposure and therefore anti-HCV may test negative. However, it is recommended that the anti-HCV test should be repeated after a few weeks prior to molecular testing as it is cost-effective as well as highly reliable for screening purposes, especially in resource-constrained countries. Molecular testing should only be used as a tool to determine viral loads at the start of treatment, periodically to monitor the response to treatment and at the completion of treatment to evaluate its effectiveness (CDC 2013; Gupta et al. 2014).

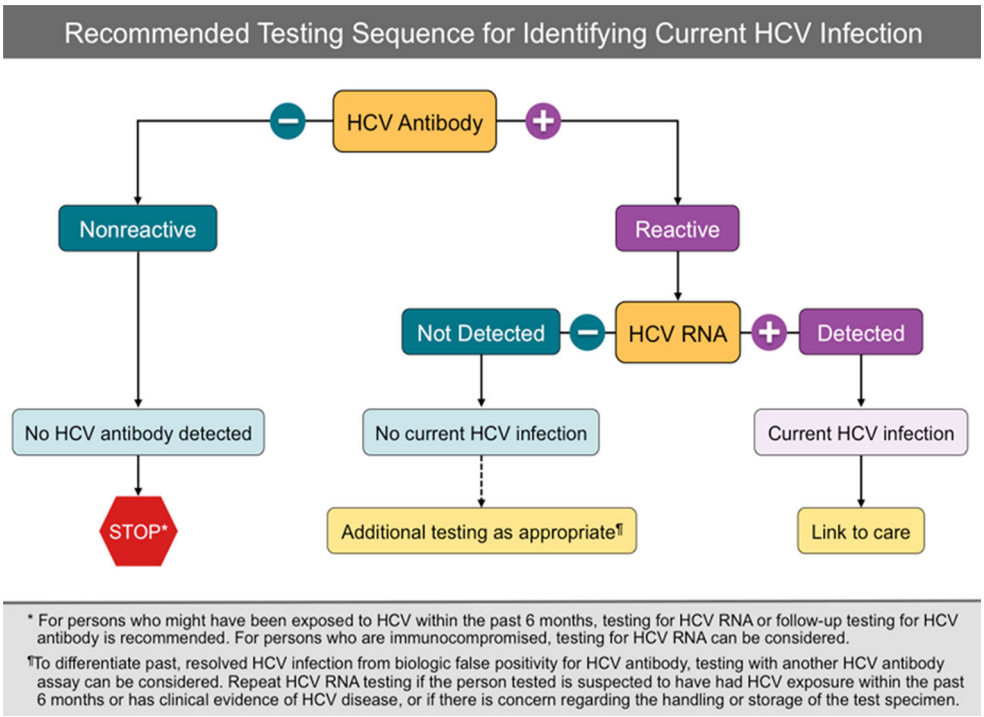

Fig. 1 The Centers for Disease Control and Prevention recommended testing sequence for identifying current hepatitis C virus (HCV) infection

The main objective of this study was to determine the diagnostic management gap in Pakistan, as evidenced by clinicians' perspectives. The appropriate or inappropriate utility of LFT, anti-HCV and molecular testing in the tertiary care setup was evaluated. The study aims to bridge the clinician knowledge gap of this aspect so that better management practices can be implemented.

Materials and methods

Study design and settings

A retrospective study was conducted at the Department of Molecular Biology, The Indus Hospital (TIH), Karachi. Patients' medical records from January 2014–January 2015 were analyzed. A total of 1758 patient records were included in the study on the basis of molecular testing, i.e., HCV RNA tests requested by clinicians. Data were stratified into positive and negative HCV RNA groups, and information on liver function and anti-HCV tests was also reviewed in both groups. Baseline demographics such as age and gender were also documented. The study was exempted from Institutional Review Board (IRB) approval because of its retrospective design.

Inclusion criteria

Patients for whom HCV RNA tests were requested with or without any laboratory parameters including LFT and anti-HCV tests were included in this study. Follow-up HCV RNA test results were excluded from the analysis.

Laboratory tests

HCV RNA quantitative assay

HCV RNA was tested by Abbott Real Time HCV assay using Abbott automated extraction analyzer m2000sp, which uses magnetic particle technology to capture nucleic acids and washes the particles to remove unbound sample components. The bound nucleic acids are eluted. The nucleic acids are then ready for amplification, which was detected by an Abbott Real Time m2000 RT analyzer. The lower limit of the assay is 12 IU/ml with the 0.5-ml sample procedure. The assay was performed according to the manufacturer's recommendations.

