NAO «West Kazakhstan Marat Ospanov Medical University»

OUTLINE
OF THE DISSERTATION WORK
for the degree of Doctor of Philosophy (PhD)

TLR3 gene polymorphism in susceptibility to chronic viral hepatitis in Kazakh population

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Relevance of the study.

INTRODUCTION

Viral hepatitis is the seventh leading cause of death from various diseases. According to recent estimates, about 7 per cent of the world's population are chronic carriers of hepatitis B virus, hepatitis C affects about 3 per cent of the world's population, which in quantitative terms is about 185 million people. In 2009, the highest incidence of viral hepatitis was recorded in Kazakhstan, with HCV reaching over 2% (0.83 per 100 thousand population) and HBV about 9% (3.21 per 100 thousand population).

The risk of severe sequelae such as cirrhosis and hepatocellular carcinoma is of particular concern. Viral cirrhosis arising from chronic hepatitis B, C and B+D accounts for a significant proportion of all cases of CKD. In the USA, viral hepatitis C has become the main cause of CKD, accounting for 26% of cases. In Russia, among non-alcohol-related liver cirrhosis, viral hepatitis B and C account for 73.3%, with hepatitis C accounting for 58.2% of cases

The innate immune system, serving as this first line of defence, is vital in recognising and neutralising pathogens. However, a delay in its ability to recognise infecting microorganisms can lead to serious consequences, including organ dysfunction, inadequate systemic responses, devastating tissue damage, life-threatening infections and even death. This is why understanding the genetic mechanisms underlying innate immunity was made possible by the completion of the global human genome decoding project at the beginning of the 21st century (Organisation 2017)This is why understanding the genetic mechanisms underlying innate immunity was made possible by the completion of the global human genome decoding project at the beginning of the 21st century (Organisation 2017).

Polymorphisms in gene SNPs play a significant role in population genetics, explaining differences in susceptibility to infections among different ethnic and racial groups. Thus, single nucleotide polymorphisms in genes associated with innate resistance to infectious diseases may serve as markers for determining susceptibility or resistance to certain infections, opening new perspectives for the development of individualised approaches in the treatment and prevention of infectious diseases.

Human-virus interactions occur through macroorganism receptors, including Toll-like receptors, which play a fundamental role in pathogen identification and immune system activation, becoming an important link in both innate and adaptive immune responses. TLR3, playing a pivotal role in the antiviral immune response and recognising double-stranded RNA of viruses, is also central to the pathophysiology of liver disease. Intracellular TLR3 recognises viral double-stranded RNA (dsRNA) and activates antiviral immune responses through the production of interferon 1 and inflammatory cytokines.

This ability of TLR3 highlights the importance of further research in developing new treatments for viral infections and related diseases, opening new perspectives for the use of TLR agonists in therapy. SNPs found in more than 1% of the population can affect gene promoter activity and induce amino acid conversions, regarding which studies have shown that patients with chronic HCV infection with certain

genotypes of Toll-like receptor 3 have significantly higher expression levels compared to healthy controls.

Purpose of the study: To study the polymorphism of TLR3 genes (rs5743305, rs5743312, rs1879026, rs3775291) in predisposition to chronic viral hepatitis B and C in the Kazakh population, their association in the clinical and laboratory course of the disease, the effectiveness of antiviral therapy and the prediction of a decrease in the degree of fibrosis.

Objectives of the study:

1. To analyse the frequency distribution of TLR3 gene rs5743305, rs 5743312, rs1879026, rs3775291 genotypes associated with chronic viral hepatitis B and C in the Kazakh population;
2. To study polymorphisms of TLR3 TLR3 genes rs5743305, rs 5743312, rs1879026, rs3775291 in comparison with clinical and laboratory parameters in chronic viral hepatitis B and C in Kazakh population;
3. To assess the contribution of TLR3 gene polymorphisms in the outcome of HBV and HCV AVT in predicting fibrosis reduction in the Kazakh population.

Scientific novelty

1. For the first time, an analysis of the frequency distribution of the TLR3 rs5743305, rs5743312, rs1879026, rs3775291 genotypes associated with chronic viral hepatitis B and C in the Kazakh ethnic group was carried out; A possible marker associated with the development of HCV in Kazakhs is the presence of the TT genotype of the rs5743312 polymorphism of the TLR3 gene.

2. Polymorphisms of TLR3 genes rs5743305, rs 5743312, rs1879026, rs3775291 were determined for the first time in comparison with clinical and laboratory parameters in chronic viral hepatitis B and C in the Kazakh ethnic group. In HBV, regression of hepatic symptoms was revealed in the presence of the CT/TT genotype, astheno-vegetative – CC rs5743312 genotype of the TLR3 gene and CT/TT rs3775291 genotype of the TLR3 gene; in CVH C - CA/AA rs1879026 genotype of the TLR3 gene.

