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Modern features of a clinical course of HCV infection at children depending on the genotype of the virus

«Pediatric infectious diseases**».**

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ABSTRACT

Virus hepatitises occupy one of leading places in infectious pathology of the person. High incidence, development of chronic forms quite often coming to an end with cirrhosis or a hepatocellular carcinoma, the high lethality and considerable economic losses define value of these infections for many countries of the world, including for Uzbekistan. It is known that for all republics of the Central Asian region of the CIS the problem of virus hepatitises is one of central. On the medical importance and size of social and economic damage of HVG take a leading place in infectious pathology

High variability of the HCV genome has led to the formation of many types of viral quasispecies and genotypes . According to recent data it is known to 6 major HCV genotypes. It is noted that the most severe course , a high percentage of chronic infections with a poor outcome, high resistance to interferon observed in patients known to have HCV genotype lb .Thus, early detection of hepatitis C in children , as well as genotyping pathogen is an important prerequisite for effective treatment of the disease.

On the basis of phylogenetic analysis of nucleotide sequences, multiple genotypes and subtypes of hepatitis C virus have been identified. Characterization of these genetic groups is likely to facilitate and contribute to the development of an effective vaccine against infection with HCV. Differences among HCV genotypes in geographic distributions have provided investigators with an epidemiologic marker that can be used to trace the source of HCV infection in a given population. HCV genotype 1 may represent a more aggressive strain and one that is less likely to respond to interferon treatment than HCV genotype 2 or 3. However, these observations require confirmation before HCV genotyping can be used in clinical settings.

CONTENTS

LIST OF ACRONYMS

AIDS - acquired immunodeficiency syndrome

AII - an acute intestinal infection

APT - Antiretroviral Therapy

CD3-T-lymphocytes

CD4-T-helper

CD8 - cytotoxic lymphocytes

CIC - circulating immune complexes

CMV - cytomegalovirus infection

CNS - central nervous system

DNA - deoxyribonucleic acid

EBV - Epstein-Barr virus

ELISA - enzyme-linked immunosorbent assay

GIT - gastro - intestinal tract

HAART - highly active antiretroviral therapy

HBV- hepatitis B virus

HCV - hepatitis C virus

HIV - Human Immunodeficiency Virus

PCR - polymerase chain reaction

RNA - ribonucleic acid

SARS - acute respiratory infection

STD - sexually transmitted diseases

TNF - tumor necrosis factor

TORCH - a group of infections caused by toxoplasma, rubella virus, herpes, cytomegalovirus

WHO - World Health Organization

INTRODUCTION

The urgency of the problem. Virus hepatitises occupy one of leading places in infectious pathology of the person. High incidence, development of chronic forms quite often coming to an end with cirrhosis or a hepatocellular carcinoma, the high lethality and considerable economic losses define value of these infections for many countries of the world, including for Uzbekistan. It is known that for all republics of the Central Asian region of the CIS the problem of virus hepatitises is one of central. On the medical importance and size of social and economic damage of HVG take a leading place in infectious pathology [E.I.Musabayev and соавт. 2008, 2010, B.M.Tadzhiyev 2010, L.N.Tuychiyev 2008]

High variability of the HCV genome has led to the formation of many types of viral quasispecies and genotypes . According to recent data it is known to 6 major HCV genotypes [Okamoto et al., 1992 ; Simmonds et al., 2004]. It is noted that the most severe course , a high percentage of chronic infections with a poor outcome [formation of cirrhosis and hepatocellular carcinoma] , high resistance to interferon observed in patients known to have HCV genotype lb .Thus, early detection of hepatitis C in children , as well as genotyping pathogen is an important prerequisite for effective treatment of the disease.

On the basis of phylogenetic analysis of nucleotide sequences, multiple genotypes and subtypes of hepatitis C virus [HCV] have been identified. Characterization of these genetic groups is likely to facilitate and contribute to the development of an effective vaccine against infection with HCV. Differences among HCV genotypes in geographic distributions have provided investigators with an epidemiologic marker that can be used to trace the source of HCV infection in a given population. HCV genotype 1 may represent a more aggressive strain and one that is less likely to respond to interferon treatment than HCV

genotype 2 or 3. However, these observations require confirmation before HCV genotyping can be used in clinical settings.

Biologists are generally not known for creativity when it comes to naming things - hence Hepatitis C virus. The most commonly used classification of Hepatitis C virus has HCV divided into the following genotypes [main types]: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11. As we've highlighted, HCV genotypes can be broken down into sub-types, some of which include:

1a, 1b, 1c

2a, 2b, 2c

3a, 3b

4a, 4b, 4c, 4d, 4e

5a

6a

7a, 7b

8a, 8b

9a

10a

11a

It is believed that theHhepatitis C virus has evolved over a period of several thousand years. This would explain the current general global patterns of genotypes and subtypes:

1a - mostly found in North & South America; also common in Australia

1b - mostly found in Europe and Asia.

2a - is the most common genotype 2 in Japan and China.

2b - is the most common genotype 2 in the U.S. and Northern Europe.

2c - the most common genotype 2 in Western and Southern Europe.

3a - highly prevalent here in Australia [40% of cases] and South Asia.

4a - highly prevalent in Egypt

4c - highly prevalent in Central Africa

5a - highly prevalent only in South Africa

6a - restricted to Hong Kong, Macau and Vietnam

7a and 7b - common in Thailand

8a, 8b & 9a - prevalent in Vietnam

10a & 11a - found in Indonesia

It's believed that of the estimated 160,000 Australians with HCV, approx. 35% have subtype '1a', 15% have '1b', 7% have '2', 35% have '3' [mostly being 3a]. The remaining people would have other genotypes.

Current scientific belief is that factors such as duration of a person's HCV infection, their HCV viral load, age, grade of liver inflammation or stage of fibrosis may play an important role in determining response to interferon treatment. Recent studies have suggested that a person's HCV subtype [or subtypes] may influence their possible response to interferon, or interferon-ribavirin combination treatment. Worldwide trials are being conducted which will soon shed more light on this belief. We'll publish any reports as they come to hand.

Purpose

To investigate the features of the circulation of the hepatitis C virus and its genotypes in the structure of infant morbidity of hepatitis C, and to determine the clinical course of hepatitis C

Research objectives:

- 1. Explore the spread of HCV among children
- 2. Reveal the nature of the clinical course of hepatitis C, depending on the genotype of the virus
- 3. Identify the nature of the biochemical features of hepatitis C, depending on the genotype of the virus

Subjects:

I. Object of study: To accomplish our clinical examination of 60 patients with HCV. Observations and clinical tests were conducted at the Institute of Virology.

All the patients were conducted clinical analyzes, blood chemistry, liver ultrasound, serology [ELISA], PCR.

II. Material for the study, blood

III. Methods:

1. Clinical and laboratory examination according to generally accepted standards;

2. Determination of viral load by polymerase chain reaction [PCR] mode real time, isolation of total RNA by HCV, from blood plasma.

Scientific novelty:

1. For the first time systematically studied clinical and biochemical and serological features of HCV infection with different genotypes in children

2. A comparative study of clinical, epidemiological and biochemical characteristics depending on the variant genotype HCV

Practical significance:

The regularities of the clinical course, biochemical changes and significance of the detection of specific serological and virological markers and clinical outcome of acute HCV infection, depending on the genotype of the virus.

CHAPTER I. LITERARY REVIEW

1.1. Healthy mother - healthy child»

Positive dynamics is largely ensured national model of maternal and child health "Healthy mother - healthy child". It covers all aspects of raising a healthy generation, from the formation of the medical culture in the society, especially among young people, promotion of physical culture and sport to create cutting-edge specialized medical centers.

Over the past 10 years, the incidence of acute infectious diseases among children 6-15 years decreased by 34.4%, pneumonia - 49.7%, bronchitis - by 32.8%, scoliosis - by 32.7%. Some infectious diseases, and even managed to eradicate, such as polio.

Doctors continue to consistently implement the State program "Year of a healthy mother and child" and other previously approved social programs that contribute to the further improvement of maternal and childhood. Work is conducted in five major directions. The company aims at providing a broad and equitable access to quality health services by strengthening reproductive health of population, protection of the health of mothers, children and adolescents at all levels of health care, improving the quality of medical and social rehabilitation of children with disabilities in the development, improvement of children and adolescents with disabilities, his creation of the necessary conditions for full participation in society. Strengthen the capacity of health personnel, particularly specialists of rural medical centers, family clinics and district associations. The infrastructure to provide qualified, specialized and high-tech health care for mothers, children and adolescents.

National model of maternal and child health "Healthy mother - healthy child" covers all aspects of raising a healthy generation, from the formation of the

medical culture in the society, promotion of physical culture and sport to create cutting-edge specialized medical centers. Another important aspect is the involvement of the population to participate in the activities and programs to improve care for mothers, newborns, children and adolescents, promoting the benefits of healthy lifestyles, the creation of strong and happy family.

Improving health information systems, ensure coordination, monitoring and evaluation of inter-agency cooperation and action in the implementation of activities in the field of reproductive health, especially for mothers and children.

The Ministry of Health announced the results of the next Month, held in March under the motto "No one will be left without attention and care!" The campaign underwent a thorough medical examination of more than 200 thousand. Inhabitants of the country. Doctors rural medical centers and family health centers, together with colleagues from the regional and national health facilities diagnosed, counseled, directed to treatment. More than half addressed women and children.

Recovery and reduce the incidence among women of childbearing age and contribute monthly targeted women of childbearing age healing Weeks, children and teens. They are carried out with the active participation of leading experts of national and regional institutions. Since 2014 within the framework of this initiative, qualified assistance received more than 1 million mothers and children.

The effectiveness of their system facilitates targeted health examinations. At all stages of the life of a person goes through consultation with doctors. One of the most important - before marriage. It allows you to create the future spouses and family planning, taking into account the health status of each of them, and if necessary - to undergo treatment.

In the framework of the "Year of a healthy mother and baby" a lot of attention is paid to improving the quality of coverage of medical examination of the intending spouses. special monitoring will be set for carrying out the diagnosis of infectious and viral diseases, including tuberculosis, AIDS and STI pathologies.

At check-ups, there is another goal. They allow physicians to have a reliable picture of the state of health of the population and to plan preventive measures.

The country continues to struggle with the diseases caused by deficient in trace elements in the environment. Prohibited the implementation of table salt without iodine enrichment took place, and the most consumed type of flour enriched with iron and folic acid. Pregnant women living in rural areas, at the expense of the State Budget provides complexes multivitamins. It is expected that participants of the program in 2016 will be more than 400 thousand. Expectant mothers.

Early detection of deviations and pathologies allows doctors to promptly appoint preventive measures. By the way, all newborns are registered in screening centers, receive free health food.[1,2,3]

1.2. The characteristic of HCV genotypes

On the basis of phylogenetic analysis of nucleotide sequences, multiple genotypes and subtypes of hepatitis C virus [HCV] have been identified. Characterization of these genetic groups is likely to facilitate and contribute to the development of an effective vaccine against infection with HCV. Differences among HCV genotypes in geographic distributions have provided investigators with an epidemiologic marker that can be used to trace the source of HCV infection in a given population. HCV genotype 1 may represent a more aggressive strain and one that is less likely to respond to interferon treatment than HCV genotype 2 or 3. However, these observations require confirmation before HCV genotyping can be used in clinical settings.

Hepatitis C virus [HCV] infection has reached epidemic proportions. Worldwide, more than one million new cases of infection are reported annually, and HCV is believed to be more prevalent than hepatitis B virus infection [HBV] [26]. In the United States alone, nearly four million persons are infected and 30,000 acute new infections are estimated to occur annually [89]. Currently, HCV is responsible for an estimated 8,000 to 10,000 deaths annually in the United States, and without effective intervention, that number is predicted to triple in the

next 10 to 20 years [89]. Furthermore, HCV is the leading reason for liver transplantation in the United States and this has major implications in the present era of organ shortage. The ultimate goal is a universally effective vaccine to prevent new cases, especially in underdeveloped countries, where HCV infection is more prevalent and treatment is financially out of reach for most patients. The development of such a vaccine has been hampered, at least partly, by the great heterogeneity of the HCV genome, which is the focus of this review.

HCV was the first virus discovered by molecular cloning without the direct use of biologic or biophysical methods. This was accomplished by extracting, copying into cDNA, and cloning all the nucleic acid from the plasma of a chimpanzee infected with non-A, non-B hepatitis by contaminated factor XIII concentrate [24]. The HCV genome is a positive-sense, single-stranded RNA genome approximately 10 kb long. It has marked similarities to those of members of the genera *Pestivirus* and *Flavivirus*. Different HCV isolates from around the world show substantial nucleotide sequence variability throughout the viral genome [25]. Based on the identification of these genomic differences, HCV has been classified into multiple strains. It is thought that genetic heterogeneity of HCV may account for some of the differences in disease outcome and response to treatment observed in HCV-infected persons.

Before proceeding with the discussion, it is important to consider the shortcomings of studies related to the clinical importance of HCV genotypes. Although several studies have specifically evaluated the role of HCV genotypes and the clinical utility of genotyping, many questions have not been answered. Investigators have used several classification systems, especially before 1995, and have adopted different methods of genotyping. Furthermore, there has been no consistency among studies in the definition of study end points to allow for comparison and “collective experience.” This was most obvious in studies that addressed the role of genotypes in liver disease progression or response to interferon therapy. The severity of liver disease was based on histologic activity in

some studies and on the development of cirrhosis or hepatocellular carcinoma in others. Similarly, in most trials before 1995, the response to interferon treatment was defined as normalization of transaminases at the end of therapy [biochemical response], but this was replaced by the virologic response, defined as the disappearance of PCR-detectable HCV RNA in plasma. Also, the clinical significance of HCV genotypes that are not common in the United States, Europe, or Japan has received minimal attention because most scientific investigations are being conducted in these countries. These genotypes, which include HCV types 4 through 9, have been found mostly in less industrialized countries [India and countries in Southeast Asia and the Middle East].

Genomic organization of HCV

The original isolate [HCV-1] was a positive-sense RNA virus with approximately 9,400 ribonucleotides, containing a poly[A] tail at the 3′ . The sequence contained a 5′ untranslated region [5′ UTR] of 341 bases, a long open reading frame coding for a polyprotein of 3,011 amino acids, and a 3′ untranslated region [3′ UTR] of about 27 bases. This RNA structure is most similar to that of the family *Flaviviridae*, which encompasses numerous arthropod-borne viruses. Consistent with the known functions of most flavivirus proteins, the three N-terminal HCV proteins are probably structural [C, E1, and E2/NS2] and the four C-terminal proteins [NS2, NS3, NS4, and NS5] are believed to function in viral replication.

