



Study the Cross-talk Between Hepatitis B Virus Infection and Interferon Regulatory Factors in Liver Transplant Patients

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Abstract

Background: Interferon regulatory factors (IRFs) as immunoregulatory molecules have a determinative antiviral role in liver transplantation outcomes and graft rejection. Hepatitis B virus (HBV) and its antigen derivatives also choose some strategies to escape from innate immune responses.

Objectives: The current study aimed at evaluating inflammatory cross-talks between pattern recognition receptors (PRRs) signaling components such as IRF3 and IRF7 with HBV infection in mRNA levels in patients undergoing liver transplantation.

Methods: The 46 HBV infected liver recipients were divided into rejection experienced (20) and not experienced (26) groups and a healthy control group was also considered. Peripheral mononuclear cells (PBMCs) were isolated from each studied patient on the days 1, 4, and 7 in post-transplant period. After RNA extraction and cDNA synthesis from each collected sample, the expression levels of IRF3 and IRF7 genes were evaluated using in-house SYBER Green based the real-time polymerase chain reaction (PCR) protocols.

Results: The overexpression of mRNA levels of IRF3 (3.37 folds) and IRF7 (1.74 folds) on the day 1 were found in patients experiencing rejection, compared with non-rejected ones, based on initial ischemia/reperfusion (I/R) injuries. But, the mRNA levels of IRF3 (0.53 folds) and IRF7 (0.74 folds) on the day 4 were downregulated in patients with rejected transplantation, compared with non-rejected ones. Finally, reducing the expression of IRF3 (0.54 fold) on the day 7 and upregulation of IRF7 (2.38 fold) on the day 7 were found in rejected liver recipients, compared with non-rejected ones in post-transplant period.

Conclusions: Downregulation of IRF3 expression in patients with HBV infection, who experienced rejection episodes in the first week post-liver transplantation indicated that they may be at higher risk for acute rejection; the hypothesis, which should be investigated in further studies.

Keywords: Hepatitis B Virus, Liver Transplantation, IRF3, IRF7

1. Background

Inflammation is the innate immunity response to infection, stress, and tissue injury. Inflammatory cells have pattern recognition receptors (PRRs), which recognize either pathogen-associated molecular patterns (PAMPs) such as viral nucleic acids and bacterial lipopolysaccharide or damage-associated molecular patterns (DAMPs). PRRs such as toll like receptors (TLRs) induce the production of proinflammatory cytokines by the activation of transcription factors such as NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B-cells), activator protein 1 (AP1), and interferon regulatory factors (IRFs) (1).

Liver inflammation occurs in acute and chronic liver diseases such as viral hepatitis, alcoholic liver disease, drug-induced liver injury, ischemia/reperfusion (I/R) injury, and other diseases, which may associate with liver damage and fibrosis (2). Among viral hepatitis such as chronic HBV infection, the liver transplant is also considered as the final treatment method, which leads to rejection or acceptance of liver graft (3-7). Accordingly, activation of inflammatory signaling pathways is reported in allograft rejection. It appears that inflammatory responses play key roles in transplant clinical outcomes. Initial I/R injury in allograft recipients leads to the production of reactive oxygen and nitrogen species. Activated innate im-

immune cells release chemokines and other proinflammatory factors that cause further oxidative stress, increased inflammation, and finally organ loss (2, 8). It is necessary to study the cross-talk between viral infections and inflammatory responses in liver graft recipients. Therefore, early prognosis of any change in immune factors such as IRFs leads to better management of post-liver transplant outcomes.

IRF family has 9 identified members in mammals: (IRF 1 - 9) (9). IRFs are the regulatory transcription factors with critical role in innate and acquired immunity responses against microbial infection, cell growth regulation, apoptosis, oncogenes, and blood cell differentiation (10). IRFs have a conserved DNA binding domain (DBD) in N-terminal area that has a helix-turn-helix structure and recognize interferon stimulated response element (ISRE) sequence in target DNA. All IRFs except IRF1 and IRF2 have an IRF-association domain (IAD) in C-terminal area, which performs homomeric and heteromeric interactions with other IRFs for gene regulation (10, 11). After infection and induction of the PRR signaling pathways, the inactivated form of IRF3 and IRF7 in cytosol are phosphorylated; they form (homomeric or heteromeric) dimmers, then translocate into nucleus and induce target gene expression (9, 11). IRF3 is activated by *TLR3*, *TLR4*, and *RIG-I/MDA5* signaling pathways and (10-13) induces the expression of *IFN-4 α / β* , *IFN*-related genes (*ISGs*), and inflammatory chemokines with critical roles in innate immunity responses, inflammation, and activation of acquired immunity (10, 14). IRF7 also induces *IFN1* production in relation to the activation of *TLR7*, *TLR9*, and *RIG1/MDA5* signaling pathways post-microbial infections (9, 11).