3. For the first time in HBV, the contribution of the TT polymorphism TLR3 rs3775291 genotype TT TLR3 rs1879026 genotype and TT TLR3rs5743312 genotype was revealed; in HCV with TT TLR3 rs3775291 genotype TT TLR3 rs5743312 genotype CT/TT TLR3 rs5743312 genotype CC and TT TLR3 rs5743305 genotype in reducing the degree of fibrosis in the outcome antiviral therapy in the Kazakh ethnic group;

4. Based on the results of genotyping, a classification tree for patients with chronic viral heptitis was developed: in hepatitis B, the greatest regression in the degree of fibrosis was observed in the TT genotype of TLR3 rs3775291 polymorphism, the smallest - CC TLR3 rs5743305; in hepatitis C, the largest - the TT TLR3 rs3775291 genotype, the smallest - the TA TLR3 rs5743305 genotype. This serves to predict a decrease in the degree of fibrosis for the purpose of a personalized therapy approach.

Theoretical and practical significance

The revealed frequency distributions of TLR3 rs5743305, rs 5743312, rs1879026, rs3775291 genotypes associated with HCV B and C in the Kazakh ethnic group supplement information about the features of the genetic aspect of the disease and help predict the course of the disease in a particular patient;

Conducting genetic testing for TLR3 rs5743305, rs5743312, rs1879026, rs3775291 gene polymorphisms in patients with HCV B and C in the Kazakh ethnic group before starting treatment will allow identifying patients with an increased risk of liver fibrosis and adapting the treatment and monitoring strategy in accordance with their genetic profile;

The integration of the results of TLR3 rs5743305, rs5743312, rs1879026, rs3775291 genotyping in the Kazakh ethnic group into the clinical practice of clinical pharmacology, gastroenterology, and infectious diseases will contribute to the development of personalized monitoring and management of the course of HCV B and C, taking into account the individual risk of complications.

Provisions for defence

It was found that the TT genotype of the rs5743312 polymorphism of the TLR3 gene can serve as a marker associated with the development of HCV C in the Kazakh ethnic group.

In CVH, regression of the hepatic symptom was noted in the presence of the CT/TT TLR3 rs5743312 genotype and the CT/TT TLR3 rs3775291 genotype, and astheno-vegetative syndrome in the presence of the CC TLR3 rs5743312 genotype. In CVH C, a similar regression was associated with the CA/Aa TLR3 rs1879026 genotype. In patients with HCV with CA/AA genotypes of the rs1879026 polymorphism of the TLR3 gene, an improvement in biochemical parameters was noted.

In hepatitis B, the greatest regression of the degree of fibrosis was observed in the TT TLR3 rs3775291 genotype, the least in the CC TLR3 rs5743305 genotype. In hepatitis C, the greatest regression was in the TT TLR3 rs3775291 genotype, the least in the TLR3 rs5743305 TA genotype.

Approbation of the work. The results of the conducted research are presented at:

1. "Abstracts of The IX Annual International Scientific-Practical Conference; Medicine Pressing Questions" May 06 - 08, 2020, Baku, Azerbaijan. "Experience of treatment of chronic viral hepatitis in Aktobe region" Nurlanova G.N. ISBN: 978-81-942709-5-9; DOI: 10.21467/abstracts.97

2. XXIV International Medical and Biological Conference of Young Researchers "Fundamental Science and Clinical Medicine - Man and his Health" 24 April 2021 St. Petersburg State University. Thesis Topic: Anaemia of patients with chronic viral hepatitis C receiving combined antiviral therapy. ISBN 978-5-6045762-2-9

3. IV International Scientific and Practical Conference "Infectious diseases at the present stage: problems and solutions" 19 April 2024, Ufa Theme: Role of TLR3 gene polymorphism in chronic viral hepatitis C.

4. III International Scientific and Practical Conference "Actual infections of the

Republic of Kazakhstan and Central Asia in emergency situations" 20-21 June 2024 Kazakhstan, Turkestan. Topic: TLR3 gene polymorphism in predisposition to chronic viral hepatitis.

Publications on the topic of the thesis. On a theme of the dissertation 5 scientific printed works are published, from them 1 article - in edition indexed in information base Scopus - "Asian pacific journal ofcancer prevention" (36 percentile in 2023); 2 article – "Scientific Research Journal of Pharmacy and Technology" (September 50, 2024); 4 articles - in editions recommended by Committee on the control in sphere of education and science of RK; 3 theses - in collections of the international conferences (including foreign - 3).

