Occult hepatitis B virus infection at patients with non viral liver disease

Bulent Cakal[1], 0000-0002-1254-844X

Alp Atasoy[2], 0000-0003-1791-897X

Bilger Cavus[2], 0000-0003-2203-4255

Mehves Poda[3], 0000-0002-1957-6072

Mesut Bulakcı[4], 0000-0003-0993-6465;

Mine Gulluoglu[5], 0000-0002-3967-0779;

Mehmet Demirci[6], 0000-0001-9670-2426

Filiz Akyuz[2], 0000-0001-7498-141X

[1] Department of Medical Microbiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey.

[2] Division of Gastroenterohepatology, Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey.

[3] Department of Genetics, Aziz Sancar Institute for Experimental Medical Research, Istanbul University, Istanbul, Turkey

[4] Department of Radiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey.

[5] Department of Pathology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey.

[6] Department of Medical Microbiology, Faculty of Medicine, Kirklareli University, Kirklareli, Turkey

***Corresponding author:** Bülent ÇAKAL PhD, Department of Medical Microbiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey. Topkapı Mahallesi, TURGUT ÖZAL CADDESİ No:118, 34093 Fatih/İSTANBUL. Tel: +90532726644. Fax: +902124142037. Email: bulentcakal@yahoo.com

Abstract

Introduction: The presence and clinical effects of OBI are not clear in patients with non-viral liver disease. The objective of this study was to determine the prevalence and serological characteristics of OBI in patients who underwent liver biopsy due to various clinical indications, and to evaluate the interaction between the clinical and histopathological characteristics of the patients and OBI.

Methodology: This study included 83 HBsAg negative patients who were followed up by the Gastroenterohepatology clinic. The presence of HBV DNA was investigated by using an in-house nested PCR method from liver parenchymal biopsy samples obtained from patients who underwent due non-viral chronic liver diseases.

Results: OBI was detected in 19 (22,9%) out of the 83 cases. OBI was defined in 11 (44%) of 25 anti-HBc positive patients and 15 (31.9%) of 47 anti-HBc and/or anti-HBs positive patients. A statistically significant correlation was found between the presence of OBI and anti-HBc (p=0.003) and anti-HBV antibody serostatus (p=0.025) of patients.

Conclusions: OBI could be associated with of individuals with a history of HBV exposure. Therefore, it is suggested that close virological monitoring of HBV can be helpful for improving the management of chronic liver disease and especially of patients anti-HBV antibody seropositive.

Keywords: Occult HBV infection, non-viral liver disease, hepatitis B core antibody, prevalence, liver biopsy

Introduction

Chronic Hepatitis B virus (HBV) infection continues to be an important worldwide public health problem due to its clinical consequences such as chronic active hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). Occult (HBV) infection (OBI) is defined as the presence of replication competent HBV DNA (i.e. episomal HBV covalently closed circular DNA [cccDNA]) in the liver and/or HBV DNA in the blood of persons testing negative for HBsAg. It is also characterized by the presence of viral replication at a low level. In the natural course of chronic HBV infection, OBI is considered as one of the likely phases which occurs as a result of the interaction of genetic, epigenetic, and immunological mechanisms that develop based on multiple factors related to the virus, the infected individual, or the environment [1-3]. The molecular basis of OBI and its pathological effects on the liver are closely linked to the HBV-specific viral lifecycle, which can be observed in the stability and long-term persistence of episomal HBV cccDNA in the nucleus of infected hepatocytes [4-7].

OBI alone may cause limited damage in the liver under immune-competent conditions. It may rather be a risk factor for the initial development and accelerated progression of cirrhotic and non-cirrhotic HCC when combined with other important causes of chronic liver damage such as cryptogenic liver disease, chronic hepatitis C virus (HCV) infection and alcoholic or non-alcoholic fatty-liver disease (NASH) [2, 8-21]. On the other hand, OBI is of clinical importance since occult virus may lead to typical new HBV infections in recipients following transmission via procedures such as blood transfusion, birth, hemodialysis, and orthotopic liver transplantation. It may further cause HBV-related liver diseases (acute and fulminant hepatitis) following reactivation in immunosuppression conditions [19, 20, 22-31]. OBI may be identified especially in residents of regions with high HBsAg seroprevalence, individuals with previous HBV and chronic HCV/HIV infection or cryptogenic hepatitis and cryptogenic cirrhosis history, HCC patients, and finally, people with hematological malignancies such as thalassemia and hemophilia [32-36].

