Cytokine Profile During Occult Hepatitis B Virus Infection In Chronic Hepatitis C Patients

Camilla Rodrigues de Almeida Ribeiro
Fundacao Oswaldo Cruz
Nathalia Alves Araújo de Almeida
Fundacao Oswaldo Cruz
Katrini Guidolini Martinelli
Universidade Federal do Espirito Santo
Marcia Amendola Pires
Gaffrée and Guinle Universitary Hospital
Carlos Eduardo Brandao Mello
Gaffrée and Guinle Universitary Hospital
Vanessa Salete De Paula
Fundacao Oswaldo Cruz
José J. Barros (barros@ioc.fiocruz.br)
Fundacao Oswaldo Cruz

Research

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Abstract

Background: The hepatitis B virus (HBV) is one of the leading causes of acute, chronic and occult hepatitis (OBI) representing a serious public health threat. Cytokines are known to be important chemical mediators that regulate the differentiation, proliferation and function of immune cells. Accumulating evidence indicate that the inadequate immune responses are responsible for HBV persistency. The aim of this study were to investigate the cytokines IFN-γ, TNF, IL-2, IL-4, IL-6, IL-10 and IL-17A in patients with OBI and verify if there is an association between the levels of these cytokines with the determination of clinical courses during HBV occult infection.

Methods: 114 patients with chronic hepatitis C were investigated through serological and molecular tests, the OBI coinfected patients were subjected to the test for cytokines using the commercial human CBA kit and the results were compared with healthy controls with no history of liver diseases.

Results: Among 114 HCV patients investigated, 11 individuals had occult hepatitis B. The levels of cytokines were heterogeneous between the groups, most of the cytokines showed higher levels of production detection among OBI individuals when compared to the control group. We found a high level of IL-17A in the control group and high levels of TNF, IL-10, IL-6, IL-4 and IL-2 in OBI patients.

Conclusion: These cytokines could be involved in the persistence of HBV DNA in hepatocytes triggers a constant immune response, inducing continuous liver inflammation, which can accelerate liver damage and favor the development of liver cirrhosis in other chronic liver diseases.

Background

Hepatitis B virus (HBV) affects 2 billion people worldwide. Of these, approximately 350 million have a chronic infection with a risk of developing a serious condition, with cirrhosis and hepatocellular carcinoma (HCC), which cause 500 to 700 thousand deaths per year in the world [1, 2]. The disease caused by the HBV can result in asymptomatic infection, acute self-limiting hepatitis, chronic hepatitis, fulminant hepatitis, occult HBV infection (OBI) and in more severe cases requires liver transplantation [3].

The persistence of replication-competent HBV DNA (i.e. episomal HBV covalently closed circular DNA [cccDNA]) in the liver tissue and/or blood of patients with negative results for the hepatitis B surface antigen (HBsAg) antigen by currently available assays is called occult HBV infection [4–6]. It can occur after the resolution of a self-limited acute infection or after a long time of infection, if there is any clinical evidence or biochemical change in liver function, using these virus carriers, with a potential risk of transmitting the infection [5, 7, 8]. In this context, the antibody against the viral core (anti-HBc) should be considered to investigating patients for OBI [9, 10].

There are three types of OBI: seropositive, seronegative and a case called “false”. Seropositive OBI is characterized by the detection of anti-HBc antibody with or without anti-HBs. The OBI seronegative is characterized by undetectable antibodies both anti-HBc and anti-HBs. OBI seropositive is responsible for

the vast majority of OBI cases, which can be attributed to the higher proportion of resolved HBV infections. However, more than 20% of individuals with OBI do not have serological markers, either due to the drop-in antibody titers over time that become undetectable, or because there has never been seroconversion, this latter case is known as OBI seronegative [11]. Thus, OBI can be found both in seropositive individuals (with the presence of anti-HBc accompanied or not by anti-HBs), and in seronegatives, making HBV-DNA the only marker of HBV infection, detectable at low levels (< 200 IU/mL) [5]. "False" OBI occurs due to the presence of mutations in the S gene (escape mutants) that produce modified HBsAg that are not recognized by commercially available detection assays [11–13].

Although the clinical implications are almost minimal in patients with OBI, the biggest concern is the fact that transmission to healthy people even with very low viral load values. There is also the possibility or ability to reactivate viral replication in the presence immunosuppression, which can lead to severe acute conditions and liver decompensation with high mortality [6, 14].

The mechanisms involved in the pathogenesis of OBI have been the subject of several studies, but they still need to be clarified. The pathogenesis of OBI can be multifactorial depending largely on the virus-host interaction, mediated by the immune response [15]. Several evidences demonstrate that the virus-host interactions are related to the induction and maintenance of the occult phase of infection by the hepatitis B virus. The host's immune response is linked to viral persistence and the immunopathogenesis of the infection [16]. Concomitant hepatitis C virus (HCV) infection and other risk factors, such as alcohol consumption, are also associated with occult HBV infection that can progress to chronic liver disease [17]. HBV reactivation has been reported in patients with chronic HCV infection under treatment with more recent direct-acting antivirals (DAA), resulting in fulminant hepatitis, liver failure and, in some cases, death [18].