Genomic organization of HCV. First generation, second generation, and third generation refer to serologic assays for detection of HCV antibodies.

The open reading frame length of each genotype is characteristically different. Whereas the open reading frame in type 1 isolates is approximately 9,400 ribonucleotides, that of type 2 isolates is typically 9,099 nucleotides and that of type 3 isolates is typically 9,063 nucleotides [12]. These differences may

potentially account for some of the phenotypic differences among genotypes discussed below.

The 5′ UTR of HCV RNA is the most highly conserved portion of the genome and thus has been used in most laboratories to develop sensitive detection assays for HCV RNA [52]. It is also thought to be important in the translation of the HCV open reading frame. The E1 and E2 regions of the HCV genome demonstrate the highest mutation rate at the nucleotide level as well as at the predicted amino acid level [49]. The finding of a rapidly evolving region within one of the envelope proteins of HCV suggests that this region is under selective pressure by the host immune system. An extraordinarily high rate of nucleotide change that frequently resulted in codon changes was found in hypervariable region 1 of the E2 protein of the HCV genome and consists of 27 amino acids [93]. The variability of this region resembles that of the V3 loop of human immunodeficiency virus [HIV], a domain that elicits anti-HIV type-specific neutralizing antibodies. Studies in chimpanzees and in patients with acute and chronic hepatitis C have demonstrated that these infected hosts mount a humoral immune response to epitopes of hypervariable region 1 of the HCV genome [110, 149]. The presence of this rapidly changing region may permit a mechanism by which HCV evades host immune surveillance and establishes and maintains persistent infection.

Multiple protein antigens encoded by the viral RNA seem to produce serologic responses in the host. Serologic responses to four of these protein antigens are used in diagnostic laboratories for the detection of HCV infection [Fig. [Fig.1].1]. The first two proteins, termed 5-1-1 and c100-3, are derived from nonstructural regions of the HCV genome, specifically the NS3 and NS4 regions, and together they form the basis of the first-generation antibody assays [enzyme-linked immunosorbent assay 1 [ELISA-1] and strip immunoassay 1 [SIA-1]]. In addition to 5-1-1 and c100-3, proteins c33 and c22 are included in the second-generation antibody assays [ELISA-2 and SIA-2]. Protein c33c is derived from the

NS3 region, which is a nonconserved region of the viral genome. However, protein c22 is derived from the highly conserved nucleocapsid [C] region [Fig. [Fig.1].1]. A recombinant NS5 antigen has been added to the above four antigens to improve the sensitivity of serologic assays for the detection of HCV antibodies [third-generation antibody assays].

After the complete HCV genome was determined by Choo et al. in 1991 [25], several HCV isolates from different parts of the world were obtained and sequenced [22, 31, 38, 60, 68, 69]. Comparison of the published sequences of HCV has led to the identification of several distinct types that may differ from each other by as much as 33% over the whole viral genome [96]. Sequence variability is distributed equally throughout the viral genome, apart from the highly conserved 5′ UTR and core regions and the hypervariable envelope [E] region [59, 95–97, 109, 126].

As different investigators developed and used their own classification system for HCV strains, a confusing literature developed [Table [Table1].1]. However, at the 2nd International Conference of HCV and Related Viruses, a consensus nomenclature system was proposed that is to be used in future studies of HCV genotypes and subtypes [P. Simmonds, A. Alberti, H. J. Alter, F. Bonino, D. W. Bradley, C. Brechot, J. T. Brouwer, S. W. Chan, K. Chayama, D. S. Chen, Q.-L. Choo, M. Colombo, H. T. M. Cuypers, T. Date, G. M. Dusheiko, J. I. Esteban, O. Fay, S. J. Hadziyannis, J. Han, A. Hatzakis, E. C. Holmes, H. Hotta, M. Houghton, B. Irvine, M. Kohara, J. A. Kolberg, G. Kuo, J. Y. N. Lau, P. N. Lelie, G. Maertens, F. McOmish, T. Miyamura, M. Mizokami, A. Nomoto, A. M. Prince, H. W. Reesink, C. Rice, M. Roggendorf, S. W. Schalm, T. Shikata, K. Shimotohno, L. Stuyver, C. Trépo, A. Weiner, P. L. Yap, and M. S. Urdea, Letter, Hepatology **19:**1321–1324, 1994]. According to this system, HCV is classified on the basis of the similarity of nucleotide sequence into major genetic groups designated genotypes. HCV genotypes are numbered [arabic numerals] in the order of their discovery [Tables [Tables11 and and2].2]. The more closely related HCV strains

within some types are designated subtypes, which are assigned lowercase letters [in alphabetic order] in the order of their discovery. The complex of genetic variants found within an individual isolate is termed the quasispecies. The quasispecies composition of HCV results

Terminology commonly used in studies related to HCV genomic heterogeneity

The genomic sequences of different HCV isolates vary by as much as 35% [96]. The degrees of difference in nucleotide sequences among isolates vary from one genomic region to another. Sequence similarities between members of the different genotypes of a 222-bp segment of the NS5 region that we used in our laboratory range between 55 and 72%, whereas identities of subtypes range from 75 to 86% [Simmonds et al., Letter, Hepatology **19:**1321–1324, 1994].

Comparative sequence analysis among HCV subtypes of a 222-nucleotide segment derived from the viral NS5 region[a]

With the rarity of severe acute or fulminant HCV infections, the significance of this infection in humans is its tendency to become persistent and to induce chronic liver disease. The mechanisms of HCV persistence are not known. However, in most human viral infections, the interaction of several arms of the immune system is important in limiting viral replication and preventing persistence. These arms of the immune system include humoral and cellular immunity.

Antibody responses are often directed against several viral proteins, although it is the antibodies directed against the viral envelope proteins that usually serve as neutralizing antibodies [90]. Neutralizing antibodies are often specific for a particular serologic type of the virus, an issue that is particularly relevant in discussions of strategies for vaccination against highly variable viruses such as HIV or HCV.

Whether infection with HCV elicits protective immunity in the host remains unclear. Farci et al. [41] attempted to neutralize HCV in vitro with plasma obtained

from a chronically infected patient. The source of HCV was the same patient during the acute phase of posttransfusion non-A, non-B hepatitis. The residual infectivity was evaluated by inoculation of seronegative chimpanzees. The authors showed that neutralization was achieved with plasma obtained 2 years after the initial exposure but not with plasma obtained 11 years later. Analysis of viral isolates for the same patient showed significant genetic divergence of HCV over time. These data support the quasispecies nature of HCV and the selection of strains to avoid immune pressure. This experiment also emphasized the possible role of genetic heterogeneity of HCV in escaping the immune system. It has been suggested that these antibodies are likely to be directed against epitopes of hypervariable region 1 located in the E2 region [41].

Similarly, cellular immune responses, particularly those mediated by cytotoxic T lymphocytes [CTLs], are important components of protective immunity against many viral infections, including hepatitis B. In HCV infection, the role of CTLs in protecting against viral persistence is unknown. HCV-specific, HLA class I-restricted CTLs were demonstrated within the liver [63, 64]. Possible targets for HCV-specific CTL recognition within the conserved core protein and additional epitopes in the more highly variable region E2 protein were also identified [63, 64]. HCV heterogeneity may also be important in escaping CTL-induced immunity. In a chronically infected chimpanzee, CTLs obtained from the liver were initially able to recognize an epitope in the NS3 protein. Over a period of several years, a new strain of the virus emerged with a mutation in the CTL epitope that was no longer recognized by the CTLs isolated earlier. Although direct evidence for the presence of CTL escape mutants in human HCV infection is lacking, it has been shown that single-amino-acid changes in CTL epitopes result in failure of recognition by HCV-specific CTLs [62]. These single-amino-acid changes are found in natural isolates of HCV, hence the need to address the problem of type specificity of immune responses.

Irrespective of the specific type of immune response [humoral or cellular] that is associated with protection and clearance of HCV after an acute exposure, the response appears to be type specific. This conclusion could be extracted from a natural experiment in children with thalassemia who had undergone numerous transfusions [66]. Lai et al. [66] reported multiple episodes of acute hepatitis C in these children. The second episode of acute hepatitis C appeared to result from infection with a different strain of HCV, suggesting that immune responses to the initial strain did not protect against an infection with another strain of HCV.

Analogously, the genetic heterogeneity of HCV is likely to make the development of an HCV vaccine difficult. A vaccine that consisted of recombinant E1 and E2 proteins of HCV-1 [genotype 1a] was tested in chimpanzees [23]. Of seven chimpanzees that were challenged with HCV-1 after vaccination, five were protected against reinfection, compared with none of four control unvaccinated chimpanzees. The finding that these proteins, derived from hypervariable regions of the HCV genome, can elicit protective immunity poses a major challenge for the development of a broadly effective vaccine for the prevention of HCV.

Because differences in geographical distribution, disease outcome, and response to therapy among HCV genotypes have been suggested, reliable methods for determining the HCV genotype may become an important clinical test. The reference standard and most definitive method for HCV genotyping is sequencing of a specific PCR-amplified portion of the HCV genome obtained from the patient, followed by phylogenetic analysis. Investigators of HCV genotyping have used sequence analysis of HCV NS5, core, E1, and 5′ UTRs. However, direct sequencing is impractical on a large scale because of the complexity of the procedure. Even with the introduction of automated sequencing methods that do not require radioactive isotopes, only a few laboratories are equipped to perform these procedures on a regular basis. Finally, sequencing of amplified DNA does not usually identify mixed infections with two different HCV genotypes.

Other methods that have been reported depend mainly on the amplification of HCV RNA from clinical specimens, followed by either reamplification with type-specific primers or hybridization with type-specific probes [70, 98, 107, 134] or by digestion of PCR products with restriction endonucleases that recognize genotype-specific cleavage site [81; D. Murphy, B. Willems, and G. Delage, Letter, J. Infect. Dis. **169:**473–475, 1994]. HCV genotyping by using type-specific primers was first introduced by Okamoto et al. [98] and used primers specific for the core region. This method lacked sensitivity and specificity [135]. Without modification, this method was able to detect subtypes 1a, 1b, 1c, 2a, 2b, and 3a. However, modifications have been introduced to improve the sensitivity and specificity of this method [94, 134], but more studies are required before the efficiency of this genotyping method can be compared with that of other methods. Several DNA hybridization assays for HCV genotyping have been described. A commercial kit [InnoLipa] for HCV genotyping has been introduced in Europe by Innogenetics [Zwijndre, Belgium] and is based on hybridization of 5′ UTR amplification products with genotype-specific probes [123]. Although the initial version of InnoLipa had lower sensitivity, the newer version is capable of discriminating among HCV subtypes 1a, 1b, 2a to 2c, 3a to 3c, 4a to 4h, 5a, and 6a [75]. It has been shown that genotyping methods using 5′ UTR, including InnoLipa, may not distinguish subtype 1a from 1b in 5 to 10% of cases and also may not distinguish between subtypes 2a and 2c [120].

Others have used restriction enzymes to determine a restriction fragment length polymorphism. In this method, a PCR-amplified DNA fragment is digested into fragments with different lengths by enzymes [restriction endonucleases] that recognize cleavage sites specific for each genotype [135]. Investigators have used different regions of the HCV genome for restriction fragment length polymorphism, including NS5 and the 5′ UTR [14, 19, 87].

Although all these methods are able to identify correctly the major genotypic groups, only direct nucleotide sequencing is efficient in discriminating among

subtypes [12, 113]. Moreover, all of these PCR-based methods have the shortcomings and advantages of PCR. They are expensive and time-consuming and require specialized facilities to ensure accurate results and prevent contamination. Their reliability may further be compromised if viral RNA is lost in the serum or plasma through storage or improper laboratory handling or if it is absent from the circulation during sample collection. The advantages of PCR-based methods include reliability if performed accurately and the ability to obtain information relevant to the molecular pathogenesis of HCV.

More recently, investigators identified genotype-specific antibodies that could be used as indirect markers for the HCV genotype [serotyping or serologic genotyping] [74, 85, 119, 130]. Serologic genotyping has several advantages that make it suitable for large epidemiologic studies. These advantages include the low risk for contamination and the simplicity of the assay. However, serologic typing seems to lack specificity and sensitivity, which limits its usefulness.

Two commercially available serologic genotyping assays have been introduced over the past 3 to 4 years. The RIBA SIA was introduced by Chiron Corp. and contained five different serotype-specific peptide sequences taken from the NS4 region and two serotype-specific peptide sequences taken from the core region of the HCV genomes for genotypes 1, 2, and 3 [34]. The second serologic genotyping assay is the Murex HCV serotyping enzyme immune assay [Murex Diagnostics Ltd.], which is based on the detection of genotype-specific antibodies directed to epitopes encoded by the NS4 region of the genomes for genotypes 1 through 6. These two assays have been compared and showed a concordance rate of more than 96% for genotypes 1, 2, and 3 [44].

A recent study by Beld et al. [4] showed high reliability of HCV serotyping by the RIBA SIA [Chiron Corp., Emeryville, Calif.] in immunocompetent individuals infected with genotype 1a. However, the assay had low sensitivity in samples containing genotype 3a or in samples from patients coinfected with HIV. These

findings suggest that the use of this assay may be limited at this time, particularly in geographic regions where genotype 1a is not prevalent. Similarly, Songsivilai et al. [122] showed that serotyping had poor sensitivity for samples from patients infected with HCV genotype 6. Unlike the two previous studies, a study conducted in the United States reported high concordance between serologic genotyping and molecular genotyping assays [44]. These findings suggest variation in the reliability of these assays based on the distribution of HCV genotypes in a specific geographic area.

The choice of typing method for HCV should be based on the expertise in a specific laboratory or institution and the goal of typing. To identify all subtypes and to identify novel sequences if present, PCR amplification followed by sequencing should be the method of choice. However, the goal in treatment trials is frequently to separate patients infected with genotype 1 from those infected with other genotypes—a task that could be done adequately by any of the methods mentioned.

1.3 Clinical relevance of HCV genotypes

At least six major genotypes of HCV, each comprising multiple subtypes, have been identified worldwide [142]. Substantial regional differences appear to exist in the distribution of HCV genotypes [Fig. [Fig.2].2]. Although HCV genotypes 1, 2, and 3 appear to have a worldwide distribution, their relative prevalence varies from

Worldwide geographic distribution of HCV genotypes and subtypes. “Others” indicate unclassified sequences.

