Which is more important and insidious in dialysis patients? Occult Hepatitis B or Occult Hepatitis C?

Diyaliz hastalarında hangisi daha önemli ve sinsidir? Okült Hepatit B veya Okült hepatit C mi?

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ABSTRACT

Objectives: The aim of this study was to investigate the presence of Occult hepatitis B infection (OBI) and Occult hepatitis C infection (OCI) in hemodialysis patients and to determine whether there is activation in follow-up or not.

Materials and Methods: Demographic data of 100 HD patients with normal alanine aminotransferase (ALT) levels included in the study, causes of renal failure, access to hemodialysis, duration of hemodialysis, ALT levels, hepatitis indicators were recorded. Serum antiHBc IgG was tested by ELISA (Architect, Abbott). Serum HBV DNA, HCV RNA (in peripheral blood mononuclear cells- PBMNH) were investigated by 'real-time' polymerase chain reaction method.

Results: AntiHBc IgG positivity was detected in 27% of patients, but there's no antiHBc IgG positivity alone. In 4% of the patients, HBV DNA positivity and OBI infection were detected. None of the patients showed HCV RNA positivity in serum and in PBMNH, therefore OCI was not detected. None of the patients developed OBI or OCI activation in a five-years follow-up. Renal transplantation was performed in one of the OBI patients and lifelong prophylaxis was planned with oral antiviral drugs.

Conclusion: Presence of OCI is lower than OBI in hemodialysis patients.

Key words: Occult Hepatitis B, occult hepatitis C, hemodialysis

ÖZ

Amaç: Bu çalışmanın amacı hemodiyaliz hastalarında Okült hepatit B enfeksiyonu (OBE) ve Okült hepatit C enfeksiyonu (OCE) varlığını araştırmak ve izlemde aktivasyon olup olmadığını tespit etmektir.

Gereç ve Yöntemler: Çalışmaya dahil edilen normal alanin aminotransferaz (ALT) düzeylerine sahip 100 HD hastasının demografik verileri, böbrek yetmezliği nedenleri, hemodiyalize erişim, hemodiyaliz süresi, ALT düzeyi, hepatit göstergeleri kaydedildi. Serum antiHBc IgG, ELISA (Architect, Abbott) ile test edildi. Serum HBV DNA, HCV RNA (periferik kan mononükleer hücrelerinde-PKMNH) "gerçek zamanlı" polimeraz zincir reaksiyonu yöntemi ile araştırıldı.

Bulgular: AntiHBc IgG pozitifliği hastaların% 27'sinde saptandı. ancak izole antiHBc IgG pozitifliği yoktu. Hastaların% 4'ünde HBV DNA pozitifliği ve OBE tespit edildi. Hiçbir hastada serum ve PBMNH'de HCV RNA pozitifliği yoktu, bu nedenle OCE saptanmadı. Beş yıllık takipte hiçbir hastada OBE veya OCE aktivasyonu gelişmedi. OBE hastalarından birinde böbrek nakli yapıldı ve oral antiviral ile yaşam boyu profilaksi planlandı.

Sonuç: Hemodiyaliz hastalarında OCE varlığı OBE'den düşüktür.

Anahtar Kelimeler: Okült hepatit B, Okült hepatit C, hemodiyaliz

Zapanalıoğlu Gazel Ö, Şener A. Which is more important and insidious in dialysis patients? Occult Hepatitis B or Occult Hepatitis C? Viral Hepat J. 2020

Introduction

In patients with chronic renal failure (CRF), infections are important causes of morbidity and mortality. These patients are particularly at risk of parenterally transmitted viral hepatitis (1). Hepatitis B and C viruses are primarily transmitted parenterally in dialysis patients. Chronic hepatitis B and C are more common infectious agents in patients with chronic renal failure compared to the normal population. These infections are also causes morbidity and mortality in patients with chronic renal failure and in patients undergoing renal transplantation (2,3). According to the Turkish Nephrology Association; hepatitis B surface antigen (HBsAg) positivity was 2,65% and antiHCV positivity was 3,94% in hemodialysis patients in Turkey at 2017 (3).

Hepatitis B virus (HBV) infection recovery defined as HbsAg disappearance with HBV DNA negativity in case of hepatitis B surface antibodies (Anti Hbs) positivity. The evaluation of serological markers for determining the infection is important but may be insufficient. Sensitive polymerase chain reaction (PCR) techniques have shown a low level of HBV DNA in some patients who have spontaneously and serologically lost their HBsAg in serum and / or liver. Therefore, this condition, which defines chronic HBV infection (by PCR) with undetectable HBsAg levels, is called occult HBV infection (OBI) (4). Occult HBV infection is divided into two groups according to antiHBc and antiHBs positivity.