The prevalence of OBI among patients with chronic hepatitis C (CHC) varies widely from 0–52% [19]. HBV can maintain its oncogenic potential in all clinical situations of the course of its infection, including OBI and it is estimated that the existence of other causes that lead to liver damage, such as HCV co-infection, accelerate this process [20]. Some studies indicate that OBI unfavorably affects the progression of liver fibrosis and the development of HCC in patients with CHC [21–23].

The aim of this study was to investigate the presence of pro and anti-inflammatory cytokines IFN-γ, TNF, IL-2, IL-4, IL-6, IL-10 and IL-17A in patients with occult hepatitis B and to verify if there is an association between the levels of these cytokines with the determination of clinical courses during HBV occult infection.

Materials And Methods

Population Studied

This is a retrospective cross-sectional study conducted in patients prior to commencement of DAA treatment who had attended the Outpatient Clinic of Liver Disease at the Gaffrée and Guinle University

Hospital (Rio de Janeiro, Brazil) from January to December 2018. All patients enrolled into the study signed an informed consent form after being provided all necessary information to make an informed decision. Socio-epidemiological data, information about infection, HBV treatment and risk behaviors were obtained from each patient record or from the questionnaire.

Serum samples were collected from a cohort of 114 consecutive HCV patients. All serum samples were HCV RNA-positive and examined for total anti-HBc and HBsAg via immunoenzymatic assays (EIA). Samples positive for anti-HBc with no HBsAg indicating seropositive OBI were further examined using real-time PCR (qPCR) and nested PCR [24]. The HBV DNA was additionally sequenced for genotyping. Occult infections were considered as those with viral DNA in the serum tissue of HBsAg-negative individuals. Of the 114 HCV patients, 11 individuals had occult hepatitis B and were included in this study. Healthy controls (10 subjects) were selected from donors of similar age and with no history of liver diseases.

Biochemical tests

Serum samples were subjected to biochemical assays of liver enzymes, such as aspartate aminotransferase (AST); alanine aminotransferase (ALT); alkaline phosphatase; total, direct and indirect bilirubin; and gamma-glutamyl transferase (GGT) via immunoenzymatic assays (EIA).

Cytokine quantification

Peripheral blood obtained from patients and healthy controls were centrifuged on 800g for 10 min at room temperature. Sera were collected and stored at −70 °C until test performance. Sera levels of IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-10 and IL-17A were evaluated using a commercial BD CBA Human Th1/Th2/Th17cytokines kit *(Becton Dickinson, USA)*. The test was performed according to the manufacturer's instructions.

Data analysis

The SPSS 2.0 program was used to perform the statistical analyzes. Descriptive statistics of the qualitative variables was determined by frequency distribution and quantitative variables by median and P25-75. Afterwards, the normality of data distribution was assessed by the Kolmogorov-Smirnov test. The Mann-Whitney nonparametric test was used to compare cytokine levels between the groups of occult HBV patients and controls. Values of $p \leq 0.05$ and 95% confidence intervals (CIs) were considered as significant for all statistical analysis. Graphs were built using Graphpad 5.0 (Graphpad software, San Diego, CA, USA).

Ethical approval

The study protocol was approved by the Research Ethics Committee of the Institute Oswaldo Cruz (CAAE 34246914.4.1001.5248 number 2.927.747/18).

Results

Sociodemographic and bioclinical characteristics of the study population

Occult HBV infection was detected in eleven patients (9,64%). The majority of the OBI patients was woman (54.5%) with a mean age of 65.17± 9.57. The patients had received treatment with pegylated interferon (80 mcg) and ribavirin (1.0 g per day) for 24 weeks without HCV clearance. No patients developed ALT and AST flareups and all were anti-HB-positive, with viral loads ranging from 207.14 to 266114.8 IU/mL ($8.5x10^2$ to 1.49x10 copies/mL). Patients with the highest viral loads were positive for S-region amplification via nested-polymerase chain reaction (nested-PCR) and HBV in these cases was classified as genotype A (A1 and A2 subtypes).

Nine patients had chronic active hepatitis C, grade 4, with cirrhosis (F4, 14.1 - 46.4 kPa; fibroscan), and two patients had chronic active hepatitis C, grade 2, with cirrhosis (F2, 6.8 - 6.9 kPa; fibroscan). After the study, all patients were re-treated with sofosbuvir (400 mg) + daclatasvir (60 mg) + ribavirin (1.0 gram), following which HCV remained undetectable. Information on gender, age distribution, viral load and genotype distribution of the OBI patients are described in table 1 (**Table 1**).