The dissertation research was carried out within the framework of funded research projects: NTP "Genetic factors of predisposition to viral infectious hepatitis in the Kazakh population of Western Kazakhstan and cytokine profile of patients in the process of antiviral therapy", funded by ZKMU named after Marat Ospanov.

Author's personal contribution

In this study, the author developed the aim and objectives to comprehensively analyse the problem under study. The collection and interpretation of data was personally carried out. A significant personal contribution was made in the process of statistical processing of the results, thanks to which it was possible to achieve objectivity and reliability of conclusions. Also, scientifically sound conclusions were formulated and practical recommendations were developed, which contributes to the further development of the scientific field of research. Analysis of the obtained data, interpretation and generalisation of the results in the form of publications, which makes a significant contribution to the theoretical and practical significance of the conducted research.

RESEARCH MATERIALS AND METHODS

The work was carried out at the Department of Infectious Diseases and Childhood Infections of the NAO "West Kazakhstan Medical University named after Marat Ospanov". Clinical and laboratory examinations of patients were carried out in the hepatological Centers of Aktobe and Atyrau. The genetic part of the work was carried out on the basis of the scientific research center of the Marat Ospanov ZKMU in Aktobe, 74 Maryeseva Street.

The study included all patients who were on outpatient treatment in the hepatological centers of Aktobe and Atyrau with a verified diagnosis of Chronic viral hepatitis B and C, provided they signed an informed consent. There were no restrictions for inclusion in the study by gender. There was no targeted equal participation of men and women. The study included people aged 18 to 60 years. The diagnosis was verified in hepatological centers and recorded in the individual records of the examined and patients (forms No. 30-1/). Potential candidates were invited to participate in the study, then, after checking compliance with the inclusion and exclusion criteria, an informed consent (IP) was signed to participate in the study.

Criteria for inclusion of patients in the study

Inclusion criteria: patients aged 18 to 60 years with a confirmed diagnosis of Chronic viral hepatitis C, HCV B or C, without concomitant pathology, belonging to ethnic Kazakhs and receiving antiviral treatment in accordance with clinical protocols (HCV B – tenofovir, HCV C-daclatasvir+sofosbuvir).

Belonging to the Kazakh nationality was established by questioning and checking with the data of the birth certificate and identity card of the respondent in three generations. This questionnaire was a questionnaire for collecting demographic information about the patient and his ancestry up to the third generation, including the nationality and place of birth of both the patient himself and his parents, grandparents, great-grandparents and great-grandparents.

Criteria for excluding patients from the study

Exclusion criteria:

• patients with HCV B, C, D pregnant, nursing mothers;

• patients with HCV B, C, D, registered in tuberculosis, neuropsychiatric, narcological dispensaries, AIDS center;

• patients with HCV B, C, D with chronic decompensated diseases of internal organs, except for cirrhosis of the liver, HCC;

• The study excluded persons with three generations, as well as relatives known to them by blood with representatives of non-Kazakh nationality

In hepatological centers, after receiving IP, patients were treated: collecting information, assessing the condition of patients, filling out questionnaires by participants (by nationality, belonging to ethnic Kazakhs, Appendix A, B). The results of clinical, laboratory and instrumental studies, information about the treatment and recommendations were written out from the medical records of patients (forms No. 30-1/).

2.2 Research methodology for the first task

Studying the frequency distribution of genotypes of TLR3 rs5743305, TLR3 rs5743312, TLR3 rs1879026, TLR3 rs3775291 genes

The design of this study is case control.

To carry out this task, patients of Kazakh nationality with a diagnosis of Chronic viral hepatitis B and C, who are registered at dispensaries in the hepatological centers of Aktobe and Atyrau, were selected.

Patients of Kazakh nationality diagnosed with chronic viral hepatitis B and C were registered in the hepatological centers of Aktobe and Atyrau regions with an established and confirmed diagnosis. The examination with verification of the pathogen and treatment were carried out in accordance with clinical protocols.

A continuous sequential sampling was used. According to the literature, the prevalence of chronic viral hepatitis B is 7%, and chronic viral hepatitis C is 14.7%. Based on these data, it was planned to include 120 patients with chronic viral hepatitis B and 232 patients with chronic viral hepatitis C.

During the allotted time period from 03/01/2020 to 08/31/2021, 187 patients were treated antivirally in hepatological centers and diagnosed with chronic viral hepatitis B (tenofovir) and C (daclatasvir+sofosbuvir) (Atyrau 86, Aktobe 101).

Of these, 16 people refused to participate in the study, 10 people did not enter the study according to the exclusion criteria. Thus, 59 patients with chronic viral hepatitis B and 102 patients with chronic viral hepatitis C (161 patients in total) were included in the study. All participants had a confirmed diagnosis of chronic viral hepatitis and met the inclusion criteria (Figure 1).

