RESEARCH ARTICLE

**TLR3 Gene Polymorphism in HCV Infection in the Kazakh Population of Western Kazakhstan**

Gulzhanat Nurlanova¹*, Galina Zhumagaliyeva², Aigerim Iskakova¹, Akezhan Astrakhanov¹, Meirambek Kurmangazin¹

Abstract

**Background and Objectives:** Hepatitis C virus (HCV) infection is a common cause of cirrhosis worldwide, leading to significant economic and social burdens. Approximately 170 million people (3% of the population) are infected with HCV, with the risk of developing complications such as cirrhosis and hepatocellular carcinoma. In the United States, HCV is the main cause of liver cirrhosis, accounting for 26% of cases. Recent studies have shown an increase in the proportion of HCV-related liver cirrhosis. **Materials and Methods:** A total of 102 patients with chronic hepatitis C in the reactivation phase from the Atyrau and Aktobe regional hepatology centers, who had not previously received antiviral therapy, were examined. A control group, matched by gender and age, included 127 practically healthy individuals of Kazakh nationality. All patients underwent a comprehensive examination, which included a complete blood count, a biochemical blood analysis and PCR for HCV. Venous blood samples were taken from all subjects for molecular genetic analysis. Genotyping of TLR3 polymorphism (rs5743312, rs5743305, rs3775291, rs5743311, rs1879026) was performed using real-time PCR. Thes study is a case control study. **Results:** In patients with cirrhosis of the liver resulting from chronic hepatitis C (HCV), the results of biochemical analysis were statistically significantly higher than in patients with HCV without liver cirrhosis: the levels of total bilirubin (p 0.017*), alkaline phosphatase (p 0.022*), and gamma-glutamyl transferase (0.041*) were elevated. The results indicated that the CC genotype of TLR3 rs1879026 was associated with the development and chronicity of HCV infection compared to practically healthy individuals (p=0.001). In the distribution of genotypes and alleles for rs5743312, rs5743305, rs3775291, and rs5743311, no significant differences were found between patients with HCV and the healthy control group. **Conclusion:** The TLR3 rs1879026 gene polymorphism plays a significant role in the predisposition to HCV infection in the Kazakh population of the Atyrau and Aktobe regions. **Keywords:** Hepatitis C- TLR3- rs5743305- rs5743312- rs5743311- rs1879026- rs3775291- Kazakhstan

Introduction

Hepatitis C virus (HCV)-infection is one of the most frequent causes of cirrhosis worldwide. It entails a significant economic and social burden and increased health care costs. According to WHO, 170 million people worldwide (3% of the population) are infected with hepatitis C virus (HCV), with the risk of long-term adverse outcomes such as cirrhosis (LC) and hepatocellular carcinoma (HCC). In the United States, viral hepatitis C is the leading cause of LC, accounting for 26% of cases. In recent years, the structure of viral LC has changed toward increasing its proportion to 30.3% in the outcome of CHC (Xiao et al., 2009). Extrahepatic manifestations of HCV infection include deterioration of quality of life and mood disorders (He et al., 2022).

There are known studies devoted to the study of genetic variants associated with the development of chronic forms of viral infectious hepatitis and its complications. According to MashaelR, Al-Anazietall found a TLR3 toll-like receptor gene polymorphism associated with the development of liver cirrhosis and HCV-ethiological hepatocellular carcinoma in the Saudi Arabian population (Al-Anazi et al., 2017). TLR3 recognize hepatitis C virus RNA and trigger primary inflammatory cascades in response to virus penetration. TLR3 is known to affect the duration and chronicity of the inflammatory process in HCV infection with subsequent pathological changes in liver cells (development of cirrhosis, fibrosis, hepatocellular carcinoma) (Graham and Hill, 2001). Many studies have shown that TLRs play an important role in the pathogenesis of many liver diseases, including viral hepatitis, nonalcoholic steatohepatitis, alcoholic hepatitis, autoimmune hepatitis, fibrogenesis and liver cirrhosis.