Table 1. Demographic and clinical data in Patients Infected with HCV and Occult Hepatitis B. Rio de Janeiro, 2018.

Categorical variables	N	%
Gender		
Female	06	54.5
Male	05	45.5
Fibrosis stage		
F2	02	18.2
F4	09	81.8
Genotype HCV		
1a	05	45.5
1b	04	36.4
3a	02	18.2
Genotype HBV		
A1	01	9.1
A2	02	18.2
ND	08	72.7
Continuos variables	**mean**	**SD**
Age (years)	65.64	6.89
Viral Load (log)	3.36	1.01
ALT*	62.91	29.92
AST*	72.09	41.95
GGT*	76.64	64.02
BT*	0.67	0.27
Copies/mL - HBV	1.76E+07	4.56E+07
IU/mL**	32,851.90	80,988.23
Continuos variables	**median**	**P25-75**
Age (years)	66	62-69
Viral Load (log)	3.26	2.61-3.71
ALT*	68	39-76
AST*	71	33-109
GGT*	65	38-94
BT*	0.80	0.40-0.90
Copies/mL - HBV	1.03E+04	2.26E+03-8.50E+04
IU/ml**	1,839.5	403-5,178.1

SD = standard deviation; ND = not determined; P25 = 25th percentile; P75 = 75th percentile; *AST (alanine aminotransferase) - Reference values in chronic hepatitis: < 31 U/L (women) and 37 U/L (men); *ALT (alkaline phosphatase) - Reference values in chronic hepatitis: <31 U/L (women) and <41 U/L (men); *GGT (gamma-glutamyl transferase) – Reference values 8-61 U/L (men) and 5-36 U/L (women); *BT (total **1 copies/ml. corresponds to 5.26 IU/ml).

Expression levels of cytokines

In the present study, a significant difference were found in IL-17A (p= 0,016); TNF (p= 0.020), IL-6 (p < 0.0001), IL-4 (p = 0.024) and IL-2 (p = 0.043) and except for IL-17A, all evaluated cytokines obtained higher medians were compared to healthy controls (**Figure 1 and Table 2**).

Table 2. Sera cytokine levels in HBV occult patients and healthy controls.

Citokyne	Control		OBI		
	median	P25-P75	median	P25-P75	P-value Mann-whitney
IL-17A	10.36	7.92-12.67	6.38	1.34-8.26	**0.016**
INF-Y	0.43	0.0-0.65	0.43	0.0-5.32	0.512
TNF	0.0	0.0-0.99	2.77	1.02-24.80	**0.020**
IL-10	0.25	0.12-0.56	2.38	0.0-2.74	0.114
IL-6	0.67	0.43-1.76	9.76	7.51-23.33	**<0.001**
IL-4	2.22	1.54-2.97	6.14	1.87-20.98	**0.024**
IL-2	0.0	0.0-1.11	1.22	0.0-8.64	**0.043**

25th percentile (P25) and 75th percentile (P75) according to different levels of cytokines.

When we compare the mean concentration found in this study, we can observe that IL-10 (p=0.031), IL-6 (p=0.025) and IL-4 (p= 0.045) were higher in HBV occult group patients compared to healthy controls. For IL-17A (0.033), a higher mean was obtained in the control group (**Table 3**).

Table 3. Mean ± standard deviation (pg/mL) values of cytokines in each group.

	IL-17A	IFN-Y	TNF	IL-10	IL-6	IL-4	IL-2
group	5.58 ± 4.96	2.92 ± 4.46	16.87 ± 32.61	2.34 ± 2.33	18.99 ± 22.39	11.63 ± 13.43	4.76 ± 7.35
l group	9.76 ± 3.00	0.35 ± 0.32	0.78 ± 1.60	0.53 ± 0.78	1.12 ± 1.06	2.31 ± 1.22	0.37 ± 0.53
lue	**0.033**	0.086	0.133	**0.031**	**0.025**	**0.045**	0.076

Discussion

Antiviral immune responses in OBI are continuously stimulated by persistent/intermittent low concentrations of HBV antigens and cytokines can play an important role in controlling HBV replication [6]. In this present study, the levels of cytokines in OBI patients were heterogeneous, showing dissemination over a wide range of values with high standard deviations. In the present study, the prevalence of OBI in patients with CHC was 9.6%. Previous studies analyzing serum samples of Brazilian HCV infected patients found frequencies of OBI ranging from 0 to 24% [25-31].

OBI patients usually have a low viral load with suppression of HBV replication and thus, most OBI patients have normal liver histology or minimal fibrosis. However, they are still at risk of developing liver cirrhosis. The prevalence of OBI in cirrhotic patients varies widely from 4 to 38% between different regions of the world [22,32].

