Association between *IRF1* Gene Expression and Liver Enzymes in HBV-infected Liver Transplant Recipients with and without Experience of Rejection

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ABSTRACT

Background: Liver function indices and anti-viral immune regulatory markers can both improve graft outcomes, which lead to better post-transplantation management and increase the possibility of surveillance in liver transplant recipients with chronic hepatitis B virus (HBV) infection.

Objective: To determine the association between the interferon regulatory factor 1 (IRF1) mRNA levels and liver enzymes in HBV-infected liver transplant recipients with and without experience of rejection.

Methods: A total of 46 chronic HBV-infected patients who had undergone liver transplant surgery was divided into 2 groups of recipients "with rejection" and "without rejection.". Blood samples were collected form each patient on days 1, 4, and 7 post-transplantation. A SYBER GREEN real-time PCR was used to evaluate the expression level of *IRF1* in liver recipients. Liver enzyme activities were also measured in all patients.

Results: The expression of *IRF1* in the patients with rejection was up-regulated at all 3 follow-up days compared with those without rejection. The serum levels of ALT and AST were more than normal levels at 3 follow-up times in both study groups. Significant differences were found in *IRF1* gene expression levels and also serum ALT levels between those with and without rejection after 7 days post-transplantation.

Conclusion: The *IRF1* expression and serum ALT levels were increased significantly in patient with rejection compared to those without rejection. IRF1, an inflammatory factor, may also intensify induction of inflammatory pathways in engrafted liver and promote liver inflammation and injuries leading to liver enzymes elevation in patients with graft rejection.

KEYWORDS: IRF1; Liver trasnplantation; Hepatitis B virus; Liver enzyme

INTRODUCTION

Liver transplantation is the final treatment for end-stage liver diseases such as advanced chronic hepatitis B virus (HBV) infection, which leads to liver cirrhosis and/or hepatocellular carcinoma. Approximately, 350 to 400 miliion people have been sufferd from chronic HBV infection in all over the world. The complicate mechanism involved in HBV pathogenesis is a serious problem during treatment of chronic viral infection and need for liver transplantation as the final recommended anti-HBV therapy [1-5].

The clinical outcomes of liver transplant depend on the recipient's pre-operative underlying disease, status of new graft and the complexity of the surgery. Despite use of immunosuppressive condition regimens and antimicrobial therapy, the liver graft may be rejected by immune reactivation of the recipi-

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ent, which may lead to graft loss and morbidity post-transplantation [6-9]. Therefore, the results of liver function tests (LFTs), which comprises the measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct bilirubin (DiBi), and albumin (ALB) with other clinical data such as radiology help to evaluate the status of the liver graft and predict rejection post-surgery [9, 10]. Among LFT tests, assessment of ALT and AST activities as liver indices are valuable. AST and ALT are aminotransferase enzymes that remove α-amino groups from aspartate and alanine to produce oxaloacetic and pyruvic acids, respectively. AST is expressed in the liver, heart, skeletal muscle, kidneys, brain and red blood cells, but ALT is mainly found in the cytosol of hepatocyte and is expressed in limited concentration in heart, skeletal muscle and kidney. Acute or chronic liver diseases such as alcoholic, autoimmune, cholestatic, and metabolic liver diseases or viral infections such as HBV and HCV lead to increased serum concentrations of aminotransferases [11-13].

In recent reports, early detection of immune response can accelerate diagnosis of liver graft rejection episodes, which leads to better management of patient's surveillance. Accordingly, a change in the expression of diverse immune factors such as proinflammatory cytokines has been evaluated post-liver transplantation [14]. Interferon regulatory factors (IRFs) are a family of transcription factors in the immune system that has nine members in mammals (IRF1–IRF9). They have important roles in the innate and acquired immunity, apoptosis, cell growth regulation and oncogenesis. Function of each IRF molecule is correlated to cell type and protein-protein interactions with other members of this family and other transcription factors or cofactors [15-18].

IRF1 is a member of IRF family which organizes gene expression during inflammation and innate and acquired immune response. At first, it was recognized as an inducer factor for expression of IFN-I and interferon-stimulated response element (ISRE) genes [17, 18]. Expression of IRF1 gene is continuously in all cell types but is up-regulated in response to IFN-I, IFN-II, dsRNA, cytokines and hormones. One of the key signaling pathways for stimulation and activation of IRF1 is IFN-γ/TLR9 pathway which in turn induces the expression of IFNβ, IL-12A, IL-12B and iNOS genes [17-20]. Defective expression of these genes in dendritic and macrophage cells with knock out IRF1 gene has been confirmed the role of IRF1 to intensify IFN-γ effects during inflammation [15, 18]. Therefore, the objective of this study was to evaluate the expression level of IRF1 mRNA and liver enzymes in HBV-infected recipients with and without experience of acute rejection post-liver transplantation.