Enzyme immunoassay

Hepatitis C antibody was briefly determined in a Vitros ECi/ECiQ immunodiagnostic system; a 5-ml serum sample was loaded on the Vitros system as per the manufacturer's instructions. The light signals are read by the system. The amount of bound HRP conjugate is indicative of the level of anti-HCV present in the sample.

Liver function profile testing

Liver function tests were performed on a Hitachi 902 automatic analyzer and Randox Imola, respectively, using a 5-ml serum sample. The assays were performed according to the manufacturer's instructions.

Diagnostic management gap evaluation

The diagnostic management gap evaluation was performed keeping the Centers for Disease Control and Prevention (CDC) recommendations for HCV RNA testing as a guideline (CDC 2009). The management gap was defined as requesting molecular testing, i.e., the HCV RNA viral loads on the basis of LFTs or without testing for LFT and HCV antibodies. Furthermore, clinicians' perspectives on the respective diagnostic management gap were assessed via verbal communications after obtaining their verbal consent. However, clinicians' answers were not analyzed statistically because of the retrospective design of the study.

Statistical analysis

Data were entered in Excel and then exported to SPSS version 19.0. The descriptive analyses such as the frequencies and mean values were determined using SPSS software.

Results

A total of 1758 patient medical records were included in the study on the basis of HCV RNA testing. Of these, 59 % (1044/1758) were females and 40 % (714/1758) males. Baseline demographics of the included patients are given in Table 1.

It was observed that of 1758 HCV RNA-tested patients, 984 (56 %) were positive, while 774 (44 %) were negative for HCV RNA. On the basis of the HCV RNA results, the data were stratified into two groups: group A was the HCV RNA-positive group and group B the HCV RNA-negative group. The results showed that in group A 51 % while in group B 62 % of the patients had not been previously tested for anti-HCV prior to HCV RNA testing. Furthermore, 36 % in group A and 9.6 % in group B had not been tested for any laboratory parameters (neither LFT nor anti-HCV) prior to the request for HCV RNA testing (Fig. 2). Of 1758 patients, 308 (17.5 %) had been tested only for LFT with direct testing for HCV RNA. Mean LFT values were found to be increased in these patients (Table 2).

Table 1 Demographic characteristics of the patients

Variables	HCV RNA detected ($n = 984$)	HCV RNA not detected ($n = 774$)
Age in years		
<15	06 (0.6 %)	08 (1 %)
15–29	179 (18 %)	205 (26 %)
30–39	287 (29 %)	229 (29 %)
40 and above	512 (52 %)	332 (43 %)
Gender		
Female	584 (59 %)	460 (59 %)
Male	400 (40 %)	314 (40 %)

The diagnostic management gap evaluation of clinicians' perspectives was performed via verbal communication with consultants, interns, residents and fellows practicing in the gastrointestinal OPD of the hospital. The majority of practicing clinicians and medical providers in the respective setting reported a lack of awareness of the international CDC guidelines for using the HCV RNA test. When asked about the justification of molecular tests for HCV patients, their views were that HCV RNA gives rapid results, molecular tests are highly specific/sensitive, and LFT values were elevated so HCV RNA was directly performed. Some clinicians suggested that since anti-HCV is non-specific as it gives negative results in acute HCV infection, therefore molecular tests were requested. Furthermore, a minority of clinicians indicated viewpoints on administrative hindrances such as the laboratory test menu being online, not being user friendly, not showing the antibody test on the menu leading to an HCV RNA test being generated, interns and residents generating the test on the consultant's behalf and getting confused between HCV RNA and HCV antibodies. A number of consultants and fellows were identified as rightly investigating for HCV RNA as they wanted to determine the disease progression as well as monitor the anti-viral therapy. However, of these, a minority were still not following the guidelines with respect to the time frame as the HCV RNA request was being generated 6–10 days apart.

Discussion

The main aim of this study was to observe the diagnostic management quality gap evaluation among clinicians practicing in a tertiary care setup in Pakistan. This is the first study from Pakistan documenting gaps in clinicians' knowledge of appropriate screening tests and utility of molecular testing for HCV diagnosis and management.