The persistence of HBV DNA in hepatocytes triggers a constant immune response, inducing mild but continuous liver inflammation, which can accelerate liver damage and favor the development of liver cirrhosis in other chronic liver diseases, such as in patients with chronic hepatitis C [22,33-35]. OBI can contribute to the development of HCC under direct and indirect mechanisms similar to those of chronic HBV infection [36].

In addition, the presence of OBI is believed to have an adverse effect on the response to treatment in IFN-based therapies [37]. A study that compared patients with chronic hepatitis C with HBV DNA positive and HBV DNA negative, the presence of OBI was associated with a decrease in the success of antiviral therapy [38].

In our study we found a significant increase in the detection of IL-2 in occult patients when compared to healthy controls, another study found overexpression of IL-2 exclusively in patients infected with OBI when compared to healthy individuals and patients who resolved HBV infection [43]. IL-2 plays an important role in the efficient development of effector cytotoxic CD8 + T cells, effector cells with a high expression of receptors for IL-2 (IL-2R) are cells that cause direct damage to the liver [44].

Baskic et al., 2017 [45] investigating the cytokine profile in chronic hepatitis C demonstrated that median levels of cytokines TNF-α, IL-2, and IL-17A were lower in patients with HCV than in controls. In our study, low levels of IL-17A were also found in OBI and HCV co-infected patients, however high levels of TNF-α and IL-2 were found when compared to controls. Some studies have already shown that IL-17A is positively regulated in chronic HBV-mediated inflammation and may be relevant for the development of liver cirrhosis and HCC [46-48]. IL-17A can also significantly stimulate monocytes and DCs to express their ligand (IL-17R) and produce pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6, etc., which are important for liver damage during progression of chronic hepatitis B [49].

Other cytokines possibly involved in OBI include increased interleukin 10 (IL-10), IL-10 can leads to reduced expression of IL-12, stromal cell-derived factor (SDF)-1α, and C-C chemokine receptor (CCR), which leads to the interruption of T and natural killer cells (NK cell) activation and the recruitment of immune cells to the infected liver [50]. Our study shows a significant difference in IL-10 levels in OBI patients compared to controls. Compared to healthy individuals, IL-10 production is also increased in patients with only chronic hepatitis C. The HCV RNA load is closely associated with IL-10 expressions, and inhibition of HCV replication was accompanied by a reduction in IL-10 [41]. However, some studies have already demonstrated reduced levels of IL-10 in HIV patients co-infected with occult HBV compared to HIV patients co-infected with chronic HBV [39].

In vivo levels of IL-6 have been associated with plasma ALT levels and the degree of liver fibrosis in patients with HCC in HCV monoinfected patients [51]. We found high levels of IL-6 in the occult group and it is well known that IL-6 can play two important roles in the pathogenesis of hepatitis B, can protect the liver from virus infections by stimulating immune responses against infected hepatocytes and can inhibit the HBV entry in hepatocytes up to 90% when cells are treated with IL-6 resulting in a marked reduction in cccDNA and HBsAg secretion [52]. But the IL-6 can also play an important role in the induction of hepatitis, cirrhosis, and HCC [53,54].

A previous study conducted with HIV co-infected individuals and in non-coinfected patients, demonstrated a low detection rate of IL-6 in patients infected with OBI when compared to healthy individuals and patients who resolved HBV infection [43].

We found levels of IL-4 significantly increased in occult patients. IL-4 is a cytokine that can suppress the Th1-type response, maintaining persistent HBV replication and promoting immune tolerance [42,55,56]. A study conducted by Zhang et al., 2014 [57] showed that patients with severe hepatitis C had higher levels of IL-4 compared to milder cases.

There were some limitations in the present study. The biggest limitation was the low number of patients with OBI, but the most of studies with OBI and cytokines analyses from 12 to 30 samples [39,43,58]; and the second limitation was that there are few studies on the cytokine production profile in patients with OBI. Therefore, our study can contribute to a better understanding of the complex response process related to cytokine production in HCV coinfection with OBI.

Conclusion

In conclusion, the results of the present study suggest that there is a significant difference in the detection of IL-17A, TNF, IL-10, IL-6, IL-4 and IL-2 in OBI patients when compared to healthy controls. However, further studies are needed to better understand the complex regulatory mechanisms of the host inflammatory response related to cytokine production during OBI infection and to understand the differences in mechanisms underlying infection resolution or the establishment of virus persistence. The expression of distorted cytokines exists in HCV co-infection with OBI and the exploration of this pattern of cytokine expression can help to develop a better understanding of the pathogenesis of chronic co-infection by OBI and HCV.