Viruses have some strategies to escape from innate immune responses. On the other hand, components of innate immunity such as IRFs are affected by viruses. Viruses interfere in IRFs activation and activity, or downregulation of IRF genes (15); most of the cell types IRF3 and IRF7 phosphorylated by *TBK1* and *IKK ϵ* kinases (10, 15, 16). Phosphorylated forms of these transcription factors are required to interact with themselves or other proteins (16, 17). Some viral proteins target *TBK1* and *IKK ϵ* to decrease the ability of innate immune system. Viral proteins inhibit or reduce function of host kinases by direct binding to *TBK1*, *IKK ϵ* , atypical phosphorylation of IRFs, deubiquitination of kinases and competition with IRF molecules to bind to kinases (15). In the review of earlier reports, *N1L* protein in vaccinia virus (*VACV*) associated with *TBK1* complex of host cell inhibited the IRF3 and IRF7 activities (18). *ORF47* (open reading frame 47) in varicella-zoster virus (*VZV*) inhibited *TBK1* by atypical phosphorylation of IRF3 (19). Deubiquitinase activity of *BPLF-1* protein in Epstein-bar virus (*EBV*) degrades *IKK α* to inhibit IRF7 activity (20). The *BGLF4* in *EBV* bound di-

rectly to IRF3 and inhibited the interaction of IRF3 with target promoters (21). *KbZIP* protein in the Kaposi sarcoma-associated herpesvirus (*KSHV*) bound to *IFN- β* promoter and inhibited IRF3 interaction with this promoter and formation of enhanceosome (22). Some viral proteins such as *Npros* in classical swine fever virus (*CSFV*) and bovine viral diarrhea virus (*BVDV*) promoted proteosomal degradation of inactivated forms of IRF molecules (23, 24). In spite of possible determinative association between pathogenesis of chronic hepatitis B virus (*HBV*) infection with IRFs, limited studies were accomplished (15). Chronic *HBV* infection can lead to the end-stage liver diseases such as hepatocellular carcinoma and cirrhosis (3-6, 25-27). Therefore, early prognosis of any change in immune factors such as IRFs molecules leads to better management of post-liver transplant outcomes and raises the possibility of graft survival. The effects of *HBV* proteins on the IRF3 and IRF7 functions are investigated in few studies. *HBV* surface antigen (*HBsAg*) in viral infected cells inhibited *TLR9*-dependent expression of IRF7 and nuclear translocation (28). Downregulation of IRF7 gene in *HBV* infected patients was indicated earlier (29). *HBV* infection also led to reduced *IFN- α* production, which is the target gene in IRF7 signaling pathway (30). *HBV* polymerase blocked phosphorylation, dimerization, and activation of IRF3 and consequently reduced the production and antiviral effect of *IFN- β* (31, 32). *HBx* as a deubiquitinating enzyme also promoted proteasome degradation of *RIG-I*, *TRAF3*, and IRF3 factors (33). Modification in IRF3 and IRF7 mRNA is not yet investigated in human or animal models of transplantation. The role of IRF3 and IRF7, as components of inflammatory signaling pathway, is important due to the early activation of innate immune system, which occurs after the liver transplantation (34).

2. Objectives

The current study aimed at evaluating the mRNA expression levels of IRF3 and IRF7 in patients with *HBV* infection undergone liver transplantation with and without experiencing rejection, compared with healthy individuals.

3. Methods

3.1. Patients

The current study investigated 46 patients with chronic *HBV* infection undergone liver transplant surgery at Transplant Unit of Namazi hospital affiliated to Shiraz University of Medical Science, Shiraz, Iran. The candidates received liver graft according to their ABO blood group

compatibility. EDTA (ethylenediaminetetraacetic acid)-treated blood samples were collected from each studied patient on the days 1, 4, and 7 post-transplantation. The presence of HBV immunologic markers were analyzed in plasma sample of all patients using the enzyme-linked immunosorbent assay (ELISA) technique prior to transplantation. The HBV recipients under study were divided into rejection (20) and non-rejection (26) patient groups, based on the pathological result. A healthy control group including 13 subjects was enrolled. All graft rejected patients were experienced rejection episodes in the first week post-transplantation. Additionally, HBV copy number was calculated based on the quantitative real-time polymerase chain reaction (PCR) protocol. All HBV infected recipients used tenofovir (300 mg oral/day) or lamivudine (50 - 100 mg oral/day) in order to prevent recurrence of HBV infection. All studied patients were negative for human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections pre-transplantation and active cytomegalovirus (CMV) infection post-transplantation. The current study was supported by the ethical committee of Shiraz University of Medical Sciences based on the ethical guidelines of the 1975 Helsinki declaration.

