PROSPECTS FOR THE USE OF PCR IN THE DETECTION OF HEPATITIS (A LITERATURE REVIEW)

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The authors declare that they have no conflicts of interest

Keywords:
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Abstract

Relevance: *According to official WHO data, currently, about 325 million people in the world live with chronic hepatitis B and C, which makes hepatitis one of the most common infectious diseases in the world. There are many scientific studies aimed at a deeper understanding of hepatitis and the development of new methods of treatment and diagnosis. With the help of polymerase chain reaction, more sensitive tests for diagnosing hepatitis have been developed, allowing faster infection detection and treatment initiation. The PCR method for diagnosing hepatitis is justified and relevant, as it is one of the most effective and modern methods for detecting viral infection, which allows you to start treatment earlier and prevent the development of complications.*

The study aimed to evaluate the PCR method's efficiency in diagnosing hepatitis.

Methods: *In this review, the causes of hepatitis were investigated, and a comparative assessment of the effectiveness of various laboratory methods for diagnosing hepatitis, including enzyme immunoassay and polymerase chain reaction, was conducted. The analysis of scientific publications revealed the factors associated with the development of hepatitis and compared the effectiveness of ELISA, used for serological analysis of antibodies to hepatitis viruses, with PCR, designed to detect DNA or RNA of hepatitis viruses. The obtained results provide important information for improving the methods of diagnosis of hepatitis and more effective control of this serious infectious pathology.*

Results: *The analyzed scientific literature has shown that PCR diagnostics has several advantages over other methods of hepatitis diagnosis, such as ELISA, including higher sensitivity and specificity, the ability to detect hidden infections, and faster results. In addition, PCR diagnostics can be used to determine a specific strain of the virus, making it possible to take measures to treat and control infection. All these advantages confirm the scientific validity of using the PCR method in detecting hepatitis.*

Conclusion: *According to global statistics, PCR has a higher sensitivity and specificity in diagnosing hepatitis than ELISA. PCR can also be used to monitor the effectiveness of hepatitis treatment and determine viral load to make decisions about the need for treatment and evaluate its effectiveness.*

Гепатитті анықтаудағы ПТР қолдану перспективалары (әдебиетке шолу)

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Түйінді сөздер:
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Түйіндеме

Өзектілігі: *ДДҰ-ның ресми мәліметтеріне сәйкес, қазіргі уақытта әлемде шамамен 325 миллион адам созылмалы В және С гепатитімен өмір сүреді, бұл гепатиттерді әлемдегі ең көп таралған жұқпалы аурулардың біріне айналдырады. Гепатитті тереңірек түсінуге және жаңа емдеу мен диагностиканы дамытуға бағытталған көптеген ғылыми зерттеулер бар. Полимеразды тізбекті реакцияның көмегімен гепатитті диагностикалау үшін сезімтал сынақтар жасалды, бұл инфекцияны тезірек анықтауға және емдеуді бастауға мүмкіндік береді. Гепатитті диагностикалауға арналған ПТР әдісі негізделген және өзекті, өйткені ол вирустық инфекцияны анықтаудың ең тиімді және заманауи әдістерінің бірі болып табылады, бұл емдеуді ертерек бастауға және асқынулардың дамуын болдырмауға мүмкіндік береді.*

Зерттеудің мақсаты – гепатитті диагностикалау үшін ПТР әдісінің мәндерін бағалау.

Әдістер: *Бұл шолуда гепатиттің себептері зерттелді және гепатиттерді диагностикалаудың*

әртүрлі зертханалық әдістерінің, соның ішінде иммуноферменттік талдау мен полимеразды тізбекті реакцияның тиімділігін салыстырмалы бағалау жүргізілді. Ғылыми жарияланымдарды талдау әдістері гепатиттің дамуына байланысты факторларды анықтауға және гепатит вирусының антиденелерін серологиялық талдау үшін қолданылатын ИФА тиімділігін гепатит вирустарының ДНҚ немесе РНҚ-сын анықтауға арналған ПТР-мен салыстыруға мүмкіндік берді. Нәтижелер гепатитті диагностикалау әдістерін жақсарту және осы ауыр инфекциялық патологияны тиімдірек бақылау үшін маңызды ақпарат береді.

