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Role of host immunity and *HBx* among inactive chronic hepatitis B patients in a highly endemic region

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ABSTRACT

The hepatitis B virus (HBV) infection has a wide range, from fulminant hepatitis to inactive chronic hepatitis B (ICB) infection. The present study evaluated critical factors in the outcomes of HBV infection in a highly endemic region of Iran (approximately 12% HBV positive). The expression of seven genes involved in host immunity (*Foxp3*, *T-bet*, *ROR-γt*, *AKT*, *CREB*, *IL-28*/or *IFN-λ2*, and *IL-28R*) and *HBx* for viral activities were evaluated using real-time PCR, TaqMan method. A total of 58 subjects were randomly chosen, including 28 ICB and 30 healthy controls (HCs) from the Esfandiar district, South Khorasan province, Iran. The expression index of *Foxp3* and *ROR-γt* was moderately up-regulated in ICBs but did not statistically significant. *T-bet* expression in ICB patients was significantly higher than in HCs ($p = 0.004$).

Furthermore, evaluating two signalling pathways in Th activation and cell survival showed that the *CREB* pathway was significantly up-regulated in ICB patients compared to HCs ($p = 0.006$), but the *AKT* did not differ. In innate immune responses, the IL-28/or *IFN-λ2* expression in ICB patients was significantly higher than in the HCs ($p = 0.02$). Surprisingly, only one ICB patient disclosed *HBx* expression, which shows deficient virus activity in these patients. The ICB condition seems to result from host immune pressure on HBV activities, up-regulation of *T-bet* and *IFN-λ*. The high expression of *CREB* may prevent Kupffer's pro-inflammatory reactions in the liver. Whereas the absence of *HBx* expression in ICB patients and, consequently, the inactivity of HBV may also confirm such immune pressure.

1. Introduction

Hepatitis B virus (HBV) infection is a major global health problem. It is the cause of lifelong chronic hepatitis, resulting in liver cirrhosis (LC) and hepatocellular carcinoma (HCC) [1]. According to the World Health Organization (WHO), countries in Africa, Asia, and South America have carrier rates as high as 8% [2], with pooled prevalence in Iran accounting for 4.8% in high-risk groups [3]. In Iran, the prevalence of HBV infection is 2%, and it has been identified as a mid-endemic area [4].

Recent studies on the general population throughout Iran have

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shown a sharp decline in HBV epidemiology [5]; however, the highest prevalence was reported in an Esfandiar district (South Khorasan province). In the Esfandiar, the prevalence of positive hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (anti-HBc) has been reported at 17.8% and 48.7%, respectively [6]. Hence, according to the WHO criteria, Esfandiar is a highly endemic area for HBV infection [7–9].

Viral factors and host immune responses, including innate and adaptive immunity, are involved in HBV infection clearance or persistence [10]. The HBV unstructured X protein (HBx) is an essential viral regulatory protein at the intersection of HBV infection, replication, pathogenesis, and possibly carcinogenesis [11]. The *HBx,* the smallest open reading frame (ORFs) in the HBV genome, initiates multiple intracellular signalling associated with cell survival and proliferation [12,13].

The host's innate immune response to HBV is the first line of viral control and is essential for the subsequent induction of adaptive immunity. Thus, both arms of the immune system are involved in disease manifestation or long-lasting protection [14]. Therefore, appropriate Th1 immunity and cytokine network are vital in developing protective immune responses against HBV [15]. However, inappropriate host inflammatory responses in the liver are implicated in the pathogenesis of fulminant, acute, chronic or HCC caused by HBV infection.

The role and expression of interleukin-28 (*IL-28* or *IFN-λ2*) can affect the course of chronic HBV infection. The three cytokine genes (IL28A, IL28B, and IL29) were encoded cytokines of the interferon lambda family (IFN-λ) [16]. IFN-λ induced an antiviral response mediated by activation of the JAK-STAT and mitogen-activated protein kinase (MAPK) signalling pathways [17].