The control group consisted of people of comparable gender and age. The control group was recruited randomly among people of Kazakh nationality who wanted to undergo a genetic examination.

Criteria for inclusion in the control group:

- The absence of a history of chronic viral hepatitis B and C was confirmed by blood tests using ELISA (HBsAg, anti-HCV) and PCR (B virus DNA and C virus RNA)

- Belonging to the Kazakh nationality was established by questioning and checking with the data of the birth certificate and identity card of the respondent in three generations .

The questionnaire was a questionnaire for collecting demographic information about the patient and his ancestry up to the third generation, including the nationality and place of birth of both the patient himself and his parents, grandparents, great-grandmothers and great-grandfathers. The questionnaire included the following sections: 1. Patient data: Patient code, date, full name, IIN, place of residence, date of birth, nationality. 2. Information about parents in 3 generations of the subject: nationality and place of birth. The questionnaire also contained fields for the signature of the research participant, the researcher and the date of completion. This questionnaire was used to analyze the patient's genealogy in order to study the ethnic factor.

A total of 136 people were examined to recruit individuals to the control group. According to the criteria for inclusion, the control group included a total of 130 people.

Determination of TLR3 gene polymorphism: venous blood was collected from patients with HCV B and C in hepatocenters, in healthy people of the control group and delivered to the laboratory of the NPC.

2.3 Research methodology for the second and third tasks

Comparison with clinical and laboratory parameters, as well as assessment of the association of polymorphisms of TLR3 genes with the outcome of HTP HCG B and C to predict a decrease in the degree of fibrosis in the Kazakh ethnic group.

The design of the study is longitudinal descriptive. All the results of the analyses of patients were entered into the IRC from the data of the individual registration card of the examined and patients with viral hepatitis B and C (forms No. 30-1/Y).

To accomplish this task, the data of objective examination, laboratory and instrumental data were analyzed before receiving HTP and after 24 weeks of monitoring.

The research methods were developed on the basis of clinical protocols for the diagnosis and treatment of HCV B and C.

The study included data collection before treatment and 24 weeks after its start, with an assessment of the following indicators: Epidemiological history, objective data, biochemical blood analysis, enzyme immunoassay, polymerase chain reaction, elastometry, molecular genetic analysis.

The total duration of the study was 1 year: the recruitment of patients was carried out from March 1, 2020 to August 31, 2020 (for six months), then the patients were monitored for six months (at 24 weeks of HTP).

2.4 Determination of gene polymorphisms

Venous blood was taken from all the subjects for molecular genetic analysis. Genotyping was performed on the basis of the scientific and practical center of the Marat Ospanov ZKMU in Aktobe, 74 Maryeseva Street.

DNA-Blood-M-100 reagent kits manufactured by TestGen LLC (Russia) were used to isolate genomic DNA from the peripheral blood of the subjects. The principle of operation of this kit is based on the reversible binding of nucleic acids to the surface of magnetic particles. This method provides high purity and concentration of DNA, which is important for subsequent genotyping. The stages of DNA isolation include lysis of blood cells, binding of DNA to magnetic particles, several stages of washing and DNA elution.

Genotyping of TLR3 gene polymorphisms (rs5743305, rs5743312, rs5743311, rs1879026, rs3775291) was performed by polymerase chain reaction (PCR) in real time on a DT-prime amplifier (DNA technologies, Russia). For this purpose, commercial reagent kits from TestGen LLC (Russia) were used. Real-time PCR is based on the application of fluorescent detection using destructible oligonucleotide probes. Each probe contains a fluorophore and a suppressor located at opposite ends of the molecule. In the initial state, the probe does not emit fluorescence, since the extinguisher absorbs the light emitted by the fluorophore.

DNA amplification includes three main phases:

1. Denaturation – heating the mixture to high temperatures (about 95 °C), at which double-stranded DNA is unwound, forming two single-stranded molecules.

2. Primer annealing – when the temperature drops to 55-65 °C, primers complementary to the target DNA sites bind to these sites.

3. Elongation – when the temperature rises to 72 °C, Taq polymerase completes a new DNA chain, starting with the primer.

Signal probes were used for genotyping, the labels of which (FAM and HEX) were specific for each polymorphism allele. During amplification, Taq polymerase destroys the probe, separating the fluorophore from the suppressor, which leads to the release of a fluorescent signal. The fluorescence intensity directly depends on the amount of the amplified product and makes it possible to determine the presence or absence of a mutation in the sample.