***Нәтижелер:** Талданған ғылыми әдебиеттер ПТР диагностикасының ИФА сияқты гепатитті диагностикалаудың басқа әдістеріне қарағанда бірқатар артықшылықтары бар екенін көрсетті, соның ішінде жоғары сезімталдық пен ерекшелік, жасырын инфекцияларды анықтау мүмкіндігі және жылдам нәтиже. Сонымен қатар, ПТР диагностикасын вирустың белгілі бір штаммын анықтау үшін қолдануға болады, бұл инфекцияны емдеу және бақылау шараларын қабылдауға мүмкіндік береді. Осы артықшылықтардың барлығы гепатиттерді анықтау кезінде ПТР әдісін қолданудың ғылыми негізділігін растайды.*

***Қорытынды:** Әлемдік статистика бойынша гепатиттердің ПТР диагностикасы ИФА диагностикасына қарағанда әдістің жоғары сезімталдығы мен ерекшелігін көрсетті. ПТР гепатитті емдеудің тиімділігін бақылау және вирустық жүктемені анықтау үшін қосымша қолданылуы мүмкін, бұл емдеу қажеттілігі туралы шешім қабылдауға және оның тиімділігін бағалауға көмектеседі.*

Перспективы использования ПЦР в выявлении гепатитов: обзор литературы

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Аннотация

***Актуальность:** Согласно официальным данным ВОЗ, в настоящее время около 325 миллионов человека в мире живут с хроническим гепатитом В и С, что делает гепатиты одной из наиболее распространённых инфекционных болезней в мире. Существует множество научных исследований, направленных на более глубокое понимание гепатитов и разработку новых методов лечения и диагностики. С помощью полимеразной цепной реакции, были разработаны более чувствительные тесты для диагностики гепатитов, что позволяет быстрее выявить инфекцию и начинать лечения. Метод ПЦР для диагностики гепатитов является обоснованным и актуальным, так как он является одним из самых эффективных и современных методов выявления вирусной инфекции, что позволяет начать лечение раньше и предотвратить развитие осложнений.*

***Цель исследования:** Оценить значения метода ПЦР для диагностики гепатитов.*

***Материал и методы:** В данном обзоре были исследованы причины возникновения гепатита и проведена сравнительная оценка эффективности различных лабораторных методов для диагностики гепатитов, включая иммуноферментный анализ и полимеразную цепную реакцию. Методы анализа научных публикаций позволили выявить факторы, связанные с развитием гепатита, и сравнить эффективность ИФА, который используется для серологического анализа антител к вирусам гепатитов, с ПЦР, предназначенной для обнаружения ДНК или РНК вирусов гепатитов. Полученные результаты предоставляют важную информацию для улучшения методов диагностики гепатитов и более эффективного контроля этой серьезной инфекционной патологии.*

***Результаты:** Проанализированная научная литература показала, что ПЦР-диагностика имеет ряд преимуществ перед другими методами диагностики гепатита, такими, как ИФА, включая более высокую чувствительность и специфичность, возможность обнаружения скрытых инфекций и более быстрый результат. Кроме того, ПЦР-диагностика может быть использована для определения конкретного штамма вируса, что позволяет принять меры по лечению и контролю инфекции. Все эти преимущества подтверждают научную обоснованность использования метода ПЦР при выявлении гепатитов.*

***Заключение:** ПЦР диагностика гепатитов по мировым статистическим данным показала более высокую чувствительность и специфичность метода, чем ИФА диагностика. ПЦР дополнительно может использоваться для мониторинга эффективности лечения гепатитов и определения вирусной нагрузки, что помогает в принятии решений о необходимости лечения и оценке его эффективности.*

Конфликт интересов:
Авторы заявляют об отсутствии конфликта интересов

Ключевые слова:
гепатиты, иммуноферментный анализ, полимеразная цепная реакция, чувствительность, обнаружение, ДНК, РНК.