The actual cause of approximately 10% of liver enzyme abnormalities is unknown. In the last decade, occult hepatitis C virus infection (OCI) has been defined with studies have been conducted to identify patients with chronic liver disease whose etiology has not been clarified. Firstly HCV RNA was detected in liver cells when anti HCV and HCV RNA were negative in serum. Thereafter HCV RNA was found in liver and in PBMNC with undiagnosed high liver function tests. Viral RNA can be detected in PBMNC over 70% of patients with OCI (5,6).

OCI defined by Castillo et al. HCV RNA is detected in liver cells, while serum anti HCV and HCV RNA was negative (6). In the following years, Fabrizi et al. defined OCI, in patients with elevated liver enzyme; serum anti HCV and HCV RNA were negative, whereas HCV RNA was detected in liver cells and PBMNC (8). Recent studies report two different types of OCI; seronegative and positive. In both types of OCI, HCV RNA is positive in liver cells of patients, and viral RNA can be detected in PBMNC with serum ultracentrifugation (9,10).

The aim of this study was to investigate the presence of OBI and OCI in hemodialysis patients in Canakkale. And follow up the reactivation of OBI and OCI.

Materials and Methods

We included 100 patients over 18 years of age and written informed consent was obtained from the patients. Patients were selected who had normal ALT levels and shows seronegativity for HbsAg and antiHCV antibody tests. The demographic data, ALT levels, hemodialysis periods, hepatitis B vaccination history, HBsAg, antiHbs and antiHCV indicators were recorded.

Peripheral venous blood samples were collected from 5 ml each of 3 separated biochemistry tubes for antiHBc IgG, HBV DNA, HCV RNA, and an amount of 9 ml blood in EDTA tube for PBMNC separation.

AntiHBc IgG test was carried out with the Architect Anti - HBc II Reagent kit. Blood samples for HBV DNA and HCV RNA isolation were centrifuged at 1500 rpm for 15 minutes. The obtained sera were stored at -20 ° C until isolation of DNA and RNA.

Whole blood (9mL) was taken into the EDTA tube for further differentiation of PBMNC. Histopaque (R)-1077 (9ML) was added to 50 ml falcon tube. Gently drop whole blood with sterile pasteur pipette from the edge of the falcon tube onto the Histopaque ®-1077. According to the manufacturer's recommendations, it was centrifuged at 400 G cycle for 30 minutes. After centrifugation, the cells in the cloud appearing in the middle of the tube were identified as PBMNC, and these cells were taken to the microvial lid cryo tubes by taking 3 ml with the help of micropipette. RNA was stored at -20 ° C until isolation.

Prepared serum and PBMNCs after DNA / RNA isolation using HBV - DNA and HCV - RNA isolation kit (Magesia ®2448 nucleic acid isolation and PCR setup robot) in Anatolia Diagnostic and Biotechnology R & D laboratory Montaina ® 4896 Real Time - PCR Bosphore® HBV / HCV quantification (analytical sensitivity is 25 IU / ml and its linear range is 1×10^1 - 1×10^8 IU / ml.) was performed using Kit V1. The Bosphore® HBV Quantification Kit v1 (analytical sensitivity of 10 IU / ml and a linear range of 1×10^1 - 1×10^8 IU / ml.)

OBI was defined as HBV DNA positivity in patients with HBsAg negative and with normal ALT levels.

OCI was defined as HCV RNA positivity in patients with antiHCV negative in patients with normal ALT levels.

Results

The study included 100 patients with normal ALT levels and HBsAg negative, antiHCV negative in one dialysis center in Canakkale province. Demographic data of the patients included in the study are given in Table 1. Fiftyeight (58%) of the patients

Table1. General characteristics of patients

Age (year), $\pm$ SD	63.5 $\pm$ 12,5
Gender (Female/Male)	42 / 58
Comorbid diases (other than CRF) N (%)	88 (% 88)
Hemodialysis way	
Catheter N (%)	15 (% 15)
A/V fistula N (%)	85 (% 85)
Total hemodialysis time (month), $\pm$ SD	67.6 $\pm$ 51,3
ALT , $\pm$ SD	10.5 $\pm$ 7,2
ALT: Alanine aminotransferase, normal range: 0 – 55 IU/ml, A/V: Arteriovenous, SD: Standart deviation	

were male and 42 (42%) were female. The mean age was 63.5 ± 12.5 years. Eightyfive (85%) patients underwent dialysis through arteriovenous fistula (AVF). Other patients underwent dialysis with a permanent hemodialysis catheter. The mean duration of dialysis was 67.6 months. 95% of the patients had dialysis 3 days a week and 5% had 2 days dialysis. When the serological tests of the patients are examined; 27 patients (27%) were showed antiHBc IgG and antiHBs positivity together. None of the patients had antiHBc IgG positivity alone. HBV DNA was positive in 4 (4/100, 4%) of all patients. OBI diagnosed patients' characteristics were shown at table 2. When the hepatitis B vaccination history were examined, it was seen that 55% had at least three doses.

