

Original paper

# Relation between levels of toll-like receptors 3 and 7 and clinical profile of Child-Pugh B cirrhotic patients

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## Abstract

**Aim of the study:** Growing data show that toll-like receptors (TLRs) have considerable roles in the pathogenesis of many liver diseases. We aimed to study the relation between TLR3 and TLR7 levels and clinical manifestations of liver decompensation among hepatitis C virus (HCV)-infected Child-Pugh B patients.

**Material and methods:** This study included 60 adult patients with Child-Pugh B liver cirrhosis on top of untreated HCV infection. We performed a two-step clustering algorithm depending on TLR-3 gene expression, TLR-7 gene expression, and other influential patients' characteristics.

**Results:** Patients were optimally divided into 2 clusters, each cluster containing 30 patients. The average silhouette score of the clustering algorithm was 0.52, indicating a good clustering power of the model. Patients in cluster 1 showed lower relative expression of TLR3 (0.188 vs. 0.29). The same was true of TLR7 (0.20 vs. 0.31). All patients within cluster 1 had lower limb edema and 93% of them had ascites. On the other hand, no one within cluster 2 had ascites or lower limb edema. The mean platelet count was lower in patients within cluster 1 (74,000 vs. 100,000 cell/mm<sup>3</sup>). The mean international normalized ratio (INR) level was higher in cluster 1 (1.61 vs. 1.3). The mean Model for End-Stage Liver Disease (MELD) score was higher in cluster 1 (15 vs. 10).

**Conclusions:** From these results, we can suggest that lower TLR3 and TLR7 can lead to worse clinical manifestations among patients with HCV-related liver cirrhosis. A deeper exploration of this point can open the door for new approaches for managing decompensated cirrhosis.

**Key words:** chronic hepatitis C, toll-like receptors, decompensated cirrhosis.

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## Introduction

Hepatitis C virus (HCV) is one of the most prevalent causes of chronic liver diseases, which results in inflammatory and wound-healing responses leading to liver fibrogenesis and hepatocellular carcinoma (HCC) development [1, 2].

Toll-like receptors (TLRs) have a major role in body immunity through identifying pathogen-associated

molecular patterns (PAMPs) [3]. Many studies have shown that TLRs have an important role in the pathogenesis of many liver diseases including viral hepatitis, non-alcoholic steatohepatitis (NASH), alcoholic hepatitis, autoimmune hepatitis, hepatic fibrogenesis, and liver carcinogenesis [4-8].

Decreased expression of TLR3 and TLR7 in cells infected with HCV has been shown [9-11]. Treatment with interferon  $\alpha$  (IFN- $\alpha$ ) was used to counteract this effect.

In a study performed by Horsmans *et al.*, treatment with intravenous isatoribine given as an agonist for TLR7 caused a significant decrease in HCV viral load [12]. TLR7 agonist was shown to decrease HCV viral load in another study [13].

TLR3 deficiency is also linked to the progression of liver fibrosis. Treatment of mice with the TLR3 ligand poly I:C was shown to inhibit liver fibrogenesis [14-16]. Also, TLR7 down-regulation was suggested to contribute to liver fibrosis progression [17].

We aimed to study the relation between TLR3 and TLR7 levels and clinical manifestations of liver decompensation among HCV-infected Child-Pugh B patients.

## Material and methods

### Materials

This study included 60 adult patients with Child-Pugh B liver cirrhosis on top of untreated HCV infection who were referred to the Internal Medicine Department, Kafr Elsheikh University Hospital. Patients with HBV or HIV co-infection and patients with hepatocellular carcinoma were excluded. Informed consent was obtained from all participants. Performance of the study was approved by Kafr Elsheikh Faculty of Medicine Ethical Committee.

### Methods

Blood samples were collected from the included patients for evaluation of complete blood picture (CBC), prothrombin time (PT), international nor-

malized ratio (INR), liver and kidney function tests, HBs antigen, anti-HCV antibody by enzyme-linked immunosorbent assay (ELISA), and serum  $\alpha$ -fetoprotein (AFP) levels. HCV RNA was assayed by reverse transcription-polymerase chain reaction (RT-PCR) to confirm ongoing viremia.

Determination of relative levels of TLR3 and TLR7 expression [18]: Relative levels of TLR3 and TLR7 gene expression were determined by Rotor-Gene Q Real-Time PCR instruments from QIAGEN USA. RNA was extracted by a QIAamp RNA Blood Mini Kit (Qiagen, Germany) on a QIAcube (Germany). After this, mRNA was transcribed into cDNA using the Omniscript reverse transcription kit (Qiagen, Dusseldorf, Germany). To quantify the relative expression of each gene, the comparative  $\Delta\Delta C_t$  method was used.

Clustering algorithm [19]: A two-step clustering algorithm was performed depending on: age, gender, presence of lower limb edema, presence of ascites, INR, platelets, total leucocyte count, hemoglobin, total bilirubin, creatinine levels, FIB-4 score [20], Model for End-Stage Liver Disease (MELD) score [21], TLR-3 gene expression and TLR-7 gene expression.

