The Role of the Clinical Laboratory in the Diagnosis of Chronic Viral Hepatitis

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Introduction

Chronic hepatitis is a clinicopathologic syndrome, which has several causes and is characterized by varying degrees of hepatocellular necrosis and inflammation. It can also be defined as chronic inflammation of the liver continuing without improvement for at least 6 months and characterized by three important histological features: chronic inflammation, hepatocellular necrosis and varying degrees of fibrosis[1].

Chronic viral hepatitis is a global problem with some five hundred million people estimated to be currently infected with hepatitis B or C. These two diseases are the cause of significant global mortality and morbidity - approximately 1 million deaths each year are attributable to them and their sequelae, liver disease and primary liver cancer.[2] Although not occurring uniformly around the world, hepatitis B and C constitute an important cause of chronic hepatitis individually or together in most parts. Surveillance of viral hepatitis varies widely from country to country but it is generally inadequate. However, it is accepted that the highest rates of hepatitis B are found in South-East Asia, Sub-Saharan Africa and parts of the Pacific Basin and Amazon Basin. Chronic liver disease is a major cause of significant morbidity and mortality in Nigeria comprising about 30% of clinical conditions seen at the gastroenterology medical outpatient unit in one tertiary health institution in Nigeria.[3] According to the WHO, countries with high rates of chronic infection with Hepatitis C are Egypt (15%), Pakistan (4.8%) and China (3.2%) [5]

Burden of the Disease

Worldwide, the major causes of chronic viral hepatitis are Hepatitis C and Hepatitis B with or without Hepatitis D infection. The World Health Organization (WHO) estimates that about 2 billion people have been infected by hepatitis B virus and approximately 350 million people are living with chronic hepatitis B infection.[4] It also estimates that about 150 million people are chronically infected with hepatitis C virus, and more than 350 000 people die every year from hepatitis C-related liver diseases.[5]

HBV infection has reached hyperendemic levels in Nigeria with sero-prevalence of HBsAg estimated to range from 10-40%[6]. HCV on the other hand is less prevalent with figures of 4.5-5% reported in most Nigerian studies.[7, 8, 9] It is believed that majority of HBV infections in

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Nigeria is acquired in childhood by vertical transmission or personal contact with subsequent development of chronic liver disease. HBV and HCV infections account for the majority of cirrhosis and primary liver cancer throughout most of the world with 57% of cirrhosis and 78% of HCC attributable to HBV and HCV globally.[10]

The place of laboratory tests in viral hepatitis

The clinical laboratory plays a pivotal role in the diagnosis and management of chronic viral hepatitis. These tests help to differentiate acute from chronic infections, and detect persistence of viremia or verify the development of immunity. These tests are used to determine the extent of disease at diagnosis and this is essential in determining the therapy offered to the patient. They may also be used to monitor responses to ongoing antiviral therapy.

Traditionally, the tests used to diagnose viral hepatitis include serological tests to identify viral markers. More recently, molecular biological methods have been added to the stable so that viraemia may be quantified at diagnosis and during treatment. Evaluation tests include biochemical tests, to assess presence and extent of hepatocyte injury and histological examination of liver biopsy tissue. In viral hepatitis, both serologic and molecular assays are used to detect early infections at the asymptomatic phase, to differentiate acute from chronic infections, and detect persistence of viraemia or verify the development of immunity.

Serology

Serological tests form the baseline investigation in the diagnosis of viral hepatitis. The persistence of these antigens and antibodies also help to differentiate between acute and chronic viral hepatitis. These tests can also be used to determine patients who have active infections. Lately, quantitative assays for some of these markers have been used to determine patients who will respond to therapy[11]. Serological tests for viral antigens and antibodies include assays for HBsAg, anti-HBs, anti-HBc (IgM, IgG), HBeAg and anti-HBe for HBV infection as well as anti-HCV for Hepatitis C virus infection.

