Case Report

Screening beyond conventional serological markers for hepatitis B and C viruses in cirrhosis: an entity overlooked

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ABSTRACT

We aimed to emphasise the role of screening beyond conventional serological markers (HBsAg and Anti HCV antibodies for chronic viral hepatitis B and C respectively) in patients with cirrhosis. Patients with cirrhosis of liver are often labelled as having cryptogenic cirrhosis (CC), if no etiology is found. In chronic viral hepatitis B and C (CHB and CHC) induced cirrhosis, HBsAg and Anti HCV antibodies respectively are usually done to rule out the viral infections however their absence have been documented in subset of patients having these infections. In this regard, we hereby present a case labelled as CC and developed HCC; later on, further evaluation turned out to be having both CHB and CHC. A 51-year-old male with diabetes presented with index episode of hematemesis. On further evaluation he was diagnosed to have cirrhosis of liver. No etiology was found and he was labeled as cirrhosis secondary to Non-alcoholic steatohepatitis (NASH)/cryptogenic cirrhosis. Later on, he developed hepatocellular carcinoma (HCC). We evaluated the patient with HBV DNA and HCV RNA levels keeping possibility of occult hepatitis B (OBI)/ seronegative hepatitis C infection despite HBsAg and Anti HCV antibodies being negative. Both levels were found to be raised and we attributed cirrhosis to dual hit by CHB and CHC. Patient was managed with antiviral drugs successfully with no recurrence of HCC and control of blood sugar levels. We hereby stress that screening beyond the HBsAg and Anti HCV antibodies should be done in all cases of liver cirrhosis in which etiology is not found on initial screening.

Keywords: Cirrhosis, Non-alcoholic Steatohepatitis, Occult Hepatitis B, Seronegative Hepatitis C.

INTRODUCTION

Chronic liver disease (CLD) is one of the most common diseases encountered worldwide. An estimated 1.2% of all hospital attendances in Indian hospitals are due to CLD alone.¹ CLD encompasses two etiological entities viz cirrhosis and non-cirrhotic. According to an Indian registry, 33.9% CLD patients had cirrhosis and almost all cirrhotics (99.4%) had decompensation at the time of diagnosis.¹ The prevalence of cirrhosis has been estimated up to 1% of population since compensated cirrhosis often goes undetected for prolonged period of time.²

Cirrhosis of liver is classically defined by the histological development of regenerative nodules surrounded by fibrous bands in response to chronic liver injury, leading to portal hypertension and end stage liver disease. Among the numerous etiological agents, alcohol consumption beyond permissible levels and hepatitis virus infections (Hepatitis B and C) have been the top charters for its causation. In Indian context, alcohol consumption was the most common etiology of cirrhosis (34.3% of the study population) while hepatitis B virus infection was the commonest cause in non-cirrhotic CLD and hepatocellular carcinoma: 40.8% and 46.8% respectively.¹ Non-alcoholic Steatohepatitis (NASH) secondary to diabetes, obesity and consequent metabolic
syndrome, is emerging fast as a cause of cirrhosis and is reportedly the most common cause of cryptogenic cirrhosis (CC) in India. In most of the cases, underlying etiology is found but in a subset of patients, no etiology is found despite extensive evaluation of all possible etiological factors and investigations and these patients are subsequently labelled as CC. In induced cirrhosis, the conventional serological markers (HBsAg, Anti HCV antibodies) respectively are usually done to rule out the viral infections however their absence have been documented in subset of patients having these infections. Screening beyond these markers for CHB and CHC has been advocated by the workers to have a clear picture of underlying etiology rather than labeling as CC. In this regard, we hereby present a case labelled as CC and developed HCC; later on further evaluation by our group turned out to be having both CHB and CHC, and was managed successfully with appropriate antiviral drugs.