There was not any clinical and laboratory activation of hepatitis B in five-years follow-up of these four patients. Serum and PBCMN were investigated by polymerase chain reaction for OCI and HCV RNA was not detected in any of the patients. The general characteristics of 27 patients with antiHBc IgG positive are in Table 2. The mean age of the patients was 57 ± 16.2 years. The mean duration of hemodialysis was 72.7 ± 37.5 months. ALT levels were within normal limits and the mean was 9.7 ± 2.3 IU/mL.

Discussion

Although HBsAg positivity was decreased in hemodialysis patients, HBV viremia (OBI) was shown by PCR tests. The prevalence of OBI varies from 1% to 87% in different regions of the world (11).

The incidence of OBI is different in every country, for example it has been reported between 0%-36.4% in blood donors in our country (12). According to other studies, OBI was reported actually between 3.4% and 19% in hemodialysis patients (13).

In this study, we investigated the presence of OBI and OCI with real time PCR in hemodialysis patients. In our study, the incidence of OBI was found 4%. But OCI was not obtained in our study group. In Egypt, Helaly et al. found antiHBc IgG positivity in all patients who had detected OBI. Therefore, in the presence of antiHBc IgG positivity, patients should be investigated for possible OBI by molecular methods (14). But in our study none of the OBI patients did not showed this antibody positivity for core antigen. In our opinion, the main cause of this situation is; core antigen production was irregular in hepatocytes. If the replication continues regularly you can detect antibody response, but if not antibody shows negativity. In hemodialysis patients this irregular synthesis might be in maximum level because of the CRF.

In the study conducted by Fontenele et al. in Brazil, 79% of 301 patients with CRF who had hemodialysis showed antiHBs positivity and isolated antiHBs positivity was detected in only 35% of the patients. They found OBI in three patients with antiHBs positivity alone (15). AntiHBs positivity was found in 95% of our patients and 68% of patients have been showed antiHBs positivity alone. All of OBI patients showed anti HBs positivity alone.

Although the exact cause is unknown, the presence of antiHBs in hemodialysis patients with OBI suggests that inadequate neutralization of virus and routine serological profiles alone are not always sufficient to define the status of HBV infection.

There are also important studies on the clinical consequences of OBI in organ transplant patients. Each hemodialysis patient is a candidate for kidney transplantation. Before solid organ transplantation, recipients should be screened for serological tests (HBsAg, Anti-HBs, Anti-HBc IgM, IgG or total, antiHCV) for possible hepatitis. In hemodialysis patients receiving immunosuppressive therapy before and after transplantation, viral activation of HCV and HBV might have seen and also cause fulminant hepatitis. Screening with appropriate methods before solid organ transplantation will increase transplant success (16). Therefore, it is suggested that these patients should be evaluated in terms of HBV DNA search by molecular methods.

One of the OBI patient has been undergone renal transplantation. Oral antiviral prophylaxis has been began and hepatitis B viral activation hasn't seen as other OBI patients in five-year follow-up.

Conclusion

In conclusion, in our study, we found the incidence of OBI 4% in seronegative hemodialysis patients, but OCI was not obtained. When the infectious properties of these patients are also taken into consideration, it is inevitable that HBV negative patients will be infected by dialysis. It is an important risk factor that may adversely affect morbidity and mortality in these patients whose quality of life has decreased significantly due to chronic renal failure. Since our key diagnostic method for detection of OBI occult HBV infection is HBV-DNA, it is essential to standardize the technique and used method. During the follow-up in dialysis units, once a year, viral DNA analysis with PCR-based method can be helpful in preventing the problems that may occur in the expected for organ transplantation.

Table 2. Occult Hepatitis B diagnosed patients' characteristics

Patient Number	Age	Gender	HD way	Total HD time (month)	HBV-DNA level (IU/ml)	Anti Hbs titer (mIU/mL)
(42)	33	M	AVF	59	61.4	1000
(58)	61	M	AVF	112	56.9	270
(98)	67	F	AVF	93	60	220
(100)	67	M	AVF	27	48.6	21

HD: Hemodialysis, AVF: Arteriovenous fistula

Study Limitations

Our study population was not enough to make a general recommendation for management of OBI in all dialysis patients.

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