In adaptive immunity, T helper type 1 (Th1), Th2, T regulatory cells (Tregs), and Th17 responses are induced by selective transcription factors such as T-box transcription factors (T-bet), GATA3, forkhead box P3 (Foxp3), and retinoic-acid-receptor-related orphan nuclear receptor gamma (RORγt) [18,19]. For an appropriate response to an infectious agent, the balance of Th1/Th2 and Th17/Treg determines the outcome of infection [20].

T-bet is a selective transcription factor that induces the Th1 immune response to produce IL-2 and IFN-γ [21,22]. This response activates the cell-mediated immunity toward eliminating viruses, intracellular bacteria and cancers. Moreover, it supports $CD8^{+}$ cytolytic T-cell killing activities by inducing IFN-γ, perforin and granzyme secretion [20,23].

Foxp3 is a member of the forkhead/winged-helix family of transcriptional regulators, which induces Treg responses to modulate immune activation [24]. On the other hand, RORγt transcription factor expression induces Th17 differentiation, promoting the inflammatory response. In the case of HBV infection, Th17 function is undeniable in the pathogenesis of acute-on-chronic hepatitis B liver failure. Cellular signalling pathways, especially cell survival and cell death pathways such as nuclear factor-κB (NF-κB), MAPK, phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), soluble forms of tumour necrosis factor receptors (TNF-Rs), and caspase by activation/inhibition of Th lymphocyte, or even cell death coordinate the output of immune responses. HBx can activate some cell lines' PI3K/AKT signalling pathway. However, it has not been evaluated whether HBx regulates PI3K/AKT signalling in normal hepatocytes [25–27].

This study evaluated the HBV-host molecular interactions in developing ICB conditions in HBsAg-positive subjects with normal liver function and undetectable HBV-DNA viral load. Therefore, the expression of the HBV regulatory molecule (*HBx*) and host transcription factors involved in inducing different T-cell responses, including *Foxp3*, *T-bet*, *ROR-γt*, *AKT* and *CREB*, were assessed. Furthermore, IL-*28* and its receptor, *IL-28R* expressions in *the IFN-λ* antiviral family, were also evaluated.

2. Material and methods

2.1. Population setting

According to the American Association for the study of liver diseases (AASLD) guideline, patients who have serum antibodies to HBeAg (anti-HBe), undetectable or low HBV DNA levels (<2000 U/mL) and normal alanine transaminase (ALT) were considered as ICB patients [28]. The study was conducted on 58 subjects with normal ALT levels (for at least 12 months), including 28 ICB patients and 30 HCs. According to the manufacturer's instructions, all participants were examined for anti-HIV-1/2, HBsAg and anti-HBc antibodies using commercial ELISA kits (DIA.PRO, Italy). The participants were 20–70 years old, without any history of autoimmune or infectious diseases and antiviral therapy.

2.2. RNA extraction and cDNA synthesis

Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from five mL EDTA-treated whole blood samples by Ficoll gradient (Sigma, USA) centrifugation. Total RNA was extracted using TriPure Isolation Reagent (Roche, Germany) according to the manufacturer's instructions. The isolated RNA was then reverse-transcribed to complementary DNA (cDNA) using the cDNA with RevertAid™ First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) according to manufacturer's manuals.

2.3. Quantitative reverse transcription-polymerase chain reaction (RT-qPCR) assays

Primers and probes were designed using Beacon Designer software version 7 (Beacon Business Systems, Australia) for genes of interest and the cellular reference gene (beta 2-microglobulin, β2-M). Table 1 shows the primers and probes nucleotide sequences.