3.2. RNA Extraction and cDNA Synthesis

Total RNA was isolated from patients' buffy coats using RNX plus (CinnaGen, Iran). The cDNA was synthesized using PrimeScript RT Reagent Kit (Takara Bio, Otsu, Shiga, Japan) according to manufacturer's instructions. The purity and concentration of total RNA was measured using NanoDrop™ (Thermo Scientific™, USA) at 260/280 nm.

3.3. Real-Time Polymerase Chain Reaction

The expression levels of IRF3 and IRF7 were analyzed in HBV infected liver recipients with and without experiencing rejection and in healthy controls using in-house SYBR Green real-time PCR protocols by StepOne-Plus™ Real-Time PCR System (ABI, StepOnePlus™, USA). To synchronize data and omit test errors of the mRNA, expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was evaluated as an internal control. The primer sequences were designed for the amplification of IRF3, IRF7, and GAPDH transcripts (NM_001197122.1; NM_001572.3, NM_001289745.1.). The PCR mix ingredients, primer sequences, product lengths, and thermocycling conditions are presented in Table 1. Melting point curves of target and internal control genes were analyzed to confirm the specificity of PCR reactions.

3.4. Molecular and Antigenic Analysis of CMV Infection

CMV genome was extracted from plasma samples of HBV infected recipients who rejected or non-rejected transplant using Invisorb® Spin Virus RNA Mini Kit (Invitex, Birkenfeld, Germany) according to the manufacturer's instruction. CMV genomic DNA load was evaluated using genisig® quantitative real-time PCR kit (Primer Design, Advanced kit, UK) according to the manufacturer's instruction. Sensitivity of the real-time PCR assay to diagnose CMV infection was as few as 10 copies of the viral genome per milliliter of sample. Detection of active cytomegalovirus infection was performed for both groups of the studied patients using antigenemia technique using CMV Brite Turbo kit (IQ Products, Groningen, the Netherlands) according to the manufacturer's instruction (35).

3.5. Statistical Analysis

Evaluation of the expression levels of IRF3 and IRF7 genes was performed by intragroup and intergroup analyses on HBV infected recipients who rejected or non-rejected liver-transplant and controls during 3 times follow-up using the $2^{-\Delta\Delta CT}$ (Livak) method. Statistical analysis was performed with SPSS version 16 (IBM Corporation, Armonk, NY, USA). $P < 0.05$ was considered significant.

4. Results

4.1. Patients

There were 26 patients in the non-rejected group with the mean age of 51.62 ± 10.6 years the age ranging 26 to 74 out of which 73% ($n = 19$) were male and 23% ($n = 7$) female. In the rejected group, there were 20 patients with the mean age of 50.95 ± 10.6 years ranging 27 to 69 out of which 85% ($n = 17$) were male and 15% ($n = 3$) female. Distribution of blood grouping in the rejected patients comprised of A⁺ (20%), B⁺ (25%), AB⁺ (5%), O⁺ (45%), and A⁻ (5%). In the non-rejected group the distribution consisted of A⁺ (30.76%), B⁺ (26.92%), AB⁺ (3.84%), O⁺ (34.61%), and O⁻ (3.84%). The most frequent blood group in both studied liver recipients was O⁺.

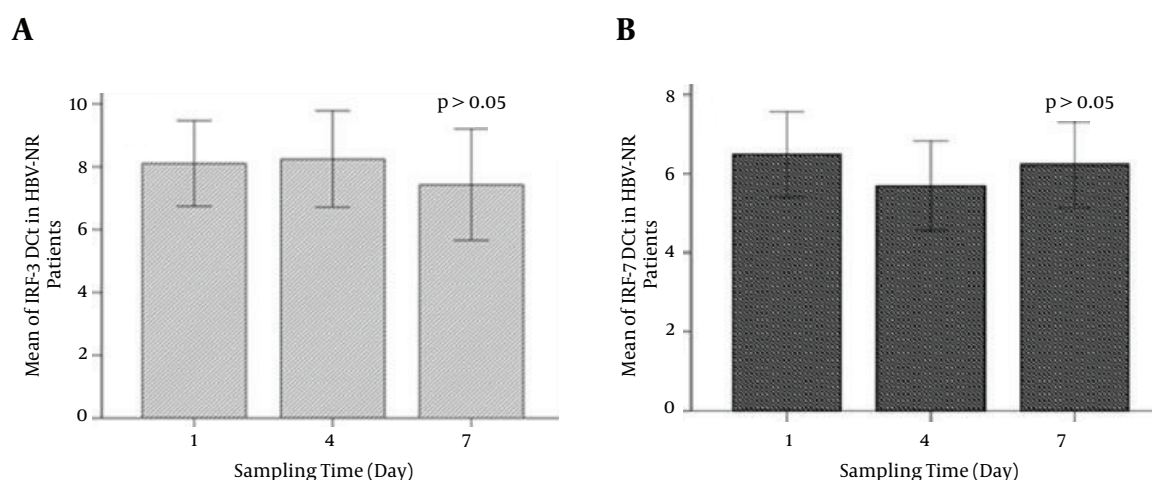
4.2. The Expression Levels of IRF3 and IRF7 Genes in HBV Infected Liver Recipients with and Without Experiencing Rejection

The mRNA expression levels of the IRF3 and IRF7 genes were compared between and within the 2 liver recipient groups on the days 1, 4, and 7 post-transplantation.