HCV subtypes 1a and 1b are the most common genotypes in the United States [Fig. [Fig.3]3] [145]. These subtypes also are predominant in Europe [35, 82, 91]. In Japan, subtype 1b is responsible for up to 73% of cases of HCV infection [124]. Although HCV subtypes 2a and 2b are relatively common in North America,

Europe, and Japan, subtype 2c is found commonly in northern Italy. HCV genotype 3a is particularly prevalent in intravenous drug abusers in Europe and the United States [101]. HCV genotype 4 appears to be prevalent in North Africa and the Middle East [1, 18], and genotypes 5 and 6 seem to be confined to South Africa and Hong Kong, respectively [17, 116]. HCV genotypes 7, 8, and 9 have been identified only in Vietnamese patients [129], and genotypes 10 and 11 were identified in patients from Indonesia [127]. There has been disagreement about the number of genotypes into which HCV isolates should be classified. Investigators have proposed that genotypes 7 through 11 should be regarded as variants of the

The geographic distribution and diversity of HCV genotypes may provide clues about the historical origin of HCV [121]. The presence of numerous subtypes of each HCV genotype in some regions of the world, such as Africa and Southeast Asia, may suggest that HCV has been endemic for a long time. Conversely, the limited diversity of subtypes observed in the United States and Europe could be related to the recent introduction of these viruses from areas of endemic infection.

Although the impact of HCV heterogeneity and genotypes on the day-to-day clinical management of chronic HCV infection has not been established, its role as an epidemiologic marker has been clearly shown. Furthermore, the sensitivity and specificity of serologic and virologic assays for the detection of HCV may be influenced by the heterogeneity of HCV. However, the exact role of genotypes in the progression of liver disease, the outcome of HCV infection, and the response to interferon therapy are much less well understood than their role as an epidemiologic marker. The study of these issues has been hampered by the long natural history of HCV infection and the lack of information about the exact time of exposure to the infection. The following subsections in this section specifically address these issues on the basis of the information available.

Genotypes and HCV Genetic Heterogeneity as Epidemiologic Markers

Because of geographic clustering of distinct HCV genotypes, genotyping may be a useful tool for tracing the source of an HCV outbreak in a given population. Examples include tracing the source of HCV infection in a group of Irish women to contaminated anti-D immunoglobulins [104]. All of these women were infected with HCV genotype 1b, a genotype identical to the isolate obtained from the implicated batch of anti-D immunoglobulin. Hohne et al. used genotyping to trace the sources of outbreaks in Germany [54]. More recently, genotyping and molecular characterization of HCV isolates provided evidence for a patient-to-patient transmission of HCV during colonoscopy [11]. The index case as well as the two other infected patients had HCV genotype 1b. Nucleotide sequencing of the NS3 region showed that the three patients had the same isolate [100% homology], strongly suggesting a common source of infection.

Suspected nonconventional routes of HCV transmission could also be investigated by molecular analysis of HCV strains from different persons. These include the vertical and sexual routes. Weiner et al. [133] showed that a single predominant HCV variant was transmitted to an infant born to a mother infected with multiple variants. Gish et al. reported similar findings [R. G. Gish, K. L. Cox, M. Mizokami, T. Ohno, and J. Y. Lau, Letter, J. Pediatr. Gastroenterol. Nutr. **22:**118–119, 1996]. A specific 12-nucleotide insertion in the E2 hypervariable region of the HCV genome was noted in the vertically transmitted sequence of an infant born to a mother infected with two different genotypes, each composed of multiple heterogeneous sequences [2]. These data may suggest a potential role of HCV heterogeneity and genotypes in mother-to-infant transmission of HCV [138].

Reports on the sexual transmission of HCV infection are conflicting. The detection of anti-HCV positivity ranged from 0% in partners of transfusion-associated hepatitis patients [40] to 8% in male homosexuals [39] and 5% in household contacts [G. Ideo, G. Bellati, E. Pedraglio, R. Bottelli, T. Donzelli, and G.

Putignano, Letter, Lancet **335:**353, 1990]. A possible explanation is that sexual transmission occurs only in association with specific HCV genotypes or in the presence of specific mutations along the HCV genome. As with vertical transmission, samples from patients with suspected sexual transmission of HCV have undergone nucleotide sequence analysis to confirm the similarity of sequences obtained from sexual partners and thus the common origin of these HCV strains [21, 51, 61]. Although the data are suggestive of a role of HCV heterogeneity in sexual transmission, this speculation needs to be confirmed.

Although Zein et al. [145] found no association between HCV genotypes and the mode of HCV acquisition in their population, others have provided evidence for such an association [7, 101, 131]. It has been suggested that genotypes 3a and 1a are closely associated with intravenous drug use and that genotype 1b is seen more often in patients who acquired HCV through blood transfusion. This information may be useful in tracing sources of HCV epidemics.

Cloning of the HCV genome led to the identification of the 5-1-1 protein that was reactive with sera obtained from patients with non-A, non-B hepatitis. Using 5-1-1 as a hybridization probe, the recombinant antigen c100 was expressed in yeast and eventually was used to develop the first screening assay [65]. The first-generation HCV antibody test approved by the Food and Drug Administration became commercially available in 1990 and was widely used. This was an ELISA that incorporated the c100 epitope from the NS4 region. Because of the high level of false positivity, SIAs were introduced as a supplemental test in patients with positive ELISA results. The ELISAs were affected by the level of circulating globulins in serum, particularly in patients with autoimmune chronic active hepatitis [79].

As more reactive recombinant antigens were identified from conserved regions of the HCV genome, newer serologic assays [second and third generation] were introduced. With improved sensitivity and specificity, the newer assays quickly

replaced the first-generation assays. These second-generation assays included the ELISA-2 and SIA-2, which were approved by the Food and Drug Administration in 1992 and 1993, respectively. In addition to the 5-1-1 and c100-3 antigens, these assays incorporated the c22-3 antigen derived from the core region of the HCV genome and the c33c antigen derived from the nonstructural region NS3. Second-generation SIAs are widely used for the validation of second-generation ELISAs in the serologic diagnosis of HCV infection. Current criteria for a positive SIA require reactivity to at least two HCV antigens encoded by different parts of the HCV genome. A common problem is that of indeterminate results, defined as reactivity to a single antigen band or reactivity to two bands derived from the same coding region [i.e., 5-1-1 and c100-3]. A significant proportion of samples [5 to 10%] that repeatedly tested reactive in ELISA-2 yielded indeterminate results when evaluated by SIA-2. Some patients with indeterminate SIA results may have detectable HCV RNA on PCR, confirming the presence of HCV viremia [46; E. A. Follett, B. C. Dow, F. McOmish, P. L. Yap, W. Hughes, R. Mitchell, and P. Simmonds, Letter, Lancet **338:**1024, 1991; P. Halfon, S. Rousseau, C. Tamalet, M. Antoni, V. Gerolami, M. Levy, M. Bourliere, R. Planells, and G. Cartouzou, Letter, J. Infect. Dis. **166:**449, 1992].

Recently, Zein et al. [140] reported on their experience with indeterminate second-generation SIAs. They found that of 720 ELISA-2-positive samples tested in the diagnostic virology laboratory between January 1994 and January 1995, 96 [13%] had indeterminate results. Of these indeterminate samples, 30 [31%] were HCV RNA positive on PCR. Next, they determined the HCV genotype distribution of SIA-2-indeterminate samples and found that it was significantly different from that of SIA-2-positive samples, suggesting that the HCV genotype affects the interpretation of these assays. Less common HCV genotypes in the United States, such as 2a, 2c, 3a, 4a, and 5a, were more prevalent in the samples with SIA-2-indeterminate results [Fig. [Fig.3];3]; this would make the serologic detection of these genotypes less efficient. These findings are consistent with an earlier report

that suggested the presence of differences in serologic reactivities to HCV antigens among HCV genotypes [146]. Reactivities to protein 5-1-1 were significantly lower for patients infected with genotype 2b or 3a than for those infected with genotypes 1a or 1b. Antibody reactivity to the c100-3 protein was also reduced in patients infected with HCV types 2b and 3a. These genotype-dependent differences may have major implications for the current use of HCV diagnostic tests, especially in geographic areas with a high prevalence of HCV genotypes that are phylogenetically distant from genotype 1a, the prototype sequence used in the development of these assays. Studies related to the efficiency of these assays should be done locally in blood banks in different regions of the world before the assay is relied on for blood screening and prevention of new infections.

Third-generation assays [ELISA-3 and SIA-3] were introduced in Europe more than 3 years ago but are still not available commercially in the United States. In these assays, a recombinant NS5 antigen has been added to the four antigens used in the second-generation assays. These third-generation assays have higher sensitivities and specificities than second-generation assays and are much less strongly influenced by the infecting genotype [57, 76, 77].

The detection of HCV RNA by reverse transcriptase PCR has become essential for the diagnosis of HCV infection and for the selection of patients before therapy. The main advantages of reverse transcriptase PCR include early diagnosis after acute infection and detection of viremia in selected patients [those with indeterminate antibody results and immunosuppressed patients]. The sensitivity of PCR for HCV RNA detection may vary according to the choice of primers and the handling of preextraction samples [13, 15]. Most laboratories use primers specific for the 5′ UTR of the HCV genome because it represents the most highly conserved region among HCV genotypes. Choosing primers from less highly conserved regions of the HCV genome is likely to compromise the sensitivity of PCR in a population in which multiple genotypes are represented.

Assays for the quantification of HCV viremia levels in serum [HCV RNA titer] have been developed and shown to be valuable in the selection of patients for interferon treatment and in the assessment of response during therapy [78, 99]. Two different methods have commonly been used for the quantitation of HCV RNA and have become commercially available. The first was developed by Roche Diagnostics Systems [Branchburg, N.J.] and is based on a competitive PCR assay for HCV RNA quantitation [Roche Monitor assay]. The second method is based on the coamplification of synthetically mutated target RNA [branched DNA [bDNA] assay; Quantiplex [Chiron Corp.]]. The quantitative results of HCV RNA detected by both methods are reliable and reproducible [73].

Earlier studies have suggested a difference in the efficiency of the bDNA assay for quantification of HCV RNA of patients infected with different HCV genotypes [10]. However, a second-generation bDNA assay was developed [Quantiplex HCV RNA 2.0] that incorporated a set of oligonucleotide probes to enhance the efficiency of binding to genotypic variants of HCV [32]. The new assay was highly sensitive and virtually unaffected by HCV genotypes. A similar variation in the efficiency of detection of different HCV genotypes was recently observed for the Roche Monitor assay [50]. These genotype-dependent differences in the efficiency of assays to accurately quantify HCV RNA may have to be considered if clinical decisions are based on the results obtained.

After initial exposure to HCV, the infection fails to resolve in the majority of patients [80%] who become chronically infected. The ability to evolve into chronic disease associated with liver damage is by far the most striking feature of HCV. The spontaneous clearance of HCV following acute infection in a small proportion of patients has been the focus of intense investigations. It has been proposed that differences in the host cellular [84] or humoral [N. N. Zein, H. Li, and D. H. Persing, Letter, Gastroenterology **117:**510, 1999] immune responses to HCV are important in spontaneous clearance, but these proposals remain unproved.

Amoroso et al. [3] specifically investigated the role of HCV genotypes in persistence of HCV infection following an acute exposure. The rate of evolution to chronicity after acute exposure to HCV was 92% in patients exposed to HCV genotype 1b infection, compared with 33% to 50% in patients exposed to other genotypes. These data provided evidence that viral factors, including the HCV genotype, may potentially play an important role in the development of chronic infection following acute exposure to HCV.

The role of HCV genotypes in the progression of liver disease is one of the most controversial areas of HCV research. There appears to be significant biologic variation in HCV disease expression in the host over the length of the infection [typically the life of the patient]. This variation among infected persons became apparent in studies on the natural history of HCV infection, for example, in a retrospective analysis of patients with chronic HCV infection whose time of HCV acquisition was known [N. N. Zein, A. S. Abdulkarim, D. Brandhagen, T. Therneau, and D. H. Persing, Abstract, Hepatology **24:**150A, 1996]. The mean times from exposure to HCV to the diagnosis of chronic active hepatitis, to compensated liver cirrhosis, to decompensated cirrhosis, and to hepatocellular carcinoma were 11, 18, 23, and 29 years, respectively [Fig. [Fig.4].**4**]. What is striking is that severe complications such as cirrhosis and hepatocellular carcinoma can occur over a short period in some persons whereas others have no complication despite a much longer period of infection [Fig. [Fig.4].**4**]. Therefore, it is likely that viral or host factors, including the infecting HCV genotype, contribute to these variations in the natural history among infected patients.

Mean time [years] between exposure to HCV and diagnosis of HCV-related complications in patients with known time of HCV acquisition.

Currently, investigators are divided into those who strongly believe in differences in pathogenicity among genotypes and those who do not. Conclusions have been derived from indirect evidence, because conducting accurate investigations to

answer these questions has been difficult. Frequently, the role of genotypes as an independent factor in the progression of liver disease cannot be separated from the roles of other cofactors such as viral load, alcohol intake, and length of time of HCV infection. Patients may not provide accurate information about drug use or the amount of alcohol intake; therefore, the time of HCV acquisition often is not known. Because of the overall slow progression of liver disease in HCV-infected patients, prospective studies frequently are not possible.

For the purpose of this discussion, the data related to HCV genotypes and progression of liver disease in patients with chronic HCV infection were examined separately from those for liver transplant recipients.

In patients with chronic HCV, infection with genotype 1b is reportedly associated with a more severe liver disease and a more aggressive course than is infection with other HCV genotypes [~~91~~; G. Pozzato, M. Moretti, F. Franzin, L. S. Croce, C. Tiribelli, T. Masayu, S. Kaneko, M. Unoura, and K. Kobayashi, Letter, Lancet **338:**509, 1991]. Similar to others [~~102~~, ~~105~~, ~~112~~], Zein et al. [~~145~~, ~~147~~] found that HCV genotype 1b was significantly more prevalent among patients with liver cirrhosis and those with decompensated liver disease requiring liver transplantation than among those with chronic active hepatitis C. Although this is indirect evidence, it suggests an association between HCV genotype 1b and the development of these complications. Furthermore, a possible link to hepatocellular carcinoma has been proposed for HCV genotype 1b. There is compelling evidence that hepatocellular carcinoma occurs more frequently or emerges earlier among HCV-infected Japanese patients [~~125~~, ~~137~~] than among HCV carriers in western countries [~~33~~, ~~55~~]. Because HCV genotype 1b is more common in Japan than in Europe or the United States, the hypothesis relating to genotype is attractive and appears to explain these differences. Furthermore, HCV genotype 1b was present in most of the patients with HCV-associated hepatocellular carcinoma studied by Zein et al. [~~143~~]. Similarly, Reid et al. determined the HCV genotypes in 28 patients with hepatocellular carcinoma and found that 19 [68%] were infected with

HCV genotype 1b and the rest were infected with a mixture of HCV genotypes that always included genotype 1b [A. E. Reid, L. J. Jeffers, K. R. Reddy, I. Aiza, E. R. Schiff, J. L. Dienstag, and T. J. Liang, Abstract, Hepatology **20:**250A, 1994].