Introduction

Hepatitis is a serious medical problem worldwide. They cause liver inflammation, which can lead to liver enlargement, cirrhosis, and even liver cancer. Hepatitis can be caused by various viruses, such as hepatitis A, B, C, D and E, as well as other factors (alcohol, toxicological, etc.).[1]

According to official data from the World Health Organization, about 325 million people worldwide are currently living with chronic hepatitis B and C, making hepatitis one of the most common infectious diseases in the world. Hepatitis C, for example, is responsible for most cases of chronic hepatitis, cirrhosis of the liver, and cancer.[2]

Thus, hepatitis is a science-based problem, and many studies are being conducted in this area to improve the diagnosis, treatment and prevention of this dangerous infectious disease.

There is a wealth of scientific research aimed at improving the understanding of hepatitis and developing new treatments and diagnostics. Using polymerase chain reaction (PCR), more sensitive tests have been developed for the diagnosis of hepatitis, which allows you to quickly detect the infection and start treatment.[3]

To detect hepatitis A, B, C, D, and Eviruses using real-time PCR, a special set of reagents (a set for quantitative and qualitative determination) is used, which contains impurities that specifically bind to viral ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). Real-time PCR allows you to monitor the growth of amplicons (increase in the number of copies of RNA or DNA in the reaction) in real time using fluorescent markers. Real-time PCR is a very sensitive and accurate method for detecting hepatitis A, B, C, D, and E viruses. PCR allows you to quickly and accurately determine the presence of the virus, as well as track the effectiveness of treatment and predict the possibility of relapse of the disease.[4]

In connection with the above, the introduction of the PCR method in the diagnosis of hepatitis is justified and relevant, as it is one of the most effective and modern methods for detecting viral infection, which allows you to start treatment earlier and prevent the development of complications.

In connection with the above, the introduction of the PCR method in the diagnosis of hepatitis is justified and relevant, as it is one of the most effective and modern methods for detecting viral infection, which allows you to start treatment earlier and prevent the development of complications.

The aim of the study was to evaluate the significance of the PCR method for the diagnosis of hepatitis.

Materials and methods: During the analysis of scientific publications, the causes leading to the occurrence of hepatitis were considered. This review also included a comparative evaluation of the effectiveness of various laboratory methods in the diagnosis of hepatitis, including enzyme immunoassay and polymerase chain reaction. The study of literature data provided information on factors associated with the development of hepatitis, and also allowed us to compare the effectiveness of these methods for detecting hepatitis-ELISA for serological analysis for detecting antibodies to hepatitis viruses and PCR for detecting DNA or RNA of hepatitis viruses.

Criteria for including sources:

- Research related to the diagnosis of hepatitis C and B.
- Studies describing PCR and ELISA methods in the context of hepatitis diagnosis.
- Articles available in English.

Criteria for excluding sources:

- Studies that use other laboratory methods to diagnose hepatitis, such as liver biopsy or scintigraphy.
- Articles that are not available in English.

Information was searched in the PubMed and Google Scholar databases for the following keywords: “hepatitis C diagnostics”, “hepatitis B diagnostics”, “polymerase chain reaction AND hepatitis”,” enzyme immunoassay AND hepatitis”,” laboratory diagnostics of hepatitis”,”methods for diagnosing viral hepatitis”.

The effectiveness of the PCR method in the diagnosis of hepatitis was evaluated according to the following parameters:

- Sensitivity: The ability of the PCR method to detect viral material even at low virus concentrations in the sample.
- Specificity: The ability of the PCR method to correctly identify the hepatitis virus and avoid false positive results.
- Positive and negative predictive values: The probability that a positive or negative PCR result corresponds to the presence or absence of hepatitis in the patient.
- Detection limit: The minimum amount of viral material that the PCR method can detect.
- Reproducibility: The possibility of repeatability of results with repeated analysis.

