Eur. Cytokine Netw. Vol. 32, n° 2, *June 2021* 23-30

ORIGINAL ARTICLE

Serum Th17 and TNF-α distinguish between patients with occult hepatitis B infection, chronic hepatitis B infection and healthy individuals

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Accepted for publication June 24, 2021

To cite this article: Sosa-Jurado F, Sánchez-Reza L, Mendoza-Torres MÁ, Meléndez-Mena D, García VHG., Guzmán-Flores B, Enciso-Moreno JA, López-Ramos JE, Balandrán JC., Vallejo-Ruiz V, Cortes-Hernández P, Reyes-Leyva J, Santos-López G. Serum Th17 and TNF-α distinguish between patients with occult hepatitis B infection, chronic hepatitis B infection and healthy individuals. *Eur. Cytokine Netw.* 2021; 32(2): 23-30. doi: 10.1684/ecn.2021.0466

ABSTRACT. Chronic hepatitis B (CHB) is classified into five phases based on virus-host interactions: immune tolerance, immune clearance, inactive carrier state, reactive phase and occult hepatitis B infection (OBI). OBI is an uncommon asymptomatic phase of CHB that can be reactivated when the immune system is compromised, occasionally giving rise to severe liver disease. Host immune factors play essential roles in all phases of the CHB infection. Cytokines may alter infection course, influencing the propensity for and the progression of CHB and thus warrant study. Three clinical groups were studied: 48 healthy individuals (HI), 28 patients with persistent positive anti-HBc serological markers and negative HBsAg over time, who were diagnosed as OBI and 12 patients with active CHB. OBI patients were defined by three independent detections of the hepatitis B virus genome through nested PCR and real-time PCR. Quantitative measurement of 20 Th1, Th2 and Th17 human cytokines was performed in the sera of HI, OBI and CHB patients. Levels of IFN-γ, TNF-β, IL-28A, IL-4, IL-5, IL-13, IL-1β, IL-6, IL-21, IL-22, IL-23, GM-CSF and MIP-3α were similar between groups. IL-2, IL-12p70, IL-10, IL-17F and TGF-β1 were similar in HI and OBI, but higher in CHB. TNF-α and the IL-17A:IL-17F ratio were significantly different between the three groups. TNF-α was progressively higher in HI, OBI and CHB ($P = 0.004$), while the IL-17A:IL-17F ratio was 1.1 in HI, 3.4 in OBI and 0.4 in CHB. Detection and levels of these pro-inflammatory cytokines in OBI patients suggest that they are undergoing a silent hepatic inflammatory process.

Key words: hepatitis B virus, cytokines, hepatitis B virus-DNA, hepatitis B surface antigen, hepatitis B core antibody, nested PCR, real-time PCR

doi: 10.1684/ecn.2021.0466

INTRODUCTION

Hepatitis B virus (HBV) infection is widespread. An estimated 350 million people are currently infected in the world, with a fraction unaware of their condition. HBV can cause acute liver infection, chronic hepatitis B (CHB), cirrhosis and hepatocellular carcinoma (HCC) [1]. CHB is classified into five phases based on virus-host interactions: immune tolerance, immune

clearance, inactive carrier state, reactive phase and occult hepatitis B infection (OBI) [2].
OBI is defined as the presence of HBV DNA in the liver or serum, coupled to the lack of detection of HBV surface antigen (HBsAg) in serum [3]. An essential OBI characteristic is a small viral load, intermittent over time [4], that leads to minimal but continuous liver inflammation in response to the persistence of covalently closed circular viral DNA within hepatocytes [2]. OBI is also related to a strong suppression of HBV replication and protein expression by immune and epigenetic mechanisms [5]. Host immune factors play essential roles in the HBV infection: specifically, helper (Th1, Th2, Th17) and regulatory T cells (Treg) secrete several cytokines that influence the course and severity of the infection [6]. Several studies have reported the association between other CHB phases and cytokine levels [7-11], but just a few studies have determined serum cytokines in OBI [12-14]. OBI is apparently very rare, but prevalence determination is complicated by the fact that it goes frequently unidentified. The purpose of this study was to detect patients with persistent anti-HBc positive/HBsAg negative results over time, without diagnosed liver disease and to identify cytokines that indicate that these patients may be undergoing a silent hepatic inflammatory process.