Some reports refute the associations mentioned above [~~6~~, ~~9~~, ~~48~~, ~~67~~, ~~88~~, ~~117~~, ~~136~~]. A possible and simple explanation may reconcile these reported discrepancies. Zein et al. [~~145~~] found that patients infected with HCV genotype 1b were older than those infected with other genotypes and that genotype 1b may have been present before the other genotypes. Thus, patients infected with genotype 1b may have been infected for a longer time [Zein et al., Abstract, Hepatology **24:**150A, 1996]. As shown in Fig. Fig.5,~~5~~, all of the patients who acquired HCV before 1955 were infected with genotype 1b. HCV genotype 1a was introduced into the United States in the late 1950s and then became the most prevalent genotype in the United States. It was not until the 1960s and 1970s that genotype 2 and genotypes 3 and 4, respectively, were introduced in the United States. After accounting for differences in the duration of HCV infection, HCV genotypes were distributed equally among patients with mild or advanced liver disease. Similar observations have been made in France and Spain [~~72~~, ~~103~~]. According to this explanation, HCV genotype 1b is a marker for more severe HCV-associated liver disease, because it reflects a longer time of infection rather than a more aggressive form of hepatitis C. Future studies are still needed to rule out other host, viral, or environmental factors that may contribute to these differences.

Zein et al. [~~147~~] and Gordon et al. [~~45~~] reported that in liver transplant recipients, HCV genotype 1b is associated with earlier recurrence and more severe hepatitis than are other genotypes. Although others have reported similar findings [~~5~~, ~~100~~, ~~111~~], some authors have suggested that there is no association between genotype and HCV recurrence after transplantation [~~8~~, ~~20~~, ~~27~~, ~~148~~]. The difference in the duration of infection that may have been a factor in non-transplant-associated HCV patients is not likely to explain the discrepancies in the literature about posttransplantation HCV. More studies are needed.

Since the discovery of HCV, considerable effort has been devoted to defining the factors that may be important in predicting the long-term response to interferon therapy [144]. The interferon dose [16], duration of treatment [58], viral RNA level [132], and liver histology [71] all seem to play a role in predicting response. It has been suggested that patients infected with HCV genotypes 1b and, to a lesser degree, 1a are less likely to have a favorable response to interferon treatment than are those infected with genotype 2 or 3. Zein et al. [145] reported a complete biochemical response at the end of 6 months of treatment with interferon in 60 to 70% of patients infected with HCV genotype 2 and in 10 to 15% of those infected with genotype 1. This difference was also present for sustained response and was independent of liver histologic features or the pretreatment HCV RNA levels. This may partly explain the higher rates of long-term response to interferon treatment that have been reported in Europe, where HCV genotype 2 is more prevalent than in the United States or Japan. However, a meta-analysis of the most relevant studies was performed recently [29], and although there was a difference, the predictive value of the non-1 HCV genotype for response to treatment was low [58% for response at the end of treatment and 55% for sustained response]. It has been suggested that the introduction of more effective therapies such as the combination of interferon and ribavirin may make the value of predictive factors for response to therapy less important and the differential response of HCV genotypes less obvious [29]. However, more recent treatment trials using interferon plus ribavirin in interferon-naive patients with chronic HCV [80] or in patients in whom previous interferon treatment failed [28] showed higher rates of sustained response to therapy in patients with HCV genotypes other than 1. Among patients with HCV genotype 1, 48 weeks of treatment was required to achieve a response similar to that of patients infected with other genotypes treated for 24 weeks [80].

Conclusions to chapter I

Multiple genotypes of HCV have been isolated throughout the world. The identification and characterization of HCV types and subtypes have major implications for HCV vaccine development. It is clear that HCV genotypes are important epidemiologic markers and may alter the sensitivity and specificity of diagnostic assays for the detection of HCV. Differences among genotypes in pathogenicity are not clear and have not been proved.

Although not efficient by itself, HCV genotyping in combination with other markers, such as quantitative evaluation of HCV RNA, may be beneficial in the management of chronic hepatitis C and in the selection of candidates for interferon treatment. At present, patients should not be excluded from treatment on the basis of the infecting genotype. However, genotype determination could potentially be used to decide the length of treatment. Patients infected with HCV genotype 1 are likely to achieve the best rate of sustained remission following a 48-week course of treatment with interferon and ribavirin. A 24-week course of therapy appears to be sufficient to achieve the maximal rate of responsiveness. More studies are needed before guidelines can be established for the routine use of genotyping outside clinical trials and research laboratories.

CHAPTER II. MATERIAL AND RESEARCH METHODS

2.1. Clinical characteristic of patients In a basis of work clinical supervision is necessary, clinical records and data of laboratory researches of 60 sick children with HCV the positive status was established and serological were confirmed the markers of parenteral virus hepatitises which were on hospitalization in scientific research institute of Virology of Republic of Uzbekistan, during the period from September, 2013 to December, 2014 The complex of researches joined an assessment of the anamnesis, the clinical status, the blood analysis, confirmation of HCV of positivity, virus loading, definition of markers of virus hepatitises, liver ultrasonography. All sick children are subjected to the clinical and developed laboratory research. Of 30 sick children of boys made 16 [53,33%], girls 14 [46,67%].

“Although 60 children were initially examined and genotyped, 30 were selected for detailed clinical and laboratory analysis due to availability of complete records and long-term observation.”

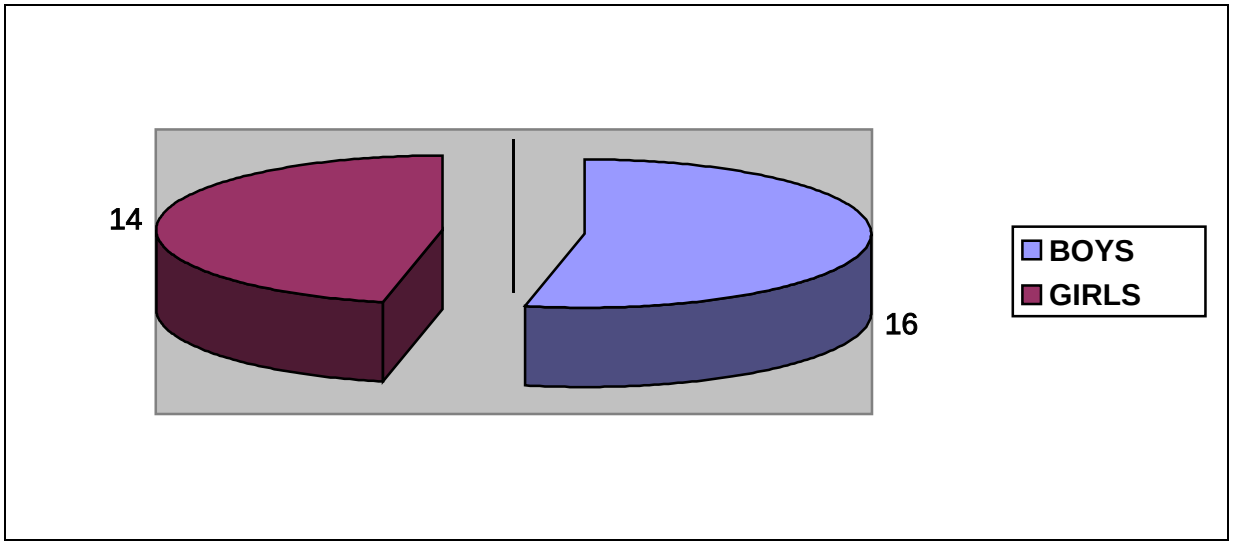


Diagram 2.1.1. **Distribution of patients on a floor**

The age of patients made from 1 year to 3 years – 16 [53,3%], 4-6 years-11 [36,6%], 7-10 years - 3[10%].

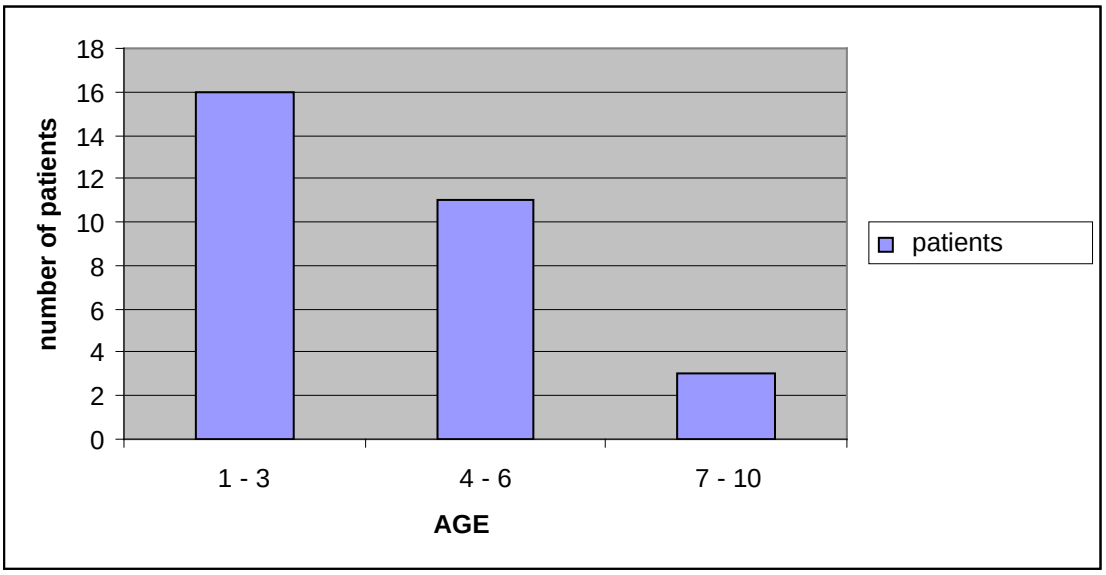


Diagram 2.1.2. **Distribution of patients on age**

By studying in detail the history of disease and interviewed the mothers of HCV-infected children, we can conclude 83% of children born to HCV + mothers, and the majority of mothers of HCV + status was installed prior to pregnancy.

2.2. Research methods

I. Considering the purpose and the research problems, to all patients were carried out:

1] Clinical inspection according to the standard standards;

2] Definition of virus loading by a Polymerase Chain Reaction [PCR] method in the Real time mode, by allocation of total RNA of HCV, HBV from blood plasma, genotypes.

Single ELISA is Adequate, Even When Results are Weak

A single enzyme linked immunosorbent assay [ELISA] determination is all that is needed for the diagnosis of hepatitis C virus infection, according to a report from France.

Researcher Jean-Michel Pawlotsky and colleagues suggest that, due to advances in the assay, confirmation of positive or weakly positive ELISAs with immunoblot-based confirmatory assays is not needed.

The first-generation group of anti-hepatitis C virus [HCV] ELISAs lacked sensitivity as well as specificity, and for this reason confirmatory assays based on immunoblot testing were developed and systematically used to confirm positive samples.

Since the early 1990s, however, ELISA assays have considerably improved, and the second- or third-generation tests available today are both highly sensitive and specific. Second- or third-generation immunoblot tests are still used for confirmation in most laboratories, however.

The aim of this study was to determine whether a double ELISA determination and confirmation of positive ELISA results with immunoblot assays are still useful in clinical laboratories performing routine diagnosis of HCV infection ["What Strategy Should Be Used for Diagnosis of Hepatitis C Virus Infection in Clinical Laboratories?" Hepatology, June 1998;27[6]:1700-1702].

Anti-HCV antibodies were sought in 3,014 consecutive unselected samples with two different ELISAs. An immunoblot-based confirmatory assay [RIBA3.0] was performed in the samples with at least one ELISA positive or weakly positive. HCV RNA was evaluated using HCV polymerase chain reaction [PCR] in the samples with a weakly positive ELISA, discrepant results of the two ELISAS, or an indeterminate RIBA3.0 pattern.

The two ELISAs gave concordant results in 2,957 [98.1 percent] of the 3,014 samples [negative in 87.9 percent, positive in 11.8 percent, and weakly positive in 0.3 percent], and discrepant results in 57 [1.9 percent].

RIBA3.0 was positive in 338 of the 350 ELISA-positive samples [96.6 percent] and indeterminate in 12. Six of them were PCR-positive.

Among the eight weakly positive samples, one was RIBA3.0-positive, six were RIBA3.0-indeterminate, and one was RIBA3.0-negative; all were PCR-negative.

Among the 57 samples with discrepant ELISA results, four were RIBA3.0-positive [none were PCR-positive], 22 were RIBA3.0-indeterminate [one was PCR-positive], and 31 were RIBA3.0-negative [six were PCR-positive]. In these cases, the clinical context and PCR detection of HCV RNA allowed for definitive classification.

Pawlotsky et al. proposed the adoption of a cost-saving diagnostic procedure for patient suspected of having HCV infection or for those with a risk for parenterally acquired viral infections. They suggested that:

† Screening should be based on one single second- or third-generation ELISA.

"Confirmation of positive ELISAs by ELISA on a second different sample might be useful to avoid false-positive results owing to sampling or processing errors," they wrote.

†No immunoblot-based confirmatory assay is needed.

† HCV RNA detection by PCR should be performed in the samples with an OD ratio in ELISA between 1 and 2, or when knowing the replicating status of HCV is needed for clinical decisions. This includes the classical indications of qualitative HCV RNA PCR, i.e., seronegative acute or chronic hepatitis of unknown cause, chronic liver disease with several possible causes including the presence of HCV antibodies, chronic hepatitis C with repeatedly normal alanine aminotransferase activity, diagnosis of HCV infection in babies born to HCV infected mothers, and antiviral therapy monitoring.

PCR [polymerase chain reaction] is a method to analyze a short sequence of DNA [or RNA] even in samples containing only minute quantities of DNA or RNA. PCR is used to reproduce [amplify] selected sections of DNA or RNA. Previously, amplification of DNA involved cloning the segments of interest into vectors for expression in bacteria, and took weeks. But now, with PCR done in test tubes, it takes only a few hours. PCR is highly efficient in that untold numbers of copies can be made of the DNA. Moreover, PCR uses the same molecules that nature uses for copying DNA:

Two "primers", short single-stranded DNA sequences that are synthesized to correspond to the beginning and ending of the DNA stretch to be copied;

An enzyme called polymerase that moves along the segment of DNA, reading its code and assembling a copy; and

A pile of DNA building blocks that the polymerase needs to make that copy.