MATERIAL AND METHODS

A total of 46 chronic HBV-infected patients who had undergone liver transplant surgery at Namazi Hospital, Shiraz University of Medical Science, Shiraz, Iran between 2012 and 2014 were recruited in this studied. Based on the pathological results, recipients were divided into two groups: those with experience of rejection (n=20) and without experience of rejection (n=26). Blood samples were collected from each studied patients on day 1, 4, and 7 post-transplantation. PBMCs and plasma were isolated from each participant using Ficol gradient and stored at -80 °C for further molecular and biochemical analyses.

Liver Enzymes Assay

Liver enzymes such as ALT and AST were also measured on day 1, 4, and 7 for each paticipant post-transplantation.

Molecular Assay

Total RNA was extracted from patient's buffy coats using RNX plus (CinnaGen, Iran). Quality and quantity of the isolated RNA was analyzed using Nanodrop at 260/280 nm. The cDNA synthesis was done using PrimeScript RT Reagent kit (Takara, Otsa, and Shiga, Japan) based on manufacturer instruction. The expression of IRF1 in recipients with and without rejection was measured using an in-house SYBR green real-time PCR protocol by

Table 1: Distribution of blood groups in HBV-infected recipients with and without experience of rejection

Blood group	With rejection (n=20)	Without rejection (n=26)
A^+	4 (20%)	8 (31%)
B^+	5 (25%)	7 (27%)
AB^+	1 (5%)	1 (4%)
O^+	9 (45%)	9 (35%)
A^-	1 (5%)	0 (0%)
O^-	0 (0%)	1 (4%)

Step One Plus Real-Time Instrument (ABI, Step One Plus, USA). Relative glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an internal control for test error elimination. The PCR mix and conditions of IRF1 and GAPDH gene transcripts have previously been described [21]. The melting point curves for GAPDH and IRF1 genes were used to investigate the specificity and contamination of PCR reactions.

Statistical Analysis

The levels of IRF1 transcripts was compared between the two study groups at three different times using the $2^{-\Delta\Delta CT}$ (Livak) method. Statistical analyses were performed using SPSS® for Windows® ver 16 (IBM Corporation, Armonk, NY, USA). A p value <0.05 was considered statistically significant.

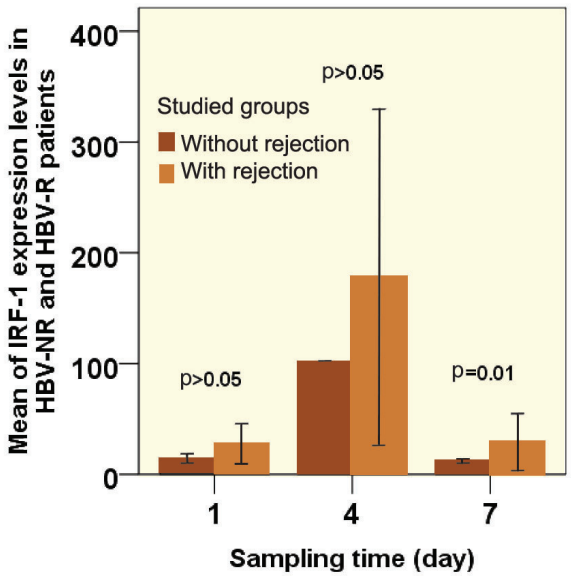

Figure 1: *IRF1* expression on days 1, 4 and 7 post-liver transplantation in HBV-infected recipients with and without experience of rejection. Error bars represent 95% CI of the mean.

RESULTS

Recipients without history of rejection had a mean±SD age of 51.6±10.6 (range: 26–74) years; the mean±SD age of those with a history of rejection was51.0±10.6 (range: 27–69) years. Nineteen (73%) of the former group and 17 (85%) of the latter group were male. The O^+ blood group was more frequent in participants (Table 1).