METHODS

Ethical regulations

This study was approved by the institutional ethics committee (registry numbers R-2010-2101-31 and R-2017-2101-1). Patients were recruited from the gastroenterology service and blood bank of the National Medical Center "General de División, Manuel Avila Camacho" of the Mexican Institute of Social Security (IMSS) in Puebla City, Mexico between January and December 2017, following international ethical regulations, based on the last actualization of Helsinki's Declaration. Individuals were informed about the study and included after signing an informed consent letter.

Patients

Healthy individuals
Blood donors were selected in the hospital's blood bank based on the following criteria: male or female, >18 years (yr), clinically healthy at the time of the study, without a history of viral hepatitis and with confirmed negative results for HBV, hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections. HI samples were integrated into six pools of eight samples.

Patients with chronic hepatitis B
Patients with clinical signs of chronic viral hepatitis, identified in the gastroenterology service, were included with the following criteria: male or female, >18 yr, with confirmed HBV infection, not coinfected with HCV or HIV and without previous treatment for HBV, HCV or HIV.

Occult hepatitis B virus infection patients
Eighty-nine patients, referred from hospital departments other than gastroenterology, were identified in the serology section of the blood bank as anti-HBc positive/HBsAg negative (probable OBI) between November 2015 and February 2017. Patients from the rheumatology and hematology services were excluded due to the possibility of cytokine alteration from other pathologies unrelated to HBV. Only patients who met the following criteria were included as OBI in the study: male or female, >18 yr, without clinical liver disease and with at least two previous HBsAg negative results, but positive to antibodies against HBV core protein (anti-HBc) in the last 5 years, not coinfected with HCV or HIV, with no previous treatment for HBV, HCV, or HIV and HBV DNA detection in serum (at least three different viral genomic regions using nested PCR [nPCR] or real-time PCR [rtPCR]) (*figure S1*).

Detection of serological markers

Serum samples of all included patients and HI were analyzed for HBsAg, for antibodies against HBc (anti-HBc), HCV (anti-HCV) and for HIV antigen p24 (anti-HIV/Agp24), with a chemiluminescent microparticle immunoassay (CMIA) (Abbot Laboratories Diagnostics Division, Abbott Park, IL, USA); HBV "e" antigen (HBeAg) (EIA 3890) and antibodies against HBeAg (EIA 3891) by ELISA (DRG International Inc., Springfield, NJ, USA).

HBV DNA detection in serum

Automated detection of viral nucleic acids
Samples of blood donors were analyzed for the detection of HIV-1 RNA, HCV RNA and HBV DNA by the ProcleixUltrio Plus System (Gen-Probe Incorporated, San Diego, CA, USA) that screens for the genetic material of all the three viruses simultaneously.

Viral load in patients with CHB

The Abbott RealTime HBV viral load kit was used in the M2000 RealTime System (Abbott Laboratories, Abbott Park, IL, USA) to quantify HBV viral genotypes A, B, C, D, E, F and H in serum or plasma. This kit has a low limit of quantification of 10 IU/mL and an upper limit of 10^9 IU/ml using 0.5 mL sample (Abbot).

HBV DNA detection in OBI patients

One milliliter of patients' sera non-reactive to HBsAg but reactive to anti-HBc (probable OBI) was used to extract total DNA with the QIAamp UltraSens Virus Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The presence of HBV DNA was confirmed by amplification of three HBV genome regions (PreS2/S, PreCore/Core and Core) by nested PCR or rtPCR using the primer sequences shown in *table S1* [15-17].

Nested PCR

DNA polymerase (Promega, Madison, WI, USA) was used to perform both the first and the nPCR with the primers given in *table S1* and described in reference [17] following manufacturer's instructions. PCR products were visualized in agarose gel electrophoresis with SYBR safe DNA green.