Comparison within non-rejected group showed that the mRNA level of IRF3 decreased on the day 4 and increased on the day 7, but vice versa the mRNA level of IRF7 increased on the day 4 and decreased on the day 7 post-liver transplants without any statistical significance (Figure 1).

Table 1. PCR Mix Ingredients and Real-Time PCR Programs

Gene	Primer	Primer Sequences (5' - 3')	PCR Product Length, bp	Thermocycling Conditions	PCR Mix, μ L; C
IRF3	IRF-3:R	5'TCCAGAATGCTTCCTGGGT3'	82	95°C/30 s, 40 cycles of 95°C/15 s, 62°C/20 s, and 72°C/30 s	SYBR Green Premix (5 μ L; 2X), SYBR Green Dye (0.2 μ L; 50X). Forward primers (0.4 μ L; 5 pM), Reverse primers (0.4 μ L; 5 pM)
	IRF-3:F	5TTGGGGACTTTTCCCAGCC3'			
IRF7	IRF-7:R	5TCGTCATAGAGGCTGTGGC 3	73	95°C/30 s, 40 cycles of 95°C/15 s, 61°C/20 s, and 72°C/30 s	
	IRF-7:F	5GTGAGGGTGTGCTTCCCTG3			
GAPDH	GAPDH:R	5CCAGTAGAGGCAGGATGAT3	199	95°C/30 s, 40 cycles of 95°C/15 s, 57.5°C/20 s, and 72°C/30 s	
	GAPDH:F	5GGACTCATGA7CCACAGTCCA3			

**Figure 1.** Comparison of the Expression Levels of IRF3 and IRF7 Genes on the Days 1, 4, and 7 Post-Liver Transplant Period in the HBV Infected Non-Rejected Group (HBV-NR).

In the rejected group, the IRF3 mRNA levels increased significantly ($P = 0.012$) during 3 follow-up days post-transplantation. The IRF7 mRNA levels did not significantly decrease on the day 4 and increased on the day 7 post-transplantation (Figure 2).

4.3. Comparison of the Expression Levels of IRF3 and IRF7 Genes Between the Liver Recipients with HBV Infection Who Rejected or Non-Rejected the Transplant

The expression levels of IRF3 and IRF7 genes were compared between the rejected and non-rejected liver recipients. The expression level of IRF3 in the rejected patients showed a significant upregulation (3.37 folds) on the day 1 ($P = 0.049$); however, downregulation on the days 4 (0.53 folds) and 7 (0.54 folds) was insignificant, compared with the non-rejected patients (Figure 3). The expression levels of IRF7 in the rejected patients showed insignificant upregulation on the days 1 (1.74 folds) and 7 (2.38 folds), but a significant downregulation ($P = 0.017$) was observed on

the day 4 (0.74 folds), compared with non-rejected patients (Figure 4).

4.4. Comparison of the Expression Levels of IRF3 and IRF7 Genes Between the HBV Infected Recipients Who Rejected or Non-Rejected the Transplant and the Healthy Controls

The expression levels of IRF3 and IRF7 genes in HBV-infected recipients who rejected or non-rejected the transplant were compared with those of the healthy controls. In rejected group, the mRNA levels of IRF3 showed upregulation on the day 1, but downregulation on the days 4 and 7 compared with those of the healthy controls. Modifications in mRNA expression were not significant on the days 1 and 7, but significant on the day 4 ($P = 0.03$) (Figure 5).