After the amplification is completed, the temperature melting (melting curve) of the amplicon and the probe is performed, which allows us to confirm the accuracy of genotyping. This stage consists in a gradual increase in temperature and tracking changes in fluorescence as the amplicon and probe duplexes dissociate. Based on

these data, the amplifier software plots the dependence of fluorescence on temperature, allowing accurate identification of various polymorphism alleles.

Methods of statistical data processing

Statistical analysis and visualisation of the obtained data were performed using the R 4.3.2 statistical computing environment (R Foundation for Statistical Computing, Vienna, Austria).

To study the prevalence of genotypes and alleles of TLR3 gene rs5743305, rs5743312, rs1879026, rs3775291 polymorphisms in patients with CVH B and C in the Kazakh population, as well as the control group, we calculated a number of statistical parameters "case-control" using SNP using the calculator Gen-Expert http://84.201.145.131/.

To study the association of polymorphisms of TLR3 TLR3 genes rs5743305, rs 5743312, rs1879026, rs3775291 in comparison with clinical and laboratory parameters in CVH B and C, three or more groups were compared; the Kraskell-Wallis test was used for quantitative variables, the Mann-Whitney test was used for comparison of two groups for quantitative variables. To examine the dynamics of quantitative variables, the Friedman test was used. Linear mixed models with the inclusion of an interaction term between the observation period and the grouping variable were used to assess differences between the groups with respect to the dynamics of quantitative indicators; dependent variables with right-sided skewness of sample distribution were included in the models after ln-transformation.

Pearson's χ^2 test and Fisher's exact test (with the minimum expected number of observations in the contingency table <5) were used to compare groups with respect to qualitative variables. Generalised linear mixed models with a logistic link function were used to analyse the dynamics of binary indicators, and generalised linear mixed models with the inclusion of an interaction term between the observation period and the grouping variable were used to analyse differences between groups with respect to the dynamics of binary indicators.

Differences were considered statistically significant at $p < 0.05$. CART classification trees were used as prognostic models, and AUC, predictive accuracy, sensitivity, specificity, predictive value of positive and negative results with corresponding 95% confidence intervals (95% CI) were used as prediction quality metrics.

OWN RESEARCH RESULTS

The study included 291 adults of Kazakh nationality. The age of the study participants ranged from 18 to 60 years. The main group included 161 patients diagnosed with CVH B (59), C (102) and 130 healthy people in the control group.

Results of frequency distribution analysis of TLR3 gene rs5743305, rs5743312, rs1879026, rs3775291 genotypes.

The polymorphism rs5743312 of TLR3 genes TT genotype was more frequent in patients with CVH C, the chance of having TT genotype was 3.1 times higher compared to the control group ($p < 0.05$). The chance of having a homozygous CC genotype was 1.4 times higher compared to controls ($p<0.05$). Similarly, the TT genotype was more frequent in CVH B, but no statistically significant differences

were found.

At TLR3 gene rs5743305 polymorphism in CVH C and B, TT genotype was frequent (55-62%), the odds of having TT genotype was higher by (1.26-1.73) times respectively compared to the control group.

The most frequent genotype ST (47%) and allele T (33%) at polymorphism of TLR3 gene rs3775291 was found in patients with hepatitis C, and the chances of having genotype ST were 1.42 times higher. Also, the most frequent heterozygous genotype ST (40%) and allele C (74%) rs3775291 of TLR3 gene are registered in patients with CVH B.

The homozygous genotype CC (62%) and allele C (79%) of rs1879026 polymorphism of TLR3 gene were most frequently observed in patients with CVH C. The odds of having the CC genotype were 1.24 times higher compared to the control group (95% CI = 0.73-2.10). In CVH B patients, homozygous genotype CC (69%) and genotype C (85%) were registered, with the chance of having genotype CC 1.67 times higher than in the control group.

The analysis of the frequency of occurrence of polymorphic variants of TLR3 gene rs5743305, rs 5743312, rs5743311, rs1879026, rs3775291 in the group of patients with CVH B and C showed that hepatitis B and C can be associated in the Kazakh population with polymorphism of TLR3 gene rs5743305, but statistically not significantly. Only in HB C polymorphism of TLR3 gene rs 5743312 the TT genotype was 3.1 times higher ($p < 0.05$), homozygous CC genotype was 1.4 times higher than in the control group ($p < 0.05$). Based on the results obtained, these genotypes may be associated with the development of CVC.

Results of association analysis of TLR3 gene polymorphisms with clinical and laboratory course in chronic viral hepatitis B and C.