HBV

The recommended tests for primary diagnosis are HBsAg, Anti-HBs, Anti HBc (total) and Anti-HBc (IgM). The two types of anti-HBC produced are IgM detected in early course of the disease and IgG produced in later course. Chronic infection is characterized by detection of HBsAg for more than 6 months and presence of anti-HBc-IgM indicates early chronic infection while HBeAg indicates high viral replication and infectivity.[12],[13] For prognostication and patient monitoring, detection of HBeAg and Anti-HBe is used in combination with molecular assay of HBV-DNA.

HCV

The diagnosis of hepatitis C infection begins by detection of anti HCV Immunoglobulin G using enzyme immunoassay. Detection of serum HCV antibodies indicates presence of recent infection, but cannot distinguish acute from chronic or resolved infection. The anti-HCV antibodies become detectable in the serum 6-8 weeks after exposure to the virus and remain positive thereafter. Anti-HCV IgM can be detected in 50 to 90% of patients with acute HCV infection and 50 to 70% of chronic cases, so they are not a reliable indicator of acute infection.[14] Recombinant Immunoblot Assay (RIBA) can differentiate true positive from false positive test, however confirmation by RIBA is needed only for low risk patients (healthy blood donors) or if high risk patient is HCV negative. It is therefore recommended that for routine testing of asymptomatic person, detection of anti HCV should include use of both enzyme immunoassay (EIA) and supplemental or confirmatory testing with additional, more specific assay and it is preferred to use supplemental testing RIBA for all positive anti-HCV result of EIA.[15]

HDV

HDV infection is generally diagnosed by assessment of total and IgM anti-HDV antibodies utilizing serologic methods. Since HDV is dependent on HBV, HBsAg sero-positivity is also a necessity for diagnosis of active HDV infection although it has been shown that HBsAg levels may be suppressed in some cases of acute HDV replication. IgM anti-HBc sero-positivity is also necessary for the diagnosis of HBV-HDV confection.[16]

Biochemical tests

These are used to– evaluate the degree of ongoing of hepatocyte injury and residual hepatocyte function in advanced cases of chronic hepatitis.

Markers of cell injury

The amount of on-going hepatocellular necrosis is determined by measurement of serum level of cytosolic liver enzymes including aspartate aminotransferase (AST), alanine aminotransferease (ALT) and lactate dehydrogenase (LDH). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most common clinically used indicators of cell necrosis. They are present in high concentration in liver cells and injury to liver cell membranes causes leakage of aminotransferases into the circulation. Aminotransferases are sensitive but relatively nonspeciûc indicators of liver cell injury as they are present in other tissues of the body and it is not always possible to distinguish acute from chronic hepatic injury. As a rule, however, serum activities are lower in chronic cell injury while acute hepatocellular injury leads to markedly elevated levels. Most patients with chronic hepatitis C, for example, have ALT values one to four times above the reference limits.[17] Generally, low levels of ALT in chronic HBV or HCV infection signify a lower risk of progression to cirrhosis. The levels of ALT in chronic HCV infection is said to be a poor indicator of disease stage or progression. This is in contrast to HBV where sustained elevation of ALT levels indicates active underlying disease and risk of disease progression. Inversion of the AST/ALT ratio (AST>ALT) may indicate underlying cirrhosis in either HBV or HCV infection. ALT, a marker of liver inflammation is used for follow up of patients with chronic HBV infection and although the level can fluctuate, sustained and intermittent elevation above the upper limit of normal is indicative of liver inflammation and correlate with disease progression.

The excretory function of the biliary system can be deranged particularly with associated liver cirrhosis and consequent biliary obstruction. This excretory function can be assessed by measurement of serum bilirubin levels (both total and conjugated), serum levels of alkaline phosphatase, gamma glutamyl transpeptidase and 5'nucleotidase.[18]

In advanced cases of chronic hepatitis, the residual hepatocyte function is readily assessed by measurement of serum levels of albumin, prothrombin time and serum ammonia.