The study evaluated the diagnostic gap in accordance with the international guidelines recommended by The International Center for Disease Control and Prevention (CDC). The results showed that a significant proportion of patients had not been tested with the recommended diagnostic tools including LFT and anti-HCV tests prior to generating the request for HCV RNA (Fig. 2). Combining data from both the HCV RNA-

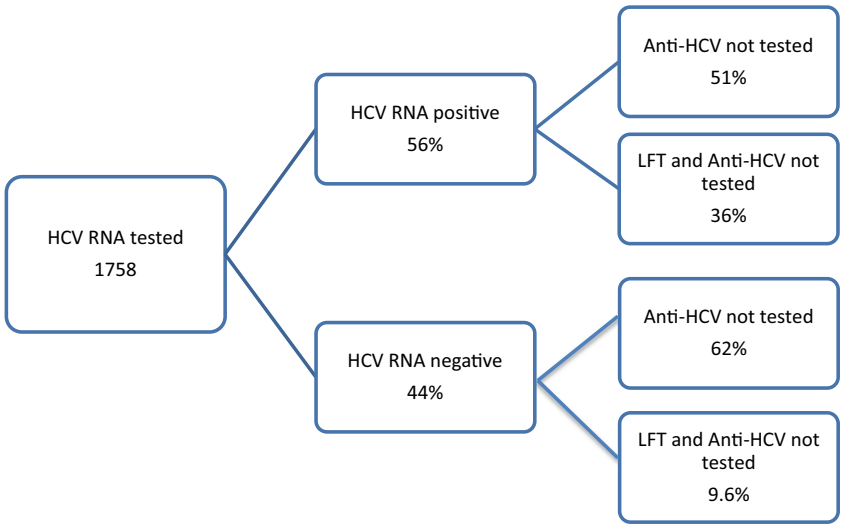

Fig. 2 Diagnostic management quality gap analysis

positive and -negative groups, it was found that 17.5 % of the patients had only been tested for LFT, while 45.6 % of the patients had neither anti-HCV tests nor LFT prior to molecular testing. These frequencies are very high and are evidence of the clinicians' perspectives of utilizing appropriate tests for HCV diagnoses. It is emphasized that molecular testing such as for HCV RNA is requested to determine active/current infection or for monitoring antiviral therapy and is not a screening tool for the presence or absence of infection. Anti-HCV is a designated screening test for HCV and needs to be utilized after the LFT profile to determine the cause of deranged LFTs prior to progressing to more sophisticated molecular testing.

It was observed that patients in whom LFT was the only test performed prior to direct HCV RNA testing showed mean elevated LFT values (Table 2). Some clinicians commented that with abnormal LFT values, a HCV RNA request was generated on this basis. However, from the clinician's point of view, the elevated values are significant, but the approach used to detect the cause of deranged LFTs is inappropriate. For abnormal LFT results, clinicians should have generated a request for anti-HCV tests, as per the recommendations, instead of HCV RNA tests so that the causative agent could be detected properly. Dysfunctional LFT ranges do not justify the direct use of molecular tests as LFT abnormalities can be multifactorial.

Table 2 Liver function test profile in patients with direct molecular testing

S. no.	Liver function tests	% Positivity ($N=308$)	Mean values
01	ALT (IU/l)	55.2	88.34
02	ALP (IU/l)	15.9	187.26
03	Direct bilirubin (mg/dl)	14.6	0.875
04	Total bilirubin (mg/dl)	5.5	1.595
05	GGT (IU/l)	17.2	110.33

ALT Alanine aminotransferase, *ALP* alkaline phosphatase, *GGT* gamma-glutamyl transferase

The major reason for the observed discrepancy between diagnostic procedure guidelines and the clinicians' diagnostic approach is clearly due to unawareness of the CDC guidelines. The residents, interns, fellows as well as some consultants used their limited knowledge of molecular testing as a reason to utilize this test. The majority rated molecular testing as a rapid test with high sensitivity and specificity as compared to anti-HCV tests. In reality, both of these reasons are inadequate for non-utilization of anti-HCV prior to HCV RNA testing. Anti-HCV is also a rapid test (especially in a high-throughput laboratory), and the specificity of anti-HCV is 99.54 %, which is comparable to HCV RNA, which has a specificity of 99.74 %. Therefore, utilization of molecular testing as a screening tool for HCV is invalid particularly because it is well known that HCV RNA detects active infection and can lead to a false sense of confidence in reporting on the infectious agent when used prior to the anti-HCV test.