How is PCR [polymerase chain reaction] done?

As illustrated in the animated picture of PCR, three major steps are involved in a PCR. These three steps are repeated for 30 or 40 cycles. The cycles are done on an automated cycler, a device which rapidly heats and cools the test tubes containing the reaction mixture. Each step -- denatauration [alteration of structure], annealing [joining], and extension -- takes place at a different temperature:

Denaturation: At 94 C [201.2 F], the double-stranded DNA melts and opens into two pieces of single-stranded DNA.

Annealing: At medium temperatures, around 54 C [129.2 F], the primers pair up [anneal] with the single-stranded "template" [The template is the sequence of DNA to be copied.] On the small length of double-stranded DNA [the joined primer and template], the polymerase attaches and starts copying the template.

Extension: At 72 C [161.6 F], the polymerase works best, and DNA building blocks complementary to the template are coupled to the primer, making a double stranded DNA molecule.

With one cycle, a single segment of double-stranded DNA template is amplified into two separate pieces of double-stranded DNA. These two pieces are then available for amplification in the next cycle. As the cycles are repeated, more and more copies are generated and the number of copies of the template is increased exponentially.

In summary:

Negative ELISA = No hepatitis C antibodies found in blood. You are probably not infected with HCV.

Positive ELISA = You may have HCV infection. However, it's possible this is a false-positive. More testing is required.

Negative RIBA = No hepatitis C antibodies found in blood. You are probably not infected with HCV.

Positive RIBA = Hepatitis C antibodies were found in your blood using a very sophisticated lab test. You probably have been infected with hepatitis C.

Negative HCV RNA = No active HCV infection.

Positive HCV RNA = Active HCV infection

HCV Genotypic Testing: Genotype refers to genetic structure or makeup of living organisms. The hepatitis C virus has more than six different genotypes, which are numbered in the order of their discovery. Each of these genotypes has many subtypes, which were lettered in the order that they were discovered. It is important to find out which hepatitis C genotype you have, because it determines both the type of treatment, and the length of treatment; HCV genotype also helps to predict the likelihood of curing HCV.

Worldwide, HCV genotype 1 is most common, accounting for 60 percent of cases. In the United States, 75 percent of all HCV infections are genotype 1; genotypes 2,3, and 4 are less common in the US, and other genotypes are rare. It is possible to infected with more than one HCV genotype; this is most likely among injection drug users, and people who received contaminated blood products before 1987 [when viral inactivation started], or a blood transfusion before 1993 [when effective screening procedures were instituted].

IL-28B Genotypic Testing: Interleukin-28B [IL-28B] is a human gene that plays a role in the immune system's defense against certain viruses. Certain inherited changes, or polymorphisms, in the gene have been linked to higher [or lower] cure rates in people with HCV genotype 1 using one of the most important drugs used to treat the virus: Pegylated interferon alpha.

Since everyone gets a gene from each parent, a person can have an IL28B "CC", "CT" or "TT" genotype. People with the IL28B CC genotype are more likely to be cured by HCV treatment than people with the TT genotype; cure rates among people with the CT genotype fall somewhere in between. Apparently, the IL28B

TT genotype is common among African Americans; it clearly contributes to lower cure rates

Knowing this information can be helpful to you and your doctor when discussing treatment options, especially with new medications that aren't affected by the IL-28B gene becoming available.

Liver Function Tests: Because hepatitis C is a liver disease, you and your doctor will want to monitor your liver's health. The easiest way to do this is to have regular blood tests that measure the levels of liver enzymes. When hepatocytes [liver cells] become damaged by HCV, these enzymes can become elevated. Some tests to know.

The Abbott RealTi*m*e HCV Genotype II is an in vitro reverse transcription-polymerase chain reaction [RT-PCR] assay for determining the genotype[s] of hepatitis C virus [HCV] in plasma and serum from HCV-infected individuals. The Abbott RealTi*m*e HCV Genotype II is not for screening blood, plasma serum or tissue donors for HCV, or to be used as a diagnostic test to confirm the presence of HCV infection.

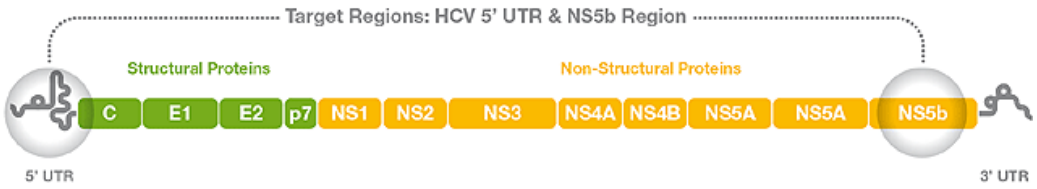
Abbott RealTime HCV Genotype performance*	
Sensitivity	500 IU/mL [0.5 mL prep]
Specificity	100% **
Target Region	5'UTR & NS5b
Genotype Detection	1a [NS5b], 1b [NS5b], 1, 2, 3, 4, 5, 6 [5'UTR]
Standardization	Second WHO International Standard for Hepatitis C Virus RNA
Internal Control	Yes; processed through sample prep with each sample
External Control	Yes; Negative, Positive
Results reported	Qualitative - Genotype Call

*Performance data shown from internal verification.

**The specificity of Abbott RealTi*m*e Genotype II was evaluated by testing 100 HCV serologically negative plasma specimens. All negative specimens tested were interpreted as HCV RNA negative by the Abbott RealTi*m*e HCV Genotype II assay, resulting in 100% specificity.

Target Region

The Abbott RealTi*m*e HCV Genotype II assay detects genotypes 1, 2, 3, 4, 5, and 6, and subtypes 1a and 1b through the use of genotype-specific fluorescent-labeled oligonucleotide probes. It targets the 5'UTR for the classification of HCV genotypes 1, 2, 3, 4, 5, and 6, and the NS5B region to accurately subtype HCV genotypes 1a and 1b.



Uses 5'UTR as well as NS5b region for broader subtype identification

The accuracy of the Abbott RealTi*m*e HCV Genotype II assay was demonstrated by testing 169 HCV genotype 1 [110 of genotype 1a, 58 of subtype 1b, and 1 genotype 1 only], 41 HCV genotype 2, 27 HCV genotype 3, 28 HCV genotype 4, 14 HCV genotype 5, and 12 HCV genotype 6 positive specimens. Nucleotide sequencing was used to determine the reference genotype of each specimen tested.

HVC Genotype/Subtype	Numbers Samples Tested	Numbers Samples Correctly Identified	Detection Rate [%]
1*	169	169	100.00
1a**	110	107	97.27
1b***	58	56	96.55
2	41	41	100.00
3	27	27	100.00
4****	28	25	89.29
5	14	14	100.00
6	12	10	83.33
1 though 6‡	291	286	98.28

* Denotes analysis based upon samples tested with *m*2000rt results: 1, 1a, 1b, 1/1a, and 1/1b.

** Three out of 110 subtype 1a samples were identified as genotype 1 only but not as subtype 1a.

*** One vendor identified subtype 1b sample was excluded from analysis because it was identified by sequencing as genotype 1 only. Two out of 58 remaining subtype 1b samples were identified as genotype 1 but not as subtype 1b.

**** Genotype 4 was detected in all 28 samples. Genotype 1 was also detected in 3 of the samples which could not be resolved due to 2-way cross-reactivity.

‡ Denotes analysis based upon genotypes 1, 2, 3, 4, 5, and 6 combined.

Genotype Detection
The assay requires three separate reactions per sample to detect genotypes 1,2,3,4,5, and 6 and subtypes 1a and 1b:

- Reaction A is designed to detect all HCV isolates, subtype 1a isolates, and genotype 3 isolates.
- Reaction B is designed to detect genotype 2 isolates, subtype 1b isolates, and genotype 1 isolates.
- Reaction C is designed to detect genotype 4 isolates, genotype 5 isolates, and genotype 6 isolates.

Conclusions to chapter II

Scientific work was carried out to scientific research institute of Virology of Tashkent where inspection and questioning was carried out at 30 patients, with the combined course of HIV and parenteral hepatitises then patients were divided into 2 groups, 1 group was made by patients of HIV+VGB, the 2nd HIV+VGC group. Analyzing age of patients it is revealed that the peak of incidence fell on age till 3 years. Distribution of surveyed mothers on age and term of establishment of HIV+ of the status, and also by training is carried out, the higher education had - 22% of women.

Analyzing methods of research came to a conclusion that the most informative method of definition of HIV of an infection is the Western blot, but tests for WB more the road, than for EIA, but possess much higher specificity. Therefore WB isn't applied to screening, and is confirming dough when receiving positive result in EIA. When carrying out inspection of the patient from which moment of infection passed >3 of months, by a standard technique with double testing in EIA and confirmation in WB sensitivity makes 99,5%. Definition of markers of virus hepatitises was carried out by the EIA method, and definition of virus loading by a method of quantitative PCR.

CHAPTER III. RESULTS OF OWN RESEARCHES AND DISCUSSIONS

3.1. Features of clinical course in chronic viral hepatitis C depending of the genotype of the virus

One of the main tasks of the laboratory diagnosis of hepatitis C is the diagnosis and differential diagnosis of acute and chronic hepatitis C using serological detection and replicative infection markers. As with other acute viral hepatitis, serological markers of HCV infection successively appear and disappear in the course of infection and after recovery. Screening test is usually linked immunosorbent assay for the detection of antibodies to HCV, but, in practice, the determination of antibodies is less sensitive in the early stages of the disease and can not differentiate between active and remission of infection. Moreover, when screening during various autoimmune processes and immunosuppressive states , there is often high incidence of false positive and false negative results. Introduction to the practice of diagnostic PCR significantly improves the ability to verify the etiological diagnosis of chronic hepatitis C.

Thus in order to verify the diagnosis HCV etiological we carried out immunological and immunogenetic study to determine HCV genotype and the viral genome in the peripheral blood serum [RNA-HCV] patients diagnosed with HCV.

In recent years, researchers discuss the importance of replication, heterogeneous ability of the HCV, the dynamics of the appearance and disappearance of infection markers in the diagnosis and prognosis of HCV.

Given the genetic heterogeneity of HCV is of great interest to determine the genotype of the virus. As a result of our genotyping revealed four major genotypes 1a, 1b, 2a and 3a, belonging to genotype group, the most common site for Eurasian

countries [12, 22]. HCV genotype distribution in patients with HCV results are presented in Table 3.1.1.

Table 3.1.1

The distribution of HCV genotypes in patients with chronic hepatitis C

			1a		**1b**		**2a**		**3a**	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Total	**60**	100	**29**	48,3	**16**	26,6	**2**	3,3	**13**	21,6
Boys	36	60	21	35	9	15	2	3,3	3	5
Girls	24	40	8	13,3	7	11,6	0	0	10	16,6

From Table 3.1.1. It shows that the most frequently encountered with HCV genotypes 1a and 1b, which were detected in 70% of patients. According to many authors [18, 43, 103], genotypes 1a and 1b are less favorable in terms of the disease and therefore efficiency of treatment, in particular IFN therapy. As for the more "favorable" genotypes 2a and 3a, it should be noted that the 2a genotype met the surveyed our patients much less frequently than the 3a genotype, but because they belong to genotype a group with a more favorable course of the disease, when further analyzing the material we We found it expedient to combine the two patients with genotypes into one group.

In analyzing the nature of the distribution of genotypes among the men and women we have found that the genotype 1a in men occurs much more frequently [44.1%] than women [25%]. At the same time, "favorable" genotypes 2a and 3a is much more common in women than in men, accounting for a total of 43.75%. As for the genotype 1b, that both men and women he had met with the same frequency.

Thus, the study of the nature of the distribution of genotypes among patients with HCV surveyed by us in most cases revealed less favorable genotypes [1a and 1b] virus [70%]. Among the examined patients with HCV genotypes 1a and 1b were more frequent in men [76.5%] than women [56.25%]. More favorable genotypes [2a and 3a] among females were detected more frequently [43.75%] than among males [23.5%].

We also examined clinical outcomes of patients with comparable groups. For ease of comparison, when considering the clinical and immunological parameters examined patients, we have divided all patients into 2 groups, the first group consisted of patients with a genotype 1a and 1b, and the second group - patients with genotypes 2 and 3. The main subjective complaints in patients with chronic hepatitis C have been signs of fatigue, complaints of fatigue, weakness, malaise. The clinical picture in patients comparison groups were observed asthenovegetative [in 84.7% of cases in patients with genotypes 2 and 3 and in 76.2% of cases in patients with genotypes 1a and 1b], dyspeptic [3.5% and 6 8%, respectively] and mixed [12.8% and 15.4%, respectively] syndromes. Artralgich syndrome was observed only in patients with genotypes 1a and 1b in 9.5% of cases. As can be seen from the data presented significant difference in performance is not marked.

Many researchers give interesting information about the existence of the relationship between genotypes and possible infection. In this regard, we decided to study the characteristics identifying the intended route of infection of the examined patients with HCV according to genotypes identified [Table 3.1.2, Fig. 3.1.1].

As can be seen from Table 3.2, the estimated number of ways to the largest percentage of infections fell on the share of blood transfusion [46%] and dental [32%] routes. According to our data, with the transfusion infection most frequently detected 1a, 2a + 3a of the HCV genotypes, which amounted to respectively 50 and 31.8%. Rarely recorded genotype 1b. When dental infection most frequently detected genotype 1b. However, in the sum of a frequency detection of genotype

1a, they accounted for 75%. When occupational exposure mainly observed genotypes 1a and 1b. When infected as a result of surgical operations has been a more frequent detection of genotype 1b, than 2a + 3a. Thus genotype 1a was not detected in any of these patients.

Table 3.1.2

HCV infection pathway in patients with HCV genotypes with different

The way of transmission of infections	**Total**		**Genotip HCV**					
			1a		**1b**		**2a+3a**	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Vertical	**33**	55	17	28,3	4	6,6	12	20
Blood transfusion	**16**	26,6	5	8.3	9	15	2	3,3
Surgery	**7**	11,6	0	0	4	6,6	3	5
Not found out	**4**	6,6	3	5	1	1,6	0	0

Thus, our analysis of the alleged tract infection patients with HCV within groups HCV genotypes showed that, unfortunately, the most common infection routes are infected as a result of blood transfusions and dental procedures, under which more often identified genotypes 1a and 1b, unfavorable both in terms of flow and in terms of prognosis [Fig. 3.1.1].

The examination of patients infected with strains of genotype 1b, according to some studies, it was found that they had a longer duration of the disease - some 10 years and more than other strains of the virus infection [25, 67, 81].