Real-time PCR

Power SYBR Green PCR Master Mix (2X) (Applied Biosystems, Foster City CA, USA) and two pairs of primers (*table S1*) were used to detect HBV sequences by qualitative rtPCR using the StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

2.5 Serum quantitative measurement of human Th1, Th2 and Th17 cytokines

Twenty cytokine concentrations were determined in serum samples using Quantibody® Human TH17 Array 1 (RayBiotech. Inc., Norcross, GE, USA): granulocyte and macrophage colony stimulating factor (GM-CSF), gamma interferon (IFN-γ), IL-1β (IL-1 F2), IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-28A (lambda interferon 2), macrophage inflammatory protein 3 alpha (MIP-3α; CCL20), transforming growth factor beta 1 (TGF-β1), tumor necrosis factor-alpha (TNF-α) and tumor necrosis factor-beta (TNF-β) (TNFSF1B). The array was read according to manufacturer's instructions with a laser scanner (GenePix Personal 4100A Molecular Device, CA, USA) and the cytokine concentrations were calculated using Ray-Biotech Quantibody Analysis Tool. Each cytokine was expressed as S/DL (sample/detection limit), defined as the proportion of cytokine concentration (pg/mL) in the sample/detection limit. When S/DL value was $\geq$1.0, it was counted as a positive detection.

Statistical analysis

For nominal variables, percentages and chi-square and Fisher's exact tests were calculated and for continuous variables, mean, standard deviation and confidence intervals were calculated. ANOVA test and unpaired *t*-test were performed with the GraphPad Prism v5.4 program.

RESULTS

Patients

Forty-eight healthy blood donors (HI), 12 patients with CHB and 28 OBI met the inclusion criteria and participated in the study. The HI group included 48 men, with the mean age of 37 years, all of them non-reactive to HIV, HCV, HBV, *Treponema pallidum, Brucella* sp and *Trypanosoma cruzi* and without detectable levels of HBV DNA, HIV, or HCV RNA. In turn, patients with CHB in the reactive phase were 10 (83.4%) men and 2 (16.6%) women, with mean age 51 years, mean anti-HBc 11.2 S/CO, 100% reactive to HBsAg, 25% reactive to anti-HBeAg and 100% non-reactive to HBeAg. All had HBV viral loads more than 2000IU/mL (*table S2*).

The 28 OBI patients represent 31.4% of the initial HBsAg negative and anti-HBc positive individuals identified as probable OBI. Only these 28 patients had all the inclusion criteria, including confirmed HBV DNA detection and willingness to take part in the study (*figure S1*). OBI patients were 22 (87.4%) men and 6 (12.6%) women, with mean age 57 years, mean anti-HBc 6.9 S/CO, 25 (89.3%) without evidence of liver disease and 3 (10.7%) with grade 1 steatosis. All OBI patients had at least three independent detections of the HBV genome through rtPCR or nPCR directed at three genome regions. All rtPCRs at the $PreS_2$/S and core regions were positive. Through nPCR, 82.1%, 89.2% and 85.7% of OBI patients had detections at $PreS_2$/S, PC/C and $PreS_2$/S regions, respectively (*table 1*). Significant differences in mean age and anti-HBc S/CO between OBI and CHB patients were observed (*tables 1 and S2*).

Detection of cytokines in serum samples of the study groups

First, we analyzed the proportion of samples with values above the detection limit for each cytokine (% samples with S/DL $\geq$1 or % of positivity) (*table 2*). Eight cytokines (IL-4, IL-10, IL-12p70, IL-17F, IL-23, GM-CSF, TGF-β1 and IFN-γ) showed a similar % of positivity in HI, OBI and CHB groups (*table 2*). IL-2 and IL-28A had higher % of positivity in HI and CHB than in OBI ($P = 0.001$ and $P = 0.007$, respectively), while TNF-β, IL-21, IL-13 and IL-17A had higher % of positivity in HI and OBI than in CHB ($P < 0.01$) (*table 2*). IL-22 and MIP-3α had no samples above the detection limit in HI and they had higher % positivity in CHB than in OBI (*table 2*). The % of positive IL-17A samples was higher than that of IL-17F in HI (83.3% *vs.* 50%) and in OBI (96.4% *vs.* 60.7%) but not in CHB (50% *vs.* 58%) (*table 2*).