CASE REPORT

51-year-old male, a known case of diabetes for last 10 yrs taking oral hypoglycemic agents (OHAs; Glimepride and Metformin) had history of 3 episodes of hematemesis in 2014 with no postural symptoms. At that time patient had no other complaints of jaundice, distension of abdomen with no peripheral signs suggestive of CLD. Pt was managed outside and upper GI endoscopy (UGIE) was done. He had high grade esophageal varices with sign of recent hemorrhage (SRH) and subsequently endoscopic variceal band ligation (EVL) was done. Ultrasonography (USG) and triple phase computed tomography (TPCT) revealed findings suggestive of liver cirrhosis with portal hypertension and splenomegaly. A diagnosis of liver cirrhosis was made and patient was further investigated. His viral markers for hepatitis B and C were negative. Autoimmune profile for liver diseases (ANA, Anti LKM1, ASMA, IgG, AMA) was negative. Iron studies were normal, which largely negated any possibility of hemochromatosis related cirrhosis. Kayser Fleischer rings were negative and serum cerulopalsmin levels were within normal range. There was no history of alcohol or any prolonged indigenous medicine intake. With a long-standing history of diabetes with current HbA1C of 7.5%, patient was labelled as having cirrhosis of liver secondary to NASH. Patient was on regular follow up with gastroenterologist and was being managed with tablet Propranolol and OHAs. Pt had regular followed up with UGIE and USG abdomen every 6 months. His blood sugar levels were having fluctuations for which OHAs dosages were optimised accordingly time to time. In Nov 2015, USG abdomen showed a hypoechoic lesion in left lobe of liver for which patient was referred to a higher centre. Triple phase CT and MRI abdomen was done and dysplastic nodules/hepatocellular carcinoma (HCC) was ruled out. His alpha feto protein (AFP) levels were within normal range (7.5; normal < 9.6 ng/ml). Patient had regular follow up as per the protocol laid down for diagnosis of HCC on background of cirrhosis. He never had any signs of decompensation of cirrhosis during follow up periods. In 2018, he had evidence of high grade dysplastic nodule on MRI scan and subsequently he underwent radiofrequency ablation (RFA) of it. Patient had no post procedural complications and came to us for further opinion regarding future course of HCC and uncontrolled blood sugar despite OHAs. Due to his uncontrolled blood sugar levels he was started with Dipeptidyl peptidase - 4 (DPP 4) inhibitors (Vildagliptin 50 mg BD) and Human insulin 30:70 twice a day in addition to previous prescribed OHAs for last 6 months.

We evaluated all previous records to find the exact etiology of underlying cirrhosis. The diagnosis of cirrhosis secondary to NASH was not biopsy proven. Patient was a moderate built male with a BMI of 21.3 Kg/m² and there was no evidence of metabolic syndrome as defined by the NCEP ATP III criterion. Though we were aware of the entity “lean NASH” which has been particularly described in Asians. We were still sceptical about this diagnosis. Patient’s uncontrolled blood sugar levels (HbA1C-10.7% at time of presentation) despite OHAs, DPP 4 inhibitors, Insulin and development of HCC lead us to further explore the underlying cause of cirrhosis. CHC and its strong association with development of diabetes/ poor control of diabetes despite therapy is well documented and accepted among the internists, gastroenterologists and endocrinologists and there is a growing experience now a days to explore the viral etiological factors beyond conventional markers especially in cirrhotics. Moreover, there is a low prevalence of HCC in NASH patients as compared to other etiological agents viz CHB, CHC and iron overload state (Hemochromatosis). Keeping these things in mind, we ordered for total Anti Hbc antibodies (a marker of hepatitis B virus infection) and quantitative HCV RNA levels. To our surprise, total Anti Hbc antibodies were positive (9.22; n<1) and HCV RNA levels were highly raised (241410 IU/ml; n < 12 IU/ml). Keeping a possibility of seropositive occult hepatitis B (total Anti HBsAb positive status with negative HBsAg), we ordered for HBV DNA levels which were higher than normal range (850 IU/ml; n<20 IU/ml). His HCV genotype turned out to be type 3. Owing to these two hidden (or rather unexplored) etiologies on background of cirrhosis, patient had likely development of HCC (both viruses can cause HCC) and deranged blood sugar levels (owing to CHC). Patient was subsequently started with tab Tenofovir Disoproxil Fumarate (300 mg) OD for CHB and tab Valpatasvir (100 mg) + Sofosbuvir (400 mg) for CHC. Patient tolerated both therapies well. After 3 months of therapy, he had undetectable HCV RNA and achieved sustained virological response (SVR; defined as undetectable HCV RNA levels 3 months after completion of therapy). He had also undetectable HBV DNA levels reflecting complete virological response. His blood sugar
levels settled with no need for insulin injection at 3 months follow up. On subsequent imaging he did not have any recurrence of HCC/any other new lesion. Currently he is stable and under endoscopic surveillance for varices every 6 months with last UGIE revealing eradicated varices, every 6 months USG and AFP, yearly triple phase MRI scan, yearly HBV DNA and HCV RNA quantification with last values being undetectable for both.

**DISCUSSION**

Cirrhosis of liver carries significant morbidity and mortality. There had been a 46% increase in CLD related mortality in the world between 1980 to 2013, with most of this increase reported from the low and low-middle income (LMIC) countries of Asia and Africa.\(^9\) In India, cirrhosis of liver accounts for approximately 2% of death due to all causes. Treatment of underlying etiological factors is the sole determining factor to prevent its progression. With the advancement of molecular biology, biochemical investigations and advanced imaging techniques, we often are able to ascertain the cause of cirrhosis in current scenario.