As a result, our study found that the average time of infection with genotype 1b was 8 ± 0.2, at 1a - 6,5 ± 1,11, with 2a and 3a - 6,7 ± 1,15 years. In addition, the total number of examinees with chronic hepatitis C in 5 patients due to frequent dental procedures are not able to identify the alleged infection period.

Thus, our analysis of the expected timing of infection is only revealed a trend towards more long-term persistence of hepatitis C virus in the group of examined patients with genotype 1b [8 ± 0,2 years].

In papers published in recent years, there is evidence that the course and outcome of hepatitis B infection with these strains determines the HCV genotype 1a and hypervariable especially genotype 1b [43,57,88]. To assess the impact of HCV genotype on during the infection process, we conducted a comparative analysis between the identified genotypes and the degree of activity of the process [tab. 3.1.3 Fig. 3.1.2], and pathologic data obtained by ultrasound [Table. 3.4], which widely used by clinicians to assess the condition of the liver parenchyma.

As shown in Table 3.3 and Figure 3.2, in patients with HCV genotype 1b was observed more frequently chronic viral hepatitis C with severe degree of activity [66.7%], the second highest frequency of occurrence held a chronic process with minimal activity [36%]. Less commonly, there is a low to moderate level of activity of chronic process.

Table 3.1.3

The level of activity og HCV	**Genotip HCV**							
	Total		**1a**		**1b**		**2a+3a**	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Minimal	**3**	5	1	1,6	2	3,3	0	0
Low	**9**	15	2	3,3	2	3,3	5	8,3
Moderate	**13**	21.6	5	8,3	3	5	5	8,3
Expressed	**35**	58,3	15	25	11	18,3	9	15
Total	**60**	100	23	38,3	18	30	19	31,6

Having analyzed the process according to the degree of activity of chronic HCV genotypes between groups, we found that patients with genotypes 1a and 1b higher [respectively 44 and 36%] than with the genotypes 2a + 3a chronic process

was observed with a minimal degree of activity. At the same time, a chronic process with a low degree of activity was observed more frequently in patients with genotypes 2a + 3a. In moderate degree of activity of chronic process 1a and 2a + 3a genotypes identified with the same frequency.

HCV persistence provides a wide range of clinical and morphological variants of the persistent evidence of active disease and ongoing liver damage with the development of further clinical multisystem suffering to the state of clinical recovery (acute infection) with very low levels of viral replication and non-progressive nature of the histological changes.

The course of chronic hepatitis C are determined, along with the level of viremia, viral genotype, additional factors that damage the liver: the presence of double, triple viral infection (HBV, HDV, viruses gerpesgruppy), alcohol abuse, taking a number of medications that cause liver damage. Of particular interest are variants of chronic hepatitis C with a normal level of alanine and aspartate aminotransferase in serum. In this form of hepatitis does not show a correlation with the level of viremia and HCV genotype, in the histological picture is dominated by minimal or moderate process activity, debated the question remains about the treatment of these options of liver damage.

In HCV-infection is observed a wide range of extrahepatic lesions, conventionally divided into three main groups: the genesis of immune extrahepatic lesions (vasculitis of various localization, cutaneous vasculitis, Raynaud's syndrome, glomerulonephritis, peripheral neuropathy periarteritis nodosa, and others.); extrahepatic changes in immune-cell and immune complex origin (arthritis, polymyositis Segre syndrome, fibrosing alveolitis, and others.); the defeat of the blood system, including B-cell malignant lymphoproliferation. It is believed that lymphotropic HCV (replication in blood cells, predominantly B cells) causes chronic stimulation of B-lymphocytes and, consequently, their activation, increased production of antibodies (autoantibodies different, poly- and monoclonal IgM rheumatoid factor with activity) to give immune complexes, including mixed cryoglobulins.

In the development of lesions extrahepatic HCV also discusses the possible role of replication in various organs and tissues (in addition to liver and hematopoietic system) with the development of cytotoxic T-adhesive accurate responses directed at antigens, autoantigens, formed due to the direct damaging effect of the virus at the cellular level.

Phase reactivity series goes into liver cirrhosis and hepatocellular carcinoma.

In 40-45% of patients, along with hepatic manifestations observed a variety of extrahepatic manifestations (Table.) Often go to the forefront of the clinical picture and in some cases determine the prognosis of the disease.

Extrahepatic manifestations of chronic HCV-infection

Endocrine	Hyperthyroidism hypothyroidism Hashimoto's thyroiditis Diabetes
Hematologic	Mixed cryoglobulinemia Idiopathic thrombocytopenia Non-Hodgkin's B-cell lymphoma * Waldenstrom's macroglobulinemia aplastic anemia
The defeat of the salivary glands and the eyes	Lymphocytic sialadenitis * Mooren corneal ulcer uveitis
Cutaneous	Cutaneous necrotizing vasculitis * Late cutaneous porphyria Lichen planus Erythema multiforme * Erythema nodosum *

	Malakoplakiya Hives*
Neuromuscular and joint	myopathic syndrome * Peripheral neuropathy * Guillain-Barré syndrome Arthritis, arthralgia *
Kidney	Glomerulonephritis * Autoimmune and other Polyarteritis nodosa
	Interstitial pulmonary fibrosis * Pulmonary vasculitis * Hypertrophic cardiomyopathy CRST-syndrome Antiphospholipid syndrome Autoimmune hepatitis type 1 and 2 Behcet's syndrome dermatomyositis

* - Often due to mixed cryoglobulinemia (1). For more details see. "Viral hepatitis", 1997, N1, str.12-16

Statistical analysis allows us to consider a proven relationship with chronic HCV-infection extrahepatic manifestations such as mixed cryoglobulinemia, glomerulonephritis membranoproliferative, late cutaneous porphyria, autoimmune thyroiditis. It is presumed link the HCV-infection with idiopathic thrombocytopenia, lichen planus, Mooren ulcers of the cornea, Sjogren's syndrome (lymphocytic sialoadenitom) and B-cell lymphoma. For other extrahepatic

manifestations have no proof of their close relationship with the HCV-infection, but further studies are needed that will, apparently, to add the list submitted.

From extrahepatic manifestations of HCV mixed cryoglobulinemia is found most frequently, especially in women of middle and old age with the duration of the current infection (for an average of 10.7 years), the presence of cirrhosis. Depending on the diagnostic methods cryoglobulinemia detected in 42-96% of patients. In 10-42% of patients have clinical manifestations of cryoglobulinemia: weakness, arthralgia, purpura, peripheral neuropathy, Raynaud's syndrome, hypertension, kidney disease. The composition cryoprecipitate detect HCV RNA and lgG anti-HCV structural and nonstructural proteins of HCV (core, E2 / NS1, NS3, NS4, NS5), lgM anti-HCV core-to protein; Complement C3 fraction. HCV RNA concentration in the cryoprecipitate is 103-105 times higher than in serum. Cryoglobulinemia HCV RNA detected in the bone marrow, peripheral blood mononuclear cells, keratinocytes, endothelial cells, and ductal epithelium. A number of patients with clinical symptoms of cryoglobulinemia have minimal histological evidence of liver injury. Role of HCV-infection in the development of cryoglobulinemia confirmed the disappearance of the clinical manifestations of cryoglobulinemia as a result of antiviral therapy with interferon alpha.

Membranoproliferative glomerulonephritis detected in 2-27% of HCV-infection, as a rule, within a mixed cryoglobulinemia type II. Kidney involvement with the development of nephrotic syndrome may be the only manifestation of HCV-associated mixed cryoglobulinemia in the absence of arthralgia, skin purpura, polyneuropathy. In most cases, unable to identify HCV RNA and anti-HCV in the kidney glomeruli, but recently there have been reports about the discovery of specific HCV-proteins in the glomeruli and tubules interstitial vessels in 66.7% of patients with glomerulonephritis membranoproliferative cryoglobulinemia due to HCV-infection . We discuss the importance of a direct pathogenetic HCV-containing immune complexes in the development of glomerulonephritis.

Extremely intriguing were reported high frequency (35%) HCV-infection detection in non-Hodgkin's B-cell lymphoma, and even more frequently it is detected (90%)

of lymphoma in combination with a mixed cryoglobulinemia (80 patients in the group).

Idiopathic thrombocytopenia, possibly caused by HCV-infection in the majority of cases than previously thought.

Endocrine disorders include various forms of thyroid dysfunction, detected in 7-12% of cases of HCV, - hypothyroidism, hyperthyroidism, Hashimoto's thyroiditis, the detection of antibodies to thyroglobulin in high titer. There were reports of frequent (50%) identify diabetes mellitus in liver cirrhosis, caused HCV.

Sialadenitis occurs in 14-57% of patients with chronic hepatitis C, but in most cases, a typical pattern of Sjogren's syndrome (clinical, histological signs, serological markers) is missing.

A variety of skin lesions are described in conjunction with the CHC, including cutaneous necrotizing vasculitis with papular or petechial rash caused by the deposition of cryoglobulins, most clearly associated with HCV-infection. Despite the fact that HCV RNA was detected in skin keratinocytes and in the pathogenesis of necrotizing vasculitis, cryoglobulinemia considered greater role than the virus replication in the vascular wall.

Neuromuscular and joint extrahepatic manifestations of chronic HCV-infection are varied and in most cases are caused by cryoglobulinemia. Muscle weakness, myopathic syndrome, myalgia, myasthenia single observation mentioned in connection with the CHC. In the debut OVGS described Guillain-Barre syndrome, but more chronic the HCV-infection is combined with peripheral polyneuropathy within cryoglobulinemia.

Systematic destruction observed in HCV-infection, reflecting generalized hepatitis C with involvement in the pathological process of many organs and tissues, making it difficult to timely diagnosis and treatment of chronic hepatitis.

It should also be noted that the chronic process with pronounced degree of activity was observed only in patients with genotypes 1a and 1b significantly more often [respectively 33% and 67%], and in patients with severe degree of activity of chronic process 2a + 3a genotypes did not specify.

3.2. Features of instrumental and biochemical examination in chronic viral hepatitis C

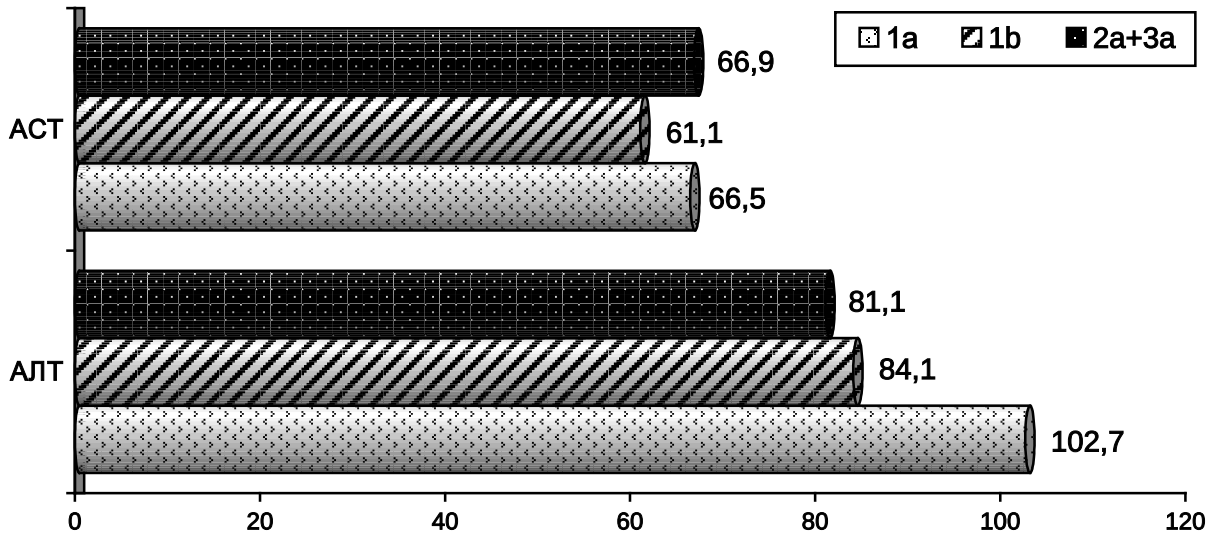
In the course of our further research, we conducted a comparative analysis between the genotypes identified by the level of viremia and liver enzymes. As a result of our analysis, it was found that biochemical parameters approximately equal severity were increased in patients with different genotypes of HCV. In addition, the biochemical parameters did not depend on the level of viral load, as they equally have been changed at high rates during viremia genotypes 1a and 1b and at the lower in patients with HCV genotypes 2a i3a [tab. 3.7].

Thus, when analyzing the levels of viremia and antibody titers in the peripheral blood serum HCV in HCV genotypes identified, we have discovered that for 1 [a, b] genotype was characterized by a high level of viremia, and high titers HCVAb. As for the group of patients with genotype 2a + 3a, they have viral load and HCV antibody titers were significantly lower.

Table 3.1.4

Biochemical parameters in patients with chronic hepatitis C, depending on the HCV genotype, M ± m

Biochemical analysis	**control**	**Found genotypes HCV**		
		1a, n=19	**1b, n=16**	**3a+2a, n=15**
ALT, ME/л	TILL 36	102,7±27,8	84,1±16	81,1±11
AST, ME/л	TILL 31	66,5±7,9	61,1±15,1	66,9±9,2



Pic.3.1.3 **Biochemical parameters in patients with chronic hepatitis C, depending on the HCV genotype**

Although there is no relationship between the level of viral load and liver enzymes in patients with chronic hepatitis C doctors need to pay attention to the need for analysis of the level of viremia, even in the presence of low biochemical parameters.

Some differences were also identified the part of the biochemical parameters. ALT levels at 1 genotype content was somewhat lower and the AST level is slightly higher than with genotypes 2 and 3, although figures were not significant differences. However, the ratio of AST / ALT, otherwise known as the coefficient of De Rytis usually determined within 0.6-0.8 in viral lesions of the liver, with HCV genotype 1 is caused by a 1.2; whereas when genotypes 2 and 3, this figure was 0.6 and 0.8 respectively. A higher ratio De Rytis in viral hepatitis C, caused by genotype 1 indicates a more profound and massive necrosis of the liver tissue.

The next stage of our study was to compare ultrasound data [US] and genotyping data. Further analysis of chronic process depending on the HCV genotype we mentioned identification signs of fibrosis, fatty liver and cirrhosis [Table 3.1.5].