Quantitative analysis of S/DL values for cytokines in serum samples of the study groups

The S/DL values of most cytokines (IFN-γ, TNF-β, IL-1β, IL-4, IL-5, IL-6, IL-13, IL-17A, IL-21, IL-22, IL-23, IL-28A, GM-CSF and MIP-3α) were similar between HI, OBI and CHB (*table 2*). Six cytokines (IL-2, IL-10, IL-12p70, IL-17A, IL-17F and TGF-β1) had similar S/DL in HI and OBI but had higher values in CHB (*table 2*, *figures 1-3*).

TNF-α values were significantly different between the groups: HI = 0.75, OBI = 13.8 and CHB = 101, $P = 0.001$ (*table 2*, *figure 2*). IL-17A and IL-17F showed similar S/DL values in HI (3.8 *vs* 3.5; $P = 0.83$), but IL-17A was higher than IL-17F in OBI (5.5 *vs* 1.62; $P = 0.001$), while IL-17F was higher than IL-17A in CHB (26.7 *vs* 11.2; $P = 0.17$ (*figure 3*). Thus, the IL-17A:IL-17F S/DL ratio distinguished between the groups (HI = 1.1, OBI = 3.4 and CHB = 0.4).

Table 1
Demographic and laboratory data of patients with occult hepatitis B virus infection (OBI)

No. Case	Demographic data		ALT	Serology				Viral DNA Detection nPCR			Viral DNA Detection rtPCR		History of liver disease
	Sex	Age (yr)	<63 IU/L	Anti-HBc S/CO >1.0	HBsAg S/CO >1.0	HBeAg ≥COV	Anti-HBeAg <COV	$PreS_2$/S nt 1-384	PC/C nt 1798-2304	$PreS_2$/S nt 331-676	$PreS_2$/S nt 92-201	Core nt 2015-2114	
1	W	58	Yes	10.0	NR	N	N	D	D	D	D	D	No
2	M	60	Yes	8.9	NR	N	N	D	D	D	D	D	No
3	M	80	Yes	9.6	NR	N	N	D	D	D	D	D	No
4	M	55	Yes	8.0	NR	N	N	D	D	D	D	D	No
5	M	60	Yes	5.3	NR	N	N	D	D	D	D	D	No
6	W	59	77	4.0	NR	N	N	D	D	D	D	D	*Yes
7	M	37	Yes	9.4	NR	N	N	ND	D	D	D	D	No
8	W	64	Yes	7.2	NR	N	N	D	ND	ND	D	D	No
9	M	62	Yes	7.3	NR	N	N	ND	D	ND	D	D	No
10	M	54	Yes	10.0	NR	N	N	D	D	D	D	D	No
11	W	56	Yes	7.4	NR	N	N	D	D	D	D	D	No
12	M	54	85	4.0	NR	N	N	ND	D	ND	D	D	*Yes
13	M	60	Yes	8.2	NR	N	N	D	ND	D	D	D	No
14	M	60	Yes	3.6	NR	N	N	ND	D	D	D	D	*Yes
15	M	58	Yes	7.1	NR	N	N	D	D	D	D	D	No
16	M	69	Yes	3.9	NR	N	N	ND	D	D	D	D	No
17	M	69	Yes	5.0	NR	N	N	D	D	D	D	D	No
18	M	58	Yes	10.4	NR	N	N	D	D	D	D	D	No
19	M	64	Yes	5.8	NR	N	N	D	D	D	D	D	No
20	M	50	Yes	2.3	NR	N	N	D	ND	ND	D	D	No
21	M	58	Yes	9.3	NR	N	N	D	D	D	D	D	No
22	W	60	Yes	6.7	NR	N	N	D	D	D	D	D	No
23	M	48	Yes	8.1	NR	N	N	D	D	D	D	D	No
24	M	42	Yes	6.1	NR	N	N	D	D	D	D	D	No
25	M	58	Yes	7.1	NR	N	N	D	D	D	D	D	No
26	M	41	Yes	8.3	NR	N	N	D	D	D	D	D	No
27	W	54	Yes	4.9	NR	N	N	D	D	D	D	D	No
28	M	54	Yes	7.9	NR	N	N	D	D	D	D	D	No
Total	12.4% (W)/ 87.4% (M)	57.1(95% CI= 54-60)	Yes = 92.8%	6.9(95% CI=6.1-7.8)	NR = 100%	N = 100%	N = 100%	D= 82.1%	D= 89.2%	D= 85.7%	D= 100%	D= 100%	No= 89.3%