Abbreviations

HBV: Hepatitis B virus; OBI: Occult hepatitis B; IFN-γ: Interferon gamma; TNF: Tumor necrosis factor; IL: Interleukin; HCV: Hepatitis C vírus; DNA: Deoxyribonucleic acid; HCC: Hepatocellular carcinoma; cccDNA: Covalently closed circular DNA; HBsAg: Hepatitis B surface antigen; anti-HBc: Antibody against the viral core; DAA: Direct-acting antivirals; EIA: Immunoenzymatic assays; PCR: Polymerase chain reaction; qPCR: Real-time PCR; AST: Aminotransferase; alanine ALT: Aminotransferase; GGT: Gamma- glutamyl transferase; CIs: Confidence intervals; SD: Standard deviation; ND: Not determined; (SDF)-1α: Stromal cell-derived factor; CCR: C-C chemokine receptor; NK cell: Natural killer cells.

Declarations

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Disclosure statement

Authors declare that they have no financial, personal, or professional interests that could be construed to have influenced this manuscript

Authors' contributions

CRAR and VSdeP: Conceptualization, Methodology; CRAR, NAAA and KGM: Formal analysis, Data Curation; CRAR: Investigation, Validation; VSdeP, MAP and CEBM: Resources; CRAR: Writing - Original Draft; CRAR, Vanessa VSdeP, CEBM and JJB: Writing - Review & Editing; CRAR: Visualization; VSdeP and JJB: Supervision; VSdeP: Project administration, Funding acquisition.

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Availability of data and materials

The dataset is available from the corresponding author.

Ethics approval and consent to participate

The study protocol was approved by the Research Ethics Committee of the Institute Oswaldo Cruz (CAAE 34246914.4.1001.5248 number 2.927.747/18).

Consent for publication

Not applicable.

Competing interests

The authors declared that, this study is without conflicts of interests.

Author details

[1]Laboratory of Molecular Virology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil; [2]Federal University of Espírito Santo, Espírito Santo, Brazil; [3]Gaffrée and Guinle Universitary Hospital, Ambulatory of Liver Disease, Rio de Janeiro State Federal University/UniRio, Rio de Janeiro, Brazil

References

1. Franco E, Bagnato B, Marino MG, Meleleo C, Serino L, Zaratti L. Hepatitis B: Epidemiology and prevention in developing countries. World J Hepatol. 2012; 74-80.

2. WHO – World Health Organization 2017. GLOBAL HEPATITIS REPORT, 2017. Available online: https://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/. (accessed on 24 April 2020).
3. Shi M, Wei J, Dong J, Meng W, Ma J, Wang T, et al. Function of interleukin17 and 35 in the blood of patients with hepatitis B related liver cirrhosis. Molecular Medicine Reports 2015; 121-126.
4. Hu KQ. Occult hepatitis B virus infection and its clinical implications. J Viral Hepat. 2002; 243–257.
5. Raimondo G, Caccamo G, Filomia R, Pollicino T. Occult HBV infection. Semin Immunopathol. 2013; 35: 39–52.
6. Raimondo G, Locarnini S, Pollicino T, Levrero M, Zoulim F, Lok AS, et al. Update of the statements on biology and clinical impact of occult hepatitis B virus infection. J Hepatol. 2019; 71:397-408.
7. Zobeiri M. Occult Hepatitis B: Clinical Viewpoint and Management. Hepat Res Treat 2013; 259148.
8. Sowole L, Labbett W, Patel M, O'Riordan A, Cross J, Davenport A, et al. The prevalence of occult hepatitis B virus (HBV) infection in a large multi-ethnic haemodialysis cohort. BMC Nephrol. 2015; 16:12.
9. Vitale F, Tramuto F, Orlando A, Vizzini G, Meli V, Cerame G, et al. Can the serological status of anti-HBc alone be considered a sentinel marker for detection of occult HBV infection? J Med Virol. 2008; 80:577–82.
10. Urbani S, F Fagnoni, Missale G, Franchini M. The role of anti-core antibody response in the detection of occult hepatitis B virus infection. Clin Chem Lab Med. 2010; 48:23-9.
11. Torbenson M, Thomas DL. Occult hepatitis B, Lancet Infect Dis. 2002; 2: 479–86.
12. Gerlich WH, Bremer C, Saniewski M. Schüttler CG, Wend UC, Willems WR, et al. Occult hepatitis B virus infection: detection and significance. Dig Dis 2010; 28.
13. Cheng HR, Kao JH, Wu HL, Chen TC, Tseng TC, Liu CH, et al. Clinical and virological features of occult hepatitis B in patients with HBsAg seroclearance post-treatment or spontaneously. Liver Int. 2014; 34:e71–9.
14. Yip TC, Wong GL. Current Knowledge of Occult Hepatitis B Infection and Clinical Implications. Semin Liver Dis. 2019; 39:249-260.
15. Said ZN. An overview of occulthepatitis Bvirus infection. World J Gastroenterol. 2011; 17;1927-38.
16. Samal J, Kandpal M, Vivekanandan P. Molecular mechanisms underlying occult hepatitis B virus infection. Clin Microbiol Rev. 2012; 25:142–63.
17. Kim MH, Kang SY, Lee WI. OccultHBVamong Anti-HBc Alone: Mutation Analysis of an HBV Surface Gene and Pre-S Gene. Yonsei Med J. 2017; 58:557-563.
18. Mücke MM, Backus LI, Mücke VT, Coppola N, Preda CM, Yeh ML, et al. Hepatitis B Virus Reactivation During Direct-Acting Antiviral Therapy for Hepatitis C: A Systematic Review and Meta-Analysis. Lancet Gastroenterol Hepatol. 2018; 3:172-180.
19. Honarkar Z, Alavian SM, Samiee S, Saeedfar K, Zali MR. Occult hepatitis B among chronic liver disease patients. Saudi Med J 2005; 26: 601–6, PMID: **15900369.**