Several indices composed of routine laboratory parameters that reflect changes in liver function have been suggested as surrogate markers of hepatic fibrosis. The *Fibrotest* consists of a panel of five markers which could best predict the stage of fibrosis: á2-macroglobulin, haptoglobin, apolipoprotein A1, GGT, and total bilirubin. Prelimanary studies using these markers have yielded positive results however the diagnostic accuracy is limited by factors such as haemolysis and recent or on-going infections.[19]

Molecular studies

HBV

Molecular assays available for the diagnosis and management of HBV infection include quantitative viral load tests, genotyping assays, drug resistance mutation tests, and core promoter/precore mutation assays.

Most practice guidelines for the management of chronic hepatitis B recommend the quantiûcation of HBV DNA in the initial evaluation of chronic hepatitis B and during

management, particularly in the decision to initiate treatment and in therapeutic monitoring. High-sensitivity molecular assays are also important for the diagnosis of HBeAg CHB and occult HBV, where viral loads can be quite low. Detectable HBV DNA in plasma or serum is one of the criteria for chronic hepatitis B in most guidelines. Assessment of HBV DNA in plasma or serum should therefore be performed along with other tests to establish this diagnosis

The infecting HBV genotype has been found to be a potentially determinant factor in the outcome of chronic hepatitis B and the success of antiviral therapy. Patients with genotype B infections tend to have a more favourable outcome than those with genotype C, including a younger age at HBeAg seroconversion, lower rates of active liver disease, and slower progression to cirrhosis. An HBV genotype-dependent response to antiviral therapy has been observed for some drugs most especially interferon-alpha. Methods used in determining HBV genotype include whole- or partial-genome sequencing, restriction fragment length polymorphism, genotype-specific PCR amplification, PCR plus hybridization, and serology. The gold standard is whole gene sequencing, however it has been found to be cumbersome and time consuming.[20]

Tests to determine the mutation that confers drug resistance are available but not routinely so, these tests may however become increasingly important in the future with the growing number of antiviral drugs available. Test formats for the detection of antiviral resistance mutations have also –been applied to the identiûcation of precore/core promoter mutations.[20]

HCV

Molecular assays play a major role in HCV diagnosis where serologic tests can document past or present infection but cannot differentiate one from the other. A variety of molecular tests can be used as sensitive (and early) detectors of viraemia, determine persistence of viraemia which is an indicator of chronic infection and monitor responses to antiviral therapy. Both qualitative and quantitative molecular assays are available, and their efficient use requires familiarity with the sensitivity and dynamic ranges of each method. Quantification of HCV-RNA can be used to monitor changes in viral load in patients undergoing alpha-interferon therapy. HCV-RNA concentrations before, during and after therapy will help to determine if the patient is responding to treatment and is this response is likely to be sustained.[21]

The molecular diagnosis of hepatitis C involves the use of reverse transcriptase polymerase chain reaction (RT- PCR) technique. HCV RNA can be detected within one or two weeks after exposure to the virus. In some patients, the detection of HCV RNA may be the only evidence of HCV infection.[15] Polymerase chain reaction (PCR) assay for HCV RNA is mostly available on research basis and because of assay variability, rigorous proficiency testing is recommended for clinical laboratories performing this assay and, the result of PCR testing should be interpreted cautiously.

Determination of hepatitis C viral genotype has become a key parameter in the management of chronic HCV infection. Direct sequencing of a specific polymerase chain reaction (PCR)-amplified portion of the viral genome obtained from a patient sample, followed by phylogenetic analysis is the standard method for determining HCV genotype. HCV genotype appears to be a strongly independent predictor of sustained virological response (SVR) to therapy. Response to Interferon-alpha has been found to differ amongst different HCV genotypes with response rates among patients with genotypes 2 & 3 observed to be two to threefold higher than individuals with genotype 1. This difference in response to treatment is important in clinical management and has been incorporated into most treatment guidelines.[22]

Liver biopsy

Liver biopsy is a valuable procedure, the purpose of which is to make a diagnosis, grade the necrosis and inflammatory activity present, stage progression of disease in terms of fibrosis, evaluate result of treatment and exclude premalignant changes.[23]

Histological features of chronic viral hepatitis include hepatocyte necrosis, mononuclear portal inflammation and portal fibrosis. In the old classification of chronic hepatitis, liver biopsy was the major tool used to diagnose and classify chronic hepatitis into the only two histologically recognized forms; the more severe chronic active hepatitis and the milder chronic persistent hepatitis which were distinguished based on presence or absence of piecemeal necrosis (inflammation and necrosis of the limiting plate of the liver; a term now referred to as 'interface hepatitis' due to the knowledge that the process of hepatocyte death is apoptosis rather than necrosis).