The mRNA expression levels of IRF7 in the rejected patients showed downregulation during all 3 follow-up periods, compared with those of the healthy controls. Modifications were significant on the days 1 ($P = 0.002$) and 4 ($P = 0.017$), but insignificant on the day 7 (Figure 6). Expression levels of IRF3 in non-rejected group showed a significant

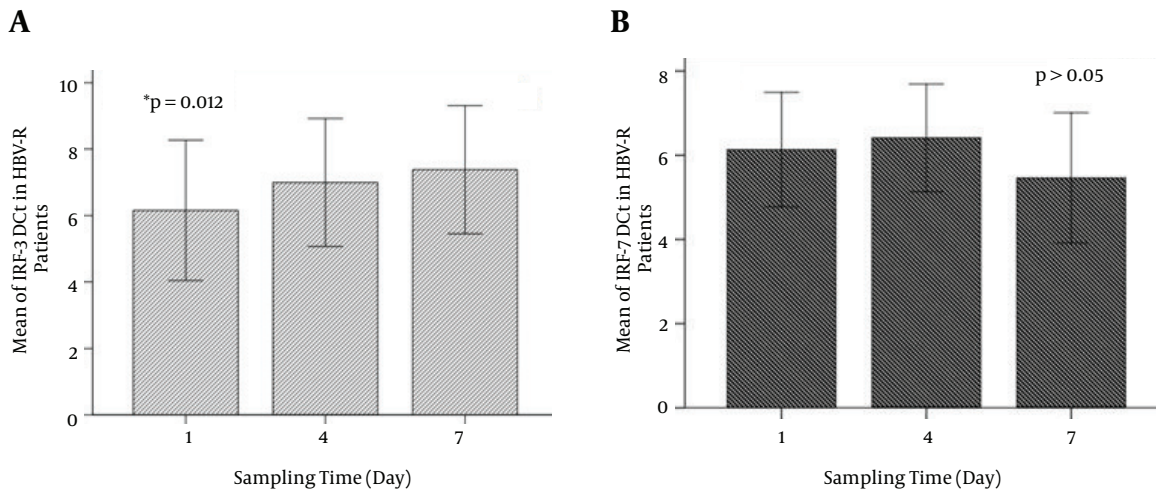


Figure 2. Comparison of the Expression Levels of IRF3 and IRF7 Genes on the Days 1, 4, and 7 Post-Liver Transplant Period in the HBV Infected Rejected Group (HBV-R).

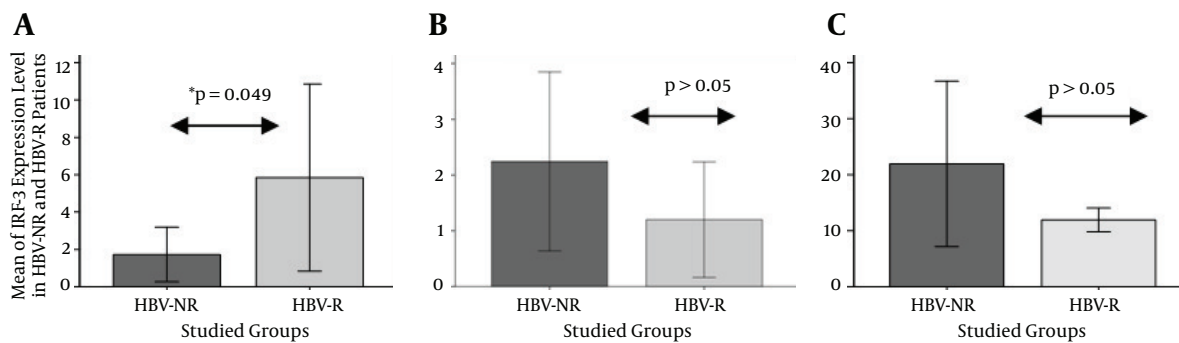


Figure 3. Comparison of the Expression Levels of IRF3 gene on the Day 1, 4, and 7 Post-Liver Transplant Period in the HBV Infected, Rejected and Non-Rejected Groups.

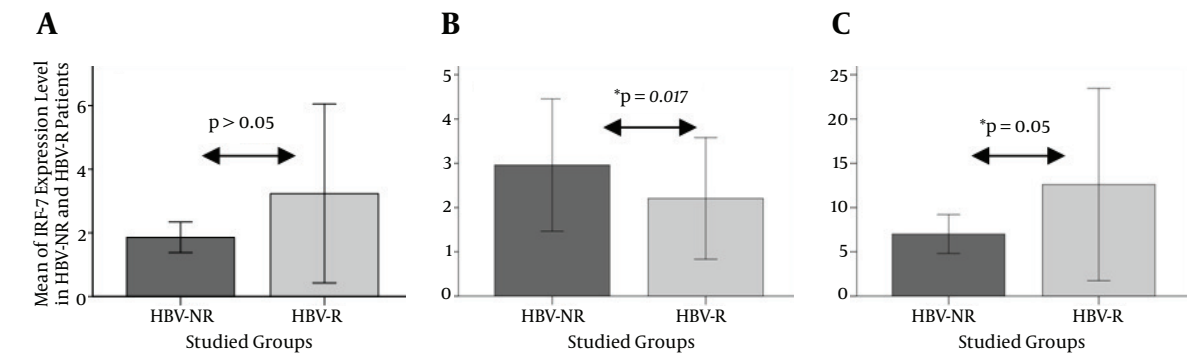


Figure 4. Comparison of the expression levels of IRF7 gene on the day 1, 4, and 7 post-liver transplant period in the HBV infected, rejected and non-rejected groups.