ALT = alanine aminotransferase (reference normal value is 16-63 IU/L); COV = average OD_{450} of negative control x factor; D = detected; M = man; N = negative; ND = not detected; nPCR = nested PCR; NR = non-reactive; nt = nucleotide in the HBV genome; positive anti-HBeAg < COV; positive HBeAg ≥ COV; reactive Anti-HBc or HBsAg samples correspond to S/CO > 1.0; rtPCR = real time PCR; S/CO = sample RLU (relative light unit)/calibrator RLU; W= woman; *yes = grade 1 liver steatosis.

DISCUSSION

OBI is mostly detected in individuals with signs of past HBV infection, such as positive anti-HBc and frequently HBV DNA can be found in the liver [2]. Although most OBI patients do not experience manifestations during their lives [2, 18], some can reactivate viral replication and viral gene expression after asymptomatic years, for instance, during immunosuppressive states, leading to severe and sometimes fulminant liver disease [5, 8]. Moreover, OBI patients can transmit HBV by blood or organ donation [5, 18] and they can develop cirrhosis or HCC [19].

Host immune response reduces HBV replication and transcription, which can lead to virus clearance or its persistence at very low viral loads as found in OBI. Thus, OBI can present with mild liver damage due to low-grade immune cytotoxicity [20]. In contrast, CHB is characterized by an intense NK activity that destroys HBV-infected hepatocytes and may contribute to the regulation or depletion of T cells [21]. The balance between helper and cytotoxic T cells is modulated by cytokines secreted during the antiviral immune response.

In general, our results showed similar cytokine profiles in OBI and CHB; however, at least six cytokines

Table 2
Quantification of serum cytokines in HI, OBI and CHB patients.

Cytokines	HI % detection**, S/DL*	OBI % detection**, S/DL*	CHB % detection**, S/DL*	*P* value
IFN-γ	50.0 1.7 ± 3.4	37.1 3.4 ± 6.5	41.0 1.9 ± 3.2	0.176 0.454
TNF-α	33.5 0.75 ± 1.17	57.1 13.8 ± 26.9	75 101 ± 127	0.001 0.004
TNF-β	83.3 7.1 ± 5.4	92.8 12.0 ± 10.4	66.6 6.7 ± 13.9	0.01 0.35
IL-2	100.0 3.8 ± 2.2	79.0 2.6 ± 3.4	100.0 23.9 ± 3.5	<0.001 0.001
IL-12p70	66.6 2.8 ± 3.1	71.1 4.0 ± 13.6	58.3 19.8 ± 27.2	0.15 0.006
IL-28A	50.0 0.85 ± 1.10	35.7 0.72 ± 0.67	58.3 2.2 ± 3.7	0.007 0.095
IL-4	66.6 3.3 ± 3.3	78.6 6.8 ± 1.9	66.6 19.0 ± 27.0	0.097 0.065
IL-5	100.0 32.9 ± 13.2	92.8 22.4 ± 21.6	83.3 51.5 ± 61.3	0.001 0.07
IL-10	100.0 15.8 ± 9.4	100.0 16.4 ± 20.4	100.0 57.1 ± 78.1	1.00 0.023
IL-13	83.3 15.3 ± 12.8	96.4 19.8 ± 14.5	66.6 41.8 ± 77.7	<0.001 0.263
GM-CSF	66.6 8.3 ± 9.9	61.0 5.4 ± 10.0	75.0 23.7 ± 43.0	0.100 0.094
IL-1 beta	66.6 7.3 ± 6.1	89.0 11.4 ± 9.8	75.0 18.2 ± 22.0	0.014 0.222
IL-6	100.0 35.7 ± 18.2	89.3 46.6 ± 41.8	91.6 46.1 ± 50.0	0.015 O.846
IL-17A	83.3 3.8 ± 1.9	96.4 5.5 ± 5.5	50.0 11.2 ± 13.9	<0.001 <0.001
IL-17F	50.0 3.5 ± 4.5	60.7 1.6 ± 2.1	58.7 26.7 ± 36.6	0.268 <0.001
IL-21	83.3 9.6 ± 3.0	78.3 12.0 ± 10.0	58.3 28.0 ± 37.4	0.010 0.07
IL-22	0.0 0.02 ± 0.03	10.7 0.18 ± 0.41	25.0 0.6 ± 1.3	<0.001 0.11
IL23	50.0 5.4 ± 2.4	53.3 19.3 ± 48.8	41.0 8.1 ± 17.0	0.583 0.608
TGF-1β	50.0 2.18 ± 1.10	39.0 1.51 ± 2.0	46.6 4.3 ± 5.3	0.270 0.021
MIP-3α	0.0 0.5 ± 0.3	37.5 1.8 ± 3.4	58.3 1.6 ± 1.5	<0.001 0.59