Studies on OBI are usually performed to identify its role in the etiopathogenesis of advanced liver injuries such as chronic hepatitis C, cirrhosis and HCC, to determine its clinical and histopathological effects, and to figure out its prevalence in specific patient

groups, blood donors, and the general population. However, in most of these studies, OBI is diagnosed through HBV-DNA detection in patient-serum samples. Hence, such studies involving patients with advanced liver diseases (cirrhosis, HCC, etc.) hoping to detect OBI based on serum-level presence are bound to have limitations.

In this study, the objective was to determine the prevalence and serological profile of OBI in patients who underwent liver biopsy due to liver-enzyme elevation for unknown reasons, fatty-liver condition, cholestatic liver disease, and other clinical indications, and to evaluate the interaction between clinical or histopathological patient characteristics and OBI.

Methodology

This study was supported by the Scientific Research Projects Coordination Unit of XXX with the project number TOA-2016-21643. Ethical approval of the study was provided by XXX Ethics Committee at XXX (No: 2015/1519). All patients included in this study gave informed consent for participation.

Patients: In this study, a total of 83 HBsAg seronegative patients were included. No patients had vaccination history and prior HBV infection. They were followed up by the Gastroenterohepatology Clinic at XXX between October 2016 and November 2019 for various clinical indications. Patient DNA was obtained from liver parenchymal biopsies conducted for histopathological diagnosis and/or treatment (Table 1). Biopsy indications in the vast majority (80%) of study participants were for liver disease (32 metabolic,16 cholestatic) and for liver-enzyme elevation due to unknown reasons (18). All patients were seronegative for Hepatitis C virus (HCV) and human immunodeficiency virus (HIV) antibodies.

Clinical materials: Liver biopsies were performed in the Interventional Radiology Unit of the Radiology Department and in the Gastroenterohepatology Department of Internal Medicine, XXX.

Specimen collection and storage: Biopsies were performed with percutaneous needle biopsy method accompanied by ultrasound imaging, using manual method (Menghini; 17-gauge, Hepafix®, B. Braun Melsungen AG 34209) in the Gastroenterohepatology Department or using automatic needles (TruCut; 18-gauge, Bard MaxCore, Covington, GA), in the Radiology Departments. One set of liver-tissue

specimens were preserved in Hollande's fixative and sent to the Pathology Department for analysis (evaluation). A second tissue fragment or another biopsy sample was immediately snap-frozen in liquid nitrogen and kept at 80°C before use for nested PCR analysis.

Serum samples: Simultaneously with biopsy procedures, patients' blood samples were taken using appropriate tubes in accordance with venous blood-collection practice. After centrifugation, the sera were separated and transferred to sterile vials to be stored at -80°C for later laboratory analyses.

Detection of intrahepatic HBV-DNA and defining OBI

DNA extraction from liver-biopsy specimens: Total DNA was extracted from each liver-tissue sample using a commercially avialable kit (QIAamp DNA Mini kit, Qiagen GmbH, Hilden, Germany). Cryopreserved tissue samples were first homogenized in a homogenization buffer with the aid of TissueRuptor (QIAGEN, Hilden, Germany) and later completely lysed with proteinase K at 56°C overnight. Lastly, the total DNA was purified according to manufacturer's recommendations. DNA concentrations were determined by using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

Nested PCR: Total DNA extracts were subjected to four different in-house nested-PCR amplification experiments to detect the existence of (1) HBV preS-S, (2) preCore-Core, (3) Polymerase, and (4) X-viral genomic regions within the sample. Nested PCR primer sets were designed so as to cover the entire HBV genome. (Table S1) [15, 37].

Hence, viral-DNA amplification was performed at the intrahepatic level. For amplifiying the target region, initially (Nested I) the nested PCR setup (25 µl) was constructed, containing 12.5 µl 2X PCR master mix (HS Prime Taq Premix; GeNet Bio) and external primers (200ng/µl) for amplification of 5µl DNA extract. Amplification was achieved using an initial denaturation for 10 min at 95ºC, and 35 PCR cycles consisting of 30 seconds of denaturation at 95ºC, 30 seconds of annealing at 55-60ºC, extension at 72ºC for 30 seconds, followed by a final extension step of 10 min at 72ºC. In the second step of the nested PCR setup, amplification was performed similarly to Nested I, using 5µl of the first PCR product and its matching internal primers for the amplification of the main target region. All PCR reactions were performed in the T100 Thermal Cycler (Bio-Rad) instrument.

