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AN UNUSUAL CASE OF CROSS REACTIVITY BETWEEN HEPATOTROPIC VIRUSES: PREVENTING A POSSIBLE MISDIAGNOSIS

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ABSTRACT **Background:** Shifting pattern in endemicity is rendering older adults vulnerable to Hepatitis A virus infection. Cross reactivity with other hepatotropic viruses such as Hepatitis-B poses a diagnostic challenge and delays appropriate clinical management. **Case Summary:** A previously non-reactive patient with no high-risk behaviour came out low reactive for HBsAg (Hepatitis-B surface antigen) on routine testing by chemiluminescence immunoassay but non-reactive by rapid diagnostic test even on repeat testing. Upon further examination, he tested positive for Hepatitis-A serology. Frequent follow ups till 1 year continuously revealed low reactivity to HBsAg (signal to cut-off ratio<50). **Conclusion:** Cross reactivity among hepatotropic viruses can lead to diagnostic challenges having grave clinical implications. Knowledge of these interactions is essential for accurate clinical diagnosis and appropriate management of viral hepatitis.

KEYWORDS : CLIA, Diagnostic test, False positive, HAV, HBsAg, Viral hepatitis

INTRODUCTION

Hepatitis A, B, C, D, and E viruses (HAV, HBV, HCV, HDV, and HEV) are the major hepatotropic viruses that affect the liver and cause a wide range of diseases ranging from cirrhosis to hepatocellular carcinoma. As per the Global Hepatitis Report of 2024 by World Health Organization (WHO), about 1.3 million people have died from viral hepatitis.[1] Annual incidence of HAV exceeds 1.5 million cases worldwide which account for 0.5% of deaths globally.[2,3] Prevalence in India is quite varied and ranges from 2.1% to 52.5% probably due to underreporting of cases.[4] HAV is traditionally considered the most common cause of acute viral hepatitis in children. It is not routinely tested in India due to its high endemicity, asymptomatic nature of infections, lack of childhood immunizations, and limited surveillance systems. However, the shifting pattern in endemicity is rendering even older adults vulnerable to this self-limiting infection.[5] This is due to reduction in seroprevalence of IgG antibodies in the paediatric population leading to increased susceptibility and severe clinical manifestations in older adults.[6,7] Evolving landscape of HAV endemicity can also be attributed to the intense hyperinflammatory response observed during the COVID-19 pandemic. The SARS CoV-2 virus (Severe Acute Respiratory Syndrome Coronavirus-2) led to substantial liver damage with subsequent infections with HAV presenting with severe manifestations.[8]

False positivity with other hepatotropic viruses particularly HBV can be when antigens or antibodies designed to target a particular part of the virus such as surface antigen (HBsAg) also react with other parts or varied antigens resulting in false positive diagnostic tests. This happens due to similar protein structures or sharing of epitopes between virus and host proteins or between different parts of the virus.[9] In the present study, a case report is described with repeated false positive HBsAg by chemiluminescence immunoassay (CLIA) in a patient with true positive Hepatitis-A infection due to cross reactivity.

Case Summary

A 50-year-old male presented to the out-patient department of our tertiary care hospital with complaints of pain abdomen, diarrhoea, yellowish discoloration of eyes and skin since past 10 days. He had a history of chronic alcoholism from past 30 years and was also diabetic. Physical examination revealed moderate ascites. No abnormality was detected in the cardiovascular, neurological, or other major systems. He had no history of blood transfusion, prolonged hospitalization, chronic diseases like tuberculosis, hypertension, systemic medications, drug abuse, dialysis, or any other high-risk behaviour. He did not give any history of cancer, autoimmune diseases, or Hepatitis-B vaccination. His samples were subjected to routine laboratory tests including haematology, biochemistry, and clinical microbiology for detailed analysis. Liver function tests were deranged with raised bilirubin and enzyme levels. Blood sugar was 250mg/dl. Ascitic fluid was extracted and was pale, with a clear appearance, no coagulum formation, showed 350 cells/mm^3, 50% each of neutrophils and lymphocytes. Background showed mesothelial cells and no atypical cells. Ascitic fluid and urine cultures were sterile during aerobic bacterial inoculation. Other laboratory parameters were unremarkable. Serum sample was non-reactive for Hepatitis-C and HIV-1/2 serology but came low reactive for Hepatitis-B surface antigen by CLIA methodology (signal to cut-off ratio<50).