20. Takeda H, Takai A, Inuzuka T, Marusawa H. Genetic basis of hepatitis virus associated hepatocellular carcinoma: linkage between infection, inflammation, and tumorigenesis. J Gastroenterol. 2017; 52:26-38.
21. Emara MH, El-Gammal NE, Mohamed LA, Bahgat MM. Occult hepatitis B infection in egyptian chronic hepatitis C patients: prevalence, impact on pegylated interferon/ribavirin therapy. Virol J. 2010; 17:324.
22. Squadrito G, Cacciola I, Alibrandi A, Pollicino T, Raimondo G. Impact of occult hepatitis B virus infection on the outcome of chronic hepatitis C. J Hepatol 2013; 59:696–700.
23. Coppola N. Role of occult hepatitis B virus infection in chronic hepatitis C. World J Gastroenterol. 2015; 21:11931-40.
24. Portilho MM, Mendonca A, Bezerra CS, Do Espirito-Santo MP, De Paula VS, Nabuco LC, et al. Usefulness of in-house real time PCR for HBV DNA quantification in serum and oral fluid samples. J Virol Methods. 2018; 256:100–6.
25. Alencar RS, Gomes MM, Sitnik R, Pinho JR, Malta FM, Mello IM, et al. Low occurrence of occult hepatitis B virus infection and high frequency of hepatitis C virus genotype 3 in hepatocellular carcinoma in Brazil. Braz. J. Med. Biol. Res. 2008; 41:235-240.
26. Branco F, Mattos AA, Coral GP, Vanderborght B, Santos DE, Franca P, et al. Occult hepatitis B virus infection in patients with chronic liver disease due to hepatitis C virus and hepatocellular carcinoma in Brazil. Arq Gastroenterol 2007; 44:58-63.
27. Fontenele AM, Gainer JB, Da Silva ESDV, Cruz Santos MD, Salgado JV, Filho N, et al. Occult hepatitis B among patients with chronic renal failure on hemodialysis from a capital city in northeast Brazil. Hemodial Int 2015; 19:353-359.
28. Motta JS, Mello FC, Lago BV, Perez RM, Gomes SA, Figueiredo FF. Occult hepatitis B virus infection and lamivudine-resistant mutations in isolates from renal patients undergoing hemodialysis. J Gastroenterol Hepatol 2010; 25:101-106.
29. Pereira JS, Goncales NS, Silva C, Lazarini MS, Pavan MH, Fais VC, et al. HBV vaccination of HCV-infected patients with occult HBV infection and anti-HBc-positive blood donors. Braz J Med Biol Res 2006; 39:525-531.
30. Peres AA, Dias EA, Chesky M, Alvares-da-Silva MR, Jobim LF, Goncalves LF, Manfro RC. Occult hepatitis B in renal transplant patients. Transpl Infect Dis 2005; 7:51-56.
31. Rosa AS, Araujo OC, Savassi-Ribas F, Fernandes CA, Coelho HS, et al. Prevalence of Occult Hepatitis B Virus Infection and Torque Teno Virus Infection and Their Association With Hepatocellular Carcinoma in Chronic Hepatitis C Patients. Virus Res 2017 15:166-172.
32. Hou J, Wang Z, Cheng J, Lin Y,Lau GK,Sun J, et al. Prevalence of naturally occurring surface gene variants of hepatitis B virus in nonimmunized surface antigen-negative Chinese carriers. Hepatology 2001; 34:1027–1034.
33. Raimondo G, Pollicino T, Squadrito G. What is the clinical impact of occult hepatitis B virus infection? Lancet 2005;365: 638–640.