For patients with overt chronic-B infection who served as positive controls, HBV plasmid (4.1 kbp) material was used to standardize nested PCR and DNA obtained from liver biopsy samples. To eliminate false-negative results and to demonstrate the existence of proper-quality DNA, the human beta-globin gene was used as target. DNA from individuals serving as negative controls without HBV infection were kept in sterile distilled water. In this study, the sensitivity limit for nested PCR was set as 10 HBV plasmid DNA copies / PCR reactions. The obtained PCR products were subjected to electrophepheresis containing 2% agarose and evaluated using a gel imaging system. The presence of HBV DNA specific to the target region and intended size was checked in comparison with a 100 bp DNA marker (100bp DNA Marker; GeNet Bio) used as a reference.

Defining OBI: After amplification with nested PCR, amplification positivity of at least 2 viral genomic regions was considered positive for OBI.

Clinical laboratory data: Demographic and pre-biopsy patient data were obtained from patient files or the hospital's electronic data management system (Table 1).

Serology markers/assays and serum HBV DNA: Serum antibodies to hepatitis B surface (anti-HBs) and hepatitis B core (anti-HBc) antigens were analyzed with Elecsys Anti-HBs II and Elecsys Anti-HBc II kit/Cobas e411® (Roche Diagnostics, Mannheim, Germany) respectively according to manufacturer's instructions. HBV DNA was extracted from serum samples using QIAsymphony DSP Virus/Pathogen Midi Kit (Qiagen GmBH, Hilden, Germany) according to manufacturers instructions and then HBV DNA viral load detection was performed by using Artus HBV QS-RGQ kit (Qiagen GmBH, Hilden, Germany) with a linear range from 31.6 to more than 2.0x107 IU/mL on the Rotor-Gene Q instrument (Qiagen GmBH, Hilden, Germany).

Liver histopathology: Histology of liver biopsy specimens was evaluated by Department of Pathology of XXX. Liver biopsy samples were fixed in a formalin solution and stained with Masson's trichrome. Pathology reports contained histological parameters including fibrosis, portal and lobular inflammation, lobular necrosis, steatosis, cholestasis, and bile duct damage. NAFLD was defined as the observation of abnormal lipid accumulation (hepatic steatosis) in more than 5% of hepatocytes. NASH was defined as the presence of lobular inflammation together with ballooning (hepatocyte damage) and hepatic steatosis, with or without fibrosis [38]. In

histopathological diagnosis, the presence of minimal portal or lobular inflammatory infiltrates, absence of fibrosis, and absence of structural changes were considered as non-specific histological findings.

Statistical analysis: For statistical data analysis, SPSS (21.0, SPSS Inc., Chicago, IL) was used. For comparisons involving categorical variables, the Chi-square test and/or Fisher's exact test was used. For non-categorical data, normality was checked using the Shapiro-Wilk test and ANOVA was used for comparisons. Results are provided as means and standard deviations. Results with p-values less than 0.05 were considered statistically significant.

Results

Prevelance of OBI in liver tissue: In this study, we have detected the presence of HBV DNA at the intrahepatic level in 19 (22.9%) of 83 HbsAg-negative patients whose liver biopsy was performed due to the clinical indications specified in Table 1. On the others hand, Serum HBV DNA was not detected in serum sample of any patients

Demographic, clinical laboratory parameters and OBI: Demographic, clinical, laboratory, serological, and clinical data of 83 patients are outlined in Table 1. 38 patients were male (45.8% of the total) and 45 female (54.2%). The mean age of the study population was 47.5, with SD of 13.8. In our study, no statistically significant correlation was found between OBI presence and patients' demographic characteristics including gender and age. Further, clinical parameters including patients' liver enzymes (ALT, AST, ALP, GGT), bilirubin levels, and INR, AFP, albumin levels were not significantly correlated with OBI presence either.