The real-time PCR TaqMan method was used for assessing *Foxp3*, *T-bet*, *ROR-γt*, *AKT*, *CREB*, *IL-28*, and *IL-28R* and SYBR green method for *the HBx* gene. The cDNA samples were applied to a Rotor-Gene *Q*-6000 machine (Qiagen, Germany) and Takara master Mix (Japan). Using Rotor-Gene software, two standard curve techniques were used to measure gene expressions. The relative quantity of every interested gene was normalised to the relative quantity of β2-M as a reference gene and reported as a gene expression index [29]. Amplification of a single product for each primer set was confirmed by electrophoresis analysis on 2% agarose gel. Then, PCR products were sequenced by Applied Biosystems (Seq Lab, Germany) to confirm the primer design validity.

2.4. Statistical analysis

Statistical analysis was performed using SPSS software ver.11.5 (SPSS, Chicago, IL). Variable normality was checked before data analysis. The Kruskal Wallis Ranked test assessed variable differences among the study groups. Mann-Whitney *U* test was used as a post-test for the variable differences between the two groups. Correlation analysis was performed using Spearman. The p-value <0.05 was considered statistically significant.

3. Results

3.1. Participant's characteristic

The subjects were 28 HBsAg positive patients and 30 HBsAg negative. Participants' mean ages were 49.36 ± 11.08 and 36.91 ± 1.44, respectively. The age and sex of the groups were matched for statistical analysis (mean ± SEM).

The main demographic and routine laboratory tests of the patients are summarised in Table 2. Data are expressed as mean ± SD for all variables, and the independent *t*-test was used for the comparison.

Table 1
The oligonucleotide and probe sequences.

Target genes	Purpose Sequence (5′ → 3′)	Product size
HBx	F: 5′-CCTACAGCCTCCTAGTAC-3′ R: 5′-CTTGGACTCTCAGCAATG-3′	124 bp
Foxp3	F: 5′- ACTACTTCAAGTTCCACAACAGC-3′ R: 5′-GAGTGTCCGCTGCTTCTCTG-3′ Probe: FAM-TCACCTACGCCACGTTCATCCCGT-BHQ-1	95 bp
T-bet	F: 5′-GTCCAACAATGTGACCCAGATG-3′ R: 5′-TGCGTGTTGGAAGCGTTG-3′ Probe: FAM-CCTCTGGCTCTCCGTCGTTCACCTCAA-BHQ-1	120 bp
Rorγt	F: 5′-GTCCAACAATGTGACCCAGATG-3′ R: 5′-TGCGTGTTGGAAGCGTTG-3′ Probe: FAM-CCTCTGGCTCTCCGTCGTTCACCTCAA-BHQ-1	145 bp
AKT	F: 5′-GTGTCAGCCCTGGACTACC -3′ R: 5′-CAGCCCGAAGTCTGTGATCTTA -3′ Probe: FAM-TCCTTGTCCAGCATGAGGTTCTCCAGC-BHQ-1	114 bp
CREB	F: 5′-ACTCCAAAAGTAAAGTCCCGTTAC-3′ R: 5′-TCCAAAGAAACAGGAAGCAGATTG-3′ Probe: FAM-TTCTCCTCCCACCGCCCTTGTCCC-BHQ-1	90 bp
IL-28/or *IFN-λ2*	F: 5′-CTGGCTGGAGAGTGATCTGG-3′ R: 5′-GAGATCCTGAGTGCCAATGC-3′ Probe: FAM-CCCCTCCTTCCAGAATGCCACCTCA-BHQ-1	90 bp
IL-28R	F: 5′-GGTGACTTTGGTGCTAGGCT-3′ R: 5′-GGCCTTCTTGAAGCTCGCTA-3′ Probe: FAM-CCACCACAACTGGGAAGGGCTGCCAC-BHQ-1	157 bp
β2-M	F: 5′-TTGTCTTTCAGCAAGGACTGG-3′ R: 5′-CCACTTAACTATCTTGGGCTGTG-3′ Probe: FAM-TCACATGGTTCACACGGCAGGCAT-BHQ-1	127 bp

Table 2
The main demographic and routine laboratory tests of the analysis and differential blood count).