Detection and possible quantification of specific antibodies in body fluids is based on the use of sandwich enzyme-linked immunosorbent assays (ELISA). Recombinant antigens are used to capture circulating antibodies in микротитровыхthe wells of microtiter plates, microbeads, or special holders adapted to automated devices. The presence of antibodies is detected byanti-antibodies labeled with an enzyme that catalyzes the conversion of the substrate to a colored compound. The ratioof the optical density(OD) of the reaction (sample OD / internal control OD) is proportional to the number of antibodies in the sample. ELISA tests are cheap, easy to use, can be fully automated, and

can be easily adapted to large-volume tests. An increase in the concentration, i.e. optical density, indicates the presence of hepatitis B, C, D and so on antibodies.

PCR requires more than just the patient's blood and other biological samples. The principle of analysis is based on recording the amplification process of a selected specific DNA fragment, RNA consisting in repeated cycles: temperature denaturation, annealing of primers with complementary sequences, and completion of polynucleotide sequences from these primers by Tagpolymerase. Fluorescence signals are recorded in each PCR cycle. Real-time PCR methods have a wide dynamic range of quantitative and qualitative determination that is well suited to clinical needs (upper range of quantitative determination: 7-8 $\log_{10}$ IU / ml). In addition, real-time PCR is more sensitive than classical PCR, with lower detection limits on the order of 10-15 IU / ml. These tests do not give false positive results due to transitory contamination, and they can be fully automated. As a result, real-time PCR has become the method of choice for detecting and quantifying hepatitis DNA and RNA in clinical practice.[3,5]

Results: PCR is one of the most accurate and sensitive methods for diagnosing hepatitis, which can detect hepatitis viruses using small amounts of viral DNA or RNA. Due to the high sensitivity and specificity of the method, it is becoming an increasingly popular method for diagnosing hepatitis.[4,6]

One of the main prospects for using PCR diagnostics in hepatitis is the ability to detect the virus even before the appearance of clinical symptoms of the disease. This allows you to start treatment at an early stage, which increases the effectiveness of treatment and reduces the risk of complications. Moreover, PCR diagnostics can detect low concentrations of viral load, which is especially important for monitoring the effectiveness of treatment and assessing the risk of infection transmission.[7]

Another perspective of PCR diagnostics is the ability to detect various types of hepatitis viruses. There are several types of hepatitis, including hepatitis A, B, C, D, and E. Each type of hepatitis has its own characteristics and requires an individual approach to diagnosis and treatment. PCR diagnostics can be used to detect all types of hepatitis, which makes it a useful tool for complex diagnosis and treatment of patients with hepatitis.[8]

It is also worth noting that PCR diagnostics is becoming more accessible and convenient. New technologies and diagnostic methods make it possible to perform PCR tests quickly and efficiently in clinical laboratories. This simplifies the diagnostic process and ensures fast results, which is important for starting treatment and controlling the spread of infection.[9]

Thus, PCR diagnostics is a promising method for diagnosing hepatitis, which can be detected in 98% of cases.

Discussion: PCR is widely used to detect hepatitis, and there are many scientific studies confirming its effectiveness. In particular, studies were conducted in which it was shown that PCR diagnostics of hepatitis A, B and C has a high sensitivity and specificity. For example, a study published in the journal PLOS One showed that PCR diagnostics have a sensitivity of 98.3% and a specificity of 99.8% for detecting hepatitis B.[10] Another study published in the journal Hepatology[11] showed that PCR diagnostics has a high sensitivity and specificity in detecting hepatitis C and is able to detect the virus in the blood even at very low concentrations, which makes this method very effective for detecting infection in the early stages. Table 1 shows the types of hepatitis and diagnostic methods based on PCR and ELISA.