OBI and anti-HBV antibody profile: The patients' anti-HBV antibody serostatus/profile is summarized in Table 1. In this sample, 25 (30.1%) were found positive for anti-HBc antibodies only, 43 (51.8%) for anti-HBs antibodies only, and 47 (56.6%) for both (seropositive) anti-HBc and anti-HBs antibodies. Among the 19 patients detected to have OBI, 11 (57.9%) were positive for anti-HBc antibodies, 15 (78.9%) positive for both anti-HBc and anti-HBs (seropositive), and 4 (21.1%) negative (seronegative) for both anti-HBV anbodies. OBI was defined in 11 (44%) of 25 anti-HBc positive patients and 15 (31.9%) of 47 anti-HBc and/or anti-HBs positive patients. In the others hand, OBI was defined in 8 (13.8%) of 58 anti-HBc negative patients and 4 (11.1%) of 36 anti-HBc and/or anti-HBs negative patients. A statistically significant

correlation was found between the presence of OBI and anti-HBc (p=0.003) and anti-HBV antibody serostatus (p=0.025) of patients. Anti-HBs antibodies were positive in 12 patients (63.2%) with OBI and in 31 (48.4%) without. There was no statistically relationship difference between OBI presence and anti-HBs antibody profile (p=0.259).

Liver Histopatology:

OBI and hepatic steatosis: Histopathological findings related to liver parenchymal biopsies of the patients are shown in Table 2. The most prevalent histopathological finding was Liver/hepatic steatosis with a total of 33 patients (39,8%), where 22 (26,5%) were NASH and 11 (13.2) NAFLD. Among these patients, OBI was detected in 7 (31,8%), 2 (18.2%) and 9 (27,3%), respectively. There was no statistically significant difference in terms of OBI occurrence between patients with and without hepatic steatosis, and patients with NASH and NAFLD (Table 3).

OBI and non-specific liver histology: Biopsy indications / clinical histories and anti-HBV serological profiles of 23 patients who exhibited non-specific histopathological / histological changes are summarized in Table 4. In this group, 7 patients (30.4%) were detected to have OBI. Liver biopsies were performed on 6 patients with a pre-diagnosis of cryptogenic liver disease, on 10 receiving immunosuppressive and/or immunosuppressive therapy due to hematological and autoimmune / autoinflammatory disease, and on 3 with elevated autoimmune antibodies. OBI was detected in 3 (50%), 3 (33.3%), and 1 (33.3%) patients, respectively. However, OBI was not detected in 2 patients with cholestatic liver disease, 1 with liver steatosis, and 1 with malignant disease (colorectal cancer). In this group, OBI was detected in 7 (77.8%) of 9 anti-HBc positive patients and in 4 (40%) of 10 anti-HBs positive patients (Table 4). Anti-HBcIgG antibodies were positive in 3 patients (100%) with cryptogenic liver disease pre-diagnosis, in 2 patients (50%) with autoimmune / autoinflammatory disease, and in 1 patient (100%) with hematological malignancy who were all determined to have OBI. One patient with OBI who underwent biopsy due to elevated autoimmune antibodies was negative for serum anti-HBcIgG antibody. In this group, four patients who were not determined to have OBI, anti-HBcIgG antibodies were negative (Table 4).

OBI and Cholestatic Liver disease: HBV DNA was not detected in the liver tissue of any of the 14 patients whose histopathological findings were consistent with cholestatic liver disease (Table 2).

OBI and other liver diseases: OBI was detected in 3 (23.1%) out of 13 patients (15,7% of the total sample), of which 4 were reported to have cirrhosis, 4 vascular liver damage, 3 toxic hepatitis, 1 autoimmune hepatitis, and 1 granulomatous liver disease consistent histopathological findings (Table 2). In this group of 3 patients with OBI, 1 had vascular liver damage, 1 had cirrhosis, and 1 had toxic hepatitis. HBV DNA was not detected in the liver parenchymal tissues of 2 patient exhibiting autoimmune hepatitis and (apparently) granulomatous liver disease.

Discussion

OBI was detected in 19 (22.9%) out of 83 patients included in this study, which was conducted to evaluate the prevalence of OBI at intrahepatic level and its association with clinical and histopathological findings in patients who underwent liver biopsy for various clinical indications. Demographic and clinical laboratory data obtained as part of this study point out to the difficulty of finding measurable specific parameters indicating or predicting the presence of OBI (Table 1).

