АКТУАЛЬНЫЕ ПРОБЛЕМЫ ТРАНСПОРТНОЙ МЕДИЦИНЫ

АКТУАЛЬНІ ПРОБЛЕМИ ТРАНСПОРТНОЇ МЕДИЦИНИ

ACTUAL PROBLEMS OF TRANSPORT MEDICINE

ISSN 1818-9385 (print)
ISSN 1818-9385 (online)

- **окружающая среда**
  навколишнє середовище
  environment

- **профессиональное здоровье**
  професійне здоров'я
  occupational health

- **патология**
  патологія
  pathology

2023
№ 4 (74)

Медицинский научный журнал

DOI http://dx.doi.org/10.5281/zenodo10417848

АКТУАЛЬНІ ПРОБЛЕМИ ТРАНСПОРТНОЇ МЕДИЦИНИ:

навколишнє середовище; професійне здоров'я; патологія

№ 4 (74), 2023 р.

НАУКОВИЙ ЖУРНАЛ

Засновники: Український науково-дослідний інститут медицини транспорту Міністерства охорони здоров'я України та Фізико-хімічний інститут ім. О.В.Богатського Національної Академії наук України

Заснований у серпні 2005 р.

Журнал є офіційним виданням Українського наукового товариства патофізіологів

Адреса редакції:
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Тел.: +380-50-988-98-94, +380-48-753-18-04
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Kanatnaya str., 92, 65039, Odessa, Ukraine
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E-mail: med_trans@ukr.net

Журнал зареєстрований Держкомітетом по телебаченню та радіомовленню України
*31 травня 2005 р. Свідоцтво: серія **КВ № 9901***
ISSN 1818-9385 (print.), ISSN 1818-9393 (online)

The Journal is registered by the State Committee on TV and broadcasting of Ukraine
*May 31, 2005. The certificate: series **КВ № 9901***
ISSN 1818-9385 (print.), ISSN 1818-9393 (online)

Журнал внесений до переліку видань, у яких можуть публікуватися результати дисертаційних робіт з біології та медицини (Категорія «Б», наказ міністра науки і освіти України № 886 від 02.07.2020)
Журнал зареєстрований в міжнародній наукометричній базі Copernicus (Польща)

Роботи, що представлені в цьому номері, рекомендовані до друку Редакційною колегією журналу після сліпого рецензування

Періодичність — 4 рази на рік
Передплатний індекс 95316
Адреси електронної версії:

http://aptm.com.ua/; http://www.medtrans.com.ua/; http://www.nbuv.gov.ua/portal/Chem_Biol/Aptm/texts.html

Підписано до друку 15.12.2023 р. Гарнітура Pragmatica. Формат 64x90 / 8. Друк офсетний. Ум. печ. лист. 15,2.
Надруковано з готового макету в друкарні "ART-V". м Одеса, вул. Комітетська, 24А.

DOI http://dx.doi.org/10.5281/zenodo10417848

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окружающая среда; профессиональное здоровье; патология

НАУЧНЫЙ ЖУРНАЛ
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№ 4 (74), 2023 г.
Основан в августе 2005 г.

DOI http://dx.doi.org/10.5281/zenodo10417848

Клінічні аспекти медицини транспорту | **Clinical Aspects of Transport Medicine**

UDC 616.36-002.12-097:575.174.015.3
DOI http://dx.doi.org/10.5281/zenodo10418041

INTERRELATION OF PARAMETERS OF BIOCHEMICAL, IMMUNOLOGICAL AND GENETIC PROFILE IN PATIENTS WITH CHRONIC HEPATITIS C

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ВЗАЄМОЗВ'ЯЗОК ПАРАМЕТРІВ БІОХІМІЧНОГО, ІМУНОЛОГІЧНОГО ТА ГЕНЕТИЧНОГО ПРОФІЛЮ У ХВОРИХ НА ХРОНІЧНИЙ ГЕПАТИТ С

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ВЗАИМОСВЯЗЬ ПАРАМЕТРОВ БИОХИМИЧЕСКОГО, ИММУНОЛОГИЧЕСКОГО И ГЕНЕТИЧЕСКОГО ПРОФИЛЯ У БОЛЬНЫХ ХРОНИЧЕСКИМ ГЕПАТИТОМ С

Усиченко К.М.
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Summary/Резюме

Relevance. Viral hepatitis C is the main cause of chronic liver disease. This is due to the fact that the hepatitis C virus is able to elude immune control, creating new antigenic variants, which contributes to the transition of acute hepatitis C to a chronic form in 70-80% of cases

Materials and methods. Biochemical, immunological, molecular-genetic, statistical.