The dynamics of severity of hepatic symptoms of CVH B and C in the course of PVT at different polymorphisms of TLR3 rs5743312, TLR3 rs5743305, TLR3 rs3775291, TLR3 rs1879026 genes was heterogeneous. At the onset of disease in both CVH B and CVH C, hepatic signs were recorded predominantly in all patients ranging from 73-85%, with the exception of 100% presence of symptoms in CVH B patients at genotype TA/AA of the TLR3 rs5743305 gene polymorphism. In patients with CVH B, the effect of PVT after 24 weeks of monitoring was observed in the minimal range of 11.1% with genotype CA/AA at TLR3 rs1879026 polymorphism to 39.1% with genotype ST/TT at TLR3 rs5743312 gene polymorphism. PVT was more effective in CVH C patients in the range of 50-60.5%: with CA/AA genotype of TLR3 rs1879026 polymorphism (60.5%), ST/TT polymorphism of TLR3 rs3775291 (58.8%), CC genotype of TLR3 rs5743312 polymorphism (55.6%).

Astheno-vegetative symptoms were most clinically pronounced in CVH B genotype TA/AA polymorphism of TLR3 gene rs5743305 (90.9%). Under the influence of PVT they regressed to a lesser extent than hepatic signs. In patients with CVH B the minimal effect of PVT was revealed by the genotype ST/TT polymorphism of TLR3 genes rs5743312 (13%), the greatest - homozygous genotype CC of TLR3 genes rs5743312 polymorphism (33,4%), genotype ST/TT of TLR3 genes rs3775291 polymorphism (33,3%). In CVH C the best effect of PVT was

observed in patients with CA/AA genotype of TLR3 rs1879026 polymorphism (44,8%), and the rest of cases were characterised by regression of astheno-vegetative symptoms in the range from 30-41,6% in the dynamics of observation.

Extrahepatic signs of liver damage in CVH B were manifested in 1/3-1.5 part of patients (16.5-38.9%), only to a greater extent in patients with TA/AA genotype of TLR3 gene polymorphism rs5743305 (45.5%). Regression of extrahepatic signs under the influence of PVT was insignificant: from 2.7-9.1%. In HCV C, extrahepatic clinical signs were detected in the range of 13.3-22.4% of cases regardless of genotypes and polymorphisms. The clinical effect of PVT was ambiguous: from symptom control in the dynamics of observation by 1.4% (genotype CC polymorphism of TLR3 genes rs5743312) and 5.3% (CA/AA polymorphism of TLR3 rs1879026), no change and on the contrary, increase in the number of patients with extrahepatic signs by 2.3%-6.7% (CT/TT polymorphism of TLR3 genes rs5743312). Consequently, PVT in CVC had practically no effect on the severity of extrahepatic signs.

The analysis of viral load in patients with CVH B depending on carriage of TLR3 rs5743312, TLR3 rs5743305, TLR3 rs3775291, TLR3 rs1879026 gene polymorphism before PVT revealed, that the greatest number of patients with low viraemia was observed when carrying polymorphism of TLR3 rs3775291 gene (CC-25%, ST-8,3%, TT-33,3%), the smallest number - polymorphism of TLR3 rs187026 gene (CC-26,8%). It should be noted that at all polymorphisms of TLR3 gene the viral load is high and above the linear range: genotype TT - 100% polymorphism of TLR3 gene rs5743312, genotype AA - 100% polymorphism of TLR3 gene rs5743305, genotype TA-89,5%, genotype -CA/AA-100% at polymorphism TLR3 rs1879026. Also at polymorphism of TLR3 gene rs3775291 genotype ST viral load is 91,7%. Probably, carriers of these genotypes and polymorphisms of the TLR3 gene are more susceptible to rapid progression of the infectious process and development of complications.

In comparison with CVD B, the viral load in blood is slower in CVD C. The greatest number of patients with low viraemia was observed in carriers of TLR3 gene polymorphism rs3775291: genotype TT-50%, genotype SS-40,9%, heterozygous genotype ST-35,4%. Carriers of TLR3 gene polymorphism rs5743312 had the same number of patients with low viraemia: CC-41.7%, ST-34.8%, TT-28.6%, and TLR3 gene polymorphism rs5743305: TA-43.2%, TT-30.3%, AA-22.2%. The lowest number of patients with low viraemia with TLR3 gene polymorphism rs1879026: CA/AA 44.7%, CC-35.9%. The greatest number of patients with high viral load with TLR3 gene polymorphism rs574305 genotype AA-77.8%; with lesser number - TLR3 gene polymorphism rs377291 genotype TT (50%), TLR3 gene polymorphism rs1879026 genotype CA/AA (44.7%).