Variables		ICBs ($n = 28$)	HCs ($n = 30$)	*P*-value
Male		18	15	NS
Female		10	15	
Education	Illiterate	4	4	NS
	Primary	13	10	
	Pre-high school	3	6	
	High school	5	8	
	University	3	2	
WBC (10^3/μL, mean ± SD)		6.25 ± 0.28	7.29 ± 0.35	0.02
RBC (10^6/μL, mean ± SD)		5.12 ± 0.96	5.03 ± 0.82	NS
Hemoglobin (g/dL, mean ± SD)		14.64 ± 0.36	14.52 ± 0.26	NS
HCT (%, mean ± SD)		46.84 ± 0.94	46.67 ± .53	NS
Platelet count (10^3/μL, median ± IQR)		201.77 (12.34)	226.51 (11.370	0.04
HbA1c (mean ± SD)		5.78 ± 0.1	6.0 ± 0.15	NS
ALT (IU/L, median ± IQR)		12.39 (0.93)	14.82 (0.980	0.07
AST (IU/L, median ± IQR)		29.44 (1.88)	25.55 (1.58)	NS
anti-HBc (IU/mL, median ± IQR)		6.49 (0.38)	4.85 (0.56)	0.02

ICB**: Inactive chronic hepatitis B**, HC: **Healthy controls,** WBC: **White blood cell**, RBC: **Red blood cell**, HbA1c: **haemoglobin A1c**, ALT: **alanine aminotransferase**, AST: **aspartate aminotransferase.**
NS: Non-significant.

However, platelet, ALT, AST, and HBcAb did not meet normal distribution; thus, median and interquartile ranges (IQR) were considered for the comparison using the Mann-Whitney test. The chi-squared test was used to analyse qualitative variables such as gender and education.

3.2. Gene expression measurements

3.2.1. HBx expression

HBx, the main HBV regulator of viral activation in liver cells, has been evaluated to assess the recurrence of acute exacerbations of chronic hepatitis B. The expression of *HBx* was detected just in one ICB patient. The ALT, AST, and platelet in this patient at sampling were 10, 23 and 239 × 10^3/μL. Two months after sampling, this patient had HBV activation leading to hospitalisation and was being treated with medication.

3.2.2. Transcription factors expression of Th subpopulations

The Mann-Whitney *U* test was used to analyse the expression of *T-bet* (Th1), *Foxp3* (Treg), and *RORγt* (Th17) due to the un-normal distribution. *T-bet* expression in ICB patients was 3.78 ± 1.2, and in controls was 1.01 ± 0.2, in which the expression in ICB patients was firmly higher than in the control group ($p = 0.004$).

While the *Foxp3* expression index in the ICB (0.93 ± 0.34) was higher than the control group (0.63 ± 0.14), there was no significant difference in the 95% confidence interval. (CI = 95%, $p = 0.7$). There was no significant difference in *ROR-γt* expression between the studied groups (1.30 ± 0.47 in chronic *vs* 0.8 ± 0.14 controls).

The Th17/Treg balance may indicate inflammation levels in chronic diseases, as measured by the index (*ROR-γt/Foxp3*). This index indicated inflammation levels were higher in ICB patients than in controls. Nevertheless, the increase was insignificant at the 95% confidence level (CI = 90%, $p = 0.1$). The CI shows that this variable should include more samples for a reliable result. Fig. 1 shows the transcription factor's expression indices of Th subpopulations.

3.2.3. AKT and CREB expression

Two main signalling pathways targeted by most viruses have been investigated. Although HBV infection significantly up-regulated CREB ($p = 0.006$), the AKT pathway did not affect ICB patients. Fig. 2 shows *AKT* and *CREB* expression in the studied groups.

3.2.4. IL-28/IL-28R expression

IFN-λs (1–4), IL28 and 29 have significant antiviral activities in innate and adaptive immunity. The Mann–Whitney *U* test showed that the *IL-28* expression level in the ICB patients was significantly higher than in HC ($p = 0.02$). The *IL-28R* expression was 0.6 ± 0.15 and 0.5 ± 0.14 in ICB and HC, respectively, which was not statistically significant. Fig. 3 shows the *IL-28* and *IL-28R* expression index in the studied groups.