Serologically, OBI may be categorised as seropositive and seronegative. According to the literature, in most OBI cases (80%) patients test positive for HBV antibodies [20, 37]. In our study, patients with OBI had a serological profile that is 78.9% seropositive and 21.1% seronegative. Therefore; the data (Table 1) obtained from serological profiles of patients with OBI support the use of anti-HBc IgG in routine clinical practice as a predictive biomarker for the prevention of possible HBV reactivations, especially for patients who are in immunosuppressed state and/or recieving immunosuppressive therapy [39].

OBI prevalence may exhibit differences depending on many factors such as the clinical profile of the patient group, geographic location (HBV endemicity), co-infection with other viruses, vaccination, immunological status, and laboratory diagnostic methods / procedures [32-36]. On the other hand, there are only a few published studies on intrahepatic HBV detection determining the prevalence and role of OBI in chronic liver disease. In a study conducted to determine HBV DNA at the intrahepatic level in patients with normal liver histology, OBI prevalence was found to be 16.3% [27]. In another study determining HBV DNA based on serum obtained from HBsAg negative healthy individuals, cryptogenic chronic liver patients, and patients with HCC, OBI prevalence was found to be 10.6%, 28.3%, and 70.4%, and anti-HBcIgG positivity

percentage 33.3%, 100%, and 86.7%, respectively [34]. In our study using patients with various clinical and histopathological characteristics as the research sample, OBI prevalence was 22.9% overall and 31.8% in the sub-group with histopathologically defined hepatocyte damage such as NASH. It is suggest that OBI prevalence could be assaociated with liver damage of patients and HBV endemicity levels.

Although OBI incidence was higher among NASH patients than NAFLD patients and other subgroups (except for patients with non-specific histopathological changes), no statistically significant difference was found OBI frequency across patient groups (Table 2 and 3). This might be due to the modest sample size. In a study conducted by Raimondo G et al. in patients with morbid obesity, OBI was detected in 4.5% of patients with NAFLD and in 20.9% of patients with NASH. In this respect, it has been suggested that OBI may be both a risk factor for the development of NASH and a cofactor that increases its progression [40]. In our study, hepatic steatosis was found in 9 (47.4%) out of 19 patients with OBI. The findings indicate that the relatively increased frequency of OBI in patients with NASH, the presence of NASH-related hepatocyte damage in the in the formation of OBI and on the other hand, OBI may increase progression of fibrosis in patients with NASH. Nuclear receptors (NR) play a critical role in hepatic lipid and glucose metabolism as well as transcriptional regulation of HBV biosynthesis. In this respect, to regulate the impaired lipid metabolism and hepatic steatosis at the hepatocyte level, NR expression increases and may activate the transcription of HBV and promote viral replication. Ultimately, futher research is needed to determine the histopathological relationship between NASH and OBI [41, 42].

In another study involving 101 patients diagnosed with cryptogenic liver disease characterized by persistent transaminase elevation and HBsAg seronegativity, 19 patients (18.8%) were reported to have OBI. Among the 49 patients who were histopathologically defined as having non-specific changes and NASH, OBI was detected in 7 (14,3%). Among 39 patients with chronic hepatitis and 13 with chirrosis, OBI was found in 30,7% and 61,5%, respectively [43]. The aforementioned study has similarities with our findings (table 2) regarding the presence and prevalance of OBI in patients with histological nonspecific changes and NASH who are monitored due to their elevated liver enzymes with unknown aetiology.

Several studies reported that the presence and frequency of OBI increase is parallel to level of liver damage in cryptogenic hepatitis, cirrhosis, cirrhotic HSC, and non-cirrhotic HSC patients and also HBV endemicity [10, 18, 31]. In our study of clinically pre-diagnosed Cryptogenic (chronic) liver disease patients, findings regarding OBI presence and anti-HBcIgG positivity taken together indicate that OBI may have a role in the etiopathogenesis of this disease. Therefore, evaluating the presence of OBI in such patients living in regions with high HBV contact risk (endemicity rate) may make clinical management more effective.

The conditions and potency of immunosuppression may be associated with OBI presense and life-threatening HBV reactivation risk [1, 19, 20, 44]. The data obtained in this study indicates that, in addition to liver damage, immunosuppression conditions may also promote OBI (Table 2 and 4). This points to the determining role of host factors including immunity, rather than viral factors in the pathogenesis of OBI. Based on these data, it may also be predicted that the neutralization capacity of anti-HBs, which is an indicator of HBV immunization, may be limited in patients with OBI [44]. ltimately, in HBsAg negative, anti-HBc seropositive immunosuppressive patients, regardless of their anti-HBs serostatus, evaluation of HBV reactivation risk might be recommended.