Since the patient had no prior history of Hepatitis-B or any high-risk behaviour, repeat testing for HBsAg was performed. Test was repeated from the same sample and even a fresh sample was also obtained from the patient. Both showed low reactivity by CLIA. However, rapid diagnostic test for the same came out to be non-reactive from each of these samples. To confirm the diagnosis, sample was sent to an outside laboratory for testing of all hepatotropic viruses since we only perform Hepatitis-B/C and HIV-1/2 serology in our facility. The sample came to be positive for Hepatitis-A virus serology. The patient was followed for a year and each time his sample came low reactive for HBsAg by CLIA but with decreasing values (signal for test sample/cutoff value).

Additionally, the patient resided in sub urban areas of Delhi and could have acquired HAV infection through consumption of contaminated drinking water or improper personal hygiene. HAV is faeco-orally transmitted but may be transmitted through sexual contact or close contact with an infected person. However, upon enquiring, none of the family members of the patient experienced similar symptoms. Since the patient was an avid visitor of fast-food joints, there is an increased likelihood of acquiring the infection from those places.

Treating clinician was informed about the final diagnosis of the patient that helped in timely management for Hepatitis A infection which could have been easily misidentified as HBV viral hepatitis. Vigilance on the part of clinical microbiologists with an emphasis on proper patient history and a keen knowledge of cross reactivity between hepatotropic viruses saved this patient from misdiagnosis and administration of incorrect treatment.

DISCUSSION

India is a highly endemic region for HAV since a large proportion of population is already exposed to this virus in childhood and have developed immunity. Due to this, many HAV infections are asymptomatic rendering mass testing less beneficial.[10] Even though we lack a comprehensive case-based surveillance system for viral hepatitis due to HAV, efforts are focused on its prevention through WASH (water, sanitation, and hygiene) practices. Owing to these measures, prevalence of HAV in children is declining rendering adolescents and adults susceptible to this infection in later stages of life. People with chronic liver disease and other immunocompromised conditions may be especially prone to HAV hepatitis making introduction of its vaccination essential.[11, 12] Having said that, routine testing of HAV should also be made mandatory when testing for other viral hepatitis. Even though it is available at almost all private healthcare facilities, government institutions like ours still perform only HBV, HCV, and HIV testing due to lack of adequate infrastructure and appropriate resources. Availability of HAV testing would ensure timely diagnosis and appropriate management of cases especially the ones presenting with cross reactivity.

As previously mentioned, antibodies raised against one Hepatitis-B antigen might also bind to other antigens or similar protein structures as they recognize broader regions of a protein, not just a single, specific

site. HBV DNA polymerase has proteins that mimic the host cell proteins such as myosin or caldesmon. These lead to possible autoantibody production and cross-reactive immunity.[13] These result in grave implications for diagnostics as well as unnecessary delay in initiation of appropriate treatment. Studies have shown that HBsAg also cross reacts with woodchuck hepatitis surface antigen (WHsAg). Similarly, antibodies against HBe antigen (HBeAg) can cross-react with the core antigen (HBcAg). This happens as HBeAg is a soluble variant of HBcAg, and they share similar epitopes.[14]

Additionally, since CLIA as a technology has more sensitivity (being a third-generation test and covering greater antibody spectrum) and rapid diagnostic tests have more specificity, samples with low reactive values in CLIA (signal to cut-off ratio<50) are often considered false reactive and reported as non-reactive by rapid diagnostic test in our set up.[15-17] Due to lack of expensive molecular tests, all reactive samples in our facility are confirmed by rapid diagnostic tests and repeat CLIA on same and/or fresh samples by protocol.

CONCLUSION

To conclude, cross reactivity among hepatotropic viruses can lead to diagnostic challenges having grave clinical implications. Knowledge of these interactions is essential for accurate clinical diagnosis and appropriate management of viral hepatitis.

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