3.3. Gene expression correlations

Based on Spearman correlation analysis in the control group, the direct correlation coefficient between *Foxp3* and *T-bet* and *ROR-γt* was

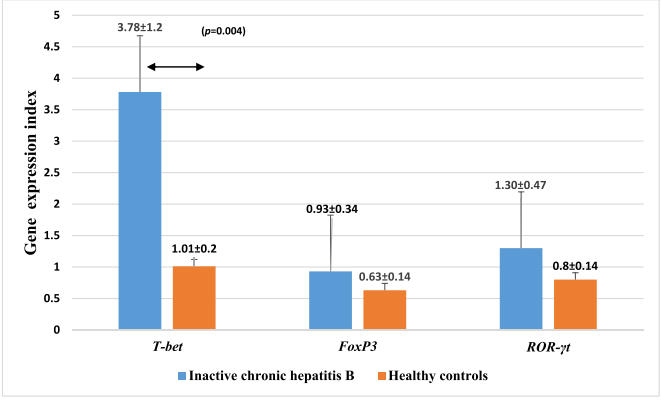

Fig. 1. Expression of Th subpopulations transcription factors in studied groups. Data are expressed as mean ± SD.

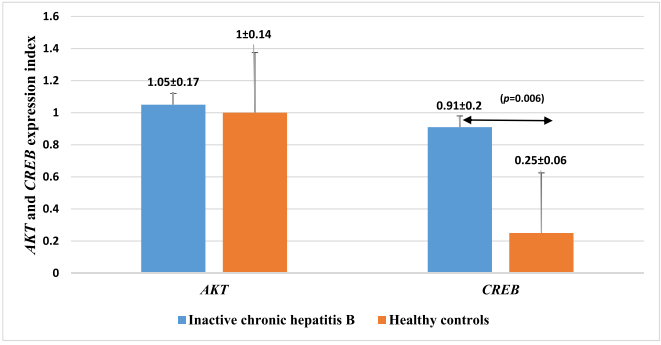

Fig. 2. *AKT* and *CREB* expression index in studied groups. Data are expressed as mean ± SD.

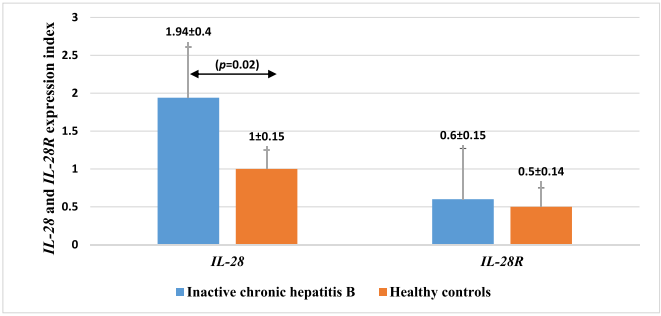

Fig. 3. *IL-28* and *IL-28R* expression index in studied groups. Data are expressed as mean ± SD.

statistically significant (r = 0.67, $p = 0.001$, and r = 0.62, $p = 0.001$, respectively). Moreover, there was a significant positive correlation between *ROR-γt* and *T-bet* (r = 0.3, $p = 0.04$). However, there was not a significant correlation between *AKT* and *CREB*. There was a positive correlation between *IL-28* and *IL-28R* (r = 0.85, $p = 0.001$).

The correlation analysis of a whole case group (Spearman Rank Order correlations) showed that *T-bet* was positively correlated with *Foxp3* (r = 0.59, $p = 0.001$) but negatively with a ratio of *ROR-γt/Foxp3* (r = −0.57, $p = 0.004$). Compared with control subjects, *AKT* was directly correlated with *CREB* (r = 0.45, $p = 0.03$). The results of correlation analysis showed that the quantitative level of HBcAb was significantly positively correlated with *ROR-γt/Foxp3* (r = 0.49, $p = 0.01$).