Other reasons communicated for the utilization of molecular tests were that there are certain circumstances when no HCV antibodies might be detectable although the patient has the disease (e.g., the early phase of acute infection) and additional testing for HCV RNA is recommended. Although this approach is acceptable, the CDC guidelines clearly recommend that if anti-HCV is negative, then molecular testing is recommended only in high-risk/immunocompromised cases in which the LFTs are also found to be abnormal, e.g., for HIV-positive patients. However, for low-moderate risk patients, even with an unexplained elevated serum alanine aminotransferase (ALT) value, clinicians need to wait a few weeks and repeat the anti-HCV testing before testing for HCV RNA in the blood. This guideline is especially true for developing countries, such as Pakistan, where resources are constrained.

Furthermore, there were a number of clinicians who were utilizing the molecular test for monitoring the response to antiviral therapy or for those patients who were candidates for antiviral therapy. For these clinicians, it was observed that they were rightly utilizing diagnostic tools such as LFT and anti-HCV prior to generating a request for HCV RNA testing. This is the correct approach as HCV RNA is a robust test for observing therapeutic outcomes especially when it is well understood that different genotypes give variable therapeutic responses in patients. Therefore, molecular testing in such cases is necessary for disease management. However, with respect to this aspect, it is worth noting that the recommended time frame for monitoring the anti-viral response is 4 weeks. In this study, a minority of clinicians were identified as using inappropriate timelines (6–10 days apart) for monitoring the antiviral response, thus raising the question about clinicians perspectives.

With the increasing burden and prevalence rate of HCV infection in Pakistan, clinician awareness of international and national guidelines for diagnostics is important. It is the clinician's responsibility to keep abreast of the guidelines in use worldwide so that patient management is not compromised. A large part of HCV infection management depends on diagnostics, and ignorance of an important parameter such as international guidelines can lead not only to poor patient management, but also increase the patient's economic burden. In developing countries, one of the most important consequences of not following guidelines is the monetary burden. In Pakistan, the cost of anti-HCV testing is approximately USD 23, while that of HCV RNA is USD 167. If clinicians correctly followed the diagnostic procedure guidelines, it would help patients save approximately USD 144. Repeat molecular testing would increase this burden many fold. Keeping in mind that the cost of HCV treatment is high in Pakistan, diagnostic testing can be cost prohibitive if the guidelines are followed appropriately.

The major victim of clinician ignorance is the patient, who not only goes through financial but also mental disturbances due to repeated and irrelevant testing that fails to diagnose the disease properly. Such patients can also serve as sources of disease transmission. Furthermore, timely care and treatment are also compromised, which may lead to clinical complications. Irrelevant testing without screening the patient for the causative agent is a medical and legal offence. Consequently, further diagnostic management of patients by clinicians with invasive and ultrasonographic testing on the basis of clinical symptoms with negative molecular tests (in cases where the diagnosis is based solely on the HCV RNA test result) can have clinical and financial repercussions.

Inappropriate testing for HCV in Pakistan is a huge dilemma that needs immediate attention at the national level by governmental health-care associated bodies so that robust guidelines are implemented. Linking prevention and control to the testing, care and treatment of hepatitis C infection require a comprehensive, cohesive approach to meet the needs of individual countries. Clinician education at forums may help to address this gap in a timely manner. Education on updated diagnostic procedures and HCV management can help reduce the management quality gap regarding HCV diagnoses. Additionally, consistent efforts by the hospital administration to enhance clinician awareness skills via continuing medical education may serve to bridge this knowledge gap.

Conclusion

Developing countries such as Pakistan face substantial barriers to the utilization of robust diagnostic tools for HCV diagnosis as a result of inappropriate testing, inadequate knowledge and awareness of international diagnostic guidelines among medical providers and clinicians. Such clinical practices serve as an important parameter of HCV transmission in Pakistan. Awareness programs, implementation of national policies and audits of clinical practices in hospitals are recommended for appropriate diagnostic management of HCV in Pakistan.

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Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest for this study.

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Informed consent For this type of study, formal consent was not required as it was a retrospective study.

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