34. Kim MJ, Kwon OS, Chung NS, Lee SY,Jung HS,Park DK, et al. The significance of anti-HBc and occult hepatitis B virus infection in the occurrence of hepatocellular carcinoma in patients with HBsAg and anti- HCV negative alcoholic cirrhosis. Korean J Hepatol 2008; 14:67–76.
35. Nishikawa H, Osaki Y. Clinical significance of occult hepatitis B infection in progression of liver disease and carcinogenesis. J Cancer 2013; 4:473–480.
36. Shi Y, Wu YH, Wu W, Zhang WJ, Yang J, Chen Z. Association between occult hepatitis B infection and the risk of hepatocellular carcinoma: a meta-analysis. Liver Int 2012; 32:231–240.
37. Fernandez-Rodriguez CM, Gutierrez ML, Lledo JL, Casas ML. Influence of occult hepatitis B virus infection in chronic hepatitis C outcomes. World J Gastroenterol 2011; 17:1558–62.
38. Mrani S, Chemin I, Menouar K, Guillaud O, Pradat P, Borghi G, et al. Occult HBV infection may represent a major risk factor of non-response to antiviral therapy of chronic hepatitis C. J Med Virol. 2007; 79: 1075–81.
39. Martin CM, Welge JA, Shire NJ, Shata MT, Sherman KE, Blackard JT. Cytokine expression during chronic versus occult hepatitis B virus infection in HIV co-infected individuals. Cytokine 2009; 47:194-198.
40. Germanidis G, Hytiroglou P, Zakalka M, Settas L. Reactivation of occult hepatitis B virus infection following treatment of refractory rheumatoid arthritis with abatacept. J Hepatol 2012; 56:1420-1421, https://doi.org/ 10.1016/j.jhep.2011.10.011.
41. Han ZQ, Huang T, Deng YZ, Zhu GZ. Expression Profile and Kinetics of Cytokines and Chemokines in Patients With Chronic Hepatitis C. Int J Clin Exp Med. 2015; 15:17995-8003, PMID: **26770394.**
42. Li Y, Wang Y, Liu Y, Wang Y, Zuo X, Li Y, Lu X. The possible role of the novel cytokines IL-35 and IL-37 in inflammatory bowel disease. Mediators Inflamm 2014; 13:63-29.
43. Fierro NA,Roman S, Realpe M, Hernandez-Nazara Z, Zepeda-Carrillo EA, Panduro A. Multiple cytokine expression profiles reveal immune-based differences in occult hepatitisB genotype H-infected Mexican Nahua patients. Mem Inst Oswaldo Cruz. 2011; 106:1007-13.
44. Xiao P, Chen QF, Yang YL, Guo ZH, Chen H. Serum soluble interleukin-2 receptor levels in patients with chronic hepatitis B virus infection and its relation with anti-HBc. World J Gastroenterol 2006; 12:482–484.
45. Baskic D, Vukovic VR,Popovic S,Djurdjevic P, Zaric M, Nikolic I, et al. Cytokine Profile in Chronic Hepatitis C: An Observation. Cytokine 2017; 96:185-188.
46. Wu JF, Wu TC, Chen CH, Ni YH, Chen HL, Hsu HY, et al. Serum levels of interleukin-10 and interleukin-12 predict early spontaneous hepatitis B virus e antigen seroconversion. Gastroenterology 2010; 138(165–172):e161–e163.
47. Fenoglio D, Bernuzzi F, Battaglia F, Parodi A, Kalli F, Negrini S, et al. Th17 and regulatory T lymphocytes in primary biliary cirrhosis and systemic sclerosis as models of autoimmune fibrotic diseases. Autoimmun Rev 2012; 12:300–304.

48. Du WJ, Zhen JH, Zeng ZQ, Zheng ZM, Xu Y, Qin LY, et al. Expression of interleukin-17 associated with disease progression and liver fibrosis with hepatitis B virus infection: IL-17 in HBV infection. Diagn Pathol 2013; 28;8:40.
49. Zhang JY, Zhang Z, Lin F, Zou ZS, Xu RN, Jin L et al. Interleukin-17-producing CD4(+) T cells increase with severity of liver damage in patients with chronic hepatitis B. Hepatology 2010; 51:81–91.
50. Arababadi MK, Ahmadabadi BN, Kennedy D. Current information on the immunologic status of occult hepatitis B infection. Transfusion 2012; 52:1819–1826.
51. Cachem FCOF,Dias AS, Monteiro C, Castro JR, Fernandes G, Delphim L, et al. The Proportion of Different interleukin-17-producing T-cell Subsets Is Associated With Liver Fibrosis in Chronic Hepatitis C. Immunology 2017; 151:167-176.
52. Bouezzedine F, Fardel O, Gripon P. Interleukin 6 inhibits HBV entry through NTCP down regulation, Virology 2015; 481:34–42.
53. Klein C, Wu¨sterfeld T, Assmus U, Roskams T, Rose-John S, Mueller M, et al. The IL-6-gp130-STAT3 pathway in hepatocytes triggers liver protection in T cell-mediated liver injury, J Clin Invest 2005; 115:860-869.
54. Barathan M, Mariappan V, Shankar EM, Abdullah BJ, Goh KL, Vadivelu J, Hypericin-photodynamic therapy leads to interleukin-6 secretion by HepG2 cells and their apoptosis via recruitment of BH3 interacting-domain death agonist and caspases. Cell Death Dis 2013; 4:e697.
55. Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE. The IL-4 receptor: signaling mechanisms and biologic functions. Annu Rev Immunol 1999; 17:701–738.
56. Yao Y, Li J, Lu Z, Tong A, Wang W, Su X, et al. Proteomic analysis of the interleukin-4 (IL-4) response in hepatitis B virus-positive human hepatocelluar carcinoma cell line HepG2.2.15. Electrophoresis 2011; 32:2004–2012.
57. Zhang L,Hao CQ, Miao L, Dou XG. Role of Th1/Th2 Cytokines in Serum on the Pathogenesis of Chronic Hepatitis C and the Outcome of Interferon Therapy. Genet Mol Res. 2014 13:9747-55.
58. Bes M, Vargas V, Piron M, Casamitjana N, Esteban JI, Vilanova N, et al. T Cell Responses and Viral Variability in Blood Donation Candidates With Occult Hepatitis B Infection. J Hepatol. 2012; 56:765-74.