Results. The presence of a relationship between transaminase activity, immunological parameters, and certain specific genotypes of IL-4 and TNFб allows using the information obtained as one of the criteria for the activity of the inflammatory process in the liver. The severity of changes in cellular immunity is an additional criterion for the degree of morphological disorders in the liver tissue.

Conclusions. The data obtained can help a practical clinician in predicting the course of chronic hepatitis C, which is directly related to an individual plan for managing a patient with chronic hepatitis C.

***Key words**: chronic hepatitis C, liver fibrosis, immunity, allelic polymorphism.*

Актуальність. Вірус гепатиту С є однією з основних причин хронічних захворювань печінки. Це пов'язано з тим, що вірус гепатиту С здатний вислизати від імунного контролю, створюючи нові антигенні варіанти, що сприяє переходу гострого гепатиту С в хронічну форму в 70-80% випадків.

Матеріали та методи. Біохімічні, імунологічні, молекулярно-генетичні, статистичні.

Результати. Наявність зв'язку між активністю трансаміназ, імунологічними показ-

DOI http://dx.doi.org/10.5281/zenodo10417848

никами та певними специфічними генотипами *IL-4* та *TNFб* дозволяє використовувати отриману інформацію як один із критеріїв активності запального процесу в печінці. Виразність змін клітинного імунітету є додатковим критерієм ступеня морфологічних порушень у тканині печінки.

Висновки. Отримані дані можуть допомогти практичному клініцисту в прогнозуванні перебігу хронічного гепатиту С, що безпосередньо пов'язано з індивідуальним планом ведення хворого на хронічний гепатит С.

***Ключові слова**: хронічний гепатит С, фіброз печінки, імунітет, алельний поліморфізм.*

Актуальность. Вирус гепатита С – одна из основных причин хронических заболеваний печени. Это связано с тем, что вирус гепатита С способен ускользать от иммунного контроля, создавая новые антигенные варианты, способствующие переходу острого гепатита С в хроническую форму в 70-80% случаев.

Материалы и методы. Биохимические, иммунологические, молекулярно-генетические, статистические.

Результаты. Наличие связи между активностью трансаминаз, иммунологическими показателями и специфическими генотипами IL-4 и TNFб позволяет использовать полученную информацию как один из критериев активности воспалительного процесса в печени. Выраженность изменений клеточного иммунитета является дополнительным критерием степени морфологических нарушений ткани печени.

Выводы. Полученные данные могут помочь практическому клиницисту в прогнозировании течения хронического гепатита С, что напрямую связано с индивидуальным планом ведения больного хроническим гепатитом С.

***Ключевые слова:** хронический гепатит С, фиброз печени, иммунитет, аллельный полиморфизм.*

Introduction

Viral hepatitis still remains one of the most pressing problems of world health, ranking 7th among the causes of mortality from all diseases. According to the latest WHO estimates, the number of patients with chronic hepatitis C in the world amounted to 71 million (1% of the world's population).

The significance of the chronic hepatitis C problem is determined not only by the disease itself, but also by the increased risk of developing long-term adverse effects - liver cirrhosis and hepatocellular carcinoma. Viral cirrhosis (in the outcome of chronic hepatitis B, C, B+D) ranges from 10% to 23.5% of all cirrhosis. In the United States, viral hepatitis C as a cause of cirrhosis came out on the 1st place and is the cause of the formation of cirrhosis in 26% of cases.

According to EASL, hepatocellular carcinoma, accounting for 70–90% of primary liver cancers, is the 5th leading cause of cancer in Europe, with 1–13 new cases and 1–10 deaths per 100,000 inhabitants per year. Hepatocellular carcinoma after the onset of cirrhosis associated with HCV-infection develops at a rate of up to 8% per year (average 1–4%). In addition, hepatocellular carcinoma may occur in the early stages of fibrosis or even without it. [1, 2].