Table 3.1.5

The results of ultrasound studies in patients with HCV

Results of Ultrasound	Total		Genotype HCV					
			1a		1b		2a+3a	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Signs of fatty liver+cirrosis	55	91,6	22	36,6	14	23,3	19	31,6
No fibrosis	5	8,3	2	3,3	3	5	0	0
Total	60	100	24	40	17	28,3	19	31,6

As shown in Table 3.1.5 and figure 3.1.4 at the time of the study, we studied patients with HCV is mainly observed during chronic infection without fibrosis [94%] and only 6% of cases, according to ultrasound revealed changes in the parenchyma liver, such as fibrosis and cirrhosis initial symptoms. A comparison and analysis of these data with the results of our genotyping revealed that the process to be diagnosed chronic fibrosis without all genotypes were detected with approximately equal frequency [when 1a- in 40.4% of cases, pri1b genotype - 27.7%, and at 2 and 3 genotypes in 31.9% of cases]. However, in all patients with the pattern of liver fibrosis and cirrhosis, which constitute in this study, 6% of patients found only genotype 1b.

Based on the comparative analysis of clinical and epidemiological data and the results of instrumental studies, the following conclusions:

In the study of the nature of the distribution of genotypes among the surveyed patients with HCV in most cases revealed less favorable genotypes [1a and 1b] virus [70%]. Among the examined patients with HCV genotypes 1a and 1b were more frequent in men [76.5%] than women [56.25%]. More favorable

genotypes [2a and 3a] among females were detected more frequently [43.75%] than among males [23.5%].

Our analysis of the alleged tract infection patients with HCV within groups HCV genotypes showed that, unfortunately, the most common infection routes are infected as a result of blood transfusions and dental procedures, under which more often identified genotypes 1a and 1b, unfavorable both in terms of flow and in terms of prognosis

Our analysis showed that patients with genotype 1 [especially 1b] characterized by minimum and severe degree of activity over a long persistence, and the presence of morphological changes in the parenchyma of the liver, such as signs of steatosis and cirrhosis of the liver, indicating that the hypervariable 1b genome genotype which contributes to an unfavorable course of chronic process.

Although there is no relationship between the level of viral load and liver enzymes in patients with chronic hepatitis C should pay attention to the need for viremia levels of analysis even in the presence of low biochemical parameters as at 1a and 1b genotypes of hepatitis C virus characterized by a high rate of viral load, even at the minimum level activity.

3.3. Characterization of immunological changes in chronic viral hepatitis C

An initial response to HCV infection is characterized by the mobilization of non-specific immune defense: interferons, natural killer cells after a few days after infection, a person develops a specific immune response directed to the elimination of free virus particles and protect against re-infection (carried out mainly humoral), on the elimination of the virus penetrated in cells, by lysis of infected cells and the inhibition of viral replication without cell lysis by cytokines (cellular link carried an immune response). HCV is a cellular parasite, so the protection of the most important cellular immune response.

HCV-specific humoral immune response characterized by the formation of antibodies directed against structural and nonstructural HCV antigens. When HCV

infection is observed specific antibody response. The possibility re HCV infection not only different, but homologous strains.

HCV-specific cellular and humoral immune response is polyclonal and multispecific character. The leading role in the immunopathogenesis of HCV have a failure and qualitative features of the T-helper (Th) CD4 + response in the early stages of infection. For the activation of T CD4 + helper necessary recognition of antigens presented by MHC molecules (HLA) II class surface antigenprezen tiruyuschih-cells (macrophages, dendritic cells, B lymphocytes). Txi are stimulators of cellular responses and secrete proinflammatory cytokines (interferon, interleukin-2, tumor necrosis factors and enhancing the cytotoxic reaction has a direct cytotoxic effect on transformed cells, induce cytotoxicity of normal macrophages. Tx2 are stimulators of antibody response and produce a number of interleukins, providing anti-inflammatory effects (interleukins-4 and -10) by suppressing activities of interferon-y.

There is a direct dependence of the activity on the duration of thorns disease at different stages of the HCV chronic-in-infection.

The most important feature of the HCV-virus infection is the way to long-term persistence in the body. Despite the presence of virus-specific immune response, it does not protect against reinfection. To date, all the factors are not established interaction between virus and host, conditional on the failure of the immune response to control the infection. Data on the biological properties of HCV and chronicity rate (85%) indicate the crucial role of viral factors, aimed at modulating the immune response of the host

In the early stages of infection inhibition plays a decisive role for inducing an immune response. The virus is able to influence the process of activating CD4 + Tx, disrupting the interaction of antigen presenting cells and T-lymphocytes.

The importance of the process of chronic HCV-infection are the mechanisms of realization of suppression of the immune response, among which the most important role acquires the avoidance of a virus the humoral and cellular immune response by mutation. Mutation HCV epitopes that are targets of cytotoxic T

lymphocytes, leads to violations of antigen processing and recognition of epitopes, CTL antagionisticheskim relationship. The lack of effective T cell immune response caused by a low level of HCV replication observed in almost 100% of hepatocytes, resulting in low expression of HLA molecules and other immunoinflammatory on the surface of infected cells.

On the outcome of and during the process is greatly influenced by the amount of infected material. The impact on the course of infection and genotype HCV degree of heterogeneity of the population has not yet been proved. The role of immunogenetic factors in the development of HCV-infection (genotype HLA II class determines the outcome of acute HCV-infection; heterozygosity for hemochromatosis gene is correlated with the degree of fibrosis; phenotype heterozygosity deficit PiMZ al-antitrypsin deficiency and genetic factors that predispose to fibrosis).

Among a host of factors affecting the outcome and course of HCV-infection, studied the value of age at the time of infection, alcohol abuse, coinfection with hepatotropic viruses, lipid metabolism, and others.

The defeat of hepatocytes infected with HCV are considered:

Direct cytopathic effect of the virus - the action of virus virion components or products on the hepatocyte cell membrane, and structures. It is shown that core-HCV protein involved in a variety of cellular processes. He is able to modulate the transcription and translation of some cellular genes and cause phenotypic changes of hepatocytes.

Immune-mediated damage directed to intracellular HCV antigens representing a direct interaction of cytotoxic T-lymphocyte to a target cell (cytotoxic response, which results in a colloid osmotic lysis of the target cells), or mediated by cytokines. Identified activated CD4- and CDS-lymphocytes in the portal tracts and inside the lobules, as well as the expression of HLA I molecules and Class II, and adhesion molecules on the surface of hepatocytes and biliary cells. There is no direct correlation between the level of viremia, HCV RNA in the liver, as well as the expression of viral antigens in the liver and liver activity process

(laboratory and histological). Patients with more active T cell immune response to HCV-infection observed lower viremia, a higher activity of hepatic process. The immune response to antigens of the virus, carried by T-lymphocytes, is a major cause of apoptosis, which is regarded as one of the main mechanisms of hepatocyte injury during the HCV-infection.

Virus-induced damage autoimmune mechanism. The participation of autoimmune mechanisms in the liver damage is proved on the basis of the high frequency of detection of serological markers of autoimmunity. Approximately 1/3 of the patients revealed Neorio-ganospetsificheskie autoantibodies.

The leading role within CHC member of the patient's immune system and backup capabilities of the organism. The results obtained indicate that distinct changes in both cellular and humoral immunity in patients with CHC. In the phase of replication of HCV is determined immune deficiency [Table 3.2.1] cellular immunity, as evidenced by severe T lymphocytopenia in 58% of cases, the reduction in the 71% content of CD4 + cases, a moderate increase in CD8 + in 49% of patients., Reduction + CD16 a and 56% increase in NKT-cells [CD3 + CD56 +] in 67%. Reducing IRI was detected in 57% of patients with chronic hepatitis C in the replication phase. Significantly lower expression of CD4 [31,9 ± 0,9, p <0.001] at the rate of 39,2 ± 0,3] in HCV patients surveyed in the replication phase with the biochemical activity indicates a rather weak T-cell proliferative response to HCV antigens. A significant increase in CD8 replication phase biochemical acute stage in patients with chronic hepatitis C in 49% of cases suggests that the cytotoxic lymphocyte response is insufficient to eliminate HCV. Important prognostic value in chronic hepatitis C has the IRI [CD4 / CD8], which is normally equal to 1,9 ± 0,01. Studies have shown that patients with chronic hepatitis C in the reactivation phase with severe biochemical activity of IRI is reduced significantly [1,5 ± 0,01, p <0,001]. A significant decrease in the level of NK-cells [CD16] indicates a weak antiviral resistance. Maximum marked reduction observed in patients in the replication phase of the stage expressed biochemical exacerbations [8,2 ± 0,1 at a rate of 10,5 ± 0,1, p <0.001], and has a

strong correlation with IRI. Reduced concentration of CD16 detects weak NK cell activity and the inadequate participation of managers in antibody-dependent cellular cytolysis.

Table 3.2.1

Performance of T-cell immunity in patients with chronic hepatitis C depending on the HCV genotype

Analays %	**Norma**	**Genotype HCV 1a**	**Genotype HCV 2a**	**Genotype HCV 2a+3a**
CD4	**35-65%**	**68,5±1,3**	**45,1±0,8**	**44,5±1,1**
CD8	**12-30%**	**11,9±1,2**	**12,4±1,1**	**14,1±1,2**
CD4/CD8 correlation	**0,9-1,9**	**5,75 ± 0,1**	**3,63 ± 0,1**	**3,15 ± 0,1**

Note: P - reliability of differences in relation to the healthy group.

NK-T-cells carry out a crucial role in the liver damage after effector mechanisms involving T cells and macrophages in the immune-mediated liver inflammation. There was a significant and meaningful increase in NK-T-cells in most HCV patients, regardless of the genotypes [Table 3.2.1]. Indicators NK-T-cells was significantly higher in patients with CHC in a phase at replication biochemical activity. In this case there is no correlation with clinical and basic laboratory syndromes, indicators of cellular immunity [CD3, CD4, CD8, NK, Iran]. Indicators NK and NK T cells have characteristic changes depending on the genotype of HCV. Thus, early fibrosis in CHC NK cell numbers decreased from 10.8 ± 0.6% to 7,3 ± 0,9%. As the progression of liver fibrosis, the number of NK-T increased to 12,8 ± 1,3%, while for the CPU [n = 6] observed NK cells rise and decline NKT-cells, which makes it possible to use these indicators as a marker of progression of fibrosis .

Our studies indicate that the level of CD95 was significantly higher in patients with chronic hepatitis C and depends on the activity of the process [tablitsa.3.2.2]. As a universal biological mechanism for CHC apoptosis may lead to excessive destruction not only of hepatocytes, but also other cell population [CD3, CD4, CD14CD95], reflecting or systemic immunological response to infection or extrahepatic viral persistence.

Table 3.2.2

Indicators of humoral immunity depending

HCV genotype

%	NORMAL	HCV 1a	HCV 2a	HCV 2a+3a
CD19	11,5± 0,9	12,9± 0,8 P>0,05	13,7± 0,8 P<0,05	14,1± 0,9× P<0,001
IgA, г/л	2,14 ± 0,3	2,2 ± 0,2 P>0,05	2,2 ± 0,2 P>0,05	2,31 ± 0,18 P>0,05
IgM, г/л	1,3 ± 0,2	1,35± 0,2 P>0,05	1,54± 0,18 P>0,05	1,7 ± 0,3 P<0,05
IgG, г/л	12,1 ± 0,3	13,9 ± 0,3 P<0,05	15,1 ± 0,5 P<0,05	17,2 ± 0,5 P<0,001
CIK, ед	101,4±7,4	141,8± 3,6 P<0,01	152,1± 4,1 P<0,001	171,6±4,9 P<0,001

Note: P - reliability of differences in relation to the healthy group.

Changes of cellular immunity, depending on the activity of the process are characterized by depression [CD14 +, CD16 +, FA] killer mechanism of cytotoxic activity, Iran, increased levels of CD3 + of CD56 +, CD8 +, the level of apoptosis of T-cells of CD95 +, the most significant changes in the group with severe clinical manifestations.

The study of humoral mmuniteta showed a significant increase of B-lymphocytes [CD19 +], IgM, IgG, CEC in patients with CHC genotype 1a. The study of humoral immunity depending on the stage of the disease are characterized by maximum manifestations in genotype 2a + 3a [Fig 3.2.2].

In the phase of reactivation in patients with CHC activated B-cell unit, as indicated by the findings of a significant increase in the level of B-lymphocytes [14,1 ± 0,9 at a rate of 11,5 ± 0,9]. It testifies to the active involvement of B cells in antiviral immunity, but not enough effective in CHC.

Number of IgM was significantly elevated in 46% of patients with chronic hepatitis C in the replication phase and in 24% of patients in the latent phase with biochemical exacerbation, its level correlates with the amount of ALT. Polyclonal immunoglobulinemiya considered as an indication of violation of the functional ability of the liver and is found in our research in 7% of cases. Elevated levels of the CEC may be indicative of a predisposition of these patients to the development of immunopathological reactions and HCV-infection association with a number of autoimmune diseases, which coincides with the opinion of some researchers [Aprosina ZG et al, 1999]. The data on T- and B-lymphocytes indicate an imbalance in the activation of the immune response by way of Th2-type dominance with CG S.

We have analyzed the immunological parameters in patients with chronic hepatitis C, depending on the genotype of hepatitis C virus

Cellular immunological parameters determined by the content of leukocytes, lymphocytes, total pool of T lymphocytes [CD3], T-helpers / inducers [CD4] and T-cytotoxic lymphocytes [CD8], CD4 / CD8 ratio [immunoregulatory index - IRI], B-lymphocytes [CD20], as well as activation markers [CD 23, CD25, CD38, CD95].

According to our sources in the peripheral blood levels of white blood cells in chronic viral C average differed from the control data [$p < 0.05$], while the genotype 1 virus leukopenia, while genotypes 2 and 3 - leukocytosis. According to the relative content of lymphocytes in patients with chronic hepatitis C and controls showed no significant difference [$p > 0.05$], but there is a tendency for lymphopenia, which is marked and in absolute terms, for which there is a significant difference between the values of HCV patients and controls data. And we should note that lymphopenia was more pronounced with genotype 1 virus

compared with genotypes 2 and 3. Thus, in patients with chronic hepatitis C was the absolute lymphocyte counts with genotype 1 virus 1776,3 ± 120,9 cells / mm; at 2 genotype - 2092,5 ± 117,8 cells / mm; at 3 - 2010,7 ± 140,7 cells / mm, whereas in the control group - 2238,1 ± 89,1 cells / mm [p <0.05].

It is known that the degree of surface expression of CD3 + receptors on the membrane of T-lymphocytes transmissive reflects its function and allows the identification of the total number of T-lymphocytes. Analysis of immunophenotype of T lymphocytes in patients with HCV showed the presence of significant suppression of the expression of CD3 + T-lymphocytes and the relative and the absolute value of it in comparison with the control group [p <0.05], which in varying degrees was noted in all genotypes of hepatitis C virus .