Table 1.
Types of hepatitis and methods of laboratory diagnostics

Hepatitis Types	PCR		ELISA PCR	
	Detection	Sensitivity	Detection	Sensitivity
A	Detection Hepatitis A RNA	Virus Detection 95 -100%	Detection of IgM and IgG antibodies to the virus	80 -90%
B	Detection of hepatitis B DNA	virus 95 -100%	Detection of HBsAg antigen and antibodies to the virus	70-80%
C	Detection of hepatitis C RNA	virus 95 -100%	Detection of IgM and IgG antibodies to the virus	80-85%
D	Detection of hepatitis D RNA	virus 95 -100%	Detection of HDVAg antigen and antibodies to the virus	75-85%
E	Detection of hepatitis E RNA	virus 95 -100%	Detection of IgM and IgG antibodies to the virus	75-85%

The table shows data for hepatitis A, C, D and E PCR diagnosis is based on the detection of viral RNA in the blood, while ELISA diagnosis is based on the detection of IgM and IgG antibodies to the virus. For hepatitis B, PCR diagnosis is based on the detection of viral DNA in the blood, and ELISA diagnosis is based on the detection of HBsAg antigen and antibodies to the virus. This table

shows the percentage of detection of different types of hepatitis using PCR and ELISA methods. As can be seen, PCR has a higher sensitivity for all types of hepatitis than ELISA. These statistics are based on many studies and meta-analyses conducted in different countries.[12,13]

Data on the number of hepatitis cases detected by PCR and ELISA diagnostics are collected and published by infectious disease control organizations, such as the World Health Organization (WHO)[14] and the Centers for Disease Control and Prevention (CDC) in the United States.[2,14]

Diseases associated with hepatitis B (HBV) can range from acute infection, asymptomatic carriage, and chronic infection to fulminant hepatitis.[15,16] Chronic infection can be associated with cirrhosis of the liver and hepatocellular carcinoma (HCC). The status of HBV infection can be determined using serological profiles; for example, the presence of HBsAg (on ELISA) is an important serological marker for diagnosing ongoing HBV infection, while the presence of only anti-HBc IgG or only anti-HBs indicates previous exposure to the virus. However, recent data indicate that routine serological profiles are not always reliable in determining the status of HBV infection. Transmission of HBV infection by HBsAg-negative blood transfusion has been documented, as well as perinatal transmission of HBV infection from HBsAg-negative mothers.[12,17,18] The presence of HBV infection with undetectable HBsAg has led to the introduction of the concept of latent, silent, or latent HBV infection. Latent HBV infection and its clinical consequences have been thoroughly studied recently.[19,20] From the use of PCR to detect HBV DNA, it is clear that low levels of HBV DNA remain detectable in the serum and liver tissue of some patients who purify HBsAg.[21,22] Moreover, the detection rate of HBV DNA was found to be highest in anti-HBc-positive/anti-HBs-negative individuals and lowest in anti-HBc-negative/anti-HBs-negative individuals.[16,19]

An international team of scientists in Zurich at a hepatitis B workshop agreed that anti-HBc testing should be mandatory for transplant donors, but with regard to blood banks, all participants agreed that national criteria should be developed that can be applied to the blood donor population. to determine whether anti-HBc screening is really necessary HBc. About 2.2% of Lebanese blood donors were positive for anti-HBc alone, but only 13% of them were positive for HBV DNA. The aim of this study was to evaluate on a national scale the frequency and clinical significance of anti-HBc in Lebanese blood donors from blood banks in various parts of the country.[23]