There are numerous factors that determine the nature of the interaction of the pathogen and the macroorganism, modify the chronicity of HCV infection, and also the rate of fibrogenesis in the liver. A special role belongs to genetic factors, it is believed that polymorphism of cytokine genes has a significant impact on the nature of the course of chronic hepatitis [3, 4].

Studies by a number of authors have shown that the genetic status of a person in terms of polymorphic variants of a num-

DOI http://dx.doi.org/10.5281/zenodo10417848

ber of cytokine genes is the most important factor determining such pathogenetic signs for the course of chronic viral hepatitis as the quantitative content of cytokines, as well as the level of blood biochemical parameters [5, 6].

Biochemical parameters of blood, such as the level of ALT, AST, bilirubin and its fractions, alkaline phosphatase, serve to determine the functional state of the liver both in normal conditions and in various pathologies. Cytolysis syndrome is assessed by determining the activity in the blood serum of the "liver" enzymes ALT and AST. The state of pigment metabolism - according to the level of bilirubin and its fractions. Synthetic function is characterized by the level of protein, albumin-globulin and prothrombin index.

It seems relevant to study the "individual" response of the patient's body to the effects of hepatitis C and B by identifying a possible association of polymorphic variants of the IL-4, IL-10, and TNFб genes with the level of biochemical parameters.

The association of polymorphism of cytokine genes with biochemical parameters in patients with chronic viral hepatitis is often contradictory and depends on the ethnic group [7]. Therefore, it seems appropriate to analyze the association of the combination of *IL-4(rs2243250), TNFб (rs1800620)* and *IL-10(rs1800896)* cytokine genes among people living in the Odessa region with clinical symptoms of the disease and basic biochemical and immunological parameters.

The aim of this work is a relationship of the polymorphism *IL-4(rs2243250), TNFб (rs1800620), IL-28B (rs8099917)* and *IL-10(rs1800896)* genes with biochemical, immunological parameters with degree of fibrosis in patients with chronic hepatitis C.

Materials and methods of research

The 120 patients with chronic hepatitis C aged 18 to 62 years were examined. All examined patients were under monitoring in the hepatological center of the Odessa Municipal Clinical Infectious Diseases Hospital. Patients are residents of the Odessa region, in the study groups there were 55% of men and 45% of women. The duration of the disease was no more than 10 years.

The control group consisted of 30 practically healthy persons, the average age of which was 32±1.05 years. The number of women and men was the same (15 people each).

All patients included in the study were given free and informed consent. The methodology of this investigation is in accordance with the requirements of the Committee on Bioethics of the Odesa National Medical University (protocol 179 of 19.11.2010).

When making a diagnosis of chronic hepatitis C, we took into account the history data and clinical indicators (weakness, fatigue, malaise, sweating, itching, decreased or absent of appetite, nausea, a feeling of heaviness in the right hypochondrium, unstable stools, jaundice, manifestations of hemorrhagic syndrome, hepatomegaly, splenomegaly). All patients were examined for complete blood count, complete urinalysis, concentration of total bilirubin in blood serum and its fractions, ALT and AST activity, concentration of total protein and its fractions, prothrombin index, alkaline phosphatase.

Confirmation of the diagnosis of chronic hepatitis C based on detection of main serological markers (anti-HCV-IgM, qualitative and quantitative determination of HCV RNA using PCR).

Assessment of fibrotic changes in the liver was determined according to the METAVIR scale using the non-invasive FibroScan method.

Determination of subpopulations of T and B lymphocytes (CD3+, CD4+, CD8+, CD16+, CD19+) was carried out by the immunofluorescence method using a set of monoclonal and polyclonal antibodies to establish differential antigens of human lymphocytes on an Eurostar immunofluorescent microscope.

DOI http://dx.doi.org/10.5281/zenodo10417848

Molecular genetic studies included the determination of polymorphic variants of the *IL-4 (rs2243250), IL-28B (rs8099917), IL-10 (rs1800896), TNFб (rs1800620)* genes. Polymorphism was studied by amplification of the corresponding regions of the genome by PCR. The structure of the primers used and the parameters of temperature cycles described in the literature and the genomic database. The studies were carried out on the basis of the German Diagnostic Center. St. Paul (Odesa).