CD4 + T-cell responses to viral proteins is an important mechanism of host defense and suppression of this mechanism reduces the effectiveness of anti-viral agent. Thus, in patients with HCV for all genotypes showed a significant suppression of the expression of CD4 + T-lymphocytes compared to control group values [p <0.001], which was manifested in the relative and absolute content of CD4 + T-helper cells in the groups studied.

It is well known that cytotoxic CD8 + T lymphocytes play a major role in the pathogenesis of viral disease that is caused, on the one hand, their ability to cause destruction of infected cells expressing the corresponding peptides of presenting MHC class 1 and, on the other hand - the ability to secrete anti-viral Factors [proinflammatory cytokines - IFN-α, TNF-α, and others] [5,9]. A content analysis of CD8 + T lymphocytes between the studied groups of patients with chronic hepatitis C patients in the treatment groups showed a significant increase in the relative and absolute number of CD8 + T lymphocytes in all genotypes of the virus when compared with the control group data [p <0.01]. Thus, in patients with HCV expression value relative CD8 + T-lymphocytes at 1 genotype was 25,4 ± 1,1%; 2 genotype at -24,4 ± 1,3%; with genotype 3 - 24,3 ± 1,5%; in the control group this index was 18,4 ± 0,5%.

During chronic viral hepatitis is essential immunoregulatory index [IRI], which is the ratio of CD4 + T-helper / inducer to the number of CD8 + T lymphocytes. It should be noted that in patients with HCV genotype, regardless of IRI virus genotype 1 was at 0.9; whereas with genotypes 2 and 3, 1.0 and 1.3 respectively, while in the control group - 1,5 ± 0,05 [p <0.05]. It is clear that the suppression of CD4 + T-helper / inducer on the background of increasing numbers of CD8 + T lymphocytes leads to IRI reduction in the groups of patients with HCV, and is an important criterion for the depth of T - cell immunodeficiency in chronic liver damage, which, as we have seen, more pronounced with genotype 1 hepatitis C virus

Natural killer cells [NK cells] are classified as the main effectors of innate immunity or natural, are capable of lysing target cells or to carry out antibody-dependent cellular cytotoxicity, and are involved in the antiviral, antiprotozoal and antimicrobial protection. It is inherent in them perform functions first line of defense before any immune T-lymphocytes and specific antibodies [1,5]. In the study of NK cells from CD16 + phenotypes observed increase in the relative number of CD16 + NK cells in groups of patients with different HCV genotypes. Significant difference in the content of CD16 + NK cells found between indicators of patients with genotype 1 chronic hepatitis C virus and the control values [p <0.01]. Whereas in groups with other genotypes of the hepatitis C virus was a trend to an increase in the relative number of CD16 + NK cells.

It is known that in addition to T-lymphocytes, B-lymphocytes are the main effectors of the immune system. Given that the function of B-lymphocytes of the organism to combat infection is the production of antibodies, the change in expression of surface receptors in the lymphocytes indicates the active participation of their antiviral response [7]. We have studied the content of B-lymphocytes expressing CD20 + receptors, which revealed the presence of significant increase in patients with HCV of all genotypes of the virus when compared with the control group values [p <0.01]. Thus, the relative number of CD20 + B lymphocytes in patients with HCV genotype 1 hepatitis C virus was

23,2 ± 0,6%; genotype at 2 - 25,5 ± 1,1%; genotype at 3 - 24,3 ± 0,9%; whereas the relative abundance of CD20 + B cells in the control group on average equal to 18,6 ± 0,6%. As we can see, with genotype 1 hepatitis C virus marked the lowest rate of participation in the cell-mediated immunity in the anti-viral response.

One of the most important biological functions of immunoglobulins is binding to the antigen and the formation of immune complexes [CIC]. An important characteristic of the CEC is their size. Thus, in patients with HCV CEC observed increase in mean values of 3% and 4% in 3.4 and 4.5 times, respectively, relative to control values. Depending on the characteristics of the virus genotype humoral immune system was characterized by a high level of fine TsIKov with genotype 1 virus than with genotypes 2 and 3. Small TsIKi bad elliminiruyutsya from the body, they may be delayed subendothelial, and are not able to activate the complement system, and show the progression of the process .

Thus, on the basis of these results it is clear that in chronic viral hepatitis C, there is a pronounced imbalance of cellular and humoral immune system are more pronounced when the genotype 1 virus. An imbalance in the cellular link immunieta expressed in suppressing immunoregulatory index by reducing the number of T-helpers / inducers and enhance cytotoxic T-lymphocytes. Circulating immune complexes large and small quantities were increased with HCV. Obviously, in chronic viral liver damage T - cell response is significantly weaker, and is directed against a smaller number of epitopes, suggesting that clonal T-cell depletion. Consequently, the identified changes in the condition of patients with HCV immunoreactivity suggest the influence of immunogenetic characteristics of the virus on the immune response and the course of infection.

Conclusions to Chapter III

Clinical and laboratory examination of 60 patients with chronic hepatitis C genotype 1-2-3.

In the study of the nature of the distribution of genotypes among the surveyed patients with HCV in most cases revealed less favorable genotypes [1a and 1b] virus [70%]. Among the examined patients with HCV genotypes 1a and 1b were more frequent in men [76.5%] than women [56.25%]. More favorable genotypes [2a and 3a] among females were detected more frequently [43.75%] than among males [23.5%].

Our analysis of the alleged tract infection patients with HCV within groups HCV genotypes showed that, unfortunately, the most common infection routes are infected as a result of blood transfusions and dental procedures, under which more often identified genotypes 1a and 1b, unfavorable both in terms of flow and in terms of prognosis

Our analysis showed that patients with genotype 1 [especially 1b] characterized by minimum and severe degree of activity over a long persistence, and the presence of morphological changes in the parenchyma of the liver, such as signs of steatosis and cirrhosis of the liver, indicating that the hypervariable 1b genome genotype which contributes to an unfavorable course of chronic process. Although there is no relationship between the level of viral load and liver enzymes in patients with chronic hepatitis C should pay attention to the need for viremia levels of analysis even in the presence of low biochemical parameters as at 1a and 1b genotypes of hepatitis C virus characterized by a high rate of viral load, even at the minimum level activity.

From these results it is clear that in chronic viral hepatitis C, there is a pronounced imbalance of cellular and humoral immune system are more pronounced when the genotype 1 virus. An imbalance in the cellular link immunieta expressed in suppressing immunoregulatory index by reducing the number of T-helpers / inducers and enhance cytotoxic T-lymphocytes. Circulating

immune complexes large and small quantities were increased with HCV. Obviously, in chronic viral liver damage T-cell response is significantly weaker, and is directed against a smaller number of epitopes, suggesting that clonal T-cell depletion. Consequently, the identified changes in the condition of patients with HCV immunoreactivity suggest the influence of immunogenetic characteristics of the virus on the immune response and the course of infection.

CONCLUSION

Today hepatitis C is a serious public health problem in many countries, including Uzbekistan. Among hepatotropic viruses cause the development of chronic hepatitis B, the first place for the development of complications such as liver cirrhosis, hepatocellular carcinoma occupies the hepatitis C virus [34, 38, 77]. According to WHO, in 2004, in the world the number of people dying from hepatitis C has exceeded the number of deaths from HIV - infection [112]. In this connection there is need to implement in clinical practice of highly effective diagnostic methods, including the identification of the virus genome.

Due to the high frequency of mutations in the HCV genome, resulting in the persistence of the virus has a long, sometimes lifelong character, is still underway to develop new methods to detect the virus.

The aim of our study was to investigate the role of genomic variants, viral load, as well as the value of mononuclear tropism of hepatitis C virus in the monitoring of patients with chronic hepatitis C interferon dynamics. To achieve this goal we have selected 50 samples of peripheral blood sera from patients with HCV positive for detection HCVAb. In these patients, HCV genotype distribution was studied and determined the effect of genotype on the clinical course of infectious diseases. Subsequently, we conducted a comparative analysis between the genotypes, levels of virus viral load titers of antibodies to HCV and efficacy of IFN therapy.

Upon completion of antiviral therapy we mononuclear tropism of the virus has been studied in patients examined and a comparative analysis between the presence of RNA-HCV in peripheral mononuclear cells identified genotype and the clinical course of the disease.

In the study, we have some immunogenetic indicators and mononuclear tropism of hepatitis C virus in 50 patients with chronic hepatitis C, held inpatient

and outpatient treatment at the clinic of infectious diseases TMA and urban Hepatology Center were studied.

Among the examined patients with HCV dominated by young persons [66%], resulting in serious social significance of HCV - infection. 68% of the patients were males. Our findings are consistent with results obtained by other researchers who have identified a high percentage of patients with HCV among males [71, 72]. Apparently, the reason for the development of chronic process in men is especially exogenous risk factors such as alcohol, eating fatty foods, etc.

Estimated period of infection able to determine in 45 of the 50 respondents. It ranged from 1 year to 15 years, with an average of 7,11 ± 0,31 years. 5 of the total number of surveyed patients with HCV because of frequent dental procedures could not name the alleged infection period. More detailed information has been obtained in the study of the ways of infection. According to our data, the most common route of infection was blood transfusion, which is the percentage of occurrence was 44%, and infection after dental procedures, which are found in 32% of the patients, which calls for timely HCV prevention - infection.

In accordance with the purpose and objectives of the study we compared the clinical variants of the disease and the replicative capacity of the virus. Thus, at 100% of the surveyed patients had replicative viral activity of them in 46% of patients with minimal activity chronic process, in patients with moderate to low disease activity despite the absence of severe symptoms, respectively at 24 and 18% of them. It should also be noted that although a minimal level of activity and moderate chronic process 6% of patients had cirrhosis and fatty liver. Minimal clinical symptoms of the disease and the lack of severe hyperenzymemia traditionally associated with the degree of hepatitis activity, not possible to estimate the real character of liver damage and determine the prognosis of the disease in these patients, who are mostly working-age persons.

Comparative analysis of activity indicators of liver enzymes and viral replication activities, we have also found that despite the high concentration of virus in the peripheral blood serum of the examined patients with moderate disease

had hyperenzymemia. Given this fact, it should be noted that in the absence of communication between viral load and the activity of liver enzymes in patients with HCV genotypes different doctors need to pay attention to the need to determine the level of viral load in patients, even with minor variations biochemical parameters.

One of the main immunogenetic characteristics of HCV is HCV genotype [67,70,80,88]. In accordance with the task we spent 6 identification of genotypes and subtypes of HCV. It was revealed 4 major HCV genotypes [1a, 1b, 2a and 3a], included in the group of genotypes, distributed on the territory of the Euro-Asian countries [54]. In most cases found are less favorable in terms of flow and treatment [1a and 1b] genotypes, which totaled 70%. These genotypes among the surveyed male patients [76.5%] were more common than among female patients [56.25%]. At the same time favorable genotypes [2a and 3a], which occurred in 30% of patients, including females were detected more frequently [43.75%] than among male patients [23.5%]. Data on the prevalence of HCV genotype 1 HCV patients in Uzbekistan are also in the works devoted to the study of the distribution of these genotypes in Uzbekistan carried out at the Institute of Immunology of the Academy of Sciences of Uzbekistan and the Nagoya University [80.104].

Upon further analysis of the distribution of HCV genotypes in patients with different infection routes we have found that genotypes 1a and 1b, in terms of adverse flow and treatment of diseases, more often detected. The findings were somewhat different from the results of studies conducted Y.M.Pawlotsky [94] and some other researchers, who note the existence of the relationship between genotypes and possible way of infection: drug addicts often met HCV type 3a, in patients who underwent blood transfusion, - 1b.

In assessing the impact of HCV genotype on the course of infection in the study, we have found that among all HCV genotypes identified in patients with genotype 1b was characterized by a long-term persistence of [8 ± 0,2 years]. These patients had marked changes in the liver parenchyma, such as steatosis and cirrhosis. Apparently, the reason for long-term persistence of the virus occurs and

thereby the development of severe complications is the high frequency of mutations in the hypervariable region [E2 / NS1] genotype 1b virus genome, which is the main target of immune attack. Our data confirm the results of the examination of patients infected with strains of genotype 1b, driven by other scientists, which revealed the characteristics of the genotype: patients had a longer duration of the disease, in this case, the researchers suggest, patients with HCV observed the development of severe complications [25, 67, 81].

With further comparative analysis of viral load in patients with HCV various genotypes we found that for patients with one [a, b] genotype was characterized by high HCVcoreAg, while in patients with 2a + 3a genotypes serum concentration peripheral blood was significantly lower, with a statistically significant difference [p = 0,01; p = 0,05]. Comparative analysis of viral load in patients with different hepatitis B virus is found an identical pattern. Thus, in patients with genotype 1 had high level of viremia, whereas in patients with genotype 2a and 3, the viral load was low concentrations [36, 100]. Apparently, the reason for the high concentration of virus in the blood serum of a high frequency of mutations in the genome of the hypervariable region 1 [a, b] genotype, located at the end of the 5th E2 / NS1 HCV-RNA gene. It is known that this gene section, which plays an important role in the virus escapes the immune response is responsible for the persistence of the virus that causes long-term nature of the persistent and long-lasting active HCV replication. Analysis of the data showed that, for patients with genotype 1 [especially 1b] is characterized by a minimum and expressed the degree of activity, more long-term persistence, as well as the presence of morphological changes in the liver parenchyma, such as signs of steatosis and cirrhosis, indicating that the hypervariable 1b genome genotype which contributes to an unfavorable course of chronic process. Also relevant is the need for analysis of the level of viremia, even in the presence of low biochemical parameters as at 1a and 1b genotypes of the hepatitis C virus characterized by a high rate of viral load, even at the minimum level of activity.

CONCLUSIONS

- The distribution of HCV genotypes in patients with chronic hepatitis C showed that genotype HCV is dominant and occur in 75% of the total surveyed.
- For genotype 1 is characterized by severe degree of activity, more long-term persistence, as well as for changes in the liver parenchyma, such as signs of steatosis and cirrhosis. It indicates the development of an unfavorable course of chronic process.
- In chronic viral hepatitis C there is a pronounced imbalance of cellular and humoral immune system, more pronounced at 1 genotype of the virus.

PRACTICE GUIDELINES

1. For the purpose of early detection of virological recommended compulsory comprehensive examination of patients with chronic hepatitis C, including the definition of the genotype of the virus, the definition of HCV RNA in serum and peripheral blood mononuclear cells and viral load.
2. Identification of patients with chronic hepatitis C genotype 1 is a poor prognostic factor for flow and outcome of the disease, and requires a thorough and personalized approach to therapy.

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