4. Discussion

Since HBV infection is an immunopathological disorder, the immune system plays a critical role in the pathological changes of liver damage. Therefore, the host's immune response is essential in controlling the spread of viral infections and also responsible for inflammatory reactions. A proper immune response can result in virus elimination and recovery in more than 90% of infected individuals. However, an excessive immune response can cause liver damage, and a tolerant response causes persistent HBV infection [30,31].

Therefore, the outcome of HBV infection reflects the viral-host interaction. For example, the consequences of HBx viral proteins and the activity of host Th-cell subpopulations determine the manifestation of infection [11]. In this study, only one patient expressed the *HBx* regulator. He was admitted to the liver hepatitis Clinic at Birjand University of Medical Sciences two months after our sampling and was treated for hepatitis B infection. At our sampling time, his liver indices were normal (ALT = 10 and AST = 23).

Some studies suggest that Th1 should be the main dual-function host cell for HBV infection [15,32]. On the one hand, its intense and uncontrolled activity causes type IV hypersensitivity, which plays a significant role in fulminant and acute hepatitis and induction of cirrhosis. On the other hand, mild activity puts pressure on the virus and prevents it from replicating in hepatocytes [33]. In the present study, *T-bet*, a selective transcription factor for Naive T-helper cells that differentiate into Th1, was enormously higher in ICB patients than in the control group.

Increased Th1 activity in ICBs shows cell-mediated immunity pressures viral activity. However, *T-bet* expression has been reported to be lower in those patients than in acute hepatitis B, which causes immunepathological damage to the liver. These findings suggest that a modest increase in *T-bet* during chronic viral infections may contribute to protecting against chronic HBV infections [23].

In contrast to Th1, Treg cells (Foxp3) are the main immune modulators of the Th subpopulation, balancing the immune response for appropriate conditions and preventing immunopathological disorders such as HBV [34]. In the present study, the *Foxp3* expression was assessed as the selective transcription factor inducing the Treg subpopulation. High numbers of Tregs in a healthy liver can create a suppressive microenvironment to prevent inflammatory responses [35]. Our results showed that *Foxp3* expression in ICB patients was similar to HC, i.e. no significant differences with controls.

Furthermore, in our study, the *ROR-γt* expression was increased in the ICB compared with the HC; however, it was not statistically significant. A previous study has demonstrated that Th17 is highly enriched in chronic patients' peripheral blood and liver. A more robust Th17 response was also correlated with liver injury in the HBV-infected individuals [13]. Th17 as an inflammatory Th subpopulation should increase more in acute hepatitis B patients than in chronic patients [36]. The proliferation of Th17 cells in the circulation and the liver positively correlates with plasma viral load, serum alanine aminotransferase levels and histological activity indices [36]. However, in our study, patients with ICB had normal AST and ALT levels, so a high level of *RORγt*, a selective transcription factor for Th17, was not expected. Accordingly, if the *RORγt/Foxp3* index is taken as the Th17/Treg balance for inflammation in chronic diseases, this index was higher in ICB patients than in the control group.

Similar to our study, Zhang et al. also demonstrated that Th17/Treg ratio was increased in chronic patients compared with HC. Moreover, an anti-HBV drug such as Entecavir could suppress liver inflammation and HBV replication, significantly reducing the Th17/Treg ratio [36].

Type I interferon families such as IFN-λ and IFN-α/β have previously been shown to inhibit HBV replication *in vivo* and *in vitro* [37]. In this study, the *IL-28* expression level in ICB patients was significantly higher than in HC. This result showed that this factor in ICB might put significant pressure on the virus, at least from innate immunity. Shi et al. study indicated that *IL28* A/B mRNA expression and IL28B protein levels are significantly lower in patients with active or advanced disease (CHB, LC, and HCC) than those with inactive disease or HC [38]. These findings were supported by the observation of greater *IL28* A/B mRNA expression and higher serum IL28B protein levels in HBeAg-positive than HBeAg-negative patients with inactive liver disease. It seems that IFN-λ applies an active pressure on HBV replication and, consequently, its harmful effects on the liver tissues. Further prospective studies are necessary to determine whether or not IL28 A/B protein levels directly affect the manifestation of different outcomes of HBV infection (CHB, LC, and HCC).

