



Original Research Article

Prevalence of Occult HBV Infection in HCV Positive Patients in Suez canal Area

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A B S T R A C T

Hepatitis B virus (HBV) is a major health problem. The term occult hepatitis B virus infection (OBI) is defined as the serologically undetectable hepatitis B surface antigen, despite of the presence of HBV DNA and played an important role in the development of hepatocellular carcinoma (HCC) in chronic hepatitis C virus (HCV) infected persons with the lack response to interferon treatment in HCV patients. Our aim was to estimate the prevalence of OBI among HCV positive patients in Suez Canal University (SCU) hospital by using the PCR (Polymerase Chain Reaction) technique and we correlated the clinical, laboratory characteristics among those patients. Fifty patients with HCV were subjected to complete blood picture, liver function, anti-schistosoma antibody, HBsAg; anti-HCV antibodies and HCV RNA. We found that the number of infected person with OBI was 3 person out of 50 which represent 6% of the studied patients. The co-infection of OBI with HCV is of great importance because of its added co-morbidity of liver enzymes elevation, increased severity of liver disease and increased risk of HCC. The highest prevalence of OBI in Egypt is similar to the scenario for classic HBV infection.

Keywords

Occult B,
Hepatitis C,
Egypt

Introduction

Hepatitis B virus (HBV) is a major health problem worldwide (Candotti & Allain (b), 2009). It is transmitted through different ways, but transfusion is the most important one that should be prevented. So, detection of hepatitis B surface antigen (HBsAg) is used as the routine screening test of blood donors in the early 1970s till now to enhance a safety transfusion (Liu et

al., 2010). However, it was found that transmission of HBsAg negative can occur (Liu et al., 2006) and remains HBV transmission the most frequent transfusion viral infection (Niederhauser et al., 2008; Calderón et al., 2009 & Kafi-abad et al., 2009); thus, the term occult hepatitis B virus infection (OBI) started to appear. OBI is defined as the serologically undetectable

hepatitis B surface antigen, despite of the presence of HBV DNA (Allain(b),2009 & De Mitri, 2010).

Several authors have defined OBI as Bremer et al in 2009 (Bremer et al.,2009) emphasized that the presence of HBV DNA in the patients liver but without detecting HBsAg in their serum which usually occurs after the progressive disappearance of HBsAg (Raimondo et al.,2007) and its persistence in low-level in carriers (Allain(a),2004). In these patients, the cause of the lack of circulating HBsAg may be due to genetic rearrangements in the HBV genome that interfere with the gene expression and lead to the production of an antigenically modified S protein (Blum et al.,1991; Carman et al.,1997 & Yamamoto et al.,1994).

The molecular basis of OBI was due to the long-lasting persistence of the viral covalently-closed-circular DNA (cccDNA) in the nuclei of hepatocytes (Levrero et al., 2009). Almost all OBI cases infected with HBV showed strong suppression of HBV replication and gene expression due to host immune-surveillance and epigenetic factors (Raimondo et al., 2008). OBI can be distinguished into seropositive and seronegative on the basis of HBV antibodies profile, the former is positive for Hbc and/or s antibodies, the latter is negative for all markers of HBV infection with very low amount of HBV DNA < 200 IU/ml (Raimondo et al., 2008).

Seronegative-OBI cases may progressively lost HBV specific antibodies after the resolution of the acute infection or, had negative results of tests from the beginning of infection (Michalak et al.,2004), as the low-dose infection was insufficient to allow the proper maturation of the antiviral protective memory response (Zerbini et al.,2008). Although OBI was associated with

the presence of HBV antibodies (Torbensohn and Thomas, 2002), the analysis of liver DNA extracts represents the gold standard for the evaluation of OBI (Raimondo et al., 2008). In all cases, it is strongly recommended to use polymerase chain reaction (PCR) or real time PCR with oligonucleotide primers specific for different HBV genomic regions in order to diagnose OBI (Raimondo et al., 2010).

Recently, it was found that OBI played an important role in some clinical phenomena like cryptogenic liver disease (Alavian et al.,2004), the poor response to antiviral treatment (Mrani et al.,2007) and the development of hepatocellular carcinoma (HCC) (Miura et al.,2008) in chronic hepatitis C virus (HCV) infected persons. In addition, the link between the occurrence of occult HBV and HCV infection is due to the same route of transmission so, the infection with both viruses can occur in areas where these viruses are endemic and among people who are at high risk for parenteral infections (Gonzalez et al.,1995; Koike et al.,1998 & Uchida et al.,1997). In the Mediterranean basin, it was clear that HBV DNA is detected in about one-third of HCV patients with negative results of HBsAg test (Torbensohn and Thomas, 2002).

At the same time, several studies have suggested that OBI is correlated with the lack response to interferon treatment in HCV patients. In addition to the low-level HBV infection not only may contribute to the severity of HCV-related liver disease but it can be of prognostic importance (Fukuda et al., 1999).