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carcinogenesis (El-Bendary et al., 2018; Khambu et al., 2019; Nasiri et al., 2020; Tong and Guo, 2019). Al-Anazi et al. (2017) identified allelic variants of SNP rs8099917 gene polymorphisms among the Central Asian group. TLR gene polymorphisms in viral infectious hepatitis vary among different nations. And virtually no genetic markers of predisposition to hepatitis C have been studied in the Kazakh population of Western Kazakhstan. The aim of the study was to examine the effect of TLR3 single nucleotide gene polymorphism (SNP) on HCV susceptibility, chronicity and outcomes (liver cirrhosis) in a Kazakh population in Western Kazakhstan.

Materials and Methods

The study protocol corresponded to the Helsinki Declaration of 1,975 and was approved by the local ethical committee of the Marat Ospanov Western Kazakhstan Medical University, protocol №65 of March 13, 2020.

One hundred two patients with CHC in the reactivation phase of Aktobe and Atyrau regional hepatology centers, who had not previously received antiviral therapy, were examined. Distribution by sex: men - 52 (51%), women - 50 (49%). The mean age of the patients was 46.8 (18-60) years with 2.65±1.8 years of infection from the moment of detection. The control group, comparable in sex and age, included 127 essentially healthy people. All patients signed an informed consent to participate in the study.

The diagnosis was made according to the “Clinical Protocol for the diagnosis and treatment of chronic HCV in adults” №23 of October 23, 2020 Хронический гепатит С у взрослых > Клинические протоколы МЗ РК - 2020 (Казахстан) > MedElement on the basis of clinical, epidemiological and laboratory data, detection of antibodies (antibodies) to HCV antigens by immunofluorescence (ELISA) and verification of HCV ribonucleic acid (RNA) by polymerase chain reaction (PCR) on a PR 4100 photometer manufactured by BIORAD. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined using an automatic biochemical analyzer “Architect-4000” (USA). HCV genotype 1 was detected in 38 (37.2%) of the 102 patients studied, genotype 2 in 15 (14.7%), and most frequently genotype 3 was detected in 49 (48%) patients. At the time of inclusion in the study, low viremia (<8x10^5 IU/ml) was observed in 38.2% (n=39) of patients, moderate (~8x10^5 IU/ml), - in 46% (n=47), high (~>2.4x10^7 IU/ml) - in 14.7% (n=15). During antiviral therapy sofosbuvir + daclatasvir at 24 weeks of follow-up, a sustained virological response was achieved in the majority of patients (89.2%).

All subjects had venous blood sampling for molecular genetic analysis. Genomic DNA isolation from peripheral blood of the subjects was performed using TestGen DNA-Blood-M-100 reagent kits. The principle of the method used in the kit is based on reversible binding of nucleic acids on the surface of magnetic particles. Genotyping of the TLR3 polymorphism (rs5743312, rs5743305, rs3775291, rs5743311, rs1879026) was performed by real-time PCR on a DT-Prim amplifier using commercial kits from TestGen using fluorescent detection based on destructible oligonucleotide probes using synthetic analogues of oligonucleotides.

Collection, accumulation, and systematization of the results of the study were carried out in a MS Excel 365 database. Statistical processing of the results was performed using Statistica 10 software. Quantitative measures were assessed for normality using the Shapiro-Wilk test (for less than 50 subjects) or the Kolmogorov-Smirnov test (for more than 50 subjects). Quantitative data with a normal distribution were described using arithmetic mean (M) and standard deviations (SD), 95% confidence interval (95% CI) limits. Categorical data were described with absolute values and percentages.

Comparison of two groups according to the quantitative indicator having normal distribution, under the condition of equality of variance, was performed using Student’s t-criterion. Comparison of percentages in the analysis of four-field contingency tables was performed using Pearson’s chi-square test (with values of the expected phenomenon greater than 10). Comparison of percentages in the analysis of multifield contingency tables was performed using Pearson’s chi-square test. A predictive model of the probability of a particular outcome was constructed using the logistic regression method. The measure of certainty, indicating the part of variance that can be explained by logistic regression, was Nigelkerk’s R² coefficient. To assess the diagnostic significance of quantitative features in predicting a particular outcome, we used the ROC-curve analysis method. The dividing value of a quantitative trait at the cut-off point was determined by the highest value of the Youden index. Results are given as: arithmetic mean values for quantitative indicators presented in the text as M±SD, where M is arithmetic mean, Me[Q₁ - Q₃], where Me is median, [Q₁ - Q₃] is upper and lower quartile, SD is standard deviation, for qualitative indicators as %, CI for fraction.