Patients with suspected acute hepatitis C infection should be tested for both HCV antibodies by ELISA and HCV RNA by real-time PCR with a lower detection limit of 10-15 IU / ml. The HCV main antigen test may be used instead of HCV RNA analysis, but diagnosis may be delayed for several days because these assays are less sensitive than HCV RNA methods. Four marker profiles can be observed depending on the presence or absence of any marker. The presence of HCV RNA in the absence of HCV antibodies strongly indicates acute HCV infection, which will be confirmed by seroconversion (i.e., the appearance of anti-HCV antibodies) after a few days or weeks. Patients with acute infection may also have both HCV RNA and anti-HCV antibodies at the time of diagnosis. In this case, it is difficult to distinguish acute hepatitis C from acute exacerbation of chronic hepatitis C or acute hepatitis of other origin in a patient with chronic hepatitis C.[16,23]

In recent years, the number of hepatitis cases detected by PCR has increased dramatically. For example, according to WHO, the number of cases of hepatitis C detected by PCR diagnostics in the world from 2010 to 2019 increased from 58 million to 71 million, while the number of cases detected by ELISA diagnostics decreased from 90 million to 59 million. Similar trends can be observed for other types of hepatitis, but specific figures may vary depending on the region and data source.[13,16]

Thus, from the available literature data, it is clear that PCR is increasingly used in the world to detect hepatitis, especially hepatitis C. For example, in the United States in 2019, 92% of all detected cases of hepatitis C were detected using PCR diagnostics. At the same time, ELISA diagnostics is still widely used to detect hepatitis.[13]

The website of the US Centers for Disease Control and Prevention has statistics on hepatitis, including the use of PCR and ELISA diagnostics. For 2019, they report that of the total number of diagnosed cases of hepatitis C in the United States, 91.9% were confirmed by a PCR test. The CDC also indicates that PCR diagnostics are becoming increasingly popular and preferred for detecting hepatitis C, as they have shown high sensitivity.[2,14]

In addition, according to the World Health Organization, statistics and data on the use of PCR and ELISA diagnostics in hepatitis are very accurate and high. According to WHO, in 2017, PCR diagnostics were used to detect hepatitis C in 86% of the world's countries, while ELISA diagnostics were used in only 39% of countries. In Kazakhstan, the ELISA method is used more than PCR, but when antibodies to hepatitis are detected, PCR confirmation is mandatory.[2,13]

Thus, the analyzed scientific literature showed that PCR diagnostics has a number of advantages over other methods of hepatitis

diagnosis, such as ELISA, including higher sensitivity and specificity, the ability to detect hidden infections, and faster results. In addition, PCR diagnostics can be used to identify a specific strain of the virus, which allows you to take measures for the treatment and control of infection. All these advantages confirm the scientific validity of using the PCR method for detecting hepatitis.

Conclusion

PCR diagnostics of hepatitis according to world statistics showed a higher sensitivity and specificity of the method than ELISA diagnostics, especially in the early stages of the disease. PCR can also be used to monitor the effectiveness of hepatitis treatment and determine viral load, which helps in making decisions about the need for treatment and evaluating its effectiveness. Based on the literature and statistical data, we have drawn several conclusions:

1. PCR diagnostics has a higher sensitivity (95-100%) than ELISA diagnostics (80-85%), and can detect the genetic material of the virus in very small amounts, which makes this method more effective for detecting infection in the early stages.

2. PCR diagnostics is highly specific and can distinguish between different strains of hepatitis viruses. This allows you to identify a specific strain of the virus and take measures to treat and control it.

3. PCR diagnostics can detect the presence of the virus directly, while ELISA diagnostics only detects the presence of antibodies to the virus. This means that PCR diagnostics can be used to determine the presence of the virus in blood, tissues, and other biological materials.

4. PCR diagnostics are usually faster than ELISA diagnostics, and results can be obtained in a few hours. This allows you to start treatment earlier and reduce the likelihood of infection spreading.

5. PCR diagnostics can detect hidden infections that may not be detected by other diagnostic methods, including ELISA diagnostics.

6. PCR diagnostics can be performed using a variety of samples of biological materials, including blood, urine, saliva, and others, while ELISA diagnostics usually require the use of blood.

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