The obtained results of immunological studies were processed by the methods of variation statistics using the Excel program. The results are given as the arithmetic mean (M) and the arithmetic mean error (±m). In order to identify correlations between individual indicators, Spearman's correlation coefficient was applied. The distribution of genotypes for the studied polymorphic loci was checked using Pearson's χ^2 test. The allele and genotype frequencies in the groups were compared using Pearson's χ^2 test with Yates' correction for continuity with the number of degrees of freedom equal to 1.

Research results and discussion

At the moment of admission to the center, asthenovegetative syndrome was observed in all patients (100%), dyspeptic syndrome – in 71,7% patients, arthralgic syndrome – in 34,7%. Jaundice was recorded rarely (9,2%), was weak and short-lived. Most patients had hepatomegaly (76,7%) and splenomegaly (37,5%).

At the moment of admission to the center, the analysis of biochemical indicators in patients with chronic hepatitis C with different degrees of process activity shows that in most of the examined persons (75%) the level of total bilirubin remained within the normal range - the average level of total bilirubin was (19,4 ± 1,3) мmol/l, the level of this indicator was 1.8 times higher than in healthy people. A significant increase in the activity of transaminases was noted in all patients: on average, the activity of ALT and AST was 6.7 times higher than in healthy individuals. The level of thymol in the sample was also increased: 2.6 times more than in healthy subjects. The content of total protein in patients with chronic hepatitis C was reduced by 1,3 times, as was the level of albumin and г-globulins (by 2,0 times and 1,6 times, respectively).

Compared to healthy persons, the level of CD3+ was 2,2 times lower, CD4+ – 1,5 times, CD16+ – 1,8 times lower in patients with chronic hepatis C: the content of CD8+ and CD19+ was increased – 1,2 times and 1,4 times respectively. That is, in patients with chronic hepatitis C, a significantly low content of CD3+, CD4+, CD16+ and an increase in the level of CD8+ and CD19+ were established in comparison with the indicators of healthy people ($p < 0.05$).

To assess the association of the activity of the inflammatory process, the degree of changes in the liver tissue, indicators of cellular immunity and allelic gene polymorphism, all patients were divided on 3 groups: with absent or minimal fibrosis (F0-F1) – 38,3%, moderate fibrosis (F2) – 25,8 % and with severe fibrosis (F3) – 19,2%.

When analyzing the dynamics of the main clinical syndromes, it was found that in patients with chronic hepatitis C with the degree of fibrosis F0-F1, a less pronounced change in the general condition was observed than in patients with the degree of fibrosis F2-F3.

In patients with minimal (F0-F1) and moderate (F2) fibrosis, the indicators of protein metabolism and liver enzymes did not change significantly, their values remained within the normal range for almost the entire period of the study. In patients with severe liver fibrosis, the highest cytolysis indicators and a significant decrease in proteinogram indicators were noted.

A certain dependence of the general condition of patients and changes in immunological parameters was established. All patients with chronic hepatitis C complained

DOI http://dx.doi.org/10.5281/zenodo10417848

of general weakness, constant fatigue and loss of working capacity, but a certain part (43 people) emphasized that such changes in general condition not only do not allow them to work fully, but also force patients to radically change their usual way of life. These patients had the lowest CD3+ (22-23%) and the highest CD8+ (28-29%) indicators. Among them were only patients with advanced liver fibrosis.