downregulation on the day 1 ($P = 0.039$), but insignificant upregulation on the days 4 and 7, compared with those of the healthy controls (Figure 7). The mRNA expression levels

of IRF7 in non-rejected patients showed a significant downregulation in all 3 follow-up time periods ($P = 0.003$, $P = 0.023$, and $P = 0.003$, respectively), compared with those of

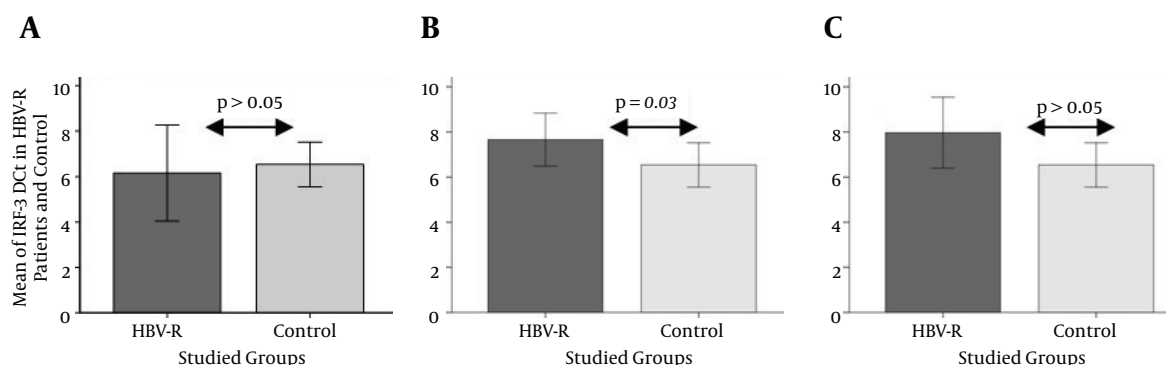


Figure 5. Comparison of the Expression Levels of IRF3 on the Day 1, 4, and 7 Post-Liver Transplant Period in Patients with Rejection and Healthy Controls.

the healthy controls (Figure 8).

5. Discussion

Transplantation is the final treatment of choice for chronic HBV infection, which causes the end-stage liver diseases (3-6, 25-27). Therefore, any changes in IFN production as an inflammatory response may influence the result of liver transplantation. The studies on the HBV infection in chimpanzees and transgenic mice showed that non-induction of intrahepatic genes such as type I and II interferon, early after HBV infection cause pervasive increase of HBV particles in liver (36-40).

In other studies, in HBV infected transgenic mouse model and in hepatoma cell line, IFN genes were induced and HBV replication was inhibited (40, 41). The expression of adaptor proteins such as TRIF and MyD88 in hepatoma cell line and activation of TLR signaling in transgenic mouse restrict and suppress HBV replication, respectively. In addition, other cytokines such as IL-12 and IL-18 controlled HBV replication mediated by IFN- γ and IFN α/β , respectively (41). These researches indicated the relationship between HBV infection and IFN production.

IRFs as components of innate immunity have antiviral effects including PRR-dependent IFN gene expression. IRF1 induces IFN α/β gene expression in cell type and time definite approach. Expression of the inflammatory chemokine by IRF5 induces IFN α/β genes in plasmacytoid dendritic cells. The antiviral effects of IRF1, IRF3, and IRF7 stimulate IFN production followed by overexpression of ISGs genes. IRF9 is one of the components of triplicate complex known as IFN-stimulated gene factor 3 (ISGF3) and induces the expression of ISGs genes (10-13, 15). Inflammation is one of the important complications occurs following the liver transplant and leads to destruction of liver graft if cannot be controlled (2). PRR-mediated inflammation increases

mRNA levels of innate immune components such as IRF3 and IRF7 genes, which induce IFN α/β production (1, 10).

On the other hand, chronic HBV infection as the main reason of liver transplant in Iran (42, 43) causes tissue inflammation and dysfunction (8) and may modify the IRF gene expression post-surgery. Viruses discipline to escape from innate immune systems involving IRFs such as IRF3 and IRF7 by managing their antiviral effect by PRR-mediated IFN gene induction (15).