Results

We analyzed the distribution of allele frequencies of eight TLR3 SNPs in patients with HCV (n=102) compared with those in essentially healthy subjects in the Kazakh population of Western Kazakhstan (n=127). Older age was significantly associated with a higher risk of liver cirrhosis in HCV outcome. Indicators of cytolytic and cholestatic syndromes: ALAT and ASAT levels, total and direct bilirubin, alkaline phosphatase, gammaglutamyl transpeptidase were evaluated in patients with HCV infection (Table 1).

The presence of liver cirrhosis in patients was characterized by statistically significant intensification of cholestatic component. Thus, the results of their biochemical study were statistically higher than in patients with HCV infection without cirrhosis: the content of total bilirubin (p 0.017*), alkaline phosphatase (p 0.022*), gammaglutamyl transpeptidase (0.041*).

We confirmed the significance of TLR3 SNPs as a marker of chronic HCV infection in patients compared to uninfected healthy control subjects. The distribution of genotypes and allele frequencies of TLR3 polymorphisms...
between HCV patients and healthy control subjects is presented in Table 2.

The results showed that the CC TLR3 genotype rs1879026 was associated with the development of HCV infection and its chronicity in comparison to essentially healthy individuals (p<0.001). There were no significant differences in the distribution of rs5743312; rs5743305; rs3775291; rs5743311 genotypes and alleles in HCV patients compared with healthy controls.

A predictive model was developed to determine the probability of a distribution group index depending on the index (alleles) of the TLR3 gene polymorphism by binary logistic regression. The observed dependence is described by the equation:

\[ P = \frac{1}{1 + e^{-z}} \times 100\% \]

where \( P \) is Probability Control, \( X_{TA} \) is rs5743305 (0 - AA, 1 - TA), \( X_{TT} \) is rs5743305 (0 - AA, 1 - TT), \( X_{CA} \) is rs1879026 (0 - CC, 1 - CA), \( X_{AA} \) is rs1879026 (0 - CC, 1 - AA).

Analysis of the predictor linkage model (alleles) with the probability of HCV infection revealed statistically significant differences (p<0.001) between the Unadjusted and Adjusted groups due to rs1879026: CA vs rs1879026: AA.

Based on the Nagelkerke determination coefficient value, the model explains 58.3% of the observed variance of the group indicator. When assessing the “rs5743305” index, the risk of HCV infection increased 3.422-fold in the presence of the TA allele and 5.450-fold in the presence of the TT allele. The “rs1879026” gene polymorphism showed a similar pattern with higher multiplicity: the CA allele genotype increased 27.102-fold, and the AA allele increased 205.737-fold. When assessing the dependence of the probability of HCV infection in patients with hepatitis C in the Kazakh population compared with healthy people, the following curve was obtained using ROC analysis (Figure 1).

ROC-curve of HCV-infection chronicity probability estimation characterizes the dependence of “group” indicator probability on the value of logistic function P. The resulting model was statistically significant (p<0.001) and the area under the ROC curve was 0.892 ± 0.021 with 95% CI: 0.850 to 0.933.

**Discussion**

Study of TLR3 gene polymorphism: rs5743312, rs5743305, rs3775291, rs5743311, rs1879026 in residents with HCV infection in Aktobe and Atyrau regions was between HCV patients and healthy control subjects is presented in Table 2.

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**Discussion**

Study of TLR3 gene polymorphism: rs5743312, rs5743305, rs3775291, rs5743311, rs1879026 in residents with HCV infection in Aktobe and Atyrau regions was