Figures

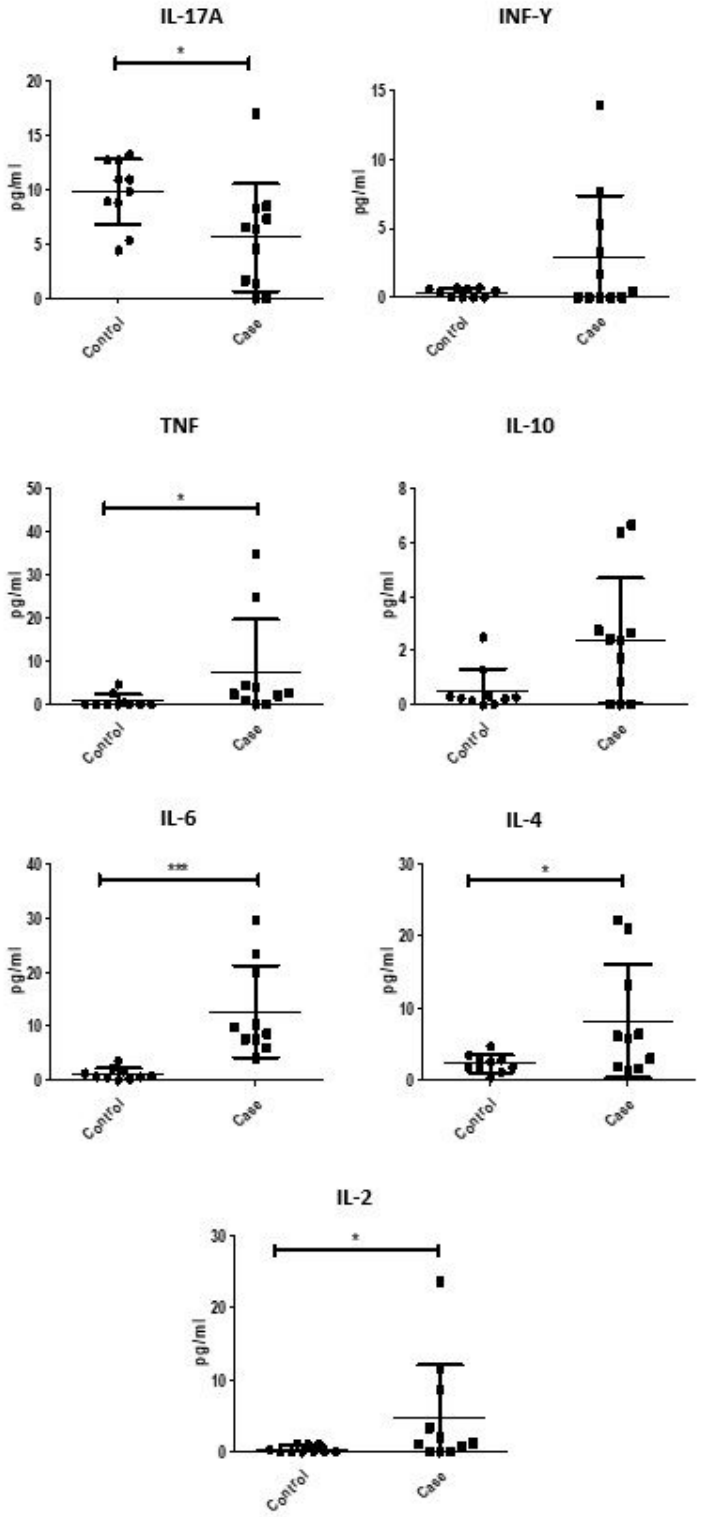

Figure 1

Expression levels of inflammatory cytokines in serum from various clinical states of hepatitis B virus infection and controls. Mann Whitney non-parametric test was used to analyze the differences between the groups

Supplementary Files

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