Although the limited number of patients with liver cirrhosis (Table 4) in our study makes it difficult to make a confident deduction, individuals with a history of high HBV contact risk and hepatocyte damage, especially in cases of cryptogenic cirrhosis, may be at risk for OBI. In studies involving patients with intrahepatic cholangiocarcinoma and autoimmune hepatitis, OBI prevalence was reported as 61.7% and 23.3%, respectively [45, 46]. The absence of OBI in our study in these subgroups may be an artifact of smaller sample size (Table 4).

In our study, serum HBV DNA was not detected in any of the patients who were OBI diagnosis. This situation supports the idea that low level of replication competence of OBI and OBI-related HBV viremia are characterized by temporal fluctuations [1-47].

The limitations of this study include the unequal distribution of study subgroups and their partially insufficient numbers.

Histopathological and clinical effects of OBI accompanying chronic liver injuries are not yet clear [48]. In a retrospective study involving patients with various clinical conditions

such as HBV exposure, immunosuppression, HIV co-infection, and liver injury, it has been stated that OBI is a heterogeneous condition associated with the specified clinical conditions [49]. In that study, attention has been drawn to the risk of HBV reactivation, especially when immunosuppression conditions are present, despite the presence of anti-HBs. Eventually, presence of persistent and low level of replication competent HBV DNA in hepatocytes may induce mild liver necroinflammation and cause liver damage. In addition, immunosuppression conditions may raise HBV reactivation risk. Other than that, OBI accompanying chronic liver damage as a cofactor may contribute to the progression of liver damage [2, 48, 50].

Conclusion

In conclusion, the results of this study suggest that a close virological monitoring of HBV may be useful for more effective clinical management of possible OBI-related complications, especially in cases where the patient has a seroprofile where HBsAg is negative and anti-HBcIgG and/or anti-HBVantibodies are positive. With such monitoring, clinical management of possible OBI-related complications could be more effective, especially in patients with chronic hepatocyte damage, patients with cryptogenic liver disease like NASH, and immunosuppressed patients.

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Table 1: Demographic, clinical laboratory, serological, virological and clinical characteristics of the patients

Data	All Patients n=83	OBI Negative n=64 (77,1%)	OBI Positive n=19 (22,9%)	P
Mean age ± SD (years)	47,48±13,81	44,42±11,63	48,39±14,35	0,274
Gender (M/F)	38/45	28/36	10/9	0,495
Clinical laboratory				
ALT (U/L), Mean±SD	114,23±141,29	102,34 ±110,90	154,26±213,78	0,161
AST (U/L), Mean±SD	96,69±148,85	88,97±128,93	122,68±204,51	0,389
ALP (U/L), Mean±SD	171,40±207,59	186,10± 231,98	121,89±67,89	0,239
GGT (U/L), Mean±SD	174,51±246,14	188,47±272,54	127,47±113,96	0,346
Total bilirubin (mg/dl), Mean±SD	1,00±1,46	1,07±1,58	0,92±0,77	0,681
Direct bilirubin(mg/dl), Mean±SD	0,59±1,28	0,67 ±1,40	0,49±0,58	0,586
Indirect bilirubin (mg/dl), Mean±SD	1,00±0,12	1,01±0,08	0,97±0,21	0,310
INR, Mean±SD	1,00±0,12	1,00±0,078	0,97±0,20	0,31
AFP (ng/ml), Mean±SD	3,64±3,38	3,79±3,78	3,16±1,34	0,473
Albumin (g/dl), Mean±SD	4,31±0,59	4,31±0,62	4,32±0,52	0,971
Platelet count (10^6/ml), Mean±SD	223,53±77,03	230,27±80,97	200,84±58,16	0,145
HBV serology				
Anti-HBc positive n (%)	25 (30,1%)	14 (21,9%)	11 (57,9%)	***0,003***
Anti-HBc negative n (%)	58 (69,9%)	50 (78,1%)	8 (42,1%)	
Anti-HBs positive n (%)	43 (51,8%)	31 (48,4%)	12 (63,2%)	0,259
Anti-HBs negative n (%)	40 (48,2%)	33 (51,6%)	7 (36,8%)	
Seropositive OBI				
Anti-HBc and/or Anti-HBs positive n (%)	47 (56,6%)	32 (50,0%)	15 (78,9%)	***0,025***
Seronegative OBI				
Anti-HBc and Anti-HBs negative n (%)	36 (43,4%)	32 (50,0%)	4 (21,1%)	
Serum HBV DNA	Not detected	Not detected	Not detected	
Liver biopsy indications/Clinical				
Metabolic liver disease*	32 (38,5)			
Cholestatic Liver disease**	16 (19,3)			
Elevated liver enzymes unknown of cause	18 (21,7)			
Elevated autoimmune antibodies	5 (6,0)			
Cirrhosis by imaging	4 (4,8)			
Vascular liver disorders***	4 (4,8)			
Toxic hepatitis	3 (3,6)			
Granulomatous liver diseases	1(1,2)			