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with chronic viral hepatitis C (n=85)</th>
<th>Patients with cirrhosis (n=17)</th>
<th>Healthy people (control) (127)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), M ± SD</td>
<td>44 ± 11</td>
<td>54 ± 6</td>
<td>45 ± 10</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Number of men, n (%)</td>
<td>46 (54.1)</td>
<td>6 (35.3)</td>
<td>80 (63.0)</td>
<td>0.067</td>
</tr>
<tr>
<td>Number of women, n (%)</td>
<td>39 (45.9)</td>
<td>11 (64.7)</td>
<td>47 (37.0)</td>
<td></td>
</tr>
<tr>
<td>ALT, Me [Q₁ - Q₃]</td>
<td>33 [19-54]</td>
<td>41 [28-114]</td>
<td>-</td>
<td>0.101</td>
</tr>
<tr>
<td>AST, Me [Q₁ - Q₃]</td>
<td>30 [16-44]</td>
<td>50 [28-110]</td>
<td>-</td>
<td>0.017</td>
</tr>
<tr>
<td>Total bilirubin, Me [Q₁ - Q₃]</td>
<td>11 [8-16]</td>
<td>14 [12-21]</td>
<td>-</td>
<td>0.017*</td>
</tr>
<tr>
<td>Direct bilirubin, Me [Q₁ - Q₃]</td>
<td>5 [3-7]</td>
<td>7 [4-9]</td>
<td>-</td>
<td>0.102</td>
</tr>
<tr>
<td>Alkaline phosphatase, M ± SD</td>
<td>86 ± 31</td>
<td>107 ± 47</td>
<td>-</td>
<td>0.022*</td>
</tr>
<tr>
<td>GGT, Me [Q₁ - Q₃]</td>
<td>42 [26-68]</td>
<td>58 [49-79]</td>
<td>-</td>
<td>0.041*</td>
</tr>
</tbody>
</table>

*, the difference of indicators in Group 1 and Group 2; **, the difference of indicators in group 2 and 3
conducted to identify genetic markers of predisposition to hepatitis C and predict its outcomes in the Kazakh population of Western Kazakhstan. Of all TLR3 polymorphism variants, TLR3 rs3775291 was the most studied. This particular variant of the TLR3 rs3775291 polymorphism corresponded to the highest value of the Juden index when assessing the dependence of the probability of HCV infection in patients with hepatitis C in the Kazakh population compared with healthy controls on the threshold value of the logistic P cut-off point function (0.545); the sensitivity and specificity were equal to 86.6% and 79.4%, respectively. This is consistent with the literature that the primary developmental point for TLR3 polymorphisms is rs3775291 (C1234T). T mutates from C in exon 4, which provokes the replacement of leucine 421 with phenylalanine. Such mutations are associated not only with HCV development but also with active chronic HBV, encephalitis, human immunodeficiency virus, and other diseases. Data from a contemporary meta-analysis on the role of the TLR3 polymorphism in HCV development show that the TLR3 rs3775291 single-nucleotide polymorphism plays a vital role in the pathological process of HBV development (Fischer et al., 2018). It is also known about the risks of developing macular degeneration when carrying the TLR3 type rs3775291 mutation (Klettner and Roider, 2021). TLR recognizes viral RNA and signals through two different pathways: through the TLR7 and TLR8 receptor via the molecular adaptor MyD88 and through the TLR3 receptor via the molecular adaptor TRIF into the cell nucleus. In the case of TLR3 mutation types rs3775291, rs13126816, rs5743305 the processes of interferon synthesis, which are responsible for viral RNA deactivation, are damaged as well as pathological changes in the expression level of other innate immunity genes.

The C allele of the three TLR3 SNPs (rs3775290, rs3775291, and rs5743312) was known to be significantly higher in HCV. However, no significant correlation was found between healthy and HCV patients, indicating a strong association of this SNP with protection against the development of chronic HCV infection. A study of the TLR3 rs3775291 gene showed no association of the polymorphism with susceptibility to HCV infection, as previously no significant difference in the C TLR3 rs3775290 allele between HCV-positive patients and healthy subjects, but the T allele was associated with late-stage fibrosis and liver cancer (El-Bendary et al., 2018). However, subgroup analysis by polymorphism sites showed a significant increase in the risk of HCV infection associated with genotypes at rs3775291. Interestingly, a significantly reduced risk was associated with the rs3775291-T allele. A strong correlation was observed in the rs3775291 and HCV infection assays. But the TT/CT genotypes accounted for the increased...
Table 4. Threshold Values of the Logistic Function P