*Corresponds to the normalized cytokine levels and expressed as concentration (pg/mL) in the sample/detection limit for that cytokine (pg/mL); mean ± standard deviations are shown, including all values, even those below the detection limit.
**Corresponds to the proportion of samples within the group that showed values above the detection limit (S/DL > 1.0)

differed significantly between groups. The main difference was in TNF-α: HI, OBI and CHB had progressively higher TNF-α values. TNF-α has been identified as a pathogenic factor in hepatitis with high concentrations associated with severe liver disease, related to its important role in inflammation [22]. In our study, TNF-α values were sevenfold higher in CHB reactive phase than in OBI and 18-fold higher in OBI than in HI (*table 2*). Other studies have reported that serum TNF-α increases significantly in patients with CHB [23]. Recently, a study reported that HBV-specific TNF-α producing CD4 T cells are associated with liver damage [24], although similar serum TNF-α concentrations have been found in patients with acute and chronic HBV infection [8, 10].

Other interesting information came from the analysis of the IL-17A:IL-17F ratio, which was near 1 in HI, but threefold higher in OBI, while CHB showed an opposite relationship, with higher IL-17F than IL-17A for an IL-17A:IL-17F ratio of 0.4 (*figure 3*). The role of these cytokines in HBV infection is not clear; much of the knowledge about them comes from autoimmune diseases where disruption of their equilibrium is associated with disease progression [25]. Higher IL-17A seems to be related to virus control or low-level persistence, while higher IL-17F suggests an

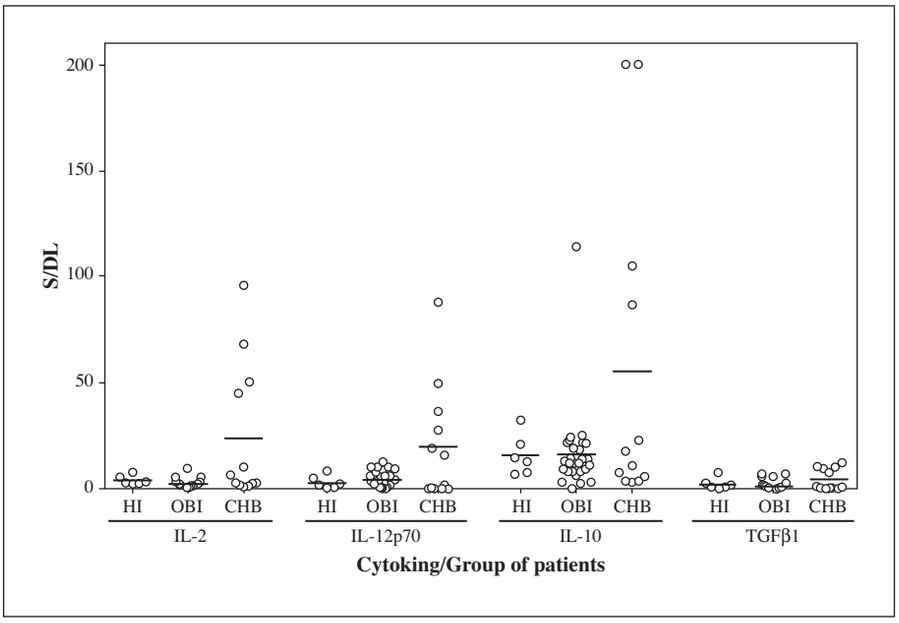

Figure 1
The figure includes cytokines with significant differences ($P < 0.05$) between OBI and CHB, except TNF-α, IL-17A and IL-17F, which are displayed in the subsequent figures. Cytokine levels are expressed as S/DL, that is, concentration (pg/mL) in the sample/ detection limit for that cytokine (pg/mL).