This study was conducted to estimate the prevalence of OBI among HCV –positive patients by using the PCR technique and we correlate the clinical, laboratory characteristics among those patients.

Patients and Methods

Patients

A total of 50 patients with Chronic Liver Disease (CLD) selected from the Internal Medicine department, Faculty of Medicine Suez Canal University (FOMSCU); Ismailia, Egypt, were enrolled in the study after obtaining informed consent from all of the patients.

Patients suffering from acute or chronic HBV infection as marked by positive hepatitis B surface antigen, other causes of liver dysfunction (e.g., primary biliary cirrhosis and autoimmune hepatitis) and being on treatment with interferon and ribavirin were excluded from the study.

All patients were subjected to the following: A complete medical history, etiology & past history of surgery, bilharziasis, blood transfusion & dental procedures. Also the following laboratory investigations were done: complete blood picture, serum alanine amino transaminase (ALT) and aspartate amino transaminase (AST), total bilirubin, prothrombin time, serum albumin, & alpha feto-protein (AFP) and markers of both HBV & HCV infections such as HBsAg, anti-HCV antibodies and HCV RNA. Moreover, anti-schistosoma antibody were done for all cases.

Result and Discussion

Demographic, clinical and laboratory characteristics of the study group are shown in (Table 1). The male subjects consisted of 26 subjects, while female subjects consisted of 24 subjects. Age mean value is 44.58 ±9.852 years. The history of presence of bilharziasis, surgery, dental procedures were 58%, 70%, and 12% respectively. Laboratory results are shown in the table,

both mean and standard deviation.

Our results showed 3 cases of OBI out of 50 which represents 6% of the studied patients. These cases showed high statistical significance with a *p value* =0.00 between OBI and the sex of patients, past history of bilharziasis, and the ANA (anti-nuclear antibody) in their serum. This significance was very clear with the viral markers in the form of the serum HBsAg, HCV antibody & HCV detected by PCR as mentioned in (Table 2).

For the laboratory findings, certain parameters showed a statistic significance in form of liver function test as (serum ALT, AST, total bilirubin), the anti-schistosomal antibody, and the abdominal ultrasonography as being clarified in (Table 3).

In the present work, HBV DNA was present in 3 patients out of 50 (6%) in HCV positive patients and negative HBsAg. The prevalence of OBI in HCV positive patients vary from 0 to 52% (Fukuda et al., 1999 & Levast et al., 2010) ring from HBV as well as HCV infections (Hollinger, 2010) and the method used for the diagnosis as PCR primers (Mrani et al., 2010).

As the diagnosis of OBI is strongly dependent on the detection of HBV DNA by using PCR technique which is an expensive tool and on the other side the Egyptian budget for health care and scientific research is very low to afford it, so many Egyptian studies depend on the anti-HBc positivity for diagnosis of OBI that lead to miss the HBV DNA positive and the anti-HBc negative cases and consequently this lead to the difficult underestimating of the problem (El-Shaarawy et al., 2007, El-sherif et al., 2012; Kamal et al., 2006 & Abu El-Makarem et al., 2012).

In spite that the gold standard for diagnosis OBI infection is the liver biopsy in order to extract DNA, Raimondo in 2007 & Levast in 2010 and their colleges reported that in certain situations it is not easily applicable to use this technique. In 1999, Cacciola reported that the usage of clinical approach of PCR due to its sensitivity and specificity start to be gold technique as the detection of HBV DNA by using liver biopsy in HBV DNA serum patients is not usually detected in the serum of patients with intra-hepatic HBV DNA.

In 2009, Morsica, advised to perform serum analysis for HBV at different time points not only once before therapy as it could not be sufficient to detect OBI infection in case of virus replication is being intermittent (Morsica et al., 2009).

El-Shaarawy et al., in 2007 (El-Shaarawy et al., 2007) reported that the prevalence of OBI in HCV patients was projected to be 50% depending on anti-HBc positivity only, although only 60% of these tested patients were positive for HBV DNA by using PCR technique which is not in agreement with the idea that HBV DNA positivity is a prerequisite test used for the diagnosis of OBI (Hassan et al., 2011). Hassan and his colleges in 2011 conducted a study on Egyptian chronic HCV patients, they discovered that serum HBV DNA was detected in 22.5% of anti-HBc positive HCV patients and nothing was detected in anti-HBc-negative HCV patients (El-sherif et al., 2012).

So that why, the serological markers could be the only way to detect OBI as only two out of the six patients with detectable HBV DNA had anti-HBc antibodies and none had anti-HBs antibodies (El-Gammal et al., 2010).

In our work, 3 OBI patients were detected out from 50 HCV positive patients as this is coherent with other studies that detected that the higher prevalence of OBI detection in HCV positive than HCV negative cases (Blum et al., 1991, Cacciola et al., 1999; El-Sherif et al., 2009 & Sagnelli et al., 2008).