## Results

Patients were optimally divided into 2 clusters, each cluster containing 30 patients. The average silhouette score of the clustering algorithm was 0.52, indicating a good clustering power of the model. Patients in cluster 1 showed lower relative expression of TLR3 (0.188 vs. 0.29). The same was true of TLR7 (0.20 vs. 0.31). All patients within cluster 1 had lower limb edema and 93% of them had ascites. On the other hand, no one within cluster 2 had ascites or lower limb edema. The mean platelet count was lower in patients within cluster 1 (74,000 vs. 100,000 cell/mm<sup>3</sup>); the same was true of mean hemoglobin levels (8.4 vs. 9.5 g/dl) and total leucocyte count (2.36 vs. 3.6  $\times 10^3$  cell/mm<sup>3</sup>). Mean INR level was higher in cluster 1 (1.61 vs. 1.3) and the same was true of creatinine (1.66 vs. 0.97 mg/dl). The mean MELD score was higher in cluster 1 (15 vs. 10). Interestingly, the mean FIB-4 score was higher in cluster 1 (5.17 vs. 4.27) (Table 1).

## Discussion

Toll-like receptors activate the innate immune system through the identification of PAMPs [3]. TLRs also play roles in activation of the adaptive immune system, regulation of inflammatory responses, cellular regeneration, and carcinogenesis [2, 4, 9, 14].

**Table 1.** Characteristics of the 2 clusters

| Item   | Cluster 1 | Cluster 2 |
|--|-----------|-----------|
| Mean age (years)                                   | 46.67     | 52.07     |
| Males (%)  | 53.3      | 53.3      |
| Lower limb edema (%)                               | 100       | 0         |
| Ascites (%)  | 93.3      | 0         |
| Mean relative levels of TLR-3 gene expression      | 0.19      | 0.29      |
| Mean relative levels of TLR-7 gene expression      | 0.21      | 0.31      |
| Mean INR level                                     | 1.61      | 1.30      |
| Mean platelet count (cell/mm <sup>3</sup> )        | 74.27     | 100.73    |
| Mean total leucocyte count (cell/mm <sup>3</sup> ) | 2.36      | 3.63      |
| Mean total bilirubin level (mg/dl)                 | 1.17      | 1.06      |
| Mean hemoglobin level (g/dl)                       | 8.47      | 9.53      |
| Mean creatinine level (mg/dl)                      | 1.66      | 0.97      |
| Mean FIB-4 score                                   | 5.17      | 4.27      |
| Mean MELD score                                    | 15.2      | 10.79     |

The ability of HCV to inhibit the immune response aiming to maintain its persistence has been revealed [13]. Decreased expression of TLR3 and TLR7 in cells infected with HCV via inhibitory effects of HCV proteins aiming to prevent the immune system from clearing the virus has been shown [17, 18]. Treatment with IFN- $\alpha$  was used to counteract this effect.

Moreover, deficiency of TLR3-mediated natural killer (NK) cell-dependent apoptosis of hepatic stellate cells (HSCs) has been associated with the progression of liver fibrogenesis in patients with alcoholic hepatitis [23]. It was suggested that treatment with the TLR3 ligand poly I:C induces NK cells which promote apoptosis of HSCs [24-26].

Lower expression of TLR7 was found in malignant hepatocytes compared with non-malignant hepatocytes among patients with HCC [27]. Also, phosphorothioate-modified TLR9 agonist was suggested to have anti-HCC activity [28]. Strangely, another trial reported different results. This trial showed that TLR7 and TLR9 were overexpressed in the HCC biopsies compared with biopsies from patients with liver cirrhosis but without HCC. The authors suggested that the inhibition of TLR7 and TLR9 with IRS-954 or chloroquine might be used for HCC prevention in susceptible patients [29].

We aimed to study the levels of TLR3 and TLR7 in patients with HCV-related decompensated cirrhosis lying within Child-Pugh class B and to determine if there is a relation between their levels and clinical manifestations of patients.

In our clusters, 100% of patients among the cluster with lower TLR3 and TLR7 had lower limb edema and 93% had ascites, while no patient within the other cluster had lower limb edema or ascites. Also, lower TLR3 and TLR7 levels were associated with lower albumin, hemoglobin, platelets, and total leucocyte count. Also, lower TLR3 and TLR7 levels were associated with higher INR, total bilirubin, FIB-4, and MELD scores.

From these results, we can suggest that lower TLR3 and TLR7 can lead to worse clinical manifestations among patients with HCV-related liver cirrhosis. These findings need to be validated on a larger population and to be explored among patients with treated HCV. Also, the pathogenesis of these findings needs to be investigated. This can open the door for new approaches for managing decompensated cirrhosis through studying the effect of TLR3 and TLR7 agonists on the clinical outcomes of these patients.

## Disclosure

The authors declare no conflict of interest.

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