The mean ALT level was higher more in those who experienced rejection compared to those without rejection at all three follow-up times. The mean±SD ALT levels on days 1, 4 and 7 post-transplantation were respectively, 923.0±917.9, 702.9±792.0, and 286.9±222.4 U/L in recipients with rejection; the values for those without rejection were 725.4±564.1, 484.1±536.0, and 182.6±167.3 U/L, respectively. The mean serum AST in those without rejection on days 1 and 7 was higher than and on day 4 was lower than that recorded for recipients with rejection. The mean±SD AST levels on days 1, 4 and 7 post-transplantation were respectively 1302.9±1364.4, 378.1±577.7, and 79.8±34.1 U/L in those with rejection, and 1415.4±1009.3, 261.6±315.1, and 81.3±84.0 U/L in recipients without rejection.

The mRNA Expression Levels of IRF1 in HBV-infected Recipients with and without Rejection

The expression of IRF1 on days 1, 4 and 7 post-liver transplantation between HBV-infected recipients with and without experience of rejection is presented in Figure 1. The expression levels of IRF1 in recipients with rejection was up-regulated at all three follow-up times compared to those without rejection. The difference was not significant on days 1 and 4, but it was significant (p=0.01) on day 7 post-liver transplantation.

Serum Level of Liver Enzymes in HBV-infected Patients Post-liver Transplantation

The mean serum activity of liver enzymes be-

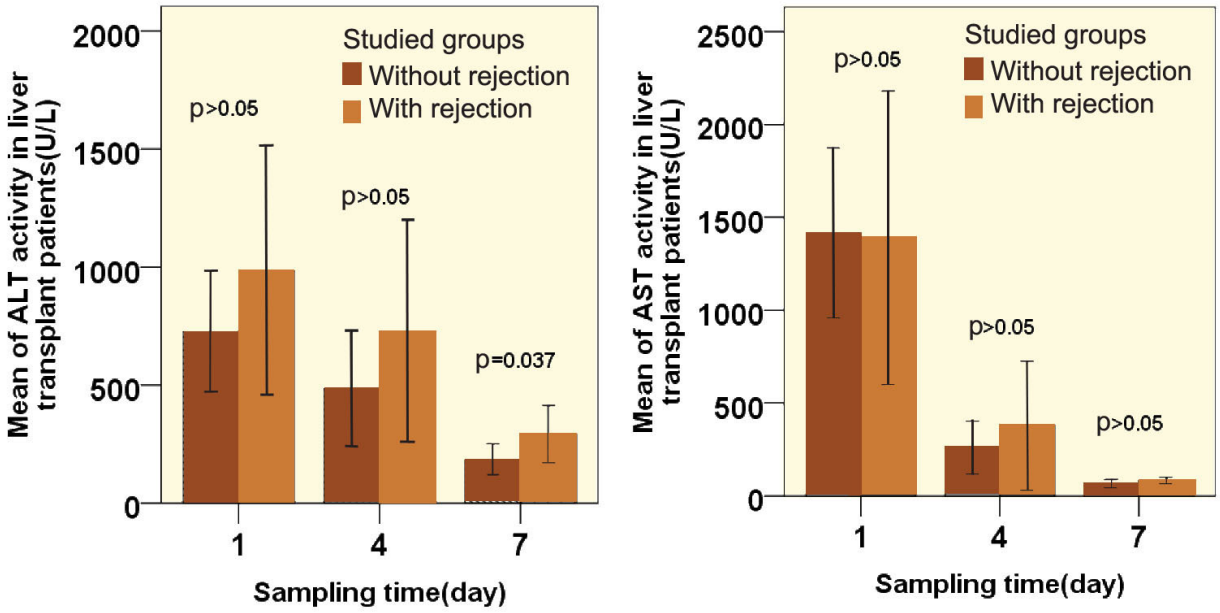

Figure 2: Mean serum activity of liver enzymes in on days 1, 4 and 7 post-liver transplantation in HBV-infected recipients with and without experience of rejection. Error bars represent 95% CI of the mean.

tween the two study groups is presented in Figure 2.

The serum levels of ALT and AST were more than normal limit (ALT: 7–56 U/L, and AST: 5–40 U/L) at three follow-up times in both study groups. However, the level of these enzymes was decreased during the first week post-liver transplantation. The mean serum AST was not significantly different at three follow-up times between the two study groups. However, the difference for serum ALT level on day 7 post-transplantation was significant (p=0.037).