For the view in Egypt the rate of positive anti-HCV is 15% (which range from 6-28%) and 5% of the community is positive for both anti-HCV and HBsAg and/or anti-HBc (El-Sayed et al., 1997). While, DNA positive OBI in Egyptian HCV patients on pegylated interferon/ribavirin therapy was observed to be 3.9%, (Michalak et al., 2004 & Emara et al., 2020) this rate is increased to be 10% in those with elevated liver enzymes while being on therapy (El-Gammal et al., 2010), and the rate can shoot to reach 20% in those who had elevated liver enzymes without being on therapy (El-sherif et al., 2012). So, The prevalence of OBI in HCV patients was in tendency to be higher in patients having either anti-HBs or anti-HBc or both (Fukuda et al., 1999 & El-Sherif et al., 2009). For the serological findings, it was found that patients with OBI and HCV co-infection 35% of people were anti-HBs positive, 42% were anti-HBc IgG positive and 22% were negative for both (Torbensohn et al., 2004).

Moreover, a strong coincidence was found between the occurrence of liver cirrhosis (Mariscal et al., 2004), increase ALT serum level and histological activity (Fukuda et al., 1999 & Uchida et al., 1997) in HCV positive patients and OBI infection. So this show that the infection with OBI is synergistic with chronic HCV infection which predispose for the occurrence of HCC (Kim et al., 1994). From the other side, Chen et al., 2010 reported

that HCV positive patients with OBI infection had lower ALT levels, liver histology activity index and fibrosis scores than those infected only with HCV infection.

Several studies concerning serum ALT levels, some showed that there were usually mild raise in OBI and HCV co-infection(Fukuda et al.,1999 & Kazemi-Shirazi et al.,2000) others showed to be either equivalent(Nirei et al.,2000) or mildly raise(El-sherif et al.,2012 ; Fukuda et al.,1999 & Stransky et al.,2000) than in HCV mono-infection. Unfortunately, the effect of OBI on liver enzyme has not been studied with large in the Egyptian literature. El-sherif et al., 2012 , found that OBI did

not influence a rise of liver enzyme in HCV patients with pegylated interferon/ribavirin therapy. Selim et al., 2011 compared the frequency of OBI in Egyptian HCV patients with normal or slightly raised liver enzymes with another group of patients with increase liver enzyme reaching up to >5 folds and found OBI in 63.3% of patients with enzymes flare in comparison to 13.3% in the normal enzymes group.

The co-infection of OBI with HCV is of great importance because of its added co-morbidity of liver enzymes elevation, increased severity of liver disease and increased risk of HCC. The highest prevalence of OBI in Egypt is similar to the scenario for classic HBV infection.

Table.1 Demographic, clinical and laboratory characteristics of the studied population

Characteristic	Patients number (n=50)
Age(years ±SD)	44.5±9.8
Males (%)	26 (52%)
Females(%)	24 (48%)
Past history of bilhasiasis [positive(n,%)]	29 (58%)
Past history of surgery [positive(n,%)]	35 (70%)
Past history of dental procedure [positive (n,%)]	12 (24%)
Total bilirubin(mg/dl)	0.76±.22
AST (IU/L)	49.6±29.9
ALT (IU/L)	53.24±36.5
Albumin (g/dL)	4.1±.35
AFP (IU/ml)	6.1±4.2
PT/second	12.11±2.4

*N.B. AST: aspartate amino transaminase, ALT: alanine amino transaminase, , AFP: Alpha fetoprotein, PT: prothrombin time.

Table.2 The relationship between HBV DNA(OBI) by PCR and the clinical and the viral markers

Characteristic	Chi square value	P value
Age	23.40	.713
Sex	58.07	.000*
Past history of bilhasiasis	59.63	.000*
HCV ab(IU/ml)	57.232	.000*
HCV PCR(IU/ml)	114.000	.001*

*N.B. Pvalue \leq 0.05 statistically significant

Table.3 The relationship between HBV DNA(OBI) by PCR and the laboratory findings

Characteristic	Chi square value	P value
CBC (HB)(mm ³)	50.000	.060
WBC (mm ³)	7.447	.995
Platelet (mm ³)	50.000	.060
ALT(IU/L)	50.000	.038*
AST(IU/L)	50.000	.022*
Total bilirubin (mg/dl)	28.723	.026*
Albumin(g/dl)	19.605	.143
AFP(IU/ml)	50.000	.012
PT/second	16.755	.669
Anti-schistosoma antibody	66.356	.000*

N.B. CBC: Complete Blood Count, HB: Hemoglobin

*P value \leq 0.05 statistically significant

Author's contribution

All authors participated in the design of the study. Fawzy has provided the patients and reviewed the manuscript. NM, OD and RH design the study, carried out the DNA isolation, performed the genotyping, written and edited the manuscript. All authors read and approved the final manuscript.

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