PVT in CVH B and C was effective in interpreting laboratory indicators. Reduction of cholestasis indicators: statistically significant reduction of alkaline phosphate, GGTP levels was found in CVH B and C with TLR3 polymorphism rs3775291 homozygotes CC at ($p=0.001$, $p<0.001$, respectively), rs1879026 ($p=0.001$ and <0.001, respectively), allele T with CVH C ($p<0.001$). Among patients with

hepatitis C, there was a statistically significant association of GGTP dynamics with T allele carriage (p<0.001). A decrease in the activity of hepatic cellular enzymes ALT, AST was observed in patients with HCV B and C regardless of genotype. Statistically significant decrease in ALT level was observed at polymorphisms of TLR3 gene polymorphisms CC genotype rs5743312 TLR3 (p<0,001), TT genotype rs5743305(p<0,001), TLR3 rs3775291 CC (p<0,001). Statistically significant decrease in AST level in patients with hepatitis B and C genotype TT rs5743305 TLR3 (p<0.001 and 0.002, respectively), CC genotype rs3775291 TLR3 (p<0.001 and 0.001, respectively).

Results of the effect of TLR3 gene polymorphisms in CVH B and C PVT outcome to predict fibrosis reduction.

Among CVH C patients with polymorphism rs5743312 of TLR3 gene TT genotype was associated with the most pronounced changes in dynamics (p < 0.001): the number of patients with fibrosis decreased twofold from 57.1% to 28.6%, while in CC/ST genotypes a slight improvement was registered, with cirrhosis regressed from 21.7% to 8.7%. In CVH B the most significant improvement after PVT was observed in patients with CC genotype, where the proportion of patients with transition to the lowest degree of fibrosis reached 83.3% of cases. And in patients with TT genotype the regression of fibrosis improved up to 75%, only 25% of patients remained unchanged. The lowest effect of PVT was observed in patients with CT genotype, who were the most resistant to treatment, as the percentage of cirrhosis did not change and was 5.3% before and after treatment.

Study of the obtained data of TLR3 gene rs5743305 polymorphism with AA genotype of TLR3 gene rs5743305 polymorphism in CVH B 100% was found to have formed liver fibrosis. Reduced fibrosis was observed among TT homozygotes (p<0.001) with a conversion to the lowest degree of fibrosis to 80.1% and a reduction in cirrhosis from 21.6% to 13.5% of cases. Among patients with hepatitis C, patients with TA genotype showed the best results, with a significant reduction in the proportion of patients with cirrhosis to 8.1%, with high-degree fibrosis resolving to the lowest degree. Patients with AA genotype were characterised by positive improvement, with disappearance of cirrhosis and minimal changes in the degree of fibrosis before and after treatment.

Carriers of CC, CT genotypes (p<0.001), as well as TT genotype (p=0.016) were characterised by a significant reduction in the severity of liver fibrosis among the patients with CVH B. The improvement of the condition in the form of a decrease in the degree of liver fibrosis was observed in 83.3% of patients with CT genotype and 75% with CC genotype. Improvement in the form of fibrosis degree reduction was observed in 83.3% of patients with CT genotype and 75% - with CC genotype. The owners of TT genotype at polymorphism rs3775291 of TLR3 gene were characterised by disappearance of fibrosis in 33,3% and transition from fibrosis 2 to fibrosis 1 in 33,3% of patients.

Among patients with hepatitis C, a statistically significant decrease in fibrosis severity was observed in patients with CC and CT genotypes (p<0.001). In TT homozygotes there were no statistically significant changes (p=0.114) in the

dynamics of PVT, indicating tolerance to antiviral drugs (sofosbuvir+daclatasvir). Analysis of patients with hepatitis C showed that for genotype CC the percentage of patients without fibrosis increased from 47.7% to 59.1% of cases after treatment, for genotype CT - from 29.2% to 41.7% of patients, while for genotype TT this indicator remained stable at 60% of cases.

At polymorphism rs1879026 of TLR3 gene the greatest formation of fibrotic changes was noted in patients with genotype CA/AA in hepatitis B and made up to 89.9%. For the CC genotype, an increase in the frequency of patients without fibrosis was recorded from 26.8% to 36.6% at the end of therapy. Notably, the percentage of patients with cirrhosis decreased from 14.6% to 9.8% of cases. Liver cirrhosis in people with CA/AA genotype decreased almost 2-fold (from 38.9% to 16.7%), with the percentage of patients without fibrosis increasing from 11.1% to 22.2% of cases. In the group of HCV C patients with CC genotype under the influence of PVT, the proportion of patients without fibrosis increased from 42.2% to 51.6%, with CA/AA genotype - from 36.8% to 50%.

We developed a classification tree of patients with CVC B and C to predict fibrosis reduction at 24 weeks of monitoring.