association between active inflammation and liver disease in CHB [26]. Accordingly, higher serum levels of IL17F than IL-17A have correlated with liver damage in alcoholic patients [27].

Significantly higher IL-17A concentrations have been found in OBI patients compared to HI [12] and in CHB compared to acute hepatitis B [28]. In partial agreement, we found slightly higher IL-17A in OBI than in HI, while CHB had the highest IL-17A levels (*table 2*, *figure 3*). As for IL-17F, similar concentrations have been reported in different CHB phases [7] and elevated IL-17F concentrations in Mexican children with hepatitis A virus have been associated with liver damage [29]. In our study, CHB patients had significantly higher IL-17F than OBI and HI ($P < 0.001$; *table 2*, *figure 3*). Thus, our findings and the previous evidence suggest that IL-17A and IL-17F and their balance play an important role in liver inflammatory damage. A recent study concluded that IL-17 may trigger liver repair mechanisms, but if the damage persists, IL-17 upregulation may contribute to liver disease progression [30].

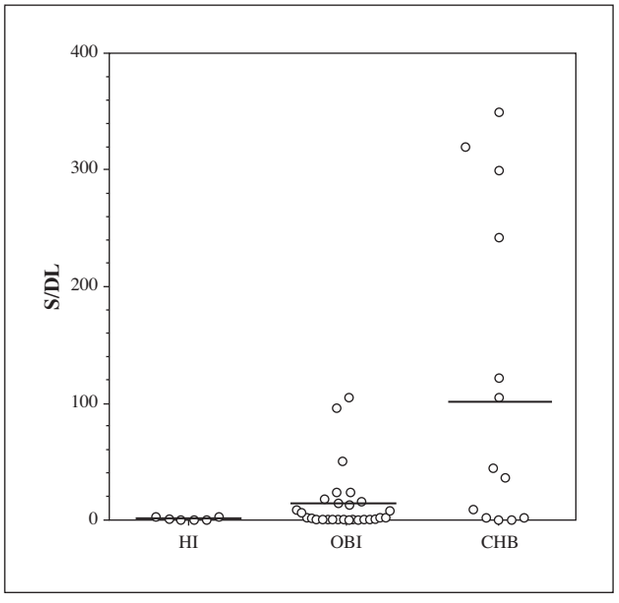

Figure 2
Significantly different TNF-α levels were found between HI, OBI and CHB (P<0.05).

In our study, IL-10, IL-2 and IL-12p70 values were similar between HI and OBI, but severalfold higher in CHB (*table 2*, *figure 1*). Some studies have reported IL-10 results similar to ours [10, 13] and others have reported high IL-10 in the inactive carrier state compared to other CHB phases [7-9, 12]. Higher IL-2 was reported in Mexican Nahua with OBI [14] and in individuals with resolved HBV [9]. In other studies, higher IL-2 was observed in CHB with respect to HI [30] but was found at similar levels in different CHB phases [7, 8]. In turn, IL-12p70 has been explored only in one study, where it was elevated in serum in the inactive carrier state with respect to other CHB phases [9]. Fierro *et al.* further reported elevated TGF-β1 in Mexican Nahua with OBI versus HI and resolved HBV [14]. Others have reported a higher TGF-β1 concentration in the active carrier state and in the acute phase of HBV infection with respect to HI [31] and yet another study positively correlated TGF-β1 with CHB severity [11]. In general accord with those reports, we found a slight TGF-β1 elevation ($P = 0.02$) in CHB with respect to OBI and HI (*table 2*, *figure 1*).

Finally, in our study, two cytokines, namely, MIP-3α (CCL20) and IL-22, were not detected in HI and showed low values in OBI and CHB. More CHB than OBI patients had these cytokines (*table 2*). Two studies reported similar serum concentrations of IL-22 at different CHB stages [7-9].