Abbreviations: ALT; alanine aminotrasferase, AST; aspartate aminotrasferase, ALP; alkaline phosphatase, GGT; gamma-glutamyl transferase, INR; international normalized ratio, AFP; serum alpha-fetoprotein.

*Fatty liver disease, Hepatic siderosis (haemochromatosis), Wilson's disease

**Cholestatic Liver disease; Biliary disease (primary biliary cholangitis; PBC, primary sclerosing cholangitis; PSC)

***Portal hypertension/ venopathy

Table 2: Liver Histopatology

	All patients n=83	OBI negative n=64	OBI positive n=19
Liver biopsy			
Liver steatosis/ Hepatic steatosis	33 (39,8)	24 (72,7)	9 (27,3)
NASH*	22 (26,5)	15 (68,2)	7 (31,8)
NAFLD**	11 (13,2)	9 (71,8)	2 (18,2)
Non-specific changes**	23 (27,7)	16 (69,6)	7 (30,4)
Others			
Cholestatic Liver disease*****	14 (16,9)	14 (100)	0 (0)
Cirrhosis	4 (4,8)	3 (75)	1(25,0)
Portal hypertension / Venopathy	4 (4,8)	3 (75)	1 (25)
Toxic hepatitis	3 (3,6)	2 (66,7)	1 (33,3)
Granulomatous liver diseases	1(1,2)	1 (100)	0 (0)
Autoimmune hepatitis	1(1,2)	1 (100)	0 (0)

*__NASH;__ Non-alcoholic steatohepatitis
**__NAFLD;__ Non-alcoholic fatty liver disease
***__Non-specific changes;__ minimal portal or lobular inflammatory infiltrates, absence of fibrosis, no structural changes in histopathological diagnosis
*****__Cholestatic Liver disease;__ Biliary disease (PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis)

Table 3: Liver steatosis and OBI frequency

Histopathology (n)	OBI negative n (%)	OBI positive n (%)	P
No Liver steatosis (50)	40 (80,0)	10 (20,0)	
Liver steatosis (33)	24 (72,7)	9 (27,3)	0,517
NASH (22)	15 (68,2)	7 (31,8)	
NAFLD (11)	9 (71,8)	2 (18,2)	0,448
No NASH (61)	49 (80,3)	12 (19,7)	0,311

Table 4: Biopsy indication/ clinical history and OBI in patients with non-specific changes

	İmmunosuppressive patients (n=10)						
n (%)	**Cryptogenic liver disease**	**Autoimmune / Autoinflammatory diseases**	**Hematological malignancy**	**Elevated autoimmune antibodies**	**Cholestatic Liver disease**	**Liver Steatosis**	**Malignant disesase****
	6 (26,1)	**6 (26,1)**	**4 (17,4)**	**3 (13,0)**	**2 (8,7)**	**1 (4,3)**	**1 (4,3)**
OBI negative (n=16)	3 (50)	4 (66,7)	3 (75)	2 (66,7)	2 (100)	1 (100)	1 (100)
OBI positive (n=7)	3 (50)	2 (33,3)	1* (25)	1 (33,3)	0	0	0
Anti-HBc positive (n=9)	4 (66,7)	3 (50)	1* (25)	1 (33,3)	0	0	0
Anti-HBs positive (n=10)	4 (66,7)	3 (50)	1* (25)	1 (33,3)	0	0	1 (100)
Anti-HBcIgG and OBI positive (n=5)	3 (100)	1 (50)	1* (100)	0	0	0	0

* Chronic myeloproliferative neoplasm ** Colorectal carcinoma