It is documented that HBV proteins such as HBV polymerase, HBx, and HBsAg interfered in IRF3 and IRF7 functions to deal with innate immune responses (28, 31-33). Despite the fact, the innate immune system reduces HBV replication and HBV-mRNA stability (44, 45). In other words, HBV and innate immunity counter with each other (15). Activation of PRR signaling pathways induce inflammation and upregulation of the mRNA levels of innate immune components such as IRFs and induce IFN production, subsequently. It is likely that, among IRF molecules, IRF3, and IRF7 play key roles to induce IFN production following inflammation (1, 10). In this regard, initial activation of inflammatory response following liver transplant may cause acute graft rejection (2). Hence, evaluation of the expression levels of inflammatory molecules in transplant recipients, early post-transplant period, may help to the better management of acute liver rejection. Thus, the current study mainly aimed at evaluating the mRNA levels of IRF3 and IRF7 in HBV infected liver recipients with and without experiencing acute rejection.

There was no direct study on cross-talk between the expression levels of IRF3 and post-transplant inflammation yet. Previous studies on *TLR4*, a receptor of IRF3 signaling pathway, in transplant animal models showed that mice with knockout *TLR4* gene were protected from ischemia-reperfusion (I/R) injuries in liver graft (46). In another re-

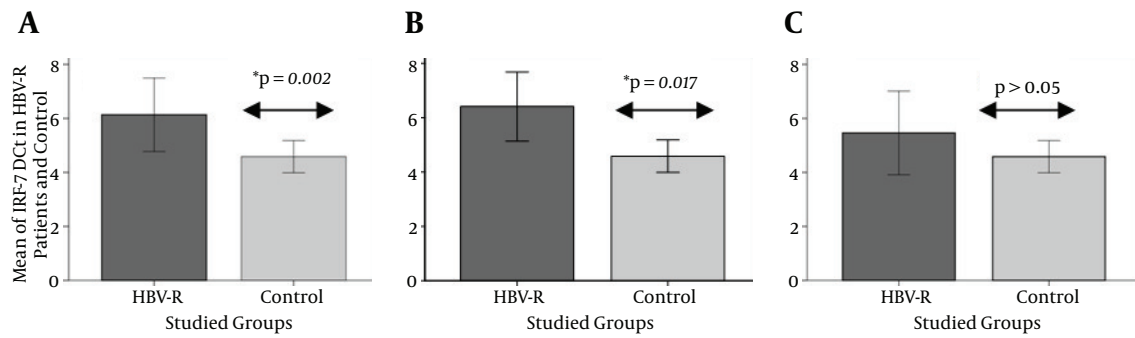


Figure 6. Comparison of the Expression Levels of IRF7 on the Days 1, 4, and 7 Post-Liver Transplant Period in Patients with Rejection and Healthy Controls.

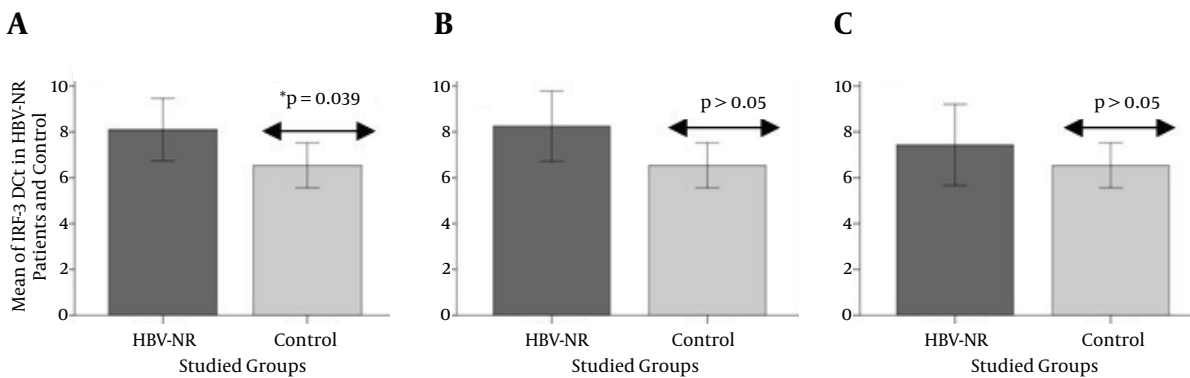


Figure 7. Comparison of the Expression Levels of IRF3 on the Days 1, 4, and 7 Post-Liver Transplant Period in Non-Rejected Patients and Healthy Controls.