The greatest reduction in the degree of fibrosis after 24 weeks of follow-up was observed in patients with CVH B with polymorphism rs3775291 of TT genotype (100% improvement), followed by CT genotype with rs1879026 (75% improvement) and TT genotype with rs5743312 (67.5% improvement). The least reduction was recorded in patients with CC variant rs5743305 (42.9% improvement) and TA/AA of the same polymorphism (60% improvement).

In CVH C at polymorphism rs3775291 of TLR3 gene, TT genotype shows the highest probability of improvement (88.6% of patients with decreased degree of fibrosis). In subsequent branching by the rs5743312 polymorphism of TLR3 gene, patients with CT and TT genotypes show improvement in 85.7%, while CC and TT genotypes show improvement in 71% of cases. The lowest percentage of improvement (46.2%) is observed in patients with TA variant by rs5743305 genotype.

CONCLUSION

Thus, the following CONCLUSIONS can be drawn based on the findings of our study:

1. For the first time, the analysis of the frequency distribution of the TLR3 rs5743305, rs5743312, rs1879026, rs3775291 genotypes associated with chronic viral hepatitis B and C in the Kazakh ethnic group was carried out; In patients with HCV, the TT genotype of the TLR3 rs 5743312 gene polymorphism was 3.1 times higher ($p < 0.05$), and the CC genotype was 1.4 times higher compared with the control group ($p < 0.05$). A possible marker associated with the development of HCV C in the Kazakh ethnic group is the presence of the TT genotype ($p < 0.05$) rs5743312 polymorphism of the TLR3 gene.

2. Clinical manifestations of HCV B (hepatic 100%, astheno-vegetative 90.9%, extrahepatic 45.5%) were found in patients with the presence of the TA/AA TLR3

rs5743305 gene genotype. In patients with HCV, regression of hepatic symptoms was revealed in the presence of the CT/TT genotype (39.1%), astheno-vegetative – CC genotype (33.4%) TLR3 rs5743312 and CT/TT genotype (33.3%) TLR3 rs3775291. In patients with HCV C, there was a more pronounced regression of clinical manifestations in the CA/AA TLR3 rs1879026 genotype: regression of hepatic signs 60.5%, asthenovegetative - 44.8%.

3. The performed antiviral therapy in patients with HCV B and C with genotype CC rs3775291, rs1879026 of the TLR3 gene caused a decrease in levels of alkaline phosphatase ($p < 0.001$), GGTP ($p < 0.001$); ALT in the presence of genotype CC rs 5743312 ($p < 0.001$), genotype TT rs5743305 ($p < 0.001$), genotype CC rs 3775291 of the TLR3 gene ($p < 0.001$); AST in the presence of genotype TT rs5743305 ($p < 0.001$), genotype CC rs3775291 of the TLR3 gene ($p < 0.001$).

4. In patients with HCV B having the TT TLR3 rs3775291 genotype, CT TLR3 rs1879026 genotype and TT TLR3 rs5743312 genotype, there was a significant decrease in the degree of fibrosis at 24 weeks of monitoring by 100%, 75% and 67.5%, respectively. In patients with HCV C with the TT TLR3 rs3775291 genotype and CT/TT TLR3 rs5743312 genotype, the degree of fibrosis decreased by 88.6% and 85.7%, respectively, and with CC and TT TLR3 rs5743305 genotypes by 71% of cases.

5. Based on the results of genotyping, a classification tree for patients with HCV was developed: in hepatitis B, the greatest regression in the degree of fibrosis was observed in the TT genotype of TLR3 rs3775291 polymorphism, the smallest - CC TLR3 rs5743305; in hepatitis C, the largest - the TT TLR3 rs3775291 genotype, the smallest - the TA TLR3 rs5743305 genotype. This serves to predict a decrease in the degree of fibrosis for the purpose of a personalized therapy approach.

PRACTICAL RECOMMENDATIONS

The revealed frequency distributions of TLR3 rs5743305, rs 5743312, rs1879026, rs3775291 genotypes associated with HCV B and C in the Kazakh ethnic group supplement information about the features of the genetic aspect of the disease and help predict the course of the disease in a particular patient;

Conducting genetic testing for polymorphisms of the TLR3 rs5743305, TLR3rs5743312, TLR3rs1879026, TLR3rs3775291 genes in patients with HCV B and C in the Kazakh ethnic group before starting treatment will allow identifying patients with an increased risk of liver fibrosis and adapting the treatment and monitoring strategy in accordance with their genetic profile;

The integration of the results of TLR3 rs5743305, rs5743312, rs1879026, rs3775291 genotyping in the Kazakh ethnic group into the clinical practice of clinical pharmacology, gastroenterology, and infectious diseases will contribute to the development of personalized monitoring and management of the course of HCV B and C, taking into account the individual risk of complications.

The obtained scientifically based conclusions and values can be used for the educational process at the university.