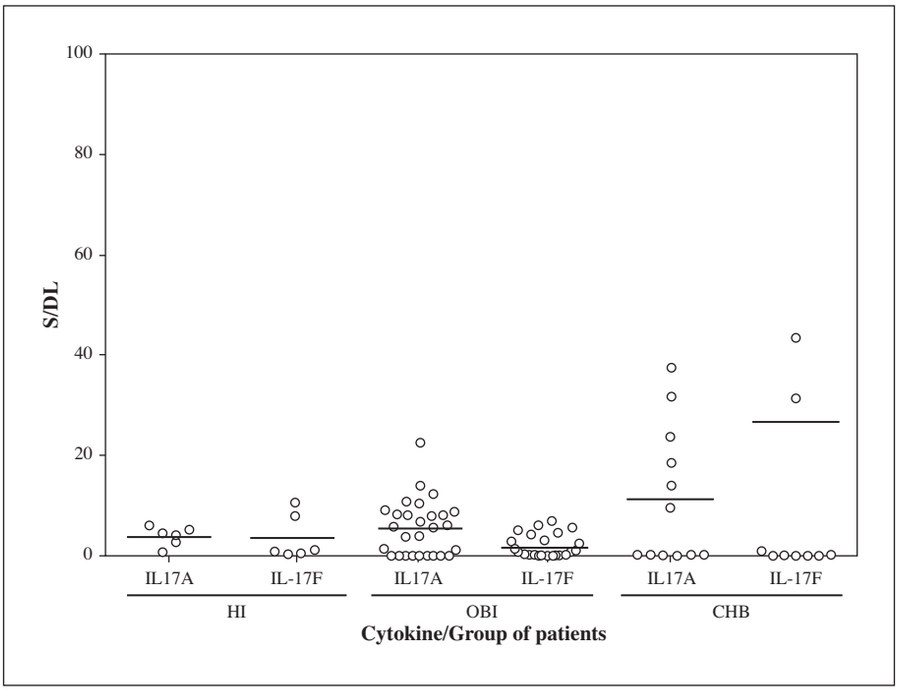

Figure 3
In OBI, IL-17A was higher than IL-17F, whereas CHB shows the opposite proportion with higher IL-17F than IL-17A.

The differences found in cytokines between this and other studies may be related to the studied population, the techniques used to quantify cytokines [32], the complex natural history of HBV infection [33] and the relationship between cytokine gene polymorphisms and HBV infection [23, 34, 35]. Although OBI patients have poor HBV replication, our results from the quantification of Th1, Th2 and Th17 cytokines suggest that they have a chronic inflammatory process and so it is relevant that individuals who have repeated positive anti-HBc and negative HBsAg results are managed as patients with CHB [36], even if they maintain normal alanine-transferase activity over time.
Mexico is among the Latin American countries considered to have a low prevalence (<2%) of HBsAg [37]; consequently, OBI is atypical and has been more studied in groups of patients with compromised immune systems [38, 39] than in individuals without apparent liver damage [18].

CONCLUSIONS

The cytokine patterns found in this study reflect the chronicity of the liver inflammatory process in HBV infection. Although several cytokines were more abundant in CHB than in OBI and HI, TNF-α and the IL-17A:IL-17F ratio distinguished between the three groups.

Acknowledgments. This study was supported by CONACYT, grant SALUD-2011-C01-161867 (FIS/IMSS/PROT/1038) and IMSS, grant number CTFIS/10RD/12/2011.

Disclosures. There is no conflict of interest to disclose.

Authors' contributions: Conceptualization and design: FS-J, GS-L and JR-L; acquisition of data, laboratory samples and clinical studies: LS-R, MAM-T, DM-M, VHGG, BG-F, JEL-R and JAE-M; determination and analysis of cytokines, curing and interpretation of data: JEL-R, JAE-M, VV-R, PC-H, JCB, GS-L and FS-J. Writing - Original draft preparation - Reviewing and Editing: VV-R, PC-H, GS-L, JR-L and FS-J. All authors read and approved the final manuscript.

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