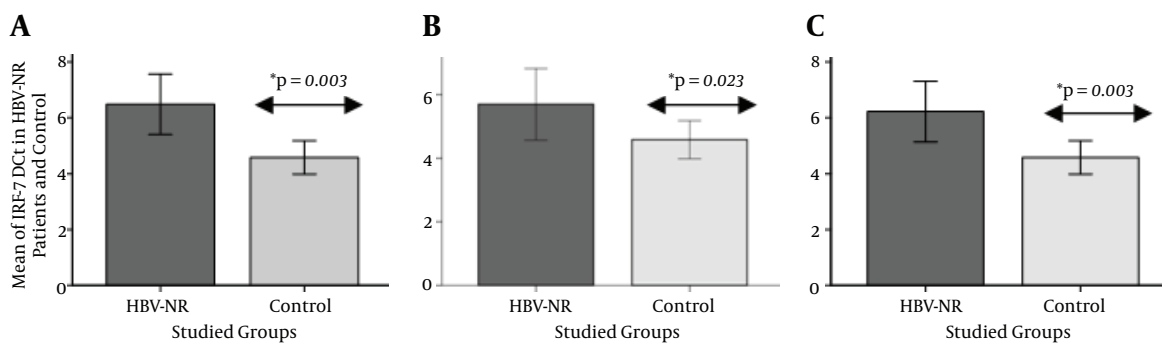


Figure 8. Comparison of the Expression Levels of IRF7 on the Days 1, 4, and 7 Post-Liver Transplant Period in Non-Rejected Patients and Healthy Control.

search, activation of innate immune system by the upregulation of IRF3 and *TLR4* genes was detected in I/R injuries of hepatic acute rejection (47). In other words, IRF3 may amplify inflammatory response in liver transplant.

The current study results demonstrated that modifications in IRF3 mRNA levels were not significant in non-

rejected and rejected recipients, separately. According to the previous studies on cross-talk between IRF3 and HBV (31-33), it seems that in the non-rejected patient group, lower levels of HBV may be the cause of IRF3 downregulation on the day 4, but use of anti-HBV drugs cause the reduction of HBV viral load and upregulation of the IRF3

on the day 7 post-surgeries. It seems that, in liver recipients experiencing acute rejection, HBV presence and I/R injuries encounter each other to decrease or increase the expression levels of IRF3, respectively. In other words, they led to change the inflammatory response following the liver transplant in HBV infected recipients. These results showed that HBV downregulated the expression of IRF3 gene during all 3 follow-up time periods, and promoted acute rejection in the patients.

In patients with liver rejection, the expression of IRF3 was upregulated on the day 1 (3.37 folds) by the effect of initial I/R injuries and downregulated on the day 4 (0.53 folds), and on the day 7 (0.54 folds) for the presence of HBV infection, compared with the none-rejected ones. Comparing the expression levels of IRF3 between patients with acute rejection and healthy controls revealed the role of I/R injuries in the upregulation of this factor on the day 1 and downregulation for the presence of HBV infection on the days 4 and 7 post-liver transplantation. The HBV infection in non-rejected patients decreased the expression of IRF3 during 3 follow-up time periods, compared with the healthy controls.

IRF7 also induces the expression of IFN in innate immune signaling pathways (12). No relationship was observed between IRF7 and any human or animal models of transplantation yet, but the cross-talk between IRF7 and HBV infection was reported in a few studies (29, 30). In a research focused on the expression levels of TLR signaling molecules such as IRF7, *IRAK1*, *IRAK4*, and *TRAF3* in chronic HBV infected patients, results indicated that IRF7 gene was downregulated (29). In other research, defective production of IFN- α , induced by IRF7, was found in chronic HBV infected patients (30).

In the current report, the expression of IRF7 was upregulated on the day 4 in non-rejected recipients after using anti-HBV drugs and activation of immune system. But, acceptance of liver graft and reduction of innate immune responses was followed by the downregulation of IRF7 on the day 7 post-surgery.

HBV infection and I/R injuries encountered each other and affected IRF7 expression in recipients who experienced rejection. In such patients, the HBV interaction with immune system leads to the downregulation of IRF7 on the day 4 post-surgery. But, initial I/R injuries promoted rejection and increased inflammation and caused overexpression of IRF7 on the day 7 post-transplants.

The comparison of expression levels of IRF7 between rejected and non-rejected patient groups and also within patients experiencing rejection showed that initial I/R injuries induced inflammatory response and overexpression of IRF7 in the rejected group especially in the day 1 (1.74 fold). However, the presence of HBV infection downreg-

ulated IRF7 mRNA levels on the day 4 (0.74 fold), promoted the rejection, increased the inflammation, and caused overexpression of IRF7 on the day 7 (2.38 folds) post-transplant. The expression of IRF7 downregulated in rejected and non-rejected liver recipients during all 3 follow-up time periods, based on the presence of HBV infection, compared with healthy controls.

Consequently, results of the current study demonstrated that the presence of HBV infection and I/R injuries can lead to decrease and increase of inflammatory responses following the liver transplantation. Evaluation of the expression levels of IRF3 and IRF7, as components of innate immune system in PBMCs of liver recipients showed different patterns in patients with and without experiencing acute rejection, but confirmed the role of HBV infection and I/R injuries during inflammatory signaling pathways in such patients. All in all, down regulation of the IRF3 gene expression, early post-transplant, in rejected patient group can present suitable candidates for acute rejection biomarker; the hypothesis requires confirmation in future studies.

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