As signalling in cell survival networks is essential in the Th cell clonal expansion and differentiation and paying attention to the pressure of the immune system on HBV in ICB subjects, the effect of the main pathways was assessed in the current study. In ICB subjects, the expression of CREB was strongly up-regulated ($p = 0.006$). However, in PI3/AKT pathway, the AKT expression did not change in ICB. The CREB/bZIP family has an essential role in the liver by regulating gluconeogenesis, lipid metabolism, and cell proliferation and has an undeniable role in the Th immune responses.

Different signals, such as inflammatory and growth factors, induce CREB. Furthermore, CREB has been suggested to inhibit NF-κB, thereby directly limiting pro-inflammatory reactions and producing anti-apoptotic signals for cell survival [39].

Therefore, it could be expected that in the presence of HBV infection in an inactive condition, higher levels of this factor can prevent pro-inflammatory reactions in the liver and help the HBV-infected cell survive. The present study showed that *CREB* was firmly higher in ICB patients. Consequently, as an immune-pathologic disease, normal liver function is expected in the studied subjects.

This study has some limitations; firstly, a larger sample size for more reliable results was needed. Secondly, if an acute hepatitis B infection group was accessible, the comparison with this group improved and fortified the results. However, this highly infected region had a limited population.

In conclusion, only in one ICB patient was the HBx regulatory factor detected, and the other cases remained undetectable without any sign of liver damage. The expression of *IFN-λ* as an antiviral agent in innate immune responses increased. In adaptive immunity, *T-bet* is the main differential transcription factor for Th1 and *CREB* as one of the leading transcription factors for appropriate T-cells and monocyte/macrophage function up-regulated. The study indicated that these factors might prepare the immune system to put pressure on HBV replication and activities toward the development of an inactive type of chronic hepatitis B infection. Therefore, HBx as an HBV regulatory factor and IFN- λ as an innate immune response driving the adaptive immunity toward Th1 can be used for monitoring HBV-infected subjects. Furthermore, the expression of *HBx* and *IFN- λ* can be targeted in immune therapy.

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Ethics approval/consent to participate

This study was performed in line with the principles of the Declaration of Helsinki. The study protocol was reviewed, approved and supervised by the Biomedical Research Ethics Committee of the Mashhad University of Medical Sciences **[IR.MUMS.REC.980930]**. The written informed consent forms were obtained and signed by all the participants. All methods were performed following relevant guidelines and regulations.

Consent for publication

Not applicable.

Availability of data and materials

All data supporting this study's findings are included in the manuscript and available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Nafise Yousefpoor: Methodology. **Malihe Mahdavian:** Methodology. **Zohre Pourbagher:** Methodology. **Sanaz Ahmadi Ghezeldasht:** Writing – review & editing, Writing – original draft. **Arman Mosavat:** Writing – review & editing, Writing – original draft. **Masood Ziaee:** Investigation. **Masoumeh Bahreini:** Investigation. **Saman Soleimanpour:** Investigation. **Mohammad Reza Sharifmoghadam:** Investigation. **Narges Valizadeh:** Investigation. **Arghvan Asghari:** Investigation. **Alijan Tabarraie:** Writing – review & editing, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Seyed Abdolrahim Rezaee:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Abbreviations

anti-HBc	hepatitis B core antibody
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HBx	HBV unstructured X protein
ICB	inactive chronic hepatitis B
IFN-λ	interferon lambda
IL-28	interleukin-28
Th1	T helper type 1
Treg	T regulatory cell

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