DISCUSSION

The liver graft may be rejected by the immune system of the recipient, so early diagnosis of the rejection episode may help better management of patient and graft survival [1]. IRF1 is one of the important regulatory factors in the immune system and has recently been considered a biomarker for acute rejection. In a study, Tsung and colleagues investigated the role of IRF1 in ischemia/reperfusion injuries post-liver transplantation. Earlier reports have been shown that IRF1 modulates the expression of some inflammatory markers and intensified ischemia/reperfusion injuries post-transplantation [22]. In another research, Ueki and colleagues got similar results [23]. In a research by Hama and colleagues the expression of IRF1 gene was investigated in an animal model of acute liver rejection. They showed that IRF1 is one of the genes induced by IFN-γ in PBMCs and acute liver graft rejection [24]. In a new list prepared by Germani and colleagues, some pro-inflammatory and modulatory cytokines are introduced as biomarkers of acute liver rejection. Among these biomarkers, IFN-γ and TNF-α, which induce IRF1 expression, are seen [14]. In this project the expression levels of IRF1 in HBV-infected liver recipients with and without experience of rejection was evaluated on days 1, 4 and 7 post-transplantation. In those with history of rejection the mRNA level of IRF1 at three follow-up times was up-regulated compared to those without rejection. This result was similar with earlier animal studies [22, 23, 25]. Activation of the IRF1 signaling pathway during acute rejection may cause up-regulation of IRF1 in these patients. IFN-γ is an inflammatory cytokine which induces IRF1 expression and causes promotion of liver rejection in animal models [17-20, 25]. Therefore, IRF1 may reflect the IFN-γ effects in recipients experienced rejection.

Measuring serum ALT and AST levels in HBV-infected recipients was done for evaluation of patient's liver status. Liver injuries cause increased serum activity of these enzymes [9, 10]. In a research by Zheng and collogues, the correlation of NK cell activity with serum ALT levels was investigated in HBV-infected patients. Results illustrated that during NK cell activation the expression of TNF-α, IFN-γ was up-regulated. However, there was no direct correlation between over-expression of pro-inflammatory cytokines (TNF-α or IFN-γ) and serum ALT levels [26]. Yu and collogues evaluated the association between the percentage of Th9 cells, levels of IL-9 and IL-10 with ALT level, could not confirm any relations [27]. In another study by Li and collogues, the expression levels of NK cell receptor (NKp64) was negatively correlated with HBV DNA and ALT levels in HBV-infected patients [28].

Acute or chronic liver injury, such as viral hepatitis infections, lead to increased serum activity of aminotransferases. Among these enzymes, ALT is accumulated in the cytoplasm whereas AST has cytosolic (20% of its total activity) and mitochondrial (80% of its total activity) transcripts in hepatocytes. After liver damage, ALT is released from injured cells, which causes an increase in serum ALT activity. So ALT is a more specific indicator for the treatment and evaluation of viral-related liver damage in clinical practice. Variation in ALT level during the HBV infection was reported by Liaw, et. al. [29-31].

Aligned with previous research in the present study, serum ALT and AST activities were increased more than the upper reference limit in all HBV-infected recipients at three follow-up times. The variation in serum ALT and AST levels can be divided into three categories: <5 times the upper reference limit (mild), 5–10 times the upper reference limit (moderate), and >10 times the upper reference limit (marked) [29]. The mean ALT level on days 1, 4 and 7 post-transplantation was respectively "marked," "marked," and "moderate" in recipients with experience of rejection, and "marked," "moderate," and "mild" in those without rejection. The mean AST level at respective days were "marked," "moderate," and "mild" in those with rejection, and "marked," "mild," and "mild" in recipients with rejection. ALT and AST levels decreased during the first week post-liver transplantation, but the rate of decrease in ALT was slower than that in AST. The serum activity of aminotransferases was more in recipients with experience of rejection compared to those without.

In this study, the expression of IRF1 and serum ALT level in recipients with rejection was increased significantly on the 7th day post-transplantation. It seems that in recipients with rejection, HBV infection stimulated IFN-α/β and IFN-γ, as the innate immune system markers. Stimulated IFNs activate Jak-STAT signaling pathway and cause over-expression of IRF1, which is a regulator of pro-inflammatory genes during liver allograft transplant inflammation [32-34]. IRF1 might intensify induction of inflammatory pathways in liver graft and promote liver inflammation and injuries in those with history of rejection post-operatively, which elevate liver aminotransferases. Elevation of the ALT and AST levels reflected the effect of IRF1 transcript elevation in HBV-infected recipients who experienced rejection.

In conclusion, IRF1 expression and serum ALT level were increased significantly in recipients with rejection compared with those without rejection 7 days after transplantation. Increasing serum AST and ALT levels in HBV-infected recipients experienced rejection is an interesting topic to be further evaluated in future studies.

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CONFLICTS OF INTEREST: None declared.

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