The new classification occasioned by advancement in knowledge about the aetiologic, pathogenetic and clinicopathologic features of this syndrome, is based primarily on aetiology to distinguish subgroups according to degree of disease activity to provide prognostic information and criteria for use of immunotherapy since the clinical course, prognosis and treatment differ among the different types of chronic hepatitis. Thus classification now involves determination of the aetiological agents, assessment of the severity of necro-inflammatory activity (grade) and degree of fibrosis (stage). While aetiology requires the use of clinical, serological and histological parameters, the grade and stage of the disease can only be determined histologicaly.

Liver biopsy remains the gold standard for fibrosis assessment and forms an important part of patient assessment for both diagnosis and treatment. Histological findings are commonly scored in clinical trials using any of the several recognized schemes. Scoring systems however are not intended to replace the detailed, descriptive, pathology report. In fact, lesions other than those scored for grading and staging may have clinical relevance and should be assessed and reported. [24]

Apart from grading and staging, presence of features of specific aetiological agents should be rported in the histological report. In chronic HCV infection, common findings include portal lymphoid aggregates, bile duct reactive changes and macrovessicular steatosis. These features differentiate Hepatitis C infection from other progressive liver diseases. Antibodies directed against HCV antigens will allow identification of viral proteins by immunohistochemistry. Hepatitis C, especially genotype 3 tends to be associated with steatosis and it is important to be able to differentiate steatosis due to HCV infection from that due to fatty liver from other causes. It has also been found out that Hepatitis C may in itself lead to increased hepatocyte and reticuloendothelial iron stores hence it is important that histochemical stains for iron be done also.

In Hepatitis B infection, particular features include the presence of 'ground glass' hepatocytes. These are hepatocytes containing viral particles. Immunostaining for hepatitis B antigens will help in differentiating hepatitis B from other possible causes of chronic progressive inflammatory liver disease.

In all forms of chronic hepatitis, irrespective of the aetiology, continued interface hepatitis and bridging necrosis between portal tracts and portal tracts-to-terminal hepatic veins, are indicators of progressive liver damage.

Features of hepatocyte dysplasia suggesting progression to HCC and that of liver cirrhosis can also be demonstrated on biopsy. Both Chronic HBV and HCV infections increase the risk for developing hepatocellular carcinoma and cellular atypia that may suggest early or possible progression to HCC may be identified on biopsy tissue. These dysplastic changes include the large cell change and small cell change. The large cell change is thought to be

malignancy associated while the small cell change is thought to be directly premalignant. Other specialized techniques can be used to demonstrate the viral agents in tissue such as immunohistochemistry, in-situ hybridization and polymerase chain reaction. In addition, features of a second contributing disease to CVH such as alcoholic liver disease, autoimmune hepatitis, and drug-induced hepatitis, haemochromatosis and alpha-1-antitrypsin deficiency may be detected on biopsy.