<table>
<thead>
<tr>
<th>Polymorphism variants</th>
<th>Threshold</th>
<th>Sensitivity (Se), %</th>
<th>Specificity (Sp), %</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs5743312</td>
<td>0.953</td>
<td>56.7</td>
<td>97.1</td>
<td>95.1</td>
<td>69.1</td>
</tr>
<tr>
<td>rs5743305</td>
<td>0.804</td>
<td>67.7</td>
<td>95.1</td>
<td>93.2</td>
<td>74.7</td>
</tr>
<tr>
<td>rs5775291</td>
<td>0.545</td>
<td>86.6</td>
<td>79.4</td>
<td>80.8</td>
<td>85.6</td>
</tr>
<tr>
<td>rs5743311</td>
<td>0.430</td>
<td>92.1</td>
<td>62.7</td>
<td>71.2</td>
<td>88.9</td>
</tr>
<tr>
<td>rs1879026</td>
<td>0.351</td>
<td>95.3</td>
<td>57.8</td>
<td>69.3</td>
<td>92.4</td>
</tr>
</tbody>
</table>

downstream of the TLR3 gene. This gene polymorphism draws particular attention, as shown by our findings on the genotypic distribution of TLR3 gene polymorphisms in patients with HCV compared to healthy individuals in the Kazakh population of Western Kazakhstan (Table 2). It was accurately associated with the development of HCV chronic infection (p<0.001) than other gene variants rs5743312, rs5743305, rs5775291, rs5743311. The high significance of TLR3 gene rs1879026 in predisposition to HCV infection, acceleration of the replicative phase and chronicity was confirmed by binary logistic regression data. It was the TLR3 rs1879026 gene variants that were statistically more different (p<0.001) between the Unadjusted and Adjusted groups in calculating the prognostic association of alleles with the probability of HCV detection (Table 3). The Nigelkerk observed variance determination coefficient showed that the risk of infection increased with the rs1879026 gene polymorphism: with the CA allele genotype 27.102-fold and the AA allele 205.737-fold. Whereas polymorphism of the “rs5743305” gene leads to the lowest risk of HCV infection: with the TA allele by 3.422 times and the TT allele by 5.450 times. We have calculated similar results by ROC-analysis of the probability of chronic HCV-infection in the Kazakh population of Western Kazakhstan, in which TLR3 gene rs1879026 was recorded by the probability dependence as the most negative (92.4%) in the trajectory of further clinical course, that is chronicity with development of liver cirrhosis. This gene variant was the least specific (57.8%) and with the highest level of sensitivity (Se) - (95.3%). Chinese scientists, when studying only the surface correlation between SNPs and susceptibility to severe hepatitis, concluded (Qiu et al., 2018), that TLR3 (e.g., rs1879026) along with other TLR2 gene polymorphism variants (e.g., rs1898830 and rs3804100), TLR4 (e.g., rs2149356) and TLR9 (e.g., rs187084, rs352139 and rs352140) may serve as risk and predictive biomarkers of neonatal severe hepatitis in the Chinese population. However, the frequency of the TLR3 gene rs1879026 allele T was significantly lower among residents of Saudi Arabia with hepatitis than residents of other countries (Al-Qahtani et al., 2012).

In conclusion, genetic typing of TLR3 genes: rs5743312, rs5743305, rs3775291, rs5743311, rs1879026 in patients with HCV of Western Kazakhstan showed that TLR3rs1879026 gene polymorphism is significant in predisposition to HBV infection in Kazakh population of Aktove and Atyrau regions. Despite the small sample size and the need for further research with larger cohorts, the results obtained are relevant for identifying the prognostic relationship of genotypes with the likelihood of HCV outcomes, to personalized approach to antiviral therapy, its optimization based on molecular

**Author Contribution Statement**

All authors participated in designing the study. All authors except AAR participated in data collection. AAR and GNN analyzed the data. GNN and AAR prepared the first draft of the manuscript. All authors provided critical comments to the first draft. All authors approved the final version of the manuscript.

DOI: 10.31557/APJCP.2023.24.11.3925

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Asian Pacific Journal of Cancer Prevention, Vol 24
Acknowledgements

The authors thank all participants of the study.

Institutional Review Board Statement

The study protocol corresponded to the Helsinki Declaration of 1975 and was approved by the local ethical committee of the Marat Ospanov Western Kazakhstan Medical University, protocol №65 of March 13, 2020.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflict of interest

The authors declare no conflict of interests.

References