CHB and CHC are the major cause of cirrhosis in India as well as worldwide. In India, HCV infection affects about 0.4%-0.7% of the population and is responsible for 20%-40% of cases with cirrhosis.\(^1\) On the other hand, prevalence of CHB in general population in India has been estimated to be between 1.4% and 2.7%.\(^9\) Among the other predominant causes, NAFLD/NASH is an emerging entity with prevalence of NAFLD varies from 9% to 35% in India.\(^12\) Indian data suggests that at least 25 million patients with NAFLD may be at risk for significant liver disease.\(^13\) NAFLD/NASH has been explored as the most common cause of cryptogenic cirrhosis in India as well as worldwide.\(^14, 15\) The diagnosis of NAFLD/NASH is usually made by ultrasound, CT or MRI scan, raised transaminase levels but histology remains the gold standard for the diagnosis.\(^12\)

Being NASH the most common cause of CC and especially with a background history of diabetes and reported mean BMI in Indian patients with NAFLD being much lower than that in the Western population (as in our case; diabetic with BMI of 21.3 kg/m\(^2\)) one would be comfortable enough to label a case as CC provided all other possible etiologies had been ruled out which was done in this case.\(^16\) At first presentation, we were also convinced with the diagnosis but the deterioration of the previously controlled blood sugar levels despite lifestyle modifications, OHAs and development of dysplastic nodule raised some queries about the diagnosis. Though we were aware of NASH leading to development of cirrhosis and subsequently development of HCC in 15-20% and 3-5% respectively but it was diagnosis of exclusion with no biopsy done to prove it in this case. Moreover, there is ample data to suggest that in cirrhosis patients, the evaluation beyond conventional viral markers have to be done before labeling it cryptogenic one.\(^17\) The HBsAg and Anti HCV antibodies have been found to be absent in cirrhotic patients despite having these infections as documented by the presence of HBV DNA and HCV RNA in serum.

Absence of anti-HCV antibodies despite the persistent presence of HCV RNA has been termed as ‘seronegative’ or ‘serosilent’ HCV infection and found to be associated with clinical conditions such as human immunodeficiency virus (HIV) co-infection, hemodialysis and organ transplantation, but sporadically also has been seen in blood donors and other patients.\(^18, 20\) The prevalence of seronegative HCV infection ranges from 0.1-0.9% among healthy blood and organ donors to upto 10-13% among HIV and hemodialysis patients.\(^18\) In addition, CHC has been associated with development of new onset diabetes in cirrhotics with a prevalence rate of 13.6-67.4% as compared to prevalence in normal HCV patients being 2.9-12.6%.\(^21\) The underlying mechanism includes the development of insulin resistance (IR) due to postreceptor insulin signalling with increasing viral load.

Similarly, HBsAg can be absent from the serum with detection of HBV DNA in the liver (with or without HBV DNA in serum), termed as occult hepatitis B infection (OBI).\(^22\) OBI can be either seropositive or seronegative depending on the presence or absence of total Anti HBC antibodies (total Anti HBcAb). The prevalence of OBI varies from 3.9% to 60% in different regions of the world with 38% reported from India.\(^23-25\) The prevalence is higher in countries that are endemic for HBV, in individuals with serologic markers of previous HBV infection, and in those with HIV or HCV infection with more common occurrence in patients with cirrhosis or HCC.\(^22, 26, 27\)

In order to rule out the seronegative Hepatitis C and OBI infection in this patient we ordered both total anti HbcAb and HCV RNA levels. Both turned out to be positive and further evaluation revealed presence of HBV DNA in serum sample confirming the diagnosis of concomitant CHB and CHC infection. We labelled the patient with cirrhosis of liver secondary to CHB + CHC with? NASH. Since HBV and HCV have similar modes of transmission, infection with both viruses is not infrequent. There is robust data of patients having these dual infections.\(^28\) On institution of specific therapy (DAA for Hepatitis C and Nucleotide analog for Hepatitis B), patient responded well with no recurrence of HCC and good control of blood sugar level.

With this case, we emphasize the importance of unexplored etiological factors in CC. The various underlying mechanism behind OBI include mutations in the HBsAg gene that prevent detection with monoclonal antibody assays, persistence of immunoglobulin-bound HBV immune complexes, blockage of HBsAg secretion, or viral interference (e.g., coinfection with HCV).\(^28\) Similarly the presence of seronegative CHC has been
attributed to delayed seroconversion which sometimes may be prolonged to several years especially in patients with immune dysfunctions such as hypogammaglobulinemia, coinfected with HIV or transplant organ recipients and intravenous drug users.18 Another reason for seronegativity has been attributed to presence of immune complexes of antibodies and viral antigens resulting in very low levels of free circulating antibodies which may occur in patients with relatively low anti-HCV response with simultaneous massive virus replication and antigen production.18 In addition, inherent properties of particular HCV strains may be responsible for Anti HCV Ab absence despite HCV infection.29,30 Seronegative infection may also develop as a result of immune tolerance to HCV antigens, acquired in early life through vertical HCV transmission.31

CONCLUSION

Screening beyond the HBsAg and Anti HCV Ab should be done in all cases of liver cirrhosis before labeling them as cryptogenic one. The detection of chronic viral hepatitis and subsequent treatment may retard the progression of cirrhosis and leads to favorable outcome.

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