References

1 Desmet VJ et al. Classification of chronic hepatitis: Diagnosis, grading & staging. Hepatology; 1994: 19(6): 1513-1520

2 World Hepatitis Alliance. Viral Hepatitis: Global policy

3 Lesi OA, Kehinde MO and Anomneze EE. Chronic liver diseases in Lagos: A clinicopathological study. The Nig. Postgrad Med J 2004; 11(2): 91-96

4 WHO Hepatitis B fact sheet. http://www.who.int/mediacentre/factsheets/fs204/en/

5 WHO Hepatitis C fact sheet. http://www.who.int/mediacentre/factsheets/fs164/en/index.html#

6 Forbi JC, Vaughan G, Purdy MA, Campo DS, Xia G-l, et al. (2010) Epidemic History and Evolutionary Dynamics of Hepatitis B Virus Infection in Two Remote Communities in Rural Nigeria. PLoS ONE 5(7): e11615. doi:10.1371/journal.pone.0011615

7 Ojo OS, Thursz M, Thomas HC, Ndububa DA, Adeodu OO, Rotimi O, Lawal AA, Durosinmi MA, Akonai AK, Fatusi AO, et al. Hepatitis B virus markers, hepatitis D virus antigen and hepatitis C virus antibodies in Nigerian patients with chronic liver disease. East Afr Med J. 1995 Nov;72(11):719-21.

8 Zaccheaus Awortu Jeremiah, Baribefe Koate, Fiekumo Buseri, Felix Emelike Prevalence of antibodies to hepatitis C virus in apparently healthy Port Harcourt blood donors and association with blood groups and other risk indicators. Blood Transfusion 2008; 6: 150-155

9. Kawo1 A. H, Bala J.A. and Dabai Y. U. Sero-prevalence study of Hepatitis C virus infection among blood donors attending selected blood banks in some Local Government Areas in Kano, Nigeria. Journal of Public Health and Epidemiology Vol. 4(6), pp. 178-183, June 2012

10 Perz JF, Armstrong GL, Farrington LA, Hutin YJF, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwideBottom of Form. J Hepatol, 2006; 45(4): 529-438.

11 Jia-Horng Kao Diagnosis of Hepatitis B Virus Infection Through Serological and Virological Markers Expert Rev Gastroenterol Hepatol. 2008;2(4):553-562.

12 Bowyer S. M, Sim JG, Webber LM. Current Laboratory diagnosis of Hepatitis B virus infection including 8 year of Retrospective laboratory Data. CME; vol. 29(5): 210-143.

13 Krajdeh M, Mc Nabb G, Petric M. The Laboratory Diagnosis of hepatitis B virus. Can J infect dis Med Microbiology 2005; 16(2): 65-72.

14 Pawlotsky J. M. Diagnostic tests for hepatitis C: J Hepato. 1999; 31 (suppl.1): 71-79

15 Linda A M, Moyer RN, Eric C, Miriam J. Hepatitis C: Part 1. Routine serologic testing and Diagnosis. A M Fam physician, 1999; 59(1): 79-87

16 Kenneth R. Harris, MD, and Anand S. Dighe, MD, PhD Laboratory Testing for Viral Hepatitis Am J Clin Pathol 2002; 118(Suppl 1): S18-S25

17 M. Desmond Burke. Liver function: test selection and interpretation of results, MD Clin Lab Med 22 (2002) 377–390

18 Clouston AD. Pathology of chronic hepatitis-viral, autoimmune & overlap syndromes; Histopath Update. www.histopath.com.au. Accessed 19th April 2011

19Rudolf E Stauber, Carolin Lackner Noninvasive diagnosis of hepatic fibrosis in chronic hepatitis C World J Gastroenterol 2007 August 28; 13(32): 4287-4294

20 Alexandra Valsamakis Molecular Testing in the Diagnosis and Management of Chronic Hepatitis B . Clin. Microbiol. Rev. 2007, 20(3):426. DOI

21 Hepatitis C—diagnosis and monitoring Mickey S. Urdea,* Linda J. Wuestehube, Patricia M. Laurenson, and Judith C. Wilber Clinical Chemistry 43:8(B) 1507–1511 (1997)

22 H James Hnatyszyn. Chronic hepatitis C and genotyping: the clinical significance of determining HCV genotypes. Antiviral Therapy 10:1–11

23 Rachel Hudacko & Neil Theise, Liver Biopsies in Chronic Viral Hepatitis. Beyond Grading and Staging. Arch Pathol Lab Med—Vol 135, October 2011

24 Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-9