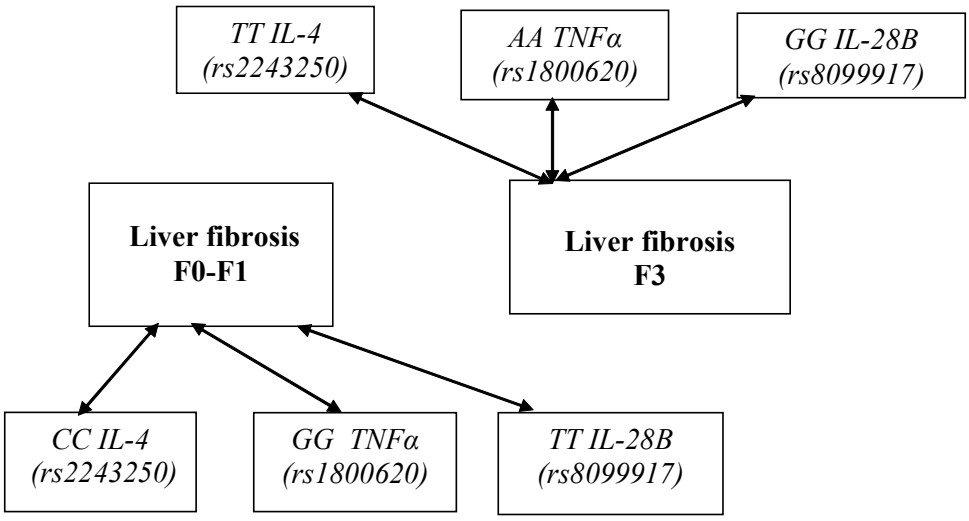
The study of allelic polymorphism of *IL-4(rs2243250), TNFб (rs1800620)* and *IL-10(rs1800896)* revealed significant differences in the control and study groups of patients. this information was presented in our other works [8].

The relationship between liver fibrosis, biochemical indexes, immunological Indicators, and allelic polymorphism of the studied genotypes was assessed using the Spearman rank correlation coefficient (Pict 1).

The presence of the following correlations has been established:

- a moderate direct correlation between the degree of fibrosis and the activity of ALT, $p < 0.01$ (in patients with a greater degree of fibrosis, higher activity of the enzyme is observed);
- a moderate direct correlation between the degree of fibrosis and the activity of AST, $p < 0.01$ (in patients with a greater degree of fibrosis, a higher activity of the enzyme is observed);
- direct correlation between *IL-4 (rs2243250)* genotypes and CD3+ content, $p<0.05$ (higher CD3+ content is noted in carriers of the CC genotype, lower CD3+ content in carriers of the TT genotype);
- direct correlation between *IL-4 (rs2243250)* genotypes and ALT and AST activity, $p<0.01$ (lower ALT and AsAt activity is noted in carriers of the CC genotype, higher ALT and AsAt activity in carriers of the TT genotype);
- inverse correlation between *TNFб (rs1800620)* genotypes and ALT and AST activity, $p<0.01$ (lower ALT and AST activity is noted in carriers of the GG *TNFб (rs1800620)* genotype, higher ALT and AST activity in carriers of the AA *TNFб (rs1800620)* genotype);
- inverse correlation between the content of CD3+ and the activity of ALT and AST (low content of CD3+ cells corresponds to a higher activity of transaminases), $p<0.05$.

The severity of the inflammatory process in the liver tissue was more significant



Pict 1. The relationship between liver fibrosis, biochemical indexes, immunological Indicators, and allelic polymorphism of the genotypes

DOI http://dx.doi.org/10.5281/zenodo10417848

with the rapid progression of fibrosis. It can be assumed that combinations of CC *IL-4 (rs2243250)* and GG *TNFб (rs1800620)* genotypes have anti-inflammatory activity, since they dominate in patients with the degree of fibrosis F0-F1 and a less pronounced imbalance of the immune status. It is possible that combinations of TT *IL-4 (rs2243250)* and AA *TNFб (rs1800620)* genotypes are associated with a profibrogenic effect, as they were found in patients with F2-F3 fibrosis.

Thus, in chronic hepatitis C, carriers of the TT IL-4 (rs2243250) and AA *TNFб (rs1800620)* genotypes are characterized by a higher level of hepatocyte damage and the severity of mesenchymal inflammation.

The presence of a relationship between transaminase activity, immunological parameters, and certain specific genotypes of IL-4 and TNFб allows using the information obtained as one of the criteria for the activity of the inflammatory process in the liver. The severity of changes in cellular immunity is an additional criterion for the degree of morphological disorders in the liver tissue.

Conclusions

The data obtained can help a practical clinician in predicting the course of chronic hepatitis C, which is directly related to an individual plan for managing a patient with chronic hepatitis C.

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Вперше надійшла до редакції 20.05.2023 р.
Рекомендована до друку на